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DEVELOPMENTS IN INTERFACING MICROBORE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY (A REVIEW)

A. P. BRUINS
State University, Department of Pharmacy, A. Deusinglaan 2, 9713 AW Groningen (The Netherlands)

INTRODUCTION

This paper is a review of methods of interfacing microbore high-performance liquid chromatography (HPLC) columns with mass spectrometers. Emphasis is given to 0.5 and 1.0 mm I.D. packed microbore columns. Narrow-bore columns (2.0 mm I.D.) are not covered, and only brief mention is made of open tubular columns. The latter have been discussed by Niessen and Poppe[1].

The operating principle of each interfacing method is discussed only if relevant in relation to microbore column flow-rates. For a complete overview the reader is referred to the bibliography of combined liquid chromatography–mass spectrometry (LC–MS)[2].

The limitations imposed by the vacuum system of a mass spectrometer are such that 5–20 μl/min of liquid can be introduced into a chemical ionization (CI) instrument equipped with differential pumping of source and analyser. A higher flow-rate (20–50 μl/min) is permissible if an additional cryopump is used[3]. Microbore columns operating at 5–100 μl/min (0.5 and 1.0 mm I.D.) are ideally suited at first sight for matching the pumping speed of a mass spectrometer.

The success of LC–MS is dependent not only on the compatibility of liquid flow-rate and pumping speed, but even more on the ability to transfer polar and labile solutes into the ion source without causing thermal degradation. On the other hand, the information desired about the molecular structure of an unknown, may not be present in the mass spectrum generated by a method involving gentle sample introduction and soft ionization. Microbore columns do not offer any advantage in this respect in comparison with 4.6 mm I.D. columns. It will be shown, however, that micro LC offers better sensitivity and/or greater flexibility in LC–MS.

The growing interest in microbore LC is reflected in the publication of a book on the subject[4]. The chapter on LC–MS by J. Henion covers the literature up to mid-1982. Duplication is avoided as far as possible in the present review by giving emphasis to more recent insights and developments.

SIMPLE CAPILLARY INTERFACES

The introduction of a liquid into a mass spectrometer via a capillary tube, as an alternative to a heated batch inlet system, has been pioneered by Talroze *et al.*[5,6].

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The flow-rate into the ion source of the mass spectrometer was extremely low and compatible with electron impact (EI) ionization. As stated in the introduction, a CI source can accept between 5 and 50 μl/min of liquid. In the first LC–MS experiments a capillary was combined with a splitter, which diverted 99% of the column effluent[7,8]. The sensitivity obtained was in the microgram range and was poor in comparison with the low nanogram full scan sensitivity of GC–MS.

Microbore columns are ideally suited for capillary interfaces. The first commercially available micro liquid chromatograph, the Jasco Familic 100, has been connected to capillary interfaces to give a full spectrum sensitivity of *ca.* 10 ng[9–15]; a 30-pg detection limit has been quoted for selected ion monitoring[10,16].

An open tubular microcolumn can be connected to the ion source of a mass spectrometer[17] in the same manner as a GC–MS system can be directly coupled to capillary columns[18]. The interface is eliminated. Flow-rates can be kept so low that the ion source can be operated in the EI mode[19]. LC–MS without an interface has also been reported by Brophy *et al.*, who incorporated a packed microbore column in a direct insertion probe[20].

Capillary interfaces have been shown to offer useful on-line LC–MS operation if applied to those samples that can be run off a conventional direct insertion probe, heated to *ca.* 200°C[9–16]. Furthermore, all publications on capillary interfaces stipulate that the capillary should be heated. A cooled capillary gives rise to severe pressure fluctuations in the ion source[12].

The situation at the end of the capillary has been the subject of calculations[5,21–23]. A liquid exposed to vacuum will evaporate at a rate depending on the vapour pressure, the temperature and the evaporation area. The liquid may evaporate inside the capillary, just at the outlet, or even form a droplet, as illustrated in Fig. 1. The rate of evaporation, G, of a liquid into vacuum, expressed in SI units, is

$$G = P_0 A \sqrt{\frac{10^{-3} M}{2\pi RT}} \text{ kg/sec}$$

The saturated vapour pressure, P_0, increases strongly with increasing temperature. The area, A, is assumed to be a hollow meniscus, a hemisphere with radius r (radius of the capillary). In the simple theoretical model, only mass transfer is considered. Limitations imposed by heat transfer have not been taken into account. The tem-

