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Nitrous Oxide versus Carbon Dioxide for Supercritical Fluid Extraction and Chromatography of Amines

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Supercritical N_2O has been evaluated and compared with supercritical CO_2 as a solvent for supercritical fluid extraction of different amines. Supercritical N_2O showed a higher solubility for amines and easily extracted both aliphatic and aromatic amines. The effect of substrate on extraction has been also examined. Supercritical N_2O has been used as a mobile phase, with different diameter columns, for supercritical fluid chromatography. The total response of flame ionization detection to supercritical N_2O using packed capillary and open tubular capillary columns demonstrated the feasibility of flame ionization detection with N_2O in a density programmed mode.

INTRODUCTION

In the past decade supercritical fluids have shown much promise for extraction and chromatographic separation of thermally labile, moderately polar, and high molecular weight compounds (1–3). The greater diffusivity and lower viscosity afforded by a supercritical fluid relative to a liquid have been demonstrated to yield faster separations and more efficient extractions. In general a supercritical fluid (SF) in order to be a useful solvent must have good solvating properties and low critical parameters and be relatively inert, nontoxic, inexpensive, and compatible with most methods of detection.

Supercritical CO_2 fulfills most of these requirements. Its compatibility has been examined with different types of flame and spectrometric detectors (flame ionization detector (FID), thermionic detector (TID), flame photometric detector (FPD), microwave induced plasma (MIP), inductively coupled plasma (ICP), UV, Fourier transform infrared (FT-IR), mass spectrometry (MS), NMR, fluorescence) (4–8). Another solvent that has characteristics similar to CO_2 is N_2O, which has low toxicity and low critical parameters (P_c = 73 atm, T_c = 37 °C). Unlike CO_2 N_2O has a permanent dipole moment. Lauer et al. (9) and later Wright et al. (10), however, have shown that supercritical CO_2 and N_2O are nearly identical in solvation properties and each elutes most solutes in the same range of densities.

Both supercritical CO_2 and N_2O have been compared (11) as mobile phases for group separation of high-boiling petroleum samples and crude oil into saturates, aromatics, and polar compounds. Supercritical N_2O was found to give less tailing of the back flush aromatics and polar compounds. The detection limit was stated to be in the microgram range with N_2O. Supercritical fluid chromatography (SFC)/FID of unsubstituted and substituted heavy carboxylic acids (C_{18}–C_{30}) and various polymer additives has been demonstrated with both CO_2 and N_2O (12). Elution from open tubular capillary columns with nonpolar methylsilicone or phenylmethylsiloxane stationary phases was achieved in less than 20 min. Leyendecker et al. (13) have studied the chromatographic behavior of a number of low boiling eluents from an un-

modified silica gel packed column with a number of supercritical fluids including N_2O. The use of N_2O for extraction of polycyclic aromatic hydrocarbons from urban dust, fly ash, and river sediment has been reported (14). N_2O with 5% methanol gave the best recoveries when compared with CO_2, N_2O, C_2H_6, and 5% methanol-modified CO_2. One of the major advantages of N_2O as a solvent (for either extraction or chromatography), which has not been greatly emphasized, is its low chemical reactivity. Since N_2O is not acidic like CO_2, it could have a real benefit for extraction or elution of organic bases, some of which tend to react with CO_2 and form insoluble carbamates.

While considerable work has previously been published regarding the merits of N_2O versus CO_2 in SF studies, its application to organic bases is minimal. This paper considers the use of supercritical N_2O as a solvent for extraction and as a mobile phase for chromatography. In the first part of this study, attention is given particularly to the extraction of different amines using both supercritical N_2O and CO_2. Separation of the extracted components was achieved via gas chromatography (GC). Extraction performance was also evaluated for various situations where the amines were adsorbed on different substrates. In the second part of the study, supercritical N_2O and CO_2 were used as mobile phases for SFC. Different size internal diameter columns were investigated along with the FID response at different N_2O pressures. The feasibility of using different spectrometric detectors (UV and FT-IR) with N_2O mobile phase was also evaluated.

