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OPTIMIZATION OF THE INSTRUMENTAL PARAMETERS OF A COMBINED LIQUID CHROMATOGRAPH–MASS SPECTROMETER, COUPLED BY AN INTERFACE FOR DIRECT LIQUID INTRODUCTION

II*. NEBULIZATION OF LIQUIDS BY DIAPHRAGMS

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SUMMARY

Direct liquid introduction interfaces for combined liquid chromatography–mass spectrometry (LC–MS) require a flow restriction to limit input liquid flow-rates to the small values tolerated by the mass spectrometer. In general, viscous type restrictions should be avoided. A non-viscous type restriction such as a 1–3-μm pinhole in a thin wall is acceptable for LC–MS as it produces a jet of small droplets of solution. However, jet instabilities affecting the mass spectral fragmentation pattern directly cannot be avoided in this simple nebulizer. Adding a hot gas to the ion source gives control of chemical ionization reactions and provides thermal energy for droplet vaporization, thus improving overall LC–MS performances. Application to analyses of monosaccharides and disaccharides illustrates the present state of the art of the technique.

INTRODUCTION

The development of combined liquid chromatography–mass spectrometry (LC–MS) is arousing much interest as the technique shows a growing capability for handling non-volatile organic substances[1,2]. A straightforward coupling method is to introduce a fraction of the liquid flow from a high-performance liquid chromatograph directly into the ion source block of a chemical ionization (CI) mass spectrometer. The study of the optimization of analytical conditions in LC–MS using an interface for direct liquid introduction (DLI) has so far been largely ignored because the feasibility of the method was not clearly demonstrated, and the technological difficulties were substantial despite the simplicity of the operating principle. Repro-

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ducible results are now routinely obtained on organic substances that are not amenable to any other technique combining chromatography and mass spectrometry. Many questions remain unsolved, but on the other hand several operating parameters are beginning to be better understood.

The optimization of vacuum equipment was discussed in Part I[3]. The maximum flow-rate of liquid that can be tolerated by the mass spectrometer is 30–70 μl/min when the effective pumping speed for vacuum, S_{eff}, around the ion source is in excess of 4000 l/sec. A cryopump of large active surface area and located close to the ion source is the method of choice for efficiently evacuating large solvent vapour throughputs. When the conductances of the ion source block apertures and of the slit between the source envelope and the analyser housing are adjusted, optimal operating pressures in the different regions of the LC–MS instrument are obtained, and they remain stable during several hours of continuous solvent introduction. Consequently, design problems are reduced to finding a suitable device for injecting liquid at low flow-rates into a tight box, which is evacuated by a constant volume flow-rate vacuum pump as long as the composition of the liquid is unchanged (Fig. 1). Methods for achieving this and pitfalls to avoid are discussed below.

Fig. 1. Simplified schematic principle of a DLI interface.

THEORETICAL

In a DLI interface, the flow-rate of liquid is usually metered into the ion source by regulating a constant pressure drop across a fixed hydraulic restriction. The inlet pressure, P_i, is from 1 bar upwards and remains in the range 1–200 bar. The outlet pressure, P_0, being 0.5–1 torr, can be neglected for flow conditions. The fixed restriction can be either a viscous type restriction, *e.g.*, a capillary tube, or a non-viscous type restriction, *e.g.*, a pinhole in a thin wall.

Viscous type restrictions

The steady laminar flow of a viscous, incompressible solvent in a long channel gives conditions for a Poiseuille flow, and the liquid pressure decreases regularly from the inlet to the outlet of the interface. The decrease is linear when the permeability of the interface is constant along its longitudinal axis. When the liquid pressure equals the vapour pressure, P_v, of the solvent, the liquid evaporates and solvent vapour flows in the remaining part of the interface.

For a capillary tube of uniform inside radius, r, the liquid flows through a portion of the tube of length, L, and the gas flows through the remainder of the tube, l[4].