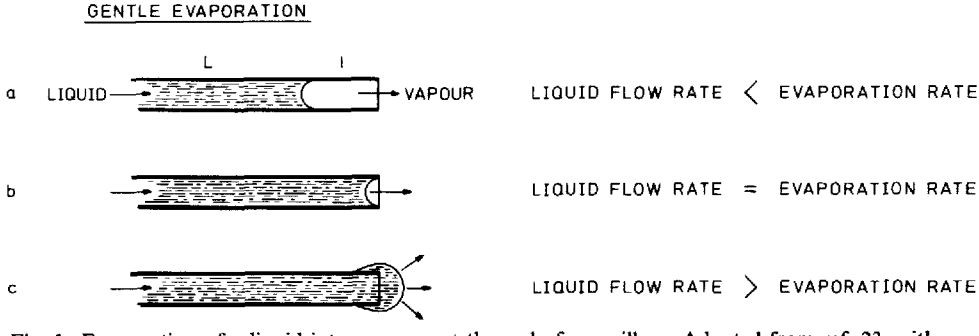

Fig. 1. Evaporation of a liquid into vacuum, at the end of a capillary. Adapted from ref. 23, with permission.

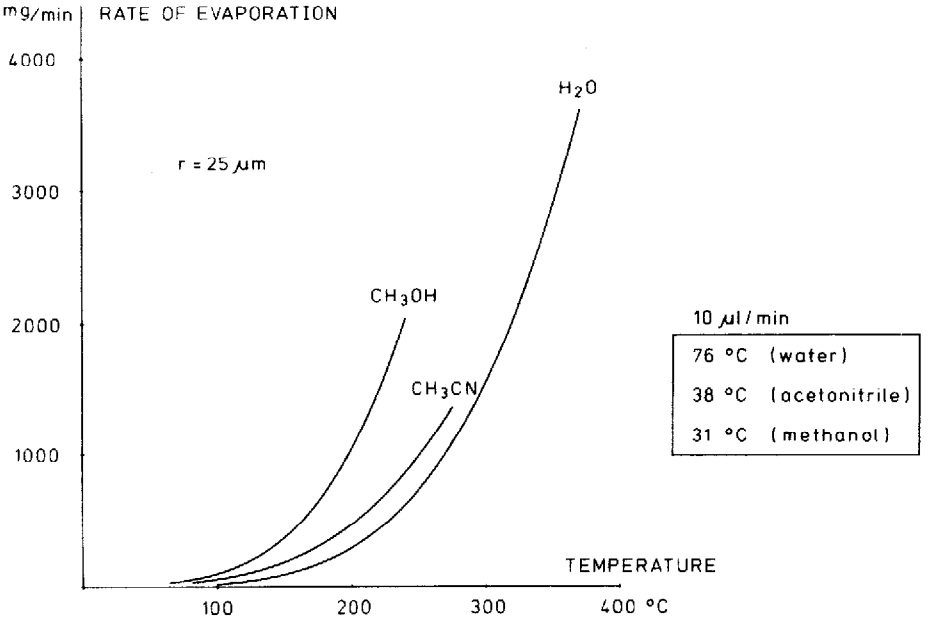

Fig. 2. Rate of evaporation of three liquids into vacuum, as a function of temperature at the liquid surface. Capillary radius, 25 µm. Adapted from ref. 23 with permission.

perature quoted is the temperature of the liquid at the meniscus. Fig. 2 shows the theoretical evaporation rate for situation b in Fig. 1. A liquid flow-rate of 10 mg/min (10 µl/min for water and 12 µl/min for methanol or acetonitrile) is in equilibrium with the evaporation rate at 76°C, 44°C and 36°C in the case of water, acetonitrile and methanol, respectively. At higher temperatures the evaporation rate increases and the solvent front retracts inside the capillary, and at lower temperatures the liquid flows out of the capillary, as depicted in Figs. 1a and 1c. Complete evaporation inside the capillary is established at moderate temperatures, far below the operating temperatures reported for stable ion source conditions (200°C and higher). Reliable and stable operation of a simple capillary evaporation interface is apparently achieved by working in situation a of Fig. 1. The major drawback of a capillary vaporizing interface becomes clear: non-volatile samples and solvent impurities will block the capillary, whereas thermally labile samples may decompose during evaporation at an elevated temperature. Yet the capillary interface is attractive by virtue of its simple and inexpensive construction, although in our hands it has sometimes been unstable for unkown reasons.

