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DETERMINATION OF NITROGEN COMPOUNDS BY SUPERCRITICAL FLUID CHROMATOGRAPHY USING NITROUS OXIDE AS THE MOBILE PHASE AND NITROGEN-SENSITIVE DETECTION

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SUMMARY

A supercritical fluid chromatography system with nitrous oxide as the mobile phase and a nitrogen sensitive gas chromatographic detector was evaluated for the determination of amines and their amide and carbamate derivatives. Special interest was focused on the detector performance. The detector sensitivity for amines was comparable with that observed when the same detector was used in gas chromatography. However, the selectivity towards hydrocarbons was 25–50 times lower, only *ca.* 200. No variation of detector sensitivity was observed for amines and their derivatives when the system pressure was varied. Methanol was found to be the best sample solvent, with a detector response more than 50 times lower than those of other solvents tested. Linear calibration graphs were obtained in the concentration range 10–1000 ppm for amide and carbamate derivatives. For free amines, however, a curvature was found below 100 ppm, at least partly depending on adsorption in the column. The precision in peak area measurements in pressure-programmed experiments at concentrations above 100 ppm was *ca.* 4%.

INTRODUCTION

The technique of using supercritical fluids, *e.g.*, carbon dioxide and nitrous oxide at high pressures, as mobile phases in combination with capillary columns with small inner diameters, is under rapid development. Several review articles discussing the merits and obstacles of supercritical fluid chromatography (SFC) as a separation technique have recently appeared[1–6].

One very interesting feature, to which special attention has been paid in this work, is that separations can be performed under conditions similar to liquid chromatography with a mobile phase of high solvating power, while ordinary gas chromatographic detectors can be employed.

Concerning the type of molecules investigated, most work has hitherto been directed towards the possibility of expanding the molecular weight range of molecules which can be separated by gas chromatography (GC). Less attention has been paid to the possibility of analyzing thermolabile substances near room temperature. Still less considered is the possibility of extending the applicability of GC to the separation of

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low concentrations of polar compounds. The concentrations of the analytes have generally been high, most often above 0.1%. In many cases the concentrations are not stated, which makes the results difficult to interpret, as adsorption effects are expected, influencing the peak shape and retention.

Investigations of polar nitrogen compounds such as amines, alkanolamines and aliphatic amides are very rare, especially those involving short-chain molecules in low concentrations. In a compilation of 335 representative chromatograms published at a recent conference[7], only about 5 chromatograms showed amines and related compounds, all using CO_2 as a carrier. Low solute concentrations were not studied in any of those five. Polar and basic compounds such as polyamines and alkanolamines are difficult or even impossible to handle at low concentrations by GC and difficult to detect with liquid chromatographic detectors. For these compounds, SFC can be a good alternative.

In this work we present an investigation concerning determinations of nitrogen compounds on columns with different polarities using nitrous oxide (N_2O) as a mobile phase and nitrogen sensitive detection. This combination has not, to our knowledge, hitherto been exploited for analysis of basic compounds. Nitrous oxide was chosen as the mobile phase instead of the most commonly used carbon dioxide, to eliminate the risk of reactions with analytes containing primary or secondary amine functions. The permanent dipole moment of nitrous oxide was also expected to give better solvation power for molecules without aromatic ring systems. A nitrogen sensitive detector was chosen instead of the more common flame ionization detector. Our hope was that the high selectivity and low detection limits obtained for nitrogen compounds with the nitrogen detector in GC with nitrogen as a carrier gas would also be obtained in SFC with nitrous oxide as the mobile phase.

EXPERIMENTAL

Equipment

The equipment is shown in Fig. 1. It consists of a pump (Model 8500; Varian, Walnut Creek, CA, U.S.A.) and a gas chromatograph (Model 3710, Varian) equipped with a thermionic, nitrogen–phosphorus detector (Model TSD, Varian). The pump was modified to work under constant pressure, as described[8]. A digital voltmeter (DVM) showed the pressure directly in bar, a digital pulse counter measured the

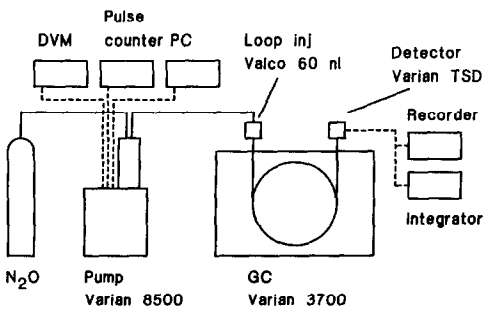

Fig. 1. Schematic diagram of the SFC equipment used in this work.

flow-rate (3600 pulses correspond to 1 ml) and a table-top computer (PC), ABC-80; Luxor, Motala, Sweden) was used for creating linear and quadratic pressure gradients. Samples were injected using a manual, 60-nl loop injector (Model CI4W; Valco, Houston, TX, U.S.A.). Chromatograms were evaluated with a digital integrator (Model 3390A; Hewlett-Packard, Palo Alto, CA, U.S.A.).