The molar flow-rate through the liquid column is

$$\frac{dn}{dt} = \frac{\pi r^4 \varrho \left(P_i - P_v + \frac{2\sigma}{r}\right)}{8\eta_l LM} \qquad (1)$$

Assuming that the flow of gas is viscous, and not molecular, the molar flow-rate through the gas column is

$$\frac{dn}{dt} = \frac{\pi r^4 M (P_v^2 - P_0^2)}{16 \eta_g l R T} \tag{2}$$

Equating eqns. 1 and 2 gives

$$\frac{l}{L} = \frac{M}{2\varrho R T} \cdot \frac{\eta_l}{\eta_g} \cdot \frac{(P_v^2 - P_0^2)}{\left(P_l - P_v + \frac{2\sigma}{r}\right)} \tag{3}$$

The liquid flows into the ion source ($l = 0$) only if $P_v = P_0$, which is never the case in LC–MS as the P_v of most HPLC solvents is 100–400 torr at ambient temperature. The distance in the gas phase can be made very small by increasing P_l, but it cannot exceed the pressure pushing the maximum tolerable flow-rate for the mass spectrometer. Increasing the temperature of the tube increases P_v, thus increasing l, which offers no advantages.

Equations similar to eqn. 3 can be derived for the case of a long capillary tube restricted at one end[5,6] by considering it as two viscous restrictions, each with a constant permeability, and connected in series, or for the case of a capillary tube with a thin coaxial metallic core wire[7,8].

Non-volatile materials dissolved in the solvent precipitate when the solvent evaporates inside the capillary tube, and change the flow conditions or even plug the interface. A small plug of non-volatile hydrocarbons ($\varrho = 0.9$), of length 100 μm, in a 100-μm I.D. tube represents 700 ng of material, which corresponds to the content of 1 ml of an analytical-reagent grade solvent rated at 1 ppm of non-volatile materials. This estimation does not take into account the possible dissolution of silica from the HPLC packing into the mobile phase[9], which may introduce several tens of micrograms of non-volatile materials per millilitre of solvent. The limited success of the first models of a DLI interface was due to the finite length of the gas column in the interface, and they were often plugged rapidly[5,6,10,11].

Non-viscous type restrictions

Melera[12] has demonstrated that replacing a viscous restriction by a small diaphragm produces a liquid jet into the ion source which breaks up into droplets soon after leaving the nozzle[13,14]. Consequently, a liquid flows through the whole interface and no accumulation of solid deposits should block the device (Fig. 2).

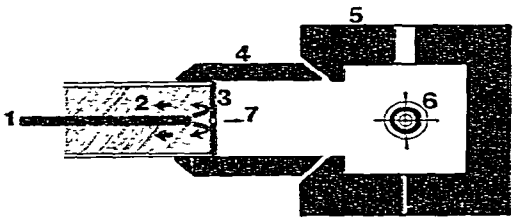


Fig. 2. Schematic diagram of a DLI interface nebulizing liquids by means of a diaphragm. 1 = Input liquid flow; 2 = output liquid flow; 3 = diaphragm; 4 = spacer; 5 = ion-source block; 6 = ionizing electron beam; 7 = liquid jet breaking into a droplet spray.

The maximal speed of a liquid jet issuing from a small orifice into a still gas is given by Bernouilli's equation:

$$u_0 = C_c C_v \sqrt{\frac{2(P_i - P_0)}{\varrho} + u_i^2} \tag{4}$$

Where C_c is the contraction coefficient of the jet and C_v is the velocity coefficient due to the action of viscosity. The outlet pressure and the initial velocity of the liquid stream, u_i, can be neglected under LC–MS conditions. For a diaphragm whose thickness is not small (10–20 μm) compared with the orifice size (1–3 μm), and the orifice is not sharp-edged[15], C_c is 1.0 and C_v is 0.8, so the discharge coefficient $C_d = C_c C_v$ is 0.8; then

$$u_0 = 0.8 \sqrt{\frac{2P_i}{\varrho}} \tag{5}$$

and the output flow-rate is

$$Q_l = 0.2\pi d^2 \sqrt{\frac{2P_i}{\varrho}} \tag{6}$$

For $Q_l = 10$ μl/min of acetonitrile and $P_i = 30$ bar, $d = 2$ μm and $u_0 = 70$ m/sec. The liquid jet issuing from the pinhole is unstable and disintegrates into small droplets[13]. Because of secondary break-up of droplets and jet turbulence, the droplets formed are not of uniform size, and a polydisperse droplet spray is obtained towards the end. The median droplet diameter is two to three times the diameter of the pinhole[14], but the geometric standard deviation is probably very large[15].

