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Chemical ionization with gaseous ammonia for normal-phase liquid chromatographic–thermospray mass spectrometric applications

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ABSTRACT

Addition of gaseous ammonia to a thermospray ion source, followed by filament-on ionization, provides chemical ionization reagent ions. The abundance of reagent ions is sufficient to obtain spectra which contain predominantly $[M+NH_4]^+$ ions. The advantage of this method of ionization lies in normal-phase LC–MS applications, where "traditional" ionization methods such as volatile buffer, filament-on or discharge-on ionization yields either no ionization or too much fragmentation, making identification very difficult or impossible. After modification of a Finnigan TSQ 70 ion source, the source pressure and effluent flow were optimized. The formation of $[M+NH_4]^+$ ions is dependent on the voltage applied to the repeller electrode. Low voltages yield spectra with predominantly $[M+NH_4]^+$ ions, whereas high voltages yield spectra showing mostly fragmentation and a small percentage of chemical ionization. The formation of the $[M+NH_4]^+$ ion of cyclohexanone is linear over two decades ranging from 0.2 to 20 ng of the compound injected into a normal-phase column. Higher concentrations give rise to a deviation of the linear relationship. Quantitative results are obtained, with a repeatability of 8% (R.S.D.).

INTRODUCTION

Liquid chromatography coupled with a thermospray interface and mass spectrometry (LC–TSP–MS) is a powerful technique for the conformation analysis and identification of polar, thermolabile and non-volatile compounds in target analysis. In TSP ionization there are three different approaches: buffer, filament-on and discharge-on ionization, each of which has its own advantages and disadvantages [1–3]. When buffer ionization is applied, the molecules of interest are ionized with aqueous solutions of ammonium buffers, as a result of either the pH of the buffer solutions (depending on the pK_a values of the compounds) or molecule–ion interactions in the ion source. Filament-on ionization and discharge-on ionization are effected by means of a beam of electrons and a plasma of ions created by an electric field, respectively.

A repeller electrode pushes these ions in the direction of the mass separator. As TSP is a "soft" ionization technique, mainly $[M+H]^+$ and $[M+NH_4]^+$ ions are formed. Depending on the repeller voltage some fragmentation occurs [3]. Therefore, the mass spectrum is dependent on both the ionization technique and repeller voltage.

Reversed-phase high-performance liquid chromatography (RP-HPLC) is the most commonly used LC technique [4]. In RP-HPLC an apolar stationary phase (*e.g.*, C_8 or C_{18}) is used in combination with a polar mobile phase *e.g.*, an aqueous buffer solution. Depending on the application, a certain percentage of organic modifier (*e.g.*, methanol or acetonitrile) is added to the mobile phase. Buffer ionization in RP-HPLC is easily applicable and normally yields mass spectra with few fragment ions. Owing to the higher electrical field applied in filament-on and discharge-on ionization, more frag-

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ment ions are found in the mass spectra obtained with these methods.

Normal-phase (NP) HPLC involves a combination of a polar stationary phase (usually modified silica) and an apolar mobile phase such as dichloromethane or *n*-hexane. Sometimes a polar modifier (*e.g.*, 2-propanol) is added to reduce the analysis time. As these apolar mobile phases are not miscible with aqueous solutions, buffer ionization is difficult to apply. For structure elucidation, $[M+H]^+$ and $[M+NH_4]^+$ ions are very useful, but in NP-HPLC, only filament-on ionization and discharge-on ionization can be applied.

The use of buffer ionization with a double-beam thermospray LC–MS interface for apolar mobile phases has been demonstrated for both LC–MS [5] and GPC–MS [6]. Increased sensitivity has been reported for both NP–HPLC–TSP applications [7] and ammonia CI of ketones [8].

When gaseous ammonia is used, mainly $[M+NH_4]^+$ ions are formed [9]. The applicability of gaseous ammonia as an ionization gas in normal-phase LC–TSP–MS will be demonstrated via the analysis of two compounds of interest in the production of polyamides, *viz.*, cyclohexanone and cyclohexanol [10].

EXPERIMENTAL

Source block modification

A slight modification to the source block was necessary to supply gaseous ammonia, as shown in Fig. 1. This was be done by making a new inlet between the aperture for the discharge electrode and the vaporizer entrance hole of the source of a TSQ-70 mass spectrometer (Finnigan MAT, San Jose, CA, USA). In this way ammonia could enter the source block via the cartridge heater and the discharge electrode opening.