The capillary column was connected with a zero dead-volume union (Valco) to a 50-μm frit restrictor (Lee Scientific, Salt Lake City, UT, U.S.A.). The restrictor end was positioned 1–2 mm below the outlet of the detector tip and swept with a make-up gas (25 ml/min of nitrogen) entering at the detector base.

Detector settings were typically: hydrogen flow-rate 1.2 ml/min, air flow-rate 210 ml/min, bead current 3.27 A (580 scale divisions) and bias voltage 7 V. For details concerning these settings, see below. Nitrous oxide was used as the mobile phase, in some experiments modified with low concentrations of methanol. Methanol mixtures, 0.1–1%, were prepared by adding small amounts of methanol directly to the pump cylinder (volume 250 ml) before pressurization of the system with nitrous oxide from the supply cylinder.

Typical flow-rates from the pump at room temperatur and 100 bar (measured by the pulse counter) were 6 μl/min. A change of the pressure from 80 to 160 bar typically increased the flow-rate by about 35%.

Columns

The columns investigated were DB-5 (95% dimethyl–5% diphenyl polysiloxane), 10 m × 50 μm I.D., film thickness 0.2 μm (J&W Scientific, Folsom, CA, U.S.A.) and DB-17 (50% methyl–50% phenyl polysiloxane), 10 m × 50 μm I.D., film thickness 0.10 μm (J&W) and SB-octyl-50 (50% *n*-octyl-50% methyl polysiloxane), 10 m × 100 μm I.D., film thickness 0.5 μm (Lee Scientific).

Chemicals

All substances used as solutes are listed in Table I together with abbreviations. The diamines (2,4-TDA, 2,6-TDA and MDA) were obtained from Merck (Darmstadt, F.R.G.). Their carbamate derivatives (2,4-TDC, 2,6-TDC and MDC) were made by reacting the corresponding diamine with ethyl chloroformate (Sigma, St. Louis, MO, U.S.A.) according to the procedure described[9]. The amide derivatives (2,4-FTDA, 2,6-FTDA and FMDA) were prepared at the Department of Occupational Medicine (University Hospital of Lund). Tri-*n*-butylamine (p.a.), tri-*n*-decylamine (purum), tri-*n*-hexylamine (>95% by GC) and tri-*n*-pentylamine (>97% by GC) were obtained from Fluka (Buchs, Switzerland). Tri-*n*-octylamine (>95% by GC) and triethanolamine (p.a.) were obtained from Merck. Solvents used were all of highest available purity (p.a. or HPLC grade).

RESULTS AND DISCUSSION

Optimization of the detector

After initial settings of the hydrogen flow-rate, air flow-rate and bead current according to our experience of the detector performance in capillary GC, the flow-rate of the make-up gas was varied in the range 10–40 ml/min with 2,6-TDC as a test substance. Within a flow-rate range of 20–30 ml/min. the peak heights for the test

TABLE I

COMPOUNDS INVESTIGATED

Name	*Abbreviation*
Dibutyl phthalate	DBP
n-Hexadecane	
2,4-Toluenediamine	2,4-TDA
2,4-Toluenediethyldicarbamate	2,4-TDC
2,4-Toluenediamine (perfluorobutyro derivative)	2,4-FTDA
2,6-Toluenediamine	2,6-TDA
2,6-Toluenediethyldicarbamate	2.6-TDC
2,6-Toluenediamine (perfluorobutyro derivative)	2,6-FTDA
4,4'-Diaminodiphenylmethane	MDA
4,4'-Diaminodiphenylmethane (diethylcarbamate derivative)	MDC
4,4'-Diaminodiphenylmethane (perfluorobutyro derivative)	FMDA
Tri-*n*-butylamine	
Tri-*n*-pentylamine	
Tri-*n*-hexylamine	
Tri-*n*-octylamine	
Tri-*n*-decylamine	
Triethanolamine	

substance were almost equal. At 10 and 40 ml/min, the signal was about 8% lower than at 25 ml/min, the value used in further experiments.