For a train of regularly spaced liquid droplets in still air, the linear velocity of the droplet decreases almost linearly along the flight except near the nozzle, because of skin friction with the air[17]. Momentum losses appear in part as heat vaporizing the liquid, and therefore droplets disappear after flying some distance. When droplets are formed under a reduced gas pressure such as in LC–MS, heat generation by friction becomes less important, but droplets are rapidly desolvated by the vacuum if the gas pressure is below P_v. Rapid cooling occurs, thus lowering P_v, which sustains the droplets along their flight. If the process is assumed to be entirely adiabatic, the temperature of the droplets decreases according to the equation

$$T_0 - T = \frac{\Delta H_v}{C_p} \cdot \log\left(\frac{V}{V_0}\right) \tag{7}$$

where V_0 is the initial volume of the droplet and V is the volume at temperature T. A droplet of acetonitrile at ambient temperature ($T_0 = 25$°C) will freeze ($T = -92$°C) after dissipating 23% of its volume.

In reality, small heat exchanges with a gas under 1 torr occur, and complete desolvation can be achieved, but this may take several milliseconds, so that the droplets may fly over long distances before disappearing because of their high initial velocity, u_0. Probably the droplets cool rapidly to the temperature where the vapour

pressure of the liquid equals the pressure, P_0, around them. For $P_0 = 1$ torr and acetonitrile, the average droplet temperature would be —44°C. Therefore, it is not surprising that thermally labile solute molecules can be transmitted unmodified into the ion source, but the experimental fact that intense protonated molecular ions are observed from solutes which may not be vaporized poses intriguing questions about the nature of the ionization process involved in DLI/LC–MS.

Another consequence of the rapid cooling of the liquid concerns the velocity of the jet at the orifice, which should be higher than the solidification speed to avoid plugging of the pinhole by solvent crystals. Therefore, the temperature of the liquid in the DLI interface should be kept between two well separated limits: below the lower limit, the liquid freezes in the diaphragm, and above the higher limit premature evaporation occurs, and both conditions create an unsteady jet or plug the pinhole.

Very few solute molecules may be dissolved in each droplet of a spray formed by atomization of an HPLC output effluent. The maximum mass flow-rate of solute in the output is

$$\left(\frac{dm}{dt}\right)_{max} = \frac{m\sqrt{N}}{t_R\sqrt{2\pi}} \tag{8}$$

For $m = 10$ ng, $N = 15{,}000$ and $t_R = 300$ sec, the mass flow-rate is $1.6 \cdot 10^{-9}$ g/sec. Assuming that the solvent flow-rate is 1 ml/min and the molecular weight is 500, the maximal molecular concentration of the solution is $1.2 \cdot 10^{14}$ molecules/cm^3. Consequently, a droplet of 2 μm contains 470 molecules and a 0.5-μm droplet contains 8 molecules. Rapid desolvation could leave most of the solute molecules in the gas phase, as suggested by Giddings *et al.*[17]. However, there are severe technological difficulties in atomizing liquids into a spray of small (0.1–0.5 μm diameter) and regularly distributed droplets. Even a small diaphragm excited at a fixed ultrasonic frequency[14] or a sophisticated pneumatic nebulizer[15] cannot realize these optimal conditions.

Because of the small diaphragm dimensions, capillary forces cause liquids to fill the orifice completely when the pressure drop across the diaphragm is zero. However, the surface tension of the liquid opposes the liquid flow through the aperture when the pressure drop is increased. The minimal pressure drop to produce a droplet of liquid is approximately

$$P_i - P_0 = \frac{2\sigma}{r} \tag{9}$$

For a 2-μm diaphragm and acetonitrile the pressure drop is 460 torr.

A practical situation often met in DLI/LC–MS is when the interface is going to be introduced into the mass spectrometer, and both P_i and P_0 are atmospheric pressure. The interface tip is then inserted into a vacuum lock for introduction into the high vacuum of the mass spectrometer. When P_0 is reduced in the vacuum lock from 760 to 10^{-1} torr, it is probable that the vapour pressure of the liquid, P_v, will be reached before the minimal pressure drop for pushing the liquid out of the diaphragm is attained, and consequently the diaphragm will become plugged in a few seconds. Attempts to unplug the pinhole by increasing P_i are generally unsuccessful; for

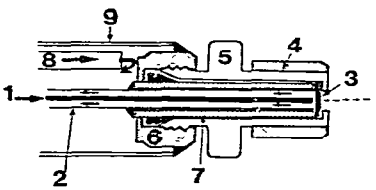
instance, a pressure of 300 bar applied on a 2-μm pinhole produces a force equivalent to that of a mass of 9 mg in the gravity field. On the other hand, the thin diaphragm wall may be damaged by high pressures. Problems are avoided by establishing a pressure drop of 1-2 bars and starting the jet before reducing the outlet pressure.