EXPERIMENTAL SECTION

The extractions were carried out by using a Suprex Model SFE/50 (Pittsburgh, PA) extractor equipped with an electronic four-port high-pressure selector valve. The extraction system was coupled to a Hewlett-Packard (Avondale, PA) Model 5890 gas chromatograph equipped with a cryogenic oven cooling option (Figure 1). The SFE/50 was coupled to the HP 5890 by a $^1/_{32}$ in. o.d. × 0.007 in. i.d. stainless steel tube. It was necessary to restrict the flow by crimping the end of the tubing to attain a decompressed flow rate of 70 mL/min at 400 atm. The tubing was inserted through the septum in the split/splitless capillary GC injector port. The temperature of the injection was maintained at 220 °C. A Suprex extraction vessel of 0.25 mL was employed.

A mixture containing three aliphatic primary amines (heptylamine, dodecylamine, and octadecylamine), a secondary aliphatic amine (N-methylheptylamine), a tertiary aliphatic amine (tributylamine), and two aromatic amines (2-ethylaniline, diphenylamine) was employed. The concentration of each component ranged from 1 to 2 $\mu g/mL$. Different substrates (celite, soil, silica) were spiked with 5 μL of the mixture, and the matrix was subsequently extracted. Two replicate extraction/gas chromatography experiments were obtained with each substrate. Supercritical CO_2 and N_2O from Scott Specialty Gases (Plumsteadville, PA) were used for both supercritical fluid extraction and SFC. Each extraction was performed at 60 °C and 400 atm (approximately 0.20 g of each fluid) for 20 min in the static mode followed by 20 min in the dynamic mode. This corresponded to
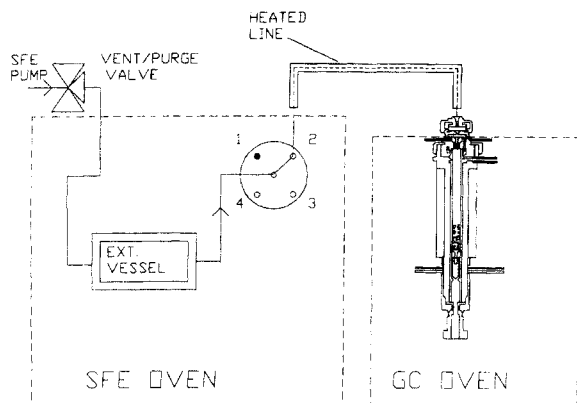
Figure 1. Schematic of SFE/GC interface.

130 μL/min flow of compressed N_2O through the cell in the dynamic mode. Since N_2O is known to support combustion, one should exercise caution in using this material as an extraction medium at relatively high pressure in a resistively heated oven. For this work no special precautions, however, were envoked since the decompressed flow rate was approximately 70 mL/min. While in the dynamic mode the extractants were collected at the head of a GC column (HP-1 cross-linked methylsilicone, 20 m × 320 μm i.d.) which was cryogenically held at different temperatures ranging from −15 to +15 °C. After 20 min of dynamic extraction/collection, an additional 2–4 min of equilibration time was needed in order for the helium carrier gas to clean the system of any residual N_2O or CO_2, so that the FID signal would stabilize. Next the GC program was started with a hold at 0 °C for 0.1 min and then temperature was programmed to 300 °C at a rate of 15 °C/min where it was held for 10 min.