The bead current was set at 3.27 A (580 scale divisions) after testing the bead under GC conditions. This value was chosen so that the sensitivity for nitrogen-containing compounds was high but still permitted an acceptable lifetime of the bead. A high bead temperature gives high sensitivity and selectivity but decreases the lifetime. When using this detector for GC, with nitrogen or helium as the carrier, a setting at 3.21–3.31 A (550–600 scale divisions) gives a bead lifetime which usually exceeds 6 months of continuous use. Each bead is manufactured manually and settings which give equal performance differs somewhat from bead to bead.

With a make-up flow-rate of 25 ml/min and a bead current of 3.27 A (580 scale divisions), the following parameters were varied: hydrogen flow-rate between 0.9 and 6.5 ml/min, air flow-rate between 135 and 275 ml/min and bias voltage between 6 and 9 V. Optimization was performed using the Simplex technique[10] with a test mixture of 2,6-TDC (A) and eicosane (B) in concentrations of 100 ppm and 0.2%, respectively. The maximum of the product of the sensitivity for A times the selectivity compared to B [(peak height of A)^2/(peak height of B)] was used as the criterion of maximum performance. All measurements were performed at a constant pressure (120 bar) of the supercritical fluid. The optimization procedure converged after eleven steps to the values 1.2 ml/min, 230 ml/min and 7.0 V, respectively. With these settings, a selectivity of about 200 was obtained.

The selectivity obtained here is much less than that in GC where values of 5000–10 000 are generally obtained for comparable compounds. This is probably due to the use of nitrous oxide as the carrier. The sensitivity for nitrogen compounds such as amines and carbamates is, within experimental uncertainties, the same as in GC. This means that the decrease in selectivity is due to an increase in sensitivity for hydrocarbons in the detector. Such an increase has been observed for the electron-

capture detector after doping the carrier gas with nitrous oxide[11]. In the nitrogen sensitive detector used here, ions are stated to be formed by a thermoionic process. However, the effects can be anticipated to be similar to those in an electron-capture detector. The different chemistry obtained around the bead when changing from nitrogen to nitrous oxide as the carrier is also reflected by the unexpected low value (1.2 ml/min) of the optimum hydrogen flow-rate compared to flow-rates of 2.5–4 ml/min normally used in GC.

Detector performance at varying system pressure

The detector optimization above was performed at constant pressure, *i.e.*, constant mass flow of nitrous oxide to the detector. The most important technique in SFC for obtaining good chromatographic behaviour, pressure programming, leads to a change of mobile phase flow-rate. A possible influence of the flow-rate of N_2O on the selectivity suggested by the experiments presented above might influence quantitation in pressure-programmed experiments. Experiments were therefore performed at different pressures, using a test mixture of 2,6-TDC (500 ppm), MDA (500 ppm) and dibutyl phthalate (10%) dissolved in methanol, on a DB-17 column.

The different peak shapes and retention times obtained at each pressure create a difficult task for a simple electronic integrator. In order to obtain the necessary accuracy in the peak area measurements, the cut-and-weigh method had to be applied. Fig. 2 shows the dependence of peak areas on the system pressure. Above 100 bar the peak areas of 2,6-TDC and MDA are independent of the pressure, while the peak area for dibutyl phthalate increases markedly as the pressure and thus the mass flow-rate of nitrous oxide to the detector is increased. As the system pressure increases, retention times decrease, leading to an increased mass flow-rate of analyte into the detector. To

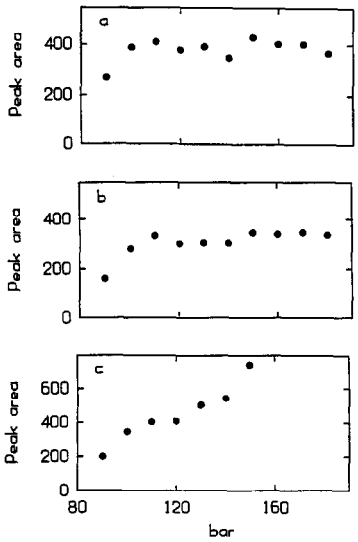

Fig. 2. Peak areas for some model substances *versus* the system pressure. (a) 2,6-TDC (500 ppm), (b) MDA (500 ppm) and (c) DBP (10%) (for abbreviations, see Table I). Sample solvent: methanol. Column: 10-m DB-17. Temperature: 130°C.