NEBULIZERS

Solute enrichment in micro LC–MS coupling was the aim of a study on the introduction of micro LC eluate into a mass spectrometer via a heated single-stage jet separator. Good peak shapes were recorded from volatile samples: aromatics and fatty acid methyl esters[24]. A few years later the same principle was adopted for ultra-micro LC–MS[25]. Sample and solute evaporate as they emerge from a capillary

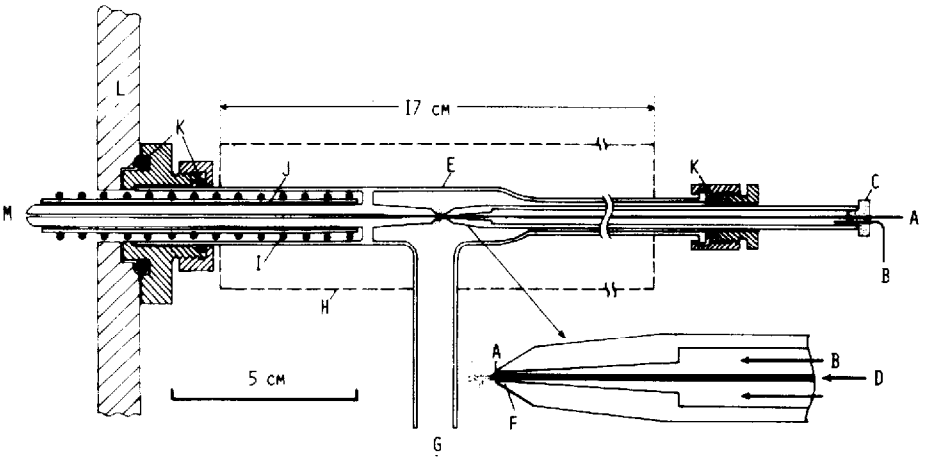

Fig. 3. Vacuum nebulizing interface. A = Capillary tube from LC; B = nebulizing gas inlet; C = silicone septum; D = effluent from LC; E = Pyrex glass; F = nebulizing tip (Pyrex); G = to rotary pump; H = heating oven; I and J = heater assembly; K = silicone O rings; L = body of mass spectrometer; M = transfer line to ion source. Reproduced from ref. 26 with permission.

located inside the heated GC inlet arm of the separator. As a consequence, application is limited to samples that might as well have been analysed by GC–MS. To overcome this drawback, the separator has been modified in several steps. First, the capillary feed tube was inserted into the jetting orifice of the separator while helium was introduced coaxially[26,27]. The interface still shows a strong resemblance to the jet separator (Fig. 3). Excess helium is pumped away through the side arm. Second, the transfer line between the jet and the ion source was made shorter[28] and finally consisted of a hole in a disk[29]. Operation was extended to less volatile samples, but stable and uniform sample introduction was only possible at an elevated temperature, and with the aid of a flow of helium of *ca.* 50 ml/min.

The operating principle was supposed to be liquid nebulization to droplets followed by complete desolvation and introduction of "nude" sample molecules into the source. However, if calculations as presented above are done on the evaporation of liquids from the exit of the capillary feed tube in the nebulizer, complete evaporation appears to be possible at slightly elevated temperatures. The temperature quoted for stable operation is much higher, so that the operation of the so-called nebulizer may be explained as total evaporation combined with sample transfer to the source, supported by helium as a carrier gas.

In its last stage of development, the nebulizer is water cooled[30] (Fig. 4), which prevents premature evaporation and makes nebulization feasible. Mass spectra and sharp peak shapes in total ion and extracted ion current profiles have been reported for less volatile compounds. The narrow peaks give an indication of efficient nebulization. However, the absence of molecular weight information in the mass spectrum of maltose[30] shows that thermal degradation[22] takes place, presumably in the heated transfer line between the nebulizer and the ion source.