The ammonia was fed through the standard available calibration gas/reagent gas inlet. A 3.18-mm stainless-steel tube was provided between the calibration gas/reagent gas inlet and the extra inlet.

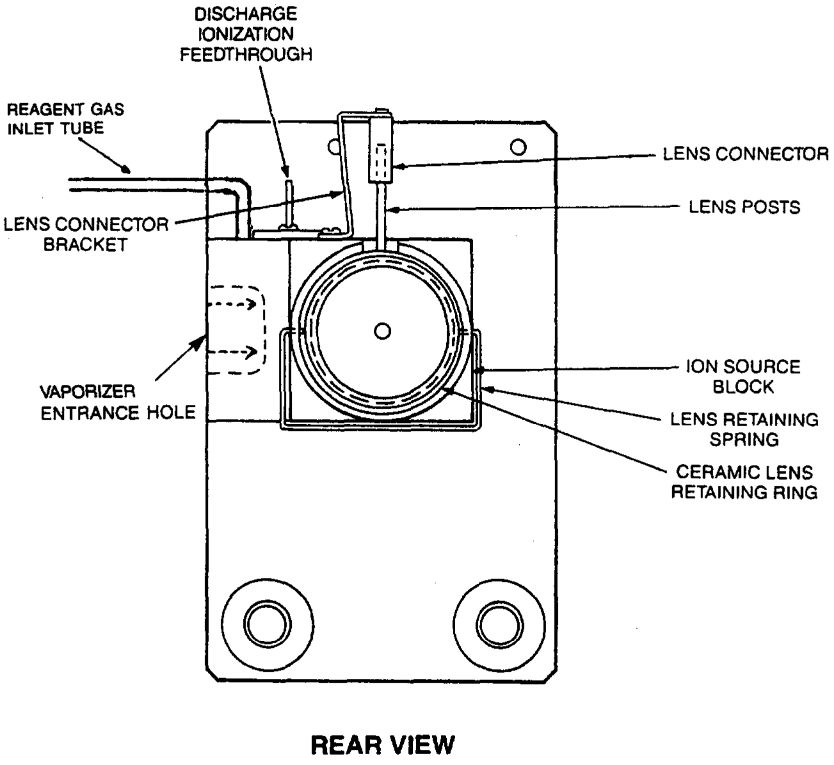

Fig. 1. Position of the reagent gas inlet tube in the modified TSP ion source. (Reprinted with permission from Finnigan MAT Benelux, Veenendaal, Netherlands.)

Instrumentation

The chromatographic system consisted of a Gilson (Villiers-le-Bel, France) Model 305 pump, a Rheodyne (Cotati, CA, USA) Model 7125 injection valve with a 20-μl loop and a Finnigan MAT TSQ-70 triple quadrupole mass spectrometer equipped with a thermospray interface. The column used was a LiChrosorb 10-Diol (250 × 4.6 mm I.D., 5 μm) from Chrompack (Middelburg, Netherlands). The flow-rate was 1 ml/min. The eluent was *n*-hexane–2-propanol (1000:15, v/v). The separations were carried out at ambient temperature. To maintain an optimum source pressure, post column addition of the eluent was effected with a Gilson Model 302 pump for discharge-on experiments only. The solution was added to the column effluent by a Lee (Frankfurt, Germany) visco-jet micromixer. The repeller voltage and ion source pressure were varied, depending on the experiment, and are indicated in the figures.

The vaporizer temperature, discharge voltage, electron energy, electron current and the source temperature were kept at 71°, 450 V, 600 eV, 50 μA and 190°C, respectively. The electron multiplier was operated at 2 kV. Scanning was performed from relative molecular mass 40 to 440 with a scan time of 1 s. Selected ion monitoring (SIM) was used for the determination of cyclohexanone at $m/z = 116$ ($[M+NH_4]^+$).

Chemicals

Cyclohexanone was obtained from DSM (Geleen, Netherlands), [$^2H_{11}$] cyclohexanol and cyclohexanol from Aldrich (Brussels, Belgium), HPLC-grade *n*-hexane and 2-propanol from Merck (Darmstadt, Germany) and gaseous ammonia from Hoek Loos (Amsterdam, Netherlands).