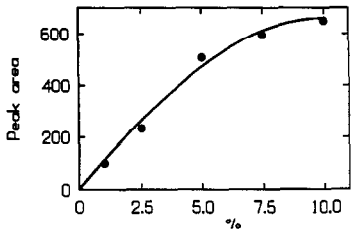

Fig. 3. Calibration curve for DBP (dibutyl phthalate). System pressure 140 bar; other chromatographic conditions as in Fig. 2.

ascertain that this effect was not responsible for the increased response for dibutyl phthalate shown in Fig. 2c, a calibration graph of peak area *versus* solute concentration was made at constant pressure. At this curve is convex due to detector overloading above 5%, as shown in Fig. 3, the effect of increased mass flow-rate of the analyte in fact leads to a decrease in the detector response. Thus, with the concentration of dibutyl phthalate used, the effect of the nitrous oxide mass flow-rate on the sensitivity is underestimated: at 140 bar, only about 70% of the real effect is observed. At lower pressures, the discrepancy is smaller.

To exclude the possibility that the oxygens in the dibutyl phthalate molecule influence the results of the experiment described above, a similar plot of peak area *versus* pressure was made with 2% *n*-hexadecane in diethyl ether on a DB-5 column. In the pressure range 90–160 bar a similar increase was observed.

The lower values of the peak areas, measured for 2,6-TDC and MDA at 90 bar, are probably due to adsorption on the column. With a reversible adsorption, which seems most likely, the decrease in peak area is an artifact in the measurements due to the difficulty in distinguishing the tail of the peak from the baseline.

At low pressure, the solvation power of nitrous oxide is relatively poor so the situation is similar to conditions in GC. In GC, with non-polar stationary phases and polar analytes, we[12] and others[13] have shown the possibility of adsorption of the analyte at the surface of the stationary phase as well as on the surface of the support material. The possibility that the low peak area at low pressures is due to poor solubility of the sample solvent in the carrier fluid, which may result in a thin film of sample solution on the walls of the injection loop, is ruled out here because of the long injection times (at least 10 s). This matter is further discussed below.

The results in Fig. 2 where the peak area increases about 2.5 times from 100 to 150 bar for dibutyl phthalate and is unchanged above 100 bar for 2,6-TDC and MDA, together with the fact that the selectivity is about 200 for these nitrogen compounds compared to dibutyl phthalate, gives information about the reactions in the detector. The results can be explained only if the increased ionization, occurring when nitrous oxide is present in the gas mixture around the detector bead, is rather non-specific. The increased ionization at the carbon chain in the nitrogen compounds will then with a selectivity of 200 contribute to an area increase of only about 1–2%, within experimental errors. This explanation is also supported by the similar results obtained for *n*-hexadecane mentioned above. Therefore it seems likely that the reactions between nitrous oxide and the compounds considered involve an attack on the carbon chain and that heteroatoms such as nitrogen and oxygen (in the phthalate) do not

contribute significantly to the ionization process induced by nitrous oxide. This is also consistent, as mentioned earlier, with the results obtained for hydrocarbons, using an electon-capture detector, doped with nitrous oxide[11]. These results also imply that quantitations of nitrogen compounds in pressure-programmed experiments will be possible without elaborate standardization procedures.

Choice of solvent

The main parameter of interest in this context is the peak height of the eluting solvent. Solvents containing nitrogen, such as acetonitrile, should obviously be avoided. Results concerning several solvents are given in Table II.

It is clear that methanol is by far the best solvent concerning detector performance, allowing determination of compounds eluting close to the solvent. The peak height of methanol normally corresponds to a concentration of less than 10 ppm of a compound such as 2,6-TDC. The reason why the signal from methanol is so low compared to other solvents is not obvious. However, many high-molecular-weight compounds, especially non-polar ones, have limited solubility in methanol. It also seems that the solubility of methanol in nitrous oxide at pressures below 100 bar is not too good. This may lead to problems of transporting the analyte from the injection loop to the column (see below). These two features should thus be considered before using methanol as a solvent.