The nebulizers described above use an intermediate pressure region, pumped by a rotary vacuum pump. A further development is the omission of the intermediate pressure region with direct nebulization into the ion source, as is done in the direct

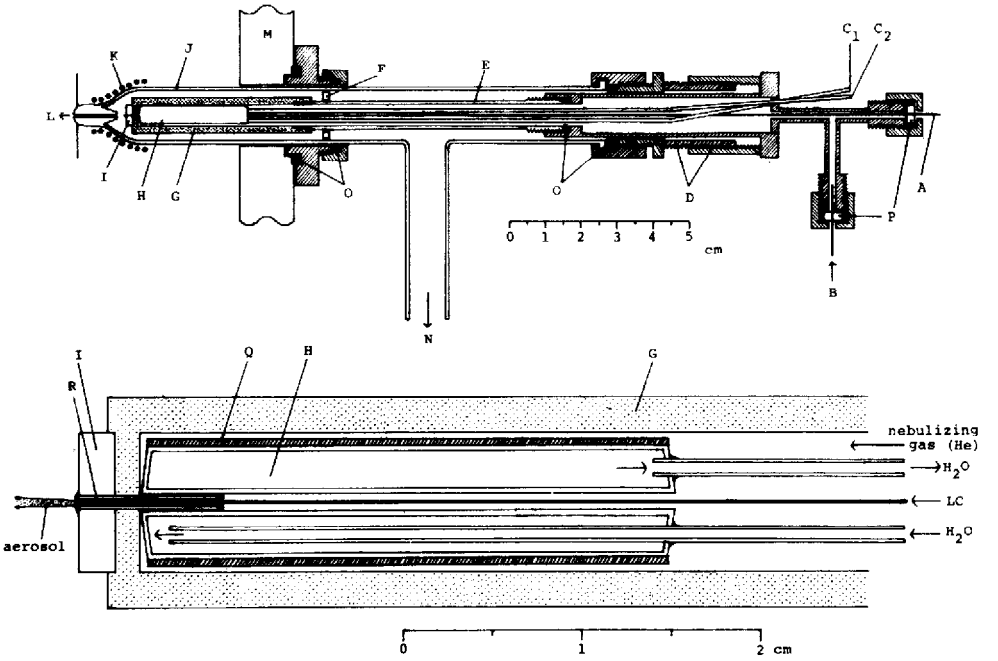

Fig. 4. Water-cooled nebulizer, A = Capillary tube from LC: B = nebulizing gas inlet; $C_{1,2}$ = cooling water connections; D = distance adjuster; E = stainless steel tube; F = centering spacer; G = housing of cooling water jacket (Macor); H = cooling jacket; I = copper disk; J = Pyrex; K = transfer line heater; L = to ion source; M = body of mass spectrometer; N = to rotary pump; O and P = silicone O ring and septum; Q = polyimide film; R = stainless steel tube. Reproduced from ref. 30 with permission.

liquid introduction (DLI) interfaces. The first publication along these lines described a "sample solution introduction" for the Finnigan 3300 CI quadrupole mass spectrometer[31]. The interface is shown in Fig. 5. A sample feeding capillary is surrounded by a gas introduction tube, methane is used as the make-up gas, and the ends of both coaxial tubes are heated. Liquid is introduced at 2 μl/min. The most favourable spectra were recorded when samples were injected as a solution in aqueous ammonia. The reactant gas mixture, ammonia–water–methane, produces NH_4^+ and solvated ammonium ions, thus giving spectra identical with those from CI with ammonia. Addition of methane to the solvent vapours increases the source pressure to a level suitable for efficient CI.

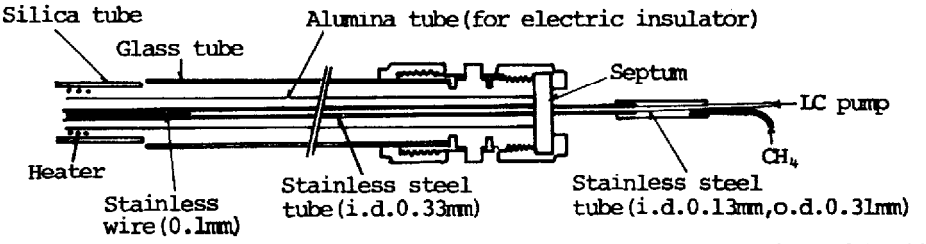

Fig. 5. Solution sample introduction interface, with coaxial gas inlet. Reproduced from ref. 31 with permission.

To return to the calculations made on simple capillary interfaces[23], complete evaporation almost certainly takes place inside the heated capillary. Degradation of labile samples is indicated by the mass spectrum of sucrose, which is identical with the spectrum recorded by DLI under the condition of a deviated jet[22].