EXPERIMENTAL

The two prototype instruments were as described previously[3]. Slight modifications were made to the apparatus at the École Polytechnique (Lucie) so that both instruments are now similar; for instance, they use the same model of vertically mounted cryopump.

A schematic diagram of the DLI interface is shown in Fig. 3. Nickel diaphragms were obtained from Nermag (Rueil-Malmaison, France). The pinhole apertures are in the range 1–3 μm, the O.D. is 3 mm and the thicknesses are 10–20 μm. The temperature of the interface is adjustable between 0 and 100°C by circulating water from a Haake (Berlin-Lichterfelde, G.F.R.) Model 4291 waterbath.

A special test chamber was designed for observing the liquid jets formed at the interface tip (Fig. 4). Two windows at right-angles permit side lighting and the direct observation of the jet. The chamber is evacuated to 0.5–1 torr by an Alcatel (Montrouge, France) Model ZM 2015 rotary pump. Pressures are read on a Pirani vacuum gauge, Model Minivac 57 (SOGEV, Orsay, France). The complete experimental set-up includes the connection in series of a Waters Assoc. (Milford, MA, U.S.A.) Model 6000 A liquid pump, a Prolabo (Paris, France) integrated valve injector–column assembly, Model Chromaflux LC 100, a Bourdon high-pressure gauge, a standard Nermag DLI interface and a Hoke (Cresskill, NJ, U.S.A.) Model 1656 G1Yfine-metering needle valve providing the back-pressure, P_i, in front of the diaphragm. The setting parallels real flow conditions into the LC–MS instrument and gives accurate control of P_i, P_0, T_0 and Q_l. The length and the orientation of the jet are observed directly.



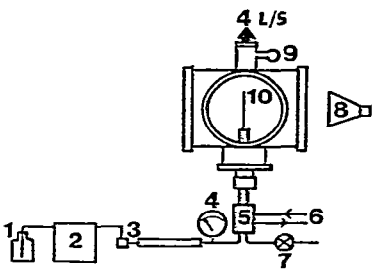


Fig. 3. Schematic cut section of the DLI interface end. 1 = Input tube (0.15 mm O.D. × 0.1 mm I.D.); 2 = output tube (1.56 mm O.D. × 1 mm I.D.); 3 = diaphragm (3 mm O.D., pinhole 1–3 μm); 4 = spacer making seal against ion source block; 5 = modified Swagelock (Cleveland, OH, U.S.A.) reducer (SS-200-R-4); 6 = Swagelock nut (SS-202-1); 7 = holder tube (3.17 mm O.D. × 1.56 mm I.D.); 8 = temperature -regulating water input; 9 = interface end shaft.

Fig. 4. Experimental set-up for observing liquid jets under simulated LC–MS conditions. 1 = Solvent reservoir; 2 = liquid pump; 3 = injector–column assembly; 4 = Bourdon gauge; 5 = interface; 6 = temperature-regulating system; 7 = fine-metering needle valve; 8 = electric lamp; 9 = Pirani vacuum gauge; 10 = droplet spray.

Real LC–MS experiments were run on prototype Carole, using a 15-cm long Rhône-Poulenc (Centre Nicolas Grillet, Vitry, France) experimental column packed with propylamine-bonded, 5-μm Spherosil XOA 600, with known aqueous mixtures of monosaccharides and disaccharides as samples at concentration of 1 mg/ml of each test compound. Pre-heated ammonia at 200–250°C was introduced through the gas chromatographic inlet into the ion source block.

RESULTS AND DISCUSSION

The first diaphragms used in this study had pinhole diameters in the range 5–10 μm. However, we found them too large for DLI/LC–MS as they often required too small a pressure drop to inject the liquid at the maximal tolerable flow-rate into the mass spectrometer; consequently the jet velocities were too low, and the holes were often plugged rapidly, perhaps because their diameters were roughly equal to that of HPLC packing materials. They were later advantageously replaced with smaller diaphragm orifices with diameters of 1–3 μm when they became routinely available. These showed improved performances and increased lifetimes.