Injection

Injection with the 60-nl loop injector was studied with 100 ppm of 2,6-TDC as a test substance with methanol as the solvent at a pressure of 90 bar on a DB-5 column. Fig. 4 shows how the peak height varies with the injection time, t_{inj}, *i.e.*, the time during which the internal sample loop is connected to the column. At 90 bar, an injection time of *ca.* 5 s is required for good reproducibility. The introduction of a sample into the column thus creates some band broadening. The mobile phase flow-rate was *ca.* 0.7 ml/h which means that during 5 s a volume of mobile phase corresponding to *ca.* 15 loop volumes has passed through the loop. Addition of ammonia (500–1500 ppm) to the test solution did not make it possible to shorten the injection time, which indicates that adsorption of the substance in the injector is not the cause of the problem. The explanation of the relatively long injection times needed is probably that a thin film of methanol, retaining a portion of the analyte, is formed at the inner walls of the injector

TABLE II

RESPONSES FOR DIFFERENT SOLVENTS

Detector: Varian TSD. System pressure: 100 bar. Temperature: 150°C. Column: 10-m DB-5.

Solvent	*Relative peak height*
Methanol	1
Diethyl ether	65
Ethanol	80
Toluene	90
Dimethyl sulphoxide	115
Diisopropyl ether	270

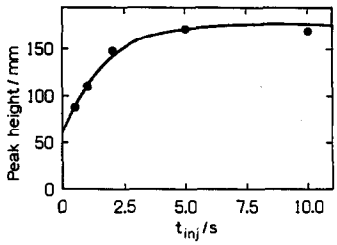

Fig. 4. Variation of peak height with injection time, t_{inj}, for 2,6-TDC (100 ppm) in methanol (for abbreviations, see Table I). System pressure: 90 bar. Column: 10-m DB-5. Temperature: 100°C.

and that this film, due to limited solubility in supercritical nitrous oxide, needs several loop volumes to be swept out. The situation is considerably improved at higher system pressures.

For very polar and very basic compounds, peak shapes may be influenced both by adsorption and by solvent solibility in the mobile phase. Peak heights become lower than expected and severe tailing occurs at lower concentrations. However, at present it is not possible to distinguish adsorption effects originating in the injector from such effects on the column.

Stationary and mobile phases

Of the columns considered, both DB-5 and DB-17 may be used for free amines as well as for the different amine derivatives. On SB-octyl-50, aromatic amines such as MDA and 2,6-TDA were not eluted in spite of the higher film thickness of this column, which otherwise was expected to minimize adsorption. Chromatograms of some test substances at two different, fairly low concentrations obtained in pressure-programmed experiments are shown in Fig. 5. For substances where the basic character of the nitrogen atom has been suppressed by derivatization, the peaks are considerably more symmetric than for the free amines. The number of theoretical plates (calculated for 2,6-TDC) was about 13 000 on both DB-17 and DB-5 columns at 150 bar and 100°C. For SB-octyl-50, plate numbers of the order of 10 000 were obtained, as expected with a thicker film and a larger column diameter. The peak shapes of the most polar solutes, the free amines (peaks 4 and 6), vary slightly with concentration, apparently due to adsorption in the chromatographic system. The observed variation of retention times of *ca.*3% over the concentration range 10–1000 ppm is consistent with the magnitude of the peak shape changes.

With proper pressure-programming conditions, even strongly basic compounds such as tertiary aliphatic amines can be chromatographed at low concentrations as shown in Fig. 6.

Ammonia was added to the solutions for controlling adsorptions on the walls of vessels and syringes according to our previous experiences[14]. Interestingly, the detector selectivity towards ammonia for the amines is only about 100, while in GC work with nitrogen as the carrier gas it is of the order of 5000–10 000.

For strongly polar and basic compounds such as triethanolamine it is obvious that adsorption will be a problem in quantification at low concentrations as illustrated in Fig. 7. Below 200 ppm, the peak tailing is severe.