A more recent paper describes a similar coaxial gas and liquid introduction interface[32]. Provision is made for water cooling, but the interface can also be used in the "hot" mode, by disconnecting the cooling water supply. Good chromatographic peak shapes with minimal band broadening were obtained for phenylurea herbicides. However, the same samples can also be analysed with a simple capillary vaporizing interface[16]. Cooling the coaxial interface gave an unstable source pressure; however, stable operation could be obtained after a 15-min period of warming up by heat transfer from the ion source[33]. Again taking into account the flow-rate, the capillary diameter and the temperature[23], this interface most probably relies on complete evaporation while sample vapour is swept into the source by a stream of helium.

The most recent nebulizer was built in the Shell Research Laboratories in Amsterdam[34]. It is water cooled and designed for a combination of open tubular LC columns with a mass spectrometer (Fig. 6). In operation, there is an extremely narrow gap between the polyimide coating and the conical nebulizer tip. A large pressure drop (from 10 bar to vacuum) causes a rapid flow of gas, although at a low volume flow-rate. A stream of droplets can be observed when the interface is operated in air. Calculations show that premature evaporation can indeed be avoided by cooling to 10–20°C. The negative ion (NI) CI spectrum of sucrose shown in Fig. 7 is identical with a NICI spectrum obtained with a jetting DLI interface[35] and shows less fragmentation than a desorption CI spectrum[36]. The results obtained with solutions of sucrose give evidence for effective nebulization, but no on-line LC–MS data of sugars and other labile molecules have been reported.

In conclusion, a vacuum nebulizer should be water cooled to prevent evaporation and to ensure nebulization. Transfer of labile samples into the source is possible. The vacuum system should be capable of pumping solvent vapour together with nebulizing gas.

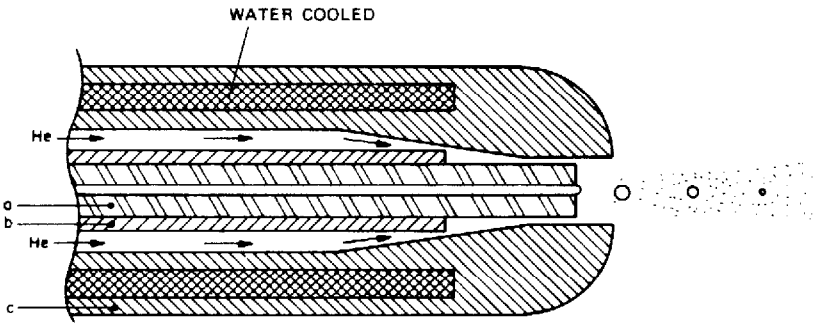

Fig. 6. Water-cooled nebulizer constructed by Shell Research, Amsterdam. Reproduced from ref. 34 with permission.

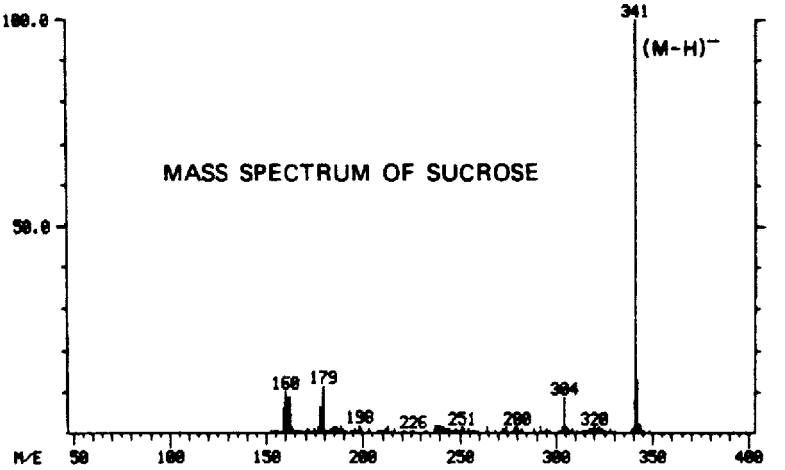

Fig. 7. Negative ion chemical ionization spectrum of sucrose, obtained by sample introduction via the nebulizer shown in Fig. 6. Eluent and reactant gas, acetonitrile–water. Reproduced from ref. 34 with permission.