A series of experiments were run in the test chamber under simulated LC–MS conditions, using acetonitrile as the solvent. Nearly every time that a vacuum was established before the jet was formed, solvent impurities plugged the interface rapidly. The surface tension of the liquid (see above) impeded the liquid flow, and solvent evaporation or rapid freezing prevailed over liquid introduction.

Next, the jet was formed by increasing P_l up to 3–6 bar with the chamber at atmospheric pressure. This was achieved by running the HPLC pump at 1 ml/min and by closing the needle valve (7 in Fig. 4). The visible portion of the jet often extended in a straight line, at right-angles to the diaphragm, over a distance of 10–15 cm. The pressure in the chamber was then reduced to 1 torr. A mist of small solid acetonitrile particles was rapidly formed and evacuated from the chamber walls. The length of the jet remained practically unchanged, but the liquid became slightly more opaque. Once it was formed a stable and constant jet could be observed for several hours, even in the absence of temperature-regulated water flowing through the interface (Fig. 5a). The nearly adiabatic cooling sustains the jet and compensates for the evaporation under vacuum. Conversely, if a jet cannot be established when the outlet is at atmospheric pressure, a vacuum will not make it better, and consequently optimization of the flow conditions through the DLI can be made outside the LC–MS set-up.

When the jet velocity was reduced because of partial plugging of the orifice, or by lowering P_l, the acetonitrile froze and appeared as a solid mass protruding at the interface tip, which swelled slowly by growing series of small (0.5–1 cm) stalactites (Fig. 5c). After a certain time, the whole mass usually broke close to the diaphragm, fell by gravity and exploded into smaller particles of solid which slowly disappeared after several tens of seconds. High and erratic pressure surges, up to 100 torr, occurred during the explosion of the solid and the vaporization of the small fragments. After this breaking and exploding, another mass of solid acetonitrile started to grow from the diaphragm.

Even when a straight and stable jet was established, its direction changed after a certain time (Fig. 5b), because of partial plugging of the orifice. Similarly,

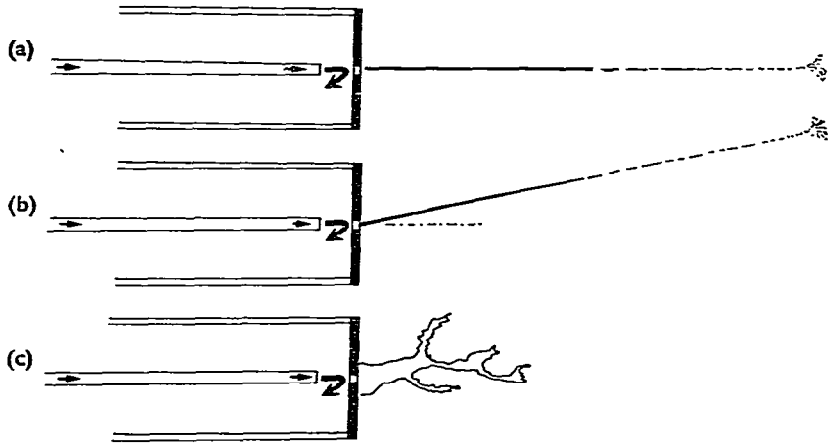


Fig. 5. Schematic representation of the appearance of a jet in the test chamber under simulated LC–MS conditions. (a) Straight jet; (b) deviated jet; (c) frozen jet.

when the interface was withdrawn from the test chamber and then re-installed, setting the jet back to its original form was sometimes either not possible or required a different inlet pressure. The direction was often not the same. Increasing P_i straightened the jet, but it also increased Q_t, which was not desired.

The reason for the change in direction of the jet is still unclear. On the one hand, small solid particles from the HPLC column packing could be still present, but this seems unlikely, or solid impurities could be introduced when assembling and disassembling the interface. On the other hand, high turbulences and gas wakes around the jet near the nozzle could bring liquid droplets back on the low-pressure side of the diaphragm, leaving solid deposits after desolvation under vacuum. This could also explain why a crown of small needles of solid crystals appeared all round the orifice when an aqueous solution of sodium chloride was injected during LC–MS runs.

The idea of using highly filtered and ultra-pure solvents for DLI/LC–MS is unrealistic because re-dissolution of a small fraction of the HPLC packing is either unavoidable or is even recommended for increasing the column lifetime[9] and, more generally, because HPLC may require buffers or highly concentrated solutions. Hence frequent cleaning or replacement of the diaphragm after several hours of use will be necessary.