Pressure programming of the mobile phase is not sufficiently efficient for

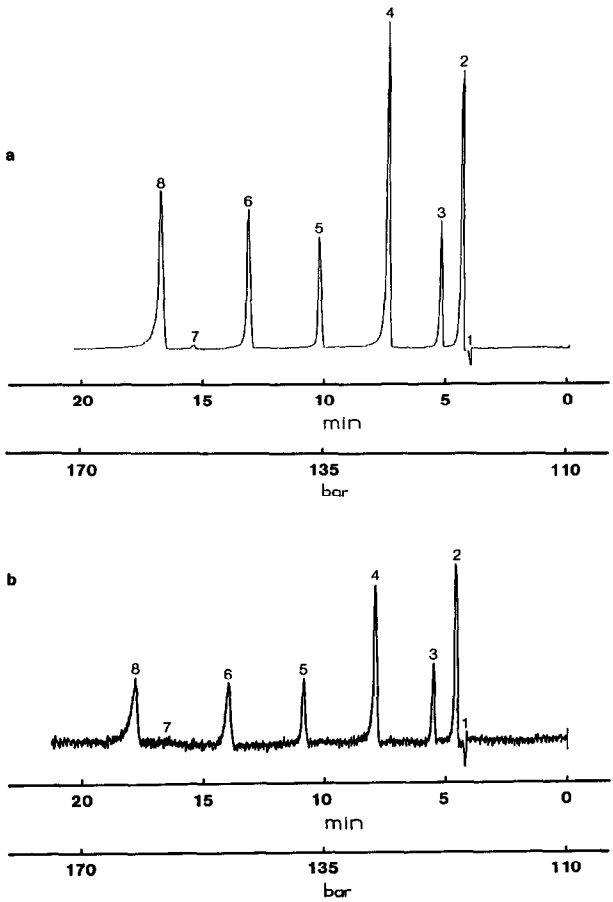

Fig. 5. Chromatograms of some model substances. Concentrations: (a) 250 ppm; $1 \cdot 10^{-11}$ A f.s.; (b) 25 ppm; $2 \cdot 10^{-12}$ A f.s. Peaks: 1 = solvent front; 2 = MDC; 3 = 2,6-FTDA; 4 = 2,6-TDA; 5 = FMDA; 6 = 2,6-TDC; 7 = impurity from MDA; 8 = MDA (for abbreviations, see Table I). Sample solvent: methanol. Injection volume: 60 nl. Pressure programme as shown. Column: 10-m DB-17. Temperature: 130°C.

improving the chromatographic performance. Another measure taken to improve the situation, namely addition of a polar modifier, methanol, to the mobile phase did not significantly improve the peak shape of triethanolamine in the methanol concentration range 0.1–0.5% but, as expected, the peak was eluted closer to the front with increasing methanol concentration. For good chromatography of basic analytes, the polar modifier should have a basic character.

Furthermore, the stability of the column may not be as good when using a mobile phase containing methanol. In such a system, with a methanol concentration of about 1% and a DB-5 column used for about 2 weeks, often above 200 bar, a drastically impaired performance was observed. Without methanol, the same column had been used at these pressures without problems for more than 2 months.