DIRECT LIQUID INTRODUCTION

The drawbacks of heated capillaries are overcome if a liquid is injected into the ion source as a stream of droplets. By pushing the liquid through a pinhole in a metal disk[22,37] or through a short narrow-bore capillary[38] a jet is formed, in which a labile sample, for example vitamin B12[39], is carried into the source without thermal degradation.

The flow-rate accepted by the mass spectrometer is between 5 and 50 μl/min, depending on the pumping capacity[3]. As with capillaries, 99% of the eluate of a standard 4.6 mm I.D. column is diverted. Narrow-bore columns (2 mm I.D.) offer a *ca.* ten-fold more favourable split ratio. A modified DLI interface can accept the full effluent of a 1-mm microbore column[40,41]. Krien *et al.* have built a 50 cm × 1 mm I.D. column inside the DLI interface[42], the outlet of the column being pressed against the diaphragm, to avoid any post-column dead volume. The analysis time was too long when this column was operated at a low flow-rate of 10 μl/min. In a second interface described in the same paper, the eluate of the microbore column was fed to the pinhole diaphragm via a 50 μm I.D. fused-silica capillary. Excess solvent could be diverted so that a trade-off between speed and resolution could be made in the split mode, which allows flexibility in adjusting the liquid flow into the mass spectrometer[42].

The disadvantage of a split outside the mass spectrometer is loss of sensitivity. Selective removal of at least part of the solvent vapour without loss of sample has been achieved with a modified desolvation chamber (Fig. 8)[43]. Sample transfer into the ion source is unaffected by a splitter incorporated in the desolvation chamber, as long as the sample is entrapped in droplets; only solvent vapour is diverted. Excess pressure inside the CI source is thus avoided, while flexibility in elution speed is enhanced without loss of sensitivity at higher flow-rates.

Christensen *et al.* have described a DLI interface that employs ultrasonic ex-

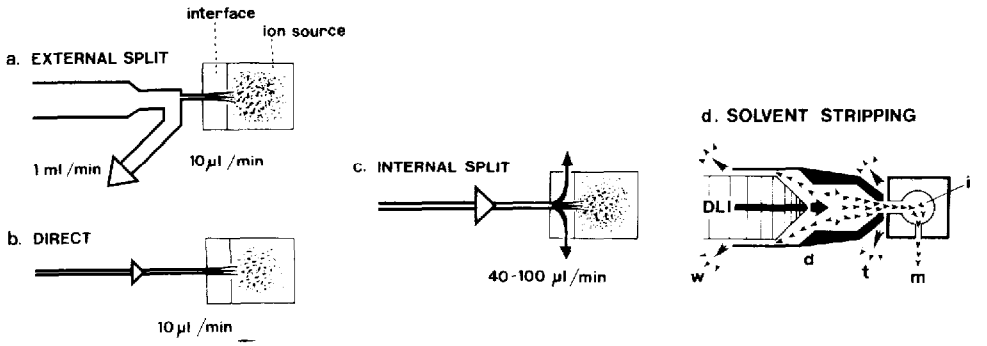

Fig. 8. Schematic diagram of direct liquid introduction in split and direct modes. Reproduced from ref. 43 with permission.

citation for droplet formation. The interface was designed to accept 5–20 µl/min of liquid, eluting from a resistance-heated stationary wire concentrator[44], but it may be adapted to microbore LC.

Open tubular columns can be inserted into a CI source. Liquid jet formation instead of evaporation is achieved by drawing the end of the column into an extremely narrow capillary[17]. However, each time the jetting orifice is blocked, a new extended tip has to be made, which was considered unsuitable for routine use[34]. An open tubular column can be used in combination with a diaphragm DLI interface if provision is made for a make-up inlet of liquid into the effluent stream to increase the flow-rate to a value required for liquid jet formation[1]. The diaphragm interface has also been incorporated in a combined open tubular super-critical fluid chromatograph mass spectrometer[45].

MOVING BELT

The operating principle of a transport interface needs no further introduction[2]. Its advantages are free choice of EI *versus* CI and free choice of reactant gas composition in CI. Problems arise, however, with reversed-phase eluents containing a high percentage of water. The aqueous eluate does not flow onto the belt in a continuous layer, but beading occurs which results in noisy ion current profiles. Complete evaporation of reversed-phase solvent mixtures inside the vacuum locks is not possible at a flow-rate above 0.1–0.3 ml/min.