The temperature of the interface seems not to be very critical for optimized conditions. With acetonitrile, rapid plugging or deviation of the jet occurred above 40°C. No serious changes were observed when the temperature was reduced to 4°C. This aspect needs to be further investigated for different solvent systems and jet conditions, but for the moment it is considered that a low temperature is to be preferred.

Results from experiments in the test chamber were helpful in accounting for data from LC–MS runs, and suggested that the angle and the length of the jet had a direct bearing on the mass spectral fragmentation patterns. For instance, the mass

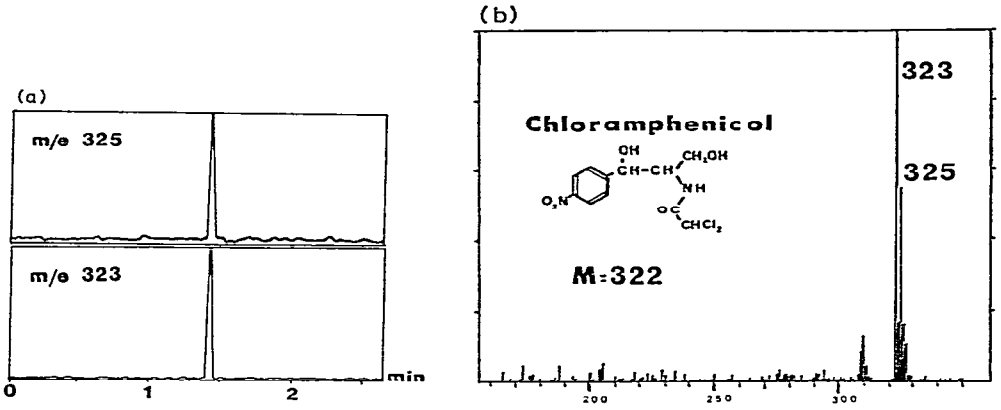


Fig. 6. LC–MS analysis of chloramphenicol in acetonitrile. (a) Extracted ion-current profile for ions at m/e = 323 and 325. (b) Mass spectrum. HPLC: 25 cm × 4 mm I.D. column packed with 5-μm C_{18}-bonded silica, eluted with acetonitrile at 1 ml/min. MS: mass range 160–360 scanned repetitively in 1.5 sec.

spectrum of chloramphenicol in acetonitrile showed an $(M + H)^+$ ion (Fig. 6) only when the jet was straight, otherwise small meaningless fragments were observed. The result was the same if the jet was straight and its length such that droplets collapsed against the opposite wall of the ion source (Fig. 7).

In other words, the jet velocity should be high enough to provide a stable and durable liquid flow-rate into the ion source, and a jet at right-angles to the diaphragm. In addition, desolvation should be sufficiently rapid to avoid droplet impacts on the ion source surfaces in any direction.

These two conditions conflict in some instances. For example, increasing droplet flights for better desolvation by setting the interface backwards increases the risk that a small change in direction will make the droplets collapse against the side walls. Consequently, the jet velocity should be high, the diaphragm located close to the ion source and the jet length kept short by supplying thermal energy to the droplets. A simple method for achieving this, needing no additional costly equipment, is to introduce a hot gas into the ion source. Because of the large pumping speed for evacuation of the instrument, the addition of a few millilitres per minute of a hot (200–250°C) gas was permitted. Also, by selecting a gas with a high proton affinity, control of CI fragmentations was possible, independent of the solvent

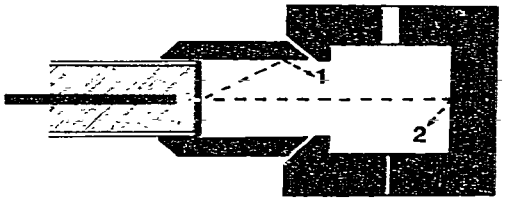


Fig. 7. Jet conditions causing thermal breakdown of sensitive solute molecules. 1 = Deviated jet; 2 = excessively long jet.

composition used for HPLC, which could be of some importance for LC–MS with HPLC under gradient elution conditions.