The SFC analyses were performed with a Suprex (Pittsburgh, PA) Model 200A supercritical fluid chromatograph equipped with a flame ionization detector. The columns used in this study included two Deltabond (Keystone Scientific, Bellefonte, PA) cyanopropyl stainless steel columns (i.e. 25 cm × 1.0 mm i.d., 5-μm particle size, and 25 cm × 0.5 mm i.d., 5-μm particle size) and a SB-25 cyanopropyl (Lee Scientific, Salt Lake City, UT) fused silica capillary column (10 m × 0.1 mm i.d., 0.1 μm film thickness). Tapered restrictors for each packed column and for the capillary column were connected to the respective column via a zero dead volume adapter (Anspec Co., Ann Arbor, MI). Average linear velocities for each column were as follows: open-tubular capillary, 23.2 cm/s; packed capillary, 6.2 cm/s; packed (1.0 mm, i.d.); 0.47 cm/s. SFC/FT-IR data were collected with a Nicolet (Madison, WI) 5SXC spectrometer equipped with a 0.6 mm i.d. × 5 mm path length (1.4 μL) high-pressure flow cell. All spectra were obtained in real time at 8-cm^{-1} resolution. UV chromatograms were obtained by using a Suprex Model 206 variable-wavelength UV detector equipped with a high-pressure flow cell (250 nL).

RESULTS AND DISCUSSION

The primary object of this research was to evaluate supercritical N_2O as a solvent for extraction and as a mobile phase for chromatography. In the first part of this study, supercritical N_2O was evaluated and compared with supercritical CO_2 for the static extraction of various aliphatic and aromatic amines spiked onto three different substrates (celite, soil, silica). In the second part of our study, supercritical N_2O was evaluated and compared with supercritical CO_2 for the separation of a standard mixture using a flame ionization detector. The use of supercritical N_2O was also evaluated with ultraviolet (UV) and Fourier transform infrared (FT-IR) spectrometric detectors. For the extraction experiments gas chromatography (GC) was used to assay the extracted components.

Initially, supercritical fluid extraction (SFE) with N_2O was performed on the amine mixture previously adsorbed onto celite. The extractables were cryogenically collected at the head of a GC column at three different temperatures (−15, 0, 15 °C). Identification of each extracted component was
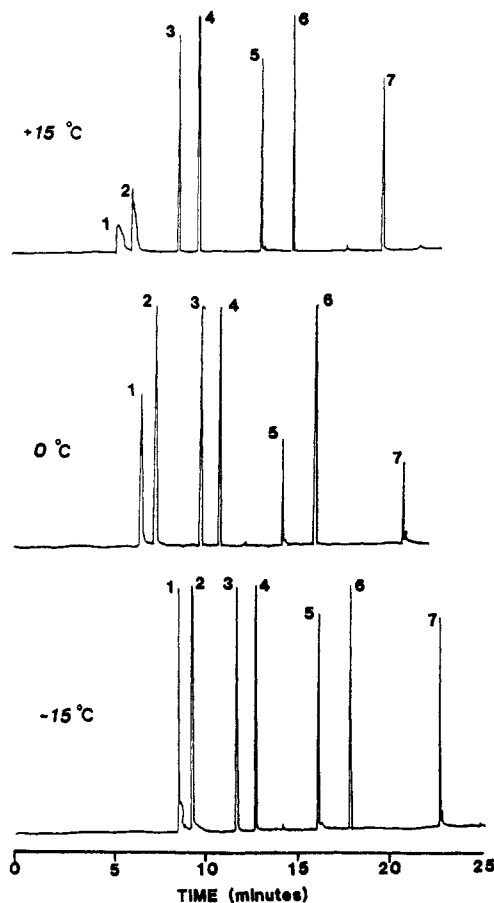

Figure 2. Effect of cryogenic trapping temperature on the SFE/GC/FID analysis of amines using supercritical N_2O as a solvent for extraction and celite as the substrate: (1) heptylamine, (2) N-methylheptylamine, (3) 2-ethylaniline, (4) tributylamine, (5) dodecylamine, (6) diphenylamine, (7) octadecylamine. See Experimental Section for chromatography parameters.

made possible by extracting and analyzing pure compounds prior to examination of the amine mixture. It can be seen (Figure 2) from the chromatograms that the most volatile (i.e. earliest eluting) compounds required a lower cryogenic collection temperature in order to obtain efficient analyte focusing and narrow GC peaks. The higher molecular weight and less volatile compounds were, however, collected efficiently even at relatively high temperatures (+15 °C). Similar results were previously reported by Hawthorne et al. (15) on the effect of cryogenic collection temperature on subsequent GC performance.