The eluate of microbore columns can be handled without problems caused by evaporation and beading provided the water content is below 50%[46,47]. Above 50% water, the addition of 0.2 ml/min of ethanol is necessary for smooth wetting of the belt. Diverting excess eluate is avoided, sensitivity is improved, and the background spectrum is reduced by the strongly reduced load of solvents and their impurities[48]. Spray deposition on the belt by means of a pneumatic nebulizer extends operation up to 90% water content and simplifies gradient elution operation. Transfer efficiency of a sample to the belt is 100%, as long as the water content in the LC eluate is below 90%[49].

In summary a moving-belt interface combined with microbore columns gives better sensitivity and signal-to-noise ratio, and a lower background level.

THERMOSPRAY

The thermospray interface[50,51] and the simple capillary vapourizers described above make use of the same device: a heated capillary tube. However, they operate in entirely different regimes. The linear liquid velocity in a thermospray interface is much higher than in a capillary vapourizer. Complete evaporation of the liquid is avoided. The liquid front inside the capillary disintegrates and a fine mist of droplets is injected into the source.

The Leidenfrost phenomenon is most probably operative in droplet formation inside the capillary[52]. A liquid poured out onto a hot plate evaporates within a short time by efficient heat transfer from the hot plate to the liquid (Fig. 9). Above a certain temperature, called the Leidenfrost temperature[53], the liquid can no longer wet the hot plate, dances and bounces and is isolated from the hot plate by a thin layer of vapour. Depending on the material and the condition of the surface of the hot plate, the Leidenfrost temperature for water is at least 180°C[54], but values up to 325°C have been reported[55].

A second condition is that ammonium acetate at a concentration of *ca.* 0.1 *M* should be present in the eluate of the liquid chromatograph, in order to achieve ionization of the sample, without the use of an electron-emitting filament[51,56]. The mechanism of ion formation is still under investigation. Thus far, two processes have been put forward[57]: CI by reactant ions in the gas phase, and evaporation of sample ions from charged droplets[58].

Proper operation is ensured by a careful selection of conditions: flow-rate, capillary diameter, temperature, solvent composition and buffer concentration[51,56,59]. The flow-rate in microbore columns requires a very narrow capillary and a lower temperature. On the one hand, complete evaporation must be avoided; on the other hand, a minimum temperature is required for the Leidenfrost phenomenon to occur. Experiments with 1 mm I.D. or smaller microbore columns and 10 μm I.D. capillaries have so far been unsuccessful[60].

The thermospray interface is very attractive in view of its simplicity of construction and operation. A serious drawback from the chromatographer's point of view is that best operation is achieved at a high water concentration combined with the presence of ammonium acetate, which may be in serious conflict with the desired chromatographic performance. Furthermore, gradient elution requires temperature programming of the sprayer. Post-column addition of an aqueous buffer solution helps to alleviate these problems[56].

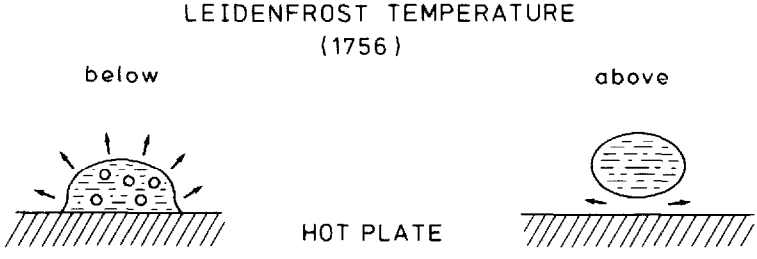

Fig. 9. Boiling and evaporation of a liquid on a hot plate. Left: nucleate boiling below the Leidenfrost temperature. Right: film boiling above the Leidenfrost temperature.