Analyses of some sugars were carried out and the results illustrate the possibilities and limitations of DLI/LC–MS with a small diaphragm and an additional hot gas. Sugars were selected because even the simpler members, *e.g.*, monosaccharides, cannot be subjected underivatized to combined gas chromatography–mass spectrometry; they are amenable to field desorption mass spectrometry[18] or "in-beam" chemical ionization[19], but they cannot be introduced through a chromatographic inlet. Hot ammonia was used as the reactant gas for chemical ionization of sugar molecules and as the thermal energy source for the droplets.

A mixture of simple monosaccharides did not pose any difficulties (Fig. 8). The four monosaccharides investigated showed identical mass spectra, with $(M + NH_4)^+$ as the base peak, and no structure-specific fragments. An ion corresponding to $(M + \text{solvent} - H_2O)^+$ was the base peak when no ammonia was added.

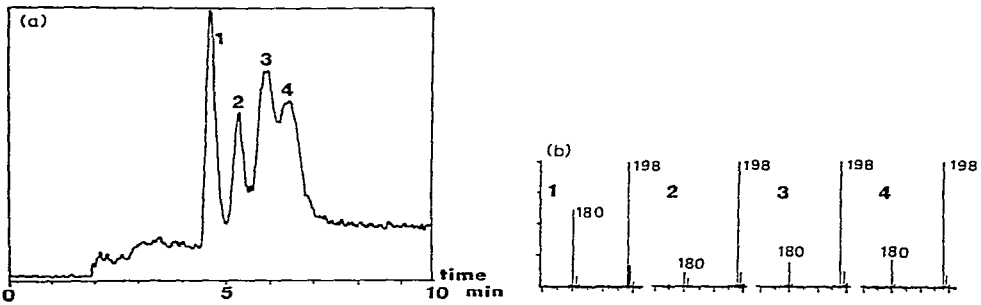


Fig. 8. LC–MS analysis of a mixture of underivatized monosaccharides. (a) Total ion chromatogram. 1 = Fructose; 2 = mannose; 3 = glucose; 4 = galactose. (b) Mass spectra recorded on top of HPLC peaks. HPLC: 15 cm × 4 mm I.D. experimental Rhône-Poulenc column, packed with propylamine-bonded, 5-μm Spherosil XOA 600. Solvent: acetonitrile–water (80:20) at 2 ml/min. Each peak corresponds to about 20 μg on the column and 400 ng in the MS ion source. MS: scan range, 150–450 at 2 msec/a.m.u.; T_{is} = 200°C; ammonia at 200°C added.

Disaccharides were more difficult to analyse and required well optimized operating parameters. For instance, in a set of two LC–MS runs (Figs. 9 and 10), the same solution of sucrose in water was employed, the same amount was injected and the gas pressures, tuning parameters and amount of ammonia were the same. However, in the first run, the jet was straight and at right-angles to the diaphragm, whereas a used diaphragm was installed in the interface tip for the second run and the jet was deviated towards the walls of the connecting tube between the diaphragm and the ion source. The former conditions gave a mass spectrum showing a base peak at m/e = 360, corresponding to $(M + NH_4)^+$, whereas a fragment ion at m/e = 180, probably corresponding to a hexose residue, was the base peak under the latter conditions (Fig. 10). In the latter instance, the ion at m/e = 360 is ten times less intense than in the first run. Peaks for sucrose on the total ion chromatograms have identical retention times and peak areas, but the peak on the second chromatogram is wider

and unsymmetrical, thus indicating possible decomposition and the slow thermal desorption of fragments from the ion source walls. The orientation of the jet is then very critical for the mass spectral fragmentation pattern, and sucrose is a cheap and convenient test sample for checking LC–MS tuning parameters before attempting to run specific non-volatile samples.