The same set of experiments was performed with CO_2 as a solvent instead of N_2O. The cryogenic collection temperature was maintained at 0 °C because it was found to be optimum for N_2O and CO_2. Only three compounds were apparently extracted by CO_2 and separated by GC, Figure 3. The peaks were identified as 2-ethylaniline, tributylamine, and diphenylamine. It is reasonable to expect that each of the other amines, which happen to be highly basic primary and secondary aliphatic amines, reacted with CO_2 thereby forming insoluble products which were not extracted by CO_2 under the conditions employed. In support of these findings, Ashraf-Khorassani et al. (16) have previously shown that aromatic and tertiary aliphatic amines can be readily eluted from packed columns with unmodified supercritical fluid CO_2. It is interesting to note the GC trace (Figure 3) on a second CO_2 extraction of the same amine/celite sample. More specifically each of the three components, 2-ethylaniline, tributylamine, and diphenylamine, are again observed in the GC analysis, indicating an incomplete first extraction. On the
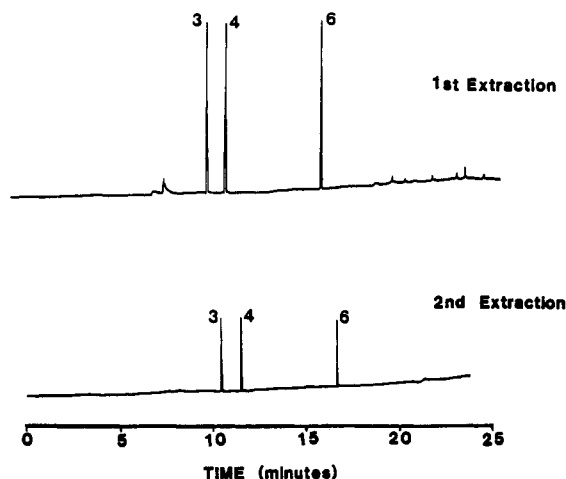
Figure 3. SFE/GC/FID analysis of amines from celite using supercritical CO_2 as a solvent for extraction. See Figure 2 for peak identification.
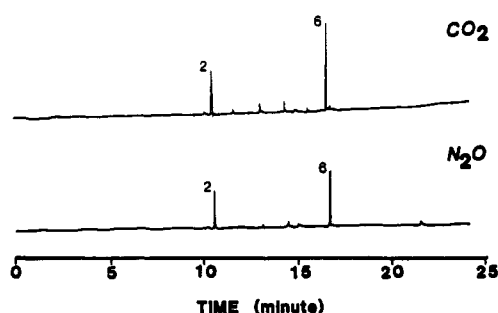

Figure 4. SFE/GC/FID analysis of amines from silica using supercritical N_2O and CO_2 for extraction. See Figure 2 for peak identification.

Figure 5. SFE/GC/FID analysis of amines from soil using supercritical N_2O and CO_2 for extraction. See Figure 2 for peak identification.

Figure 6. Separation of (1) diphenylamine, (2) naphthol, and (3) caffeine on SB-25 cyanopropyl capillary and Deltabond cyanopropyl packed capillary columns with 100% N_2O and FID at 100 °C.

other hand, the GC trace of a second supercritical N_2O extraction of the amine/celite sample under the same extraction and chromatography conditions as employed for the supercritical CO_2 extraction did not show any evidence of an incomplete first extraction of amines. These results suggest that supercritical N_2O has higher solubility than supercritical CO_2 for the extraction of these amines from this matrix. No doubt the inertness of N_2O toward amines is a major contributing factor since both CO_2 and N_2O are known to have similar solvating power.