Above *ca.* 0.8% methanol, the signal from the analyte rapidly decreased. This

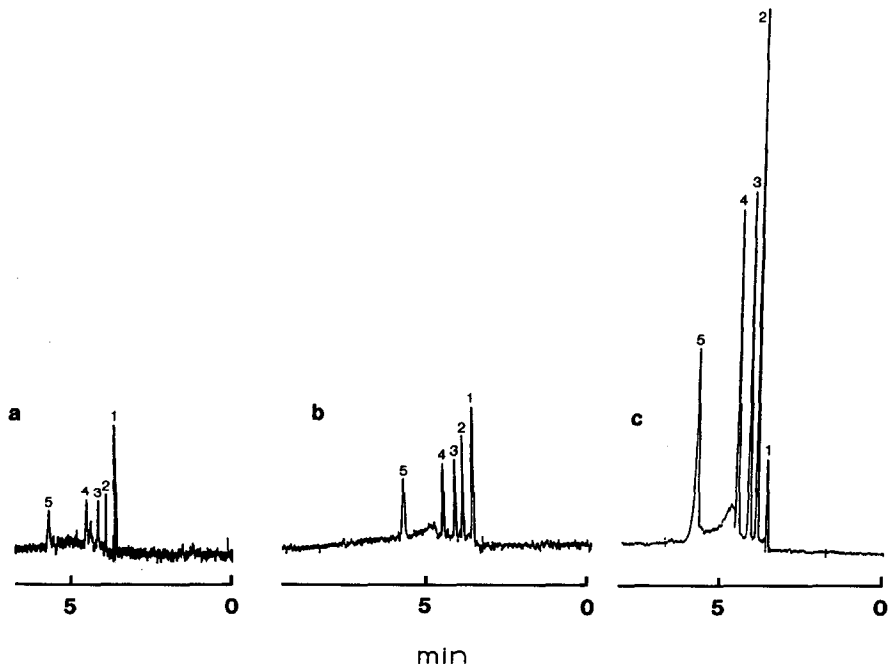

Fig. 6. Chromatograms of tertiary aliphatic amines. Concentrations: (a) 2.5 ppm; $1 \cdot 10^{-12}$ A f.s.; (b) 10 ppm; $2 \cdot 10^{-12}$ A f.s.; (c) 100 ppm; $4 \cdot 10^{-12}$ A f.s. Peaks: 1 = ammonia; 2 = tri-*n*-butylamine; 3 = tri-*n*-pentylamine; 4 = tri-*n*-hexylamine; 5 = tri-*n*-octylamine. Sample solvent: methanol with *ca.* 1000 ppm NH_3. Injection volume: 60 nl. Pressure programme: 95–130 bar, 5 bar/min. Column: 10-m DB-5. Temperature: 100°C.

behaviour is in accordance with results discussed above, where it was shown that nitrous oxide increased the detector signal for the hydrocarbon part of the molecule. With carbon dioxide as the mobile phase, the detector would most probably have tolerated an higher methanol concentration. Greibrokk *et al.*[6] used 7% methanol in carbon dioxide for the determination of 2,4-nitroaniline with nitrogen sensitive detection, and an Hewlett-Packard thermionic detector.

The combination of a polar mobile phase with a constant dipole moment, somewhat polar columns such as DB-17 and polar solutes, with possibilities for specific interactions, implies that changes in pressure and temperature are expected to give changes in resolution. This is illustrated by the observation that a change of the system pressure from 86 to 100 bar increased the resolution between 2,4-TDC and 2,6-TDC from 1.3 to 2.1 with an increase in retention time from about 10 to 15 min. At the same time, the occurrence of specific interactions makes the retention behaviour very difficult to interpret as shown by the following example: if tridecylamine is included in the sample of tertiary amines, shown in Fig. 6, a shorter retention time is obtained for this compound than for trihexylamine. Evidently, only small changes in hydrophobicity contra polarity may give large and unexpected effects. Much work remains before a deeper insight in the retention mechanisms of SFC is reached. However, recent work by Yonker and co-workers concerning the influence of temperature[15], density[16], solute concentration[17] and modifier concentration[18] on

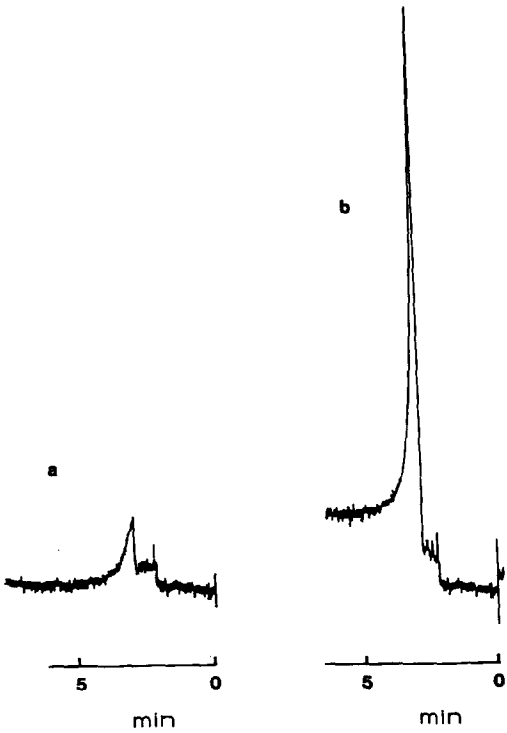

Fig. 7. Chromatograms of triethanolamine. Concentrations: (a) 100 ppm; (b) 250 ppm. Attenuation: $1 \cdot 10^{-12}$ A f.s. Sample solvent: methanol. Injection volume: 60 nl. Pressure programme: 95–160 bar, 5 bar/min. Column: 10-m DB-5. Temperature: 100°C.

retention has provided some insight in the underlying mechanisms determining the retention in SFC.

The columns investigated here are non-polar to slightly polar. The choice has been governed by our knowledge concerning capillary GC for the determination of basic nitrogen compounds as free aromatic amines and carbamates. In such systems, the best behaviour has always been obtained for relatively non-polar columns, while more polar columns, such as those based on polyethylene glycol have given adsorption problems probably due to the presence of acidic sites.