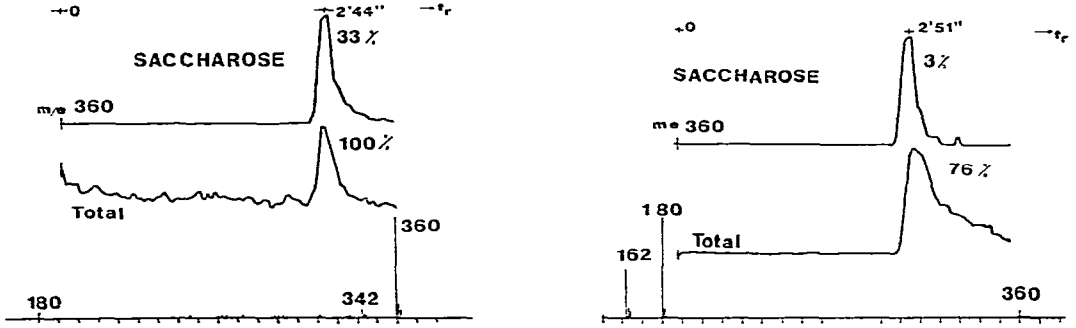


Fig. 9. LC–MS analysis of saccharose in methanol at 1 ml/min using a straight jet. Reacting ion species are from methanol (solvent) and ammonia (added gas). Top trace: extracted ion current profile for ion at $m/e = 360$ corresponding to $(M + NH_4^+)$. Middle trace: total ion current. Bottom trace: mass spectrum taken at maximal peak height on the chromatogram. Peak heights are expressed as a percentage of the total ion count for the maximal peak height on the total ion current.

Fig. 10. LC–MS analysis of the same amount of sucrose as in Fig. 9, with identical tuning and pressure parameters, but with a deviated jet. Traces as in Fig. 9. Peak heights are expressed as a percentage of total ion count for the maximal peak height on the total ion chromatogram in Fig. 9.

CONCLUSION

Viscous type restrictions should not be used in a DLI interface when the outlet pressure is below P_v, but two-stage systems can be considered. A diaphragm is, for the moment, a simple and convenient method for nebulizing HPLC output effluents into a spray of small droplets. Diaphragms of 1–3 μm, smaller than those used previously (5–10 μm), have shown longer lifetimes and greater ease of use, but no efforts were made to estimate whether the basic sensitivity of the instrument was related to the droplets diameters. Reasonably stable and reproducible conditions can be achieved over periods of several hours. Simple cleaning in an ultrasonic tank was often effective for regenerating plugged diaphragms but they may be cheap enough to be changed frequently. Under the described experimental set-up there was no way of avoiding erratic changes in the directions of the jets. Many organic substances were not sensitive to some impacts with the ion source walls and gave good spectra, on the other hand several thermally labile substances gave no response under these conditions. A present this is the main limitation of the device, and efforts are being made to improve this situation. Pneumatic nebulizers[16] such as those being used for LC–MS by Japanese workers[20–22], diaphragms excited at ultrasonic frequencies[14,23] combined oxy-hydrogen flame–nozzle–skimmer systems[24], or electrostatic atomizers[25] have been effective in some instances, suggesting several interesting

approaches for direct LC–MS. Moreover, nebulization problems encountered in DLI/LC–MS appear to be similar to those found in atomic-absorption spectrometry and inductively coupled plasma–atomic emission spectrometry, indicating that a similar approach to the nebulization problem could be adopted.

These problems should not shadow the fact that DLI/LC–MS with a diaphragm, although a slightly acrobatic technique, has already been applied successfully to practical problems[2]. The previous need for CI reactant species imposed by HPLC solvents has been severely criticized in the past. Now this can be avoided by adding a gas with a high thermal energy and a high proton affinity, and this was successful for analysing underivatized monosaccharides. It could provide a more general LC–MS method for several classes of non-volatile substances.

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SYMBOLS

dn/dt	molar flow-rate
dm/dt	mass flow-rate
r	radius of capillary tube
d	pinhole diameter of diaphragm
η_l	absolute viscosity of liquid solvent
η_g	absolute viscosity of solvent vapour
σ	solvent surface tension
ϱ	density
M	molecular weight
R	gas constant
P_i	liquid inlet pressure
P_v	vapour pressure of solvent
P_0	outlet pressure and ion-source block pressure
l	length of viscous interface occupied by solvent vapour
L	length of viscous interface occupied by liquid solvent
C_c	contraction coefficient
C_v	velocity coefficient
C_d	discharge coefficient

u_0 maximal stream velocity at diaphragm outlet
u_i initial stream velocity into the interface
Q_l liquid flow-rate of solvent
T_0 interface temperature and initial droplet temperature
T droplet temperature in the ion source
ΔH_v heat of vaporization
C_p heat capacity
V_0 initial droplet volume
V droplet volume
N column plate number
m mass of solute
t_R retention time
S_{eff} effective pumping speed for vacuum at ion-source location
C total conductance for vacuum of ion-source apertures
T_{is} temperature of ion source block

ABBREVIATIONS

CI chemical ionization
DLI interface for direct liquid introduction
HPLC high-performance liquid chromatography
LC–MS combined liquid chromatography–mass spectrometry

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