This study was further extended to determine the effect of different substrates (celite, silica, and soil) on the extraction of the same amine mixture using supercritical N_2O and CO_2. Figures 4 and 5 show the SFE/GC of amines from silica and soil matrices. GC traces from both the supercritical N_2O and CO_2 extraction of the silica matrix showed only two peaks (ethylaniline and diphenylamine). It is believed that all of the more basic aliphatic amines were irreversibly adsorbed onto the silica and not extracted. In support of this explanation, it has been previously reported (17) that silica has strong adsorption for most basic compounds. SFE/GC of the amine mixture with individually supercritical N_2O and CO_2 from soil are shown in Figure 5. Results obtained from the extraction of amines from soil were similar to the results obtained for celite (i.e. all the amines were extracted from soil by using supercritical N_2O and only tertiary aliphatic and aromatic amines were extracted with supercritical CO_2). The spiked soil extract, however, exhibited a prolific background of GC peaks which were not identified.

In the second part of this research, three columns of different internal diameter (100 μm capillary, 500 μm packed capillary, and 1.0 mm packed microbore) were used with flame ionization detection (FID) and supercritical N_2O as the mobile phase for the separation of a mixture containing diphenyl-
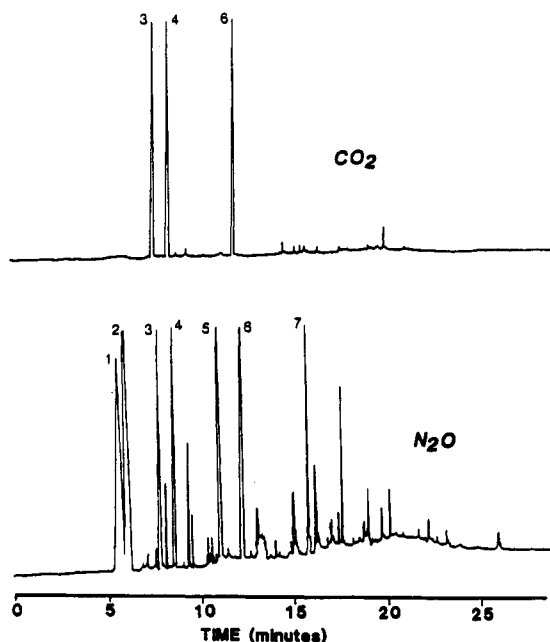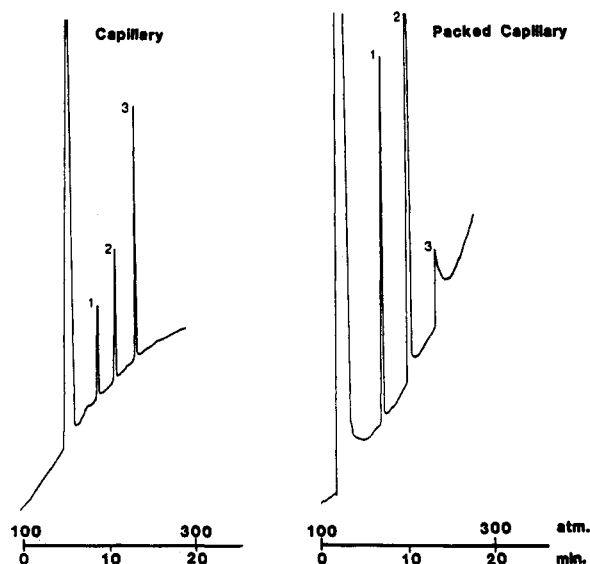
amine, naphthol, and caffeine. It was observed that as the diameter of the column increased, the overall FID response increased due to the higher relative concentration of N_2O in the flame. In SFC, both packed capillary and wall coated open tubular capillary columns have very low flow rates compared to a microbore packed column; therefore, the FID response was not as large for the former two columns. The increased response is due to the fact that N_2O is an oxidizer; consequently, increasing the N_2O pressure changes the fuel/oxidizer ratio which causes the base-line rise. Figure 6 shows the separation of diphenylamine, naphthol, and caffeine with the open tubular capillary and packed capillary columns. The microbore packed column was not effective in this case since the extremely high base-line rise precluded detection of the separated components. These data, nevertheless, demonstrate the feasibility of pressure-programmed separations involving N_2O and FID. For comparison the same mixture was separated on these two columns plus the microbore column using supercritical CO_2 as a mobile phase and FID as a detector.
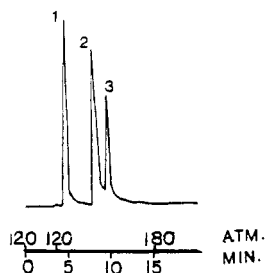
Figure 7. Separation of (1) diphenylamine, (2) naphthol, and (3) caffeine on Deltabond cyanopropyl packed column (10 cm × 1.0 mm i.d.) with 100% CO_2 and UV (254 nm) detection at 75 °C.