Quantitative analysis

Calibration graphs in the range of 10–1000 ppm were obtained for a mixture of four test substances: 2,6-TDA, 2,6-TDC, MDA and FMDA (see Table I) representing free amines as well as their carbamate and amide derivatives. The chromatographic conditions were as in Fig. 5. As shown in Fig. 8, the graphs seem to be virtually linear over the concentration range considered. The concentration is expressed in ppm, *i.e.*, weight units. If it is instead expressed in moles of nitrogen, the graphs for TDA and TDC will overlap, as will those of MDA and FMDA. The detector response is thus caused mainly by the nitrogen atom, and seems to be relatively independent of derivatization.

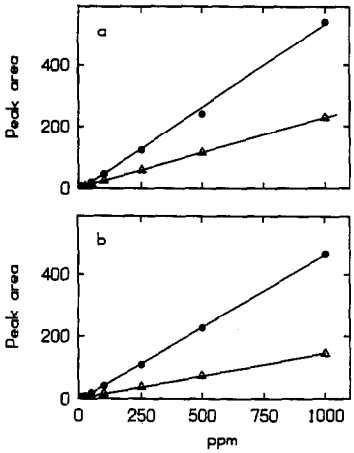

Fig. 8. Calibration graphs in pressure-programmed experiments. Peak area *versus* concentration. (a) ● = 2,6-TDA; △ = 2,6-TDC; (b) ● = MDA; △ = FMDA. (for abbreviations, see Table I). Chromatographic conditions as in Fig. 5.

The correlation coefficients were 0.9989, 0.9997, 0.99990 and 0.99991, respectively. For 2,6-TDA and MDA, negative intercepts were obtained, but the 95% confidence interval did not include the origin only in the case of MDA. The occurrence of negative intercepts indicates that other retention mechanisms, *e.g.*, adsorption may be present. In such cases the behaviour of these test solutes at low concentrations is interesting. Instead of drawing calibration lines as above, it is better to normalize the response by dividing by the solute concentration. This is illustrated in Fig. 9, where the relative response has been calculated as the normalized response divided by the normalized response at 1000 ppm.

No significant variation of the relative response with concentration was found for the two derivatives, while for the two free amines a marked decrease occurred

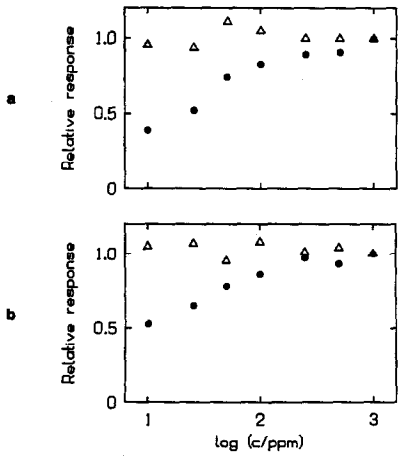

Fig. 9. Relative response as defined in the text *versus* logarithm of concentration (*c*). Details as in Fig. 8.

below 100 ppm. These results imply that derivatization may in many cases be advantageous. The results also show that for free amines in the low concentration range, a calibration graph should only cover a small concentration range, say one decade, to improve the accuracy in the quantitation.

The precision in peak area measurements, based on triple injections for the four test substances in the pressure-programmed experiments described above, was about equal (*ca.* 4% R.S.D.) above a concentration of 100 ppm. At low concentrations the precision was, as expected, better for the two derivatives, 2,6-TDC and FMDA. At a concentration of 25 ppm it was on average *ca.* 6%, compared to an average of *ca.* 12% for the free amines 2,6-TDA and MDA.

The precision has also been investigated at different constant pressures (110–180 bar) and temperatures (100–150°C) for 2,6-TDC at a concentration of 100 ppm. Triple injection gave in this case slightly better results, *ca.* 3% at 100°C and 110 bar, the starting pressure in the pressure-programmed experiments. The precision varied insignificantly in the investigated pressure and temperature ranges.

The precision obtained in this work, with manual direct injection of 60 nl at a solute concentration of 100 ppm, can be compared with that obtained by Richter *et al.*[19], using time-split injection introducing approximately the same sample volume (70 nl) at a concentration of 200 ppm. The solutes used in their investigation were long-chain *n*-alkanes which should behave ideally in the chromatographic system. Depending on the choice of valve, the precision varied between 2 and 4%, in agreement with our results.

CONCLUSION

We have shown that a combination of supercritical nitrous oxide as the mobile phase and a nitrogen selective detector can be used for quantitation of small, polar nitrogen compounds even at relatively low concentrations with acceptable precision. Derivatization is expected to improve the chromatographic behaviour, especially for compounds with primary or secondary amine functions.

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