RESULTS AND DISCUSSION

Modification and optimization of the mass spectrometer

Chemical ionization with gaseous ammonia (NH_3-CI) is possible in both the filament-on and discharge-on modes. After ionization of the ammonia molecules, the ions can react with the compounds of interest. Discharge-on with postcolumn addition of effluent can also be used for ionization, but it causes a large increase in chemical noise due to clustering and fragmentation. The signals of the ions formed by ammonia (*m/z* 18 and 35) and 2-propanol (*m/z* 78) were selectively removed from the scan table, resulting in a very low noise level compared with buffer or discharge-on ionization. Hence the signal-to-noise ratios are much better with NH_3-CI.

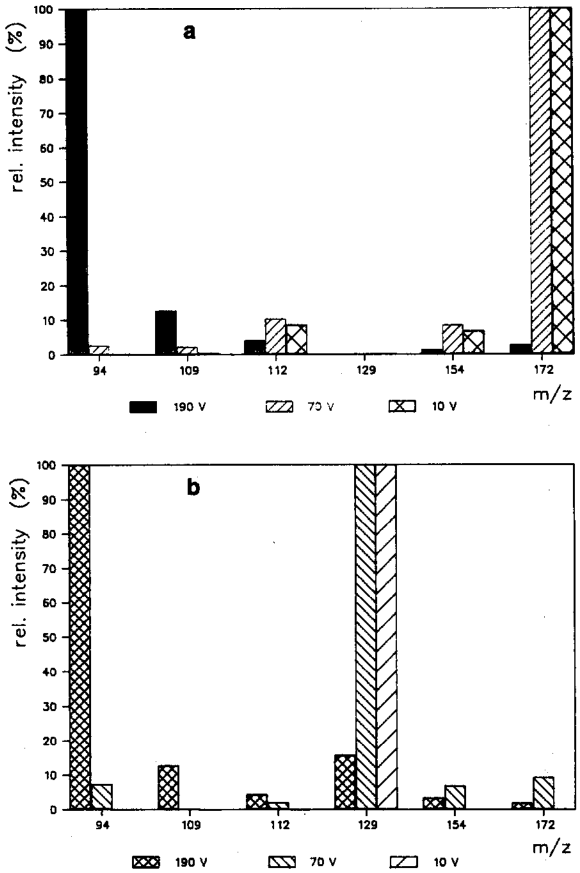

Fig. 2. Relative intensities of characteristic ions of [$^2H_{11}$]cyclohexanol at different repeller voltages. (a) Discharge-on; (b) NH_3-CI/filament-on ionization.

In our experiments we used an ion source pressure of about 120 Pa (0.9 Torr). This was achieved by supplying 67–80 Pa (0.5–0.6 Torr) of ammonia to the ion source (filament-on) or post column addition of 0.5 ml/min of effluent (discharge-on). An ion source pressure of 120 Pa (0.9 Torr) proved to give an optimum yield of ammonium ions. Below 107 Pa (0.8 Torr) instability of the signals occurred.