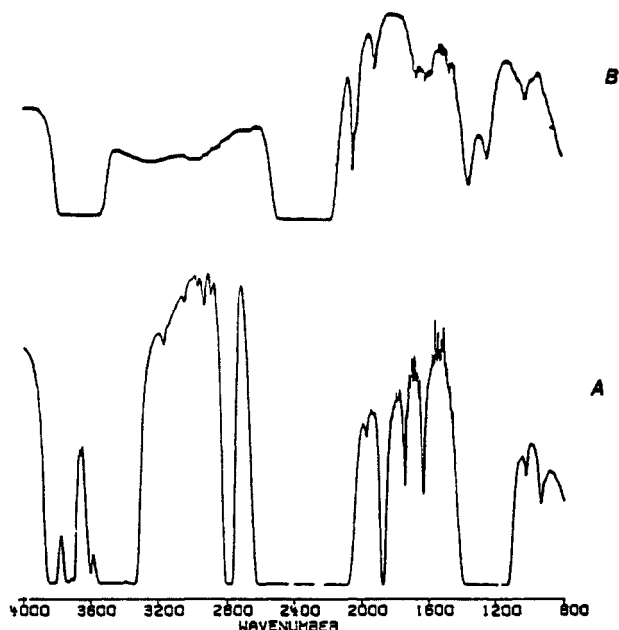**Figure 8.** FT-IR spectra of supercritical fluid (A) N_2O and (B) CO_2 at 2000 psi and 34 °C.

The same elution order with comparable elution time as supercritical N_2O was observed.

In the last part of this study the feasibility of supercritical N_2O and CO_2 with various spectrometric detectors was compared. Using fixed wavelength UV, we were able to detect each separated component (i.e. diphenylamine, naphthol, caffeine) employing a 10 cm × 1.0 mm i.d. packed column and both supercritical N_2O and CO_2 as the mobile phase (Figure 7). No base-line drift was observed due to pressure programming in any case. Previously supercritical N_2O and CO_2 had been compared with a UV detector with similar results, but this study was performed under isobaric/isothermal conditions (9). Next FT-IR spectra of both neat N_2O and CO_2 were compared under supercritical conditions (i.e. 140 atm

and 35 °C). While the FT-IR spectrum of supercritical CO_2 has been previously reported, we have found no spectrum of N_2O under supercritical conditions. Figure 8 shows the IR spectra of both supercritical fluids. The poor transparency through the mid-IR region suggests that supercritical N_2O may not be a suitable mobile phase for separations using on-line FT-IR as a detector.

In conclusion, N_2O has been examined and compared with CO_2 in regard to (a) solvent for supercritical fluid extraction of amines, (b) total response to flame ionization detection at different pressures using different size internal diameter columns, and (c) usage with different spectrometeric detectors. The extraction study demonstrates great potential of supercritical N_2O for the extraction of all types of amines, although the nature of the matrix is critical if complete extraction is desired. Flame ionization detection showed a base-line rise with pressure programming of supercritical N_2O; however, because the flow of N_2O from packed and open tubular capillary columns is very small, its use with FID is quite feasible. Finally, the use of a UV spectrometric detector with N_2O was reasonably good. Given the wide usage of organic bases, N_2O rather than CO_2 appears to be a viable candidate for performing SFE/SFC studies.

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