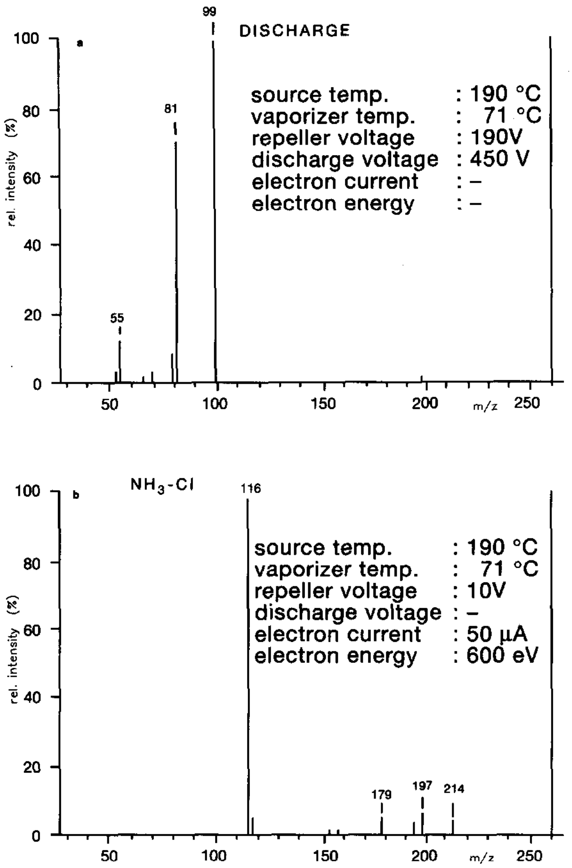

Fig. 3. (a) Discharge-on and (b) NH_3-CI/filament-on ionization spectra from cyclohexanone.

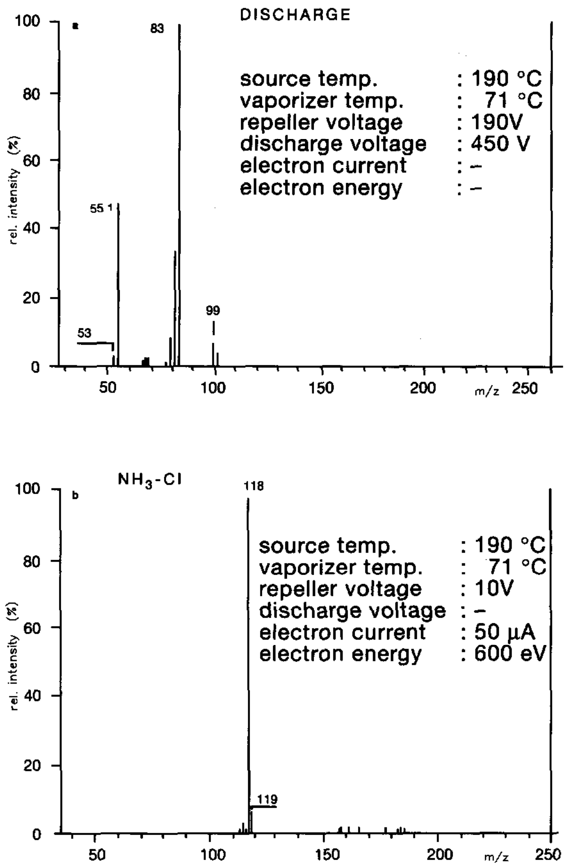

Fig. 4. (a) Discharge-on and (b) NH_3-CI/filament-on ionization spectra from cyclohexanol.

Dependence of mass spectra on repeller voltage

The mass spectra of [$^2H_{11}$]cyclohexanol were investigated at different repeller voltages, both with discharge-on ionization and with NH_3-CI. The relative intensities of the characteristic ions formed are shown in Fig. 2. A decrease in repeller voltage in the discharge-on mode results in less fragmentation and more cluster ion formation. These cluster ions are $[2M+H]^+$ at m/z 223, $[M+C_3H_7OH_2]^+$ at m/z 172 and $[M+C_3H_7]^+$ at m/z 154. The $[M+NH_4]^+$ ion with m/z 129 is the base peak obtained by NH_3-CI at low repeller voltages, as is shown in Fig. 2b.

As demonstrated in Fig. 2, the molecular mass can be obtained from NH_3-CI at low repeller voltages and structural information from discharge-on experiments at high repeller voltages.

Discharge-on ionization versus ammonia chemical ionization

For both discharge-on ionization and NH_3-CI, mass spectra were recorded under the optimum conditions. The spectra of cyclohexanone, cyclohexanol and [$^2H_{11}$]cyclohexanol are shown in Figs. 3, 4 and 5, respectively. Table I gives the formulae of the ions detected. As can be seen in Figs. 3–5, fragmentation occurs with discharge-on ionization at high repeller voltages, whereas an intense $[M+NH_4]^+$ ion is obtained with NH_3-CI at low repeller voltages. Owing to the relatively high concentration of cyclohexanone, cluster ions are present in the mass spectrum (Fig. 3b). These cluster ions are $[2M+NH_4]^+$ at m/z 214, $[2M+H]^+$ at m/z 197, $[2M+NH_4-H_2O]^+$ at m/z 196 and $[2M+H-H_2O]^+$ at m/z 179.

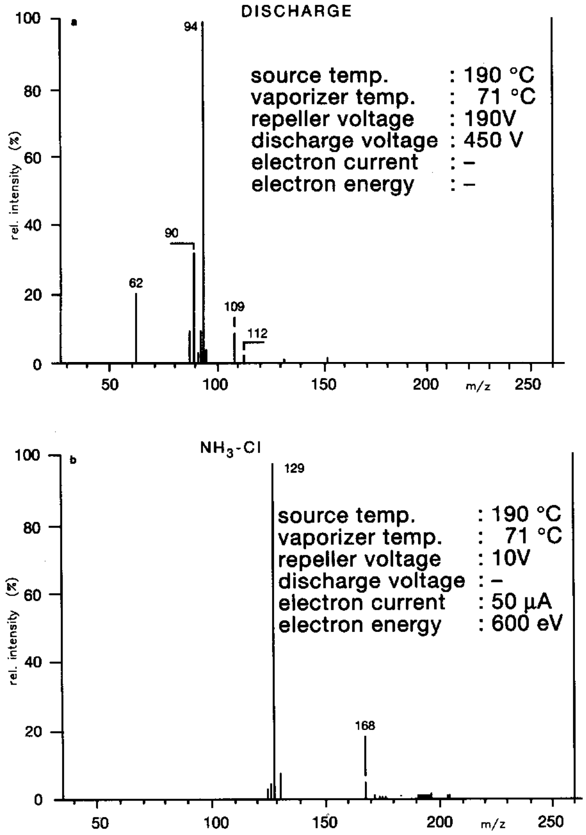

Fig. 5. (a) Discharge-on and (b) NH_3-CI/filament-on ionization spectra from [$^2H_{11}$]cyclohexanol.

TABLE I

CHARACTERISTIC IONS FROM CYCLOHEXANONE, CYCLOHEXANOL AND [$^2H_{11}$]CYCLOHEXANOL OBTAINED BY DISCHARGE-ON IONIZATION

Cyclohexanone		Cyclohexanol		[$^2H_{11}$]Cyclohexanol	
m/z	Ion	*m/z*	Ion	*m/z*	Ion
99	$[M+H]^+$	101	$[M+H]^+$	112	$[M+H]^+$
97	$[C_6H_9O]^+$	99	$[C_6H_{10}OH]^+$	109	$[C_6{}^2H_{10}OH]^+$
		83	$[C_6H_{11}]^+$	94	$[C_6{}^2H_{11}]^+$
81	$[C_6H_9]^+$	81	$[C_6H_9]^+$	90	$[C_6{}^2H_9]^+$
79	$[C_6H_7]^+$	79	$[C_6H_7]^+$		
55	$[C_4H_7]^+$	55	$[C_4H_7]^+$	62	$[C_4{}^2H_7]^+$
53	$[C_4H_5]^+$	53	$[C_4H_5]^+$		

Quantitative analysis

A linear relationship ($r^2 = 0.999$) was found over the range 0.2–20 ng of cyclohexanone. A detection limit of 200 pg of cyclohexanone was found at a signal-to-noise ratio of 3. The repeatability of the method, calculated from repeated injections of 0.8 mg/l of cyclohexanone, is 8.0% (R.S.D.) ($n = 11$).

CONCLUSIONS

Through a simple modification of the ion source of a Finnigan MAT TSQ-70 mass spectrometer, ionization with gaseous ammonia was made possible for normal-phase LC–TSP–MS applications.

Ionization with gaseous ammonia, in the filament-on mode and at low repeller voltages, results in spectra with predominantly the $[M+NH_4]^+$ peak. Upon additional discharge-on ionization, without NH_3 and at a high repeller voltage, fragment ions are obtained. Hence information on both molecular mass and structure of the compound of interest is obtained in normal-phase LC separations. Structure elucidation of unknown structures thus becomes much easier, provided there is little background interference, as in our case.

Scanning from low masses (*e.g.*, *m/z* 20), which is a problem with other ionization techniques, is possible.

Chemical ionization with ammonia is very sensitive. In the SIM mode, a detection limit of 200 pg of cyclohexanone (signal-to-noise ratio = 3) was found. Quantitative analysis also gives good results. The calibration graphs are linear over two decades. The repeatability is 8.0% (R.S.D.).

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