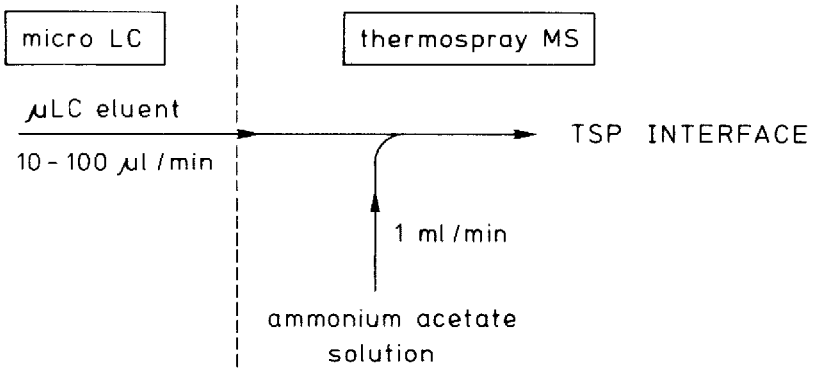

Fig. 10. Post-column buffer addition in micro LC–thermospray MS.

The ability to handle 1–2 ml/min of aqueous solutions might indicate that there is no need for microbore columns in thermospray LC–MS. However, the principle of post-column addition of a buffer can be taken to the extreme when microbore columns are used (Fig. 10). The eluent from the column then constitutes less than 10% of the liquid entering the interface and, consequently, the performance of the thermospray interface has become largely independent of the conditions in the micro LC. Gradient elution no longer requires temperature programming of the interface, and chromatographic conditions can be selected freely as long as eluent and sample can be dissolved in aqueous ammonium acetate. As indicated schematically in Fig. 10, the buffer solution has essentially become part of the mass spectrometer, rather than the liquid chromatograph.

Reports of a micro LC–thermospray combination have not yet appeared but it should be attractive.

LIQUID ION EVAPORATION AND ELECTROSPRAY

Emission of ions from charged droplets, which is one of the ionization mechanisms in thermospray, has previously been described to take place at atmospheric pressure[58,61] and may even occur in thunderclouds[62]. Droplets are generated by pneumatic nebulization at ambient temperature and charge is induced on the sprayed droplets by means of a small electrode held at 2–3 kV, placed close to the sprayer. A positive high voltage on the induction electrode generates negatively charged drop-

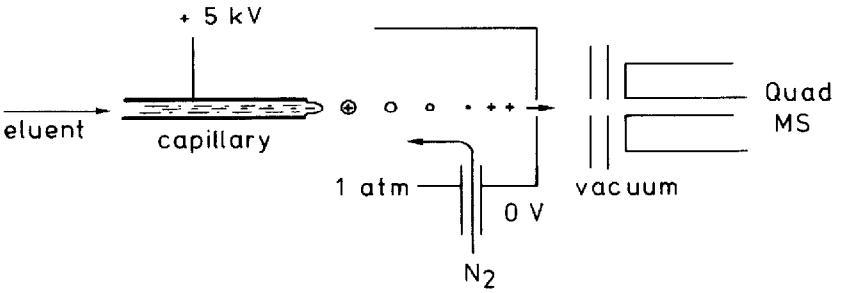

Fig. 11. Schematic diagram of an atmospheric pressure electrospray interface for LC–MS.

lets[63], which are introduced into the source of a Sciex quadrupole mass spectrometer, designed to sample ions from a region at atmospheric pressure. The atmospheric pressure ion source and nebulizer can accept the effluent of a standard 4.6 mm I.D. column, so at present there seems to be no need for microbore columns.

Electrospraying a solution is a most efficient means of creating charged droplets. A simplified diagram is shown in Fig. 11. In the original experiments the electric field pulls the liquid out of the capillary[64,65], the liquid flow-rate being dependent on the dimensions of the capillary and on the nature of the solvent. However, the solvent can be fed into the electrosprayer at a predetermined flow-rate. Its potential as an LC–MS interface method has already been claimed in patents[66–68]. Mass analysis of electrosprayed solutions of macromolecules (MW > 20 000) has been reported[69].

Electrospray LC–MS with microbore columns has been described by two research groups. A flow-rate of 5–15 μl/min was compatible with the liquid consumption of the electrosprayer[70–74]. The solution is sprayed into nitrogen at atmospheric pressure. Sample ions are drawn into the mass spectrometer after complete or nearly complete desolvation, a process identical with the ionization mechanism of liquid ion evaporation. The sensitivity quoted was such that a concentration of 10^{-5} *M* gramicidin S in methanol–water (50:50) gives a discernible mass spectrum[71]. Fig. 12 gives an example of combined LC–electrospray MS.

Electrospray LC–MS offers some attractive features[72]: no critical temperature control is required for droplet formation, as long as premature evaporation of solvent is avoided; the interface does not degrade the LC separation; the system is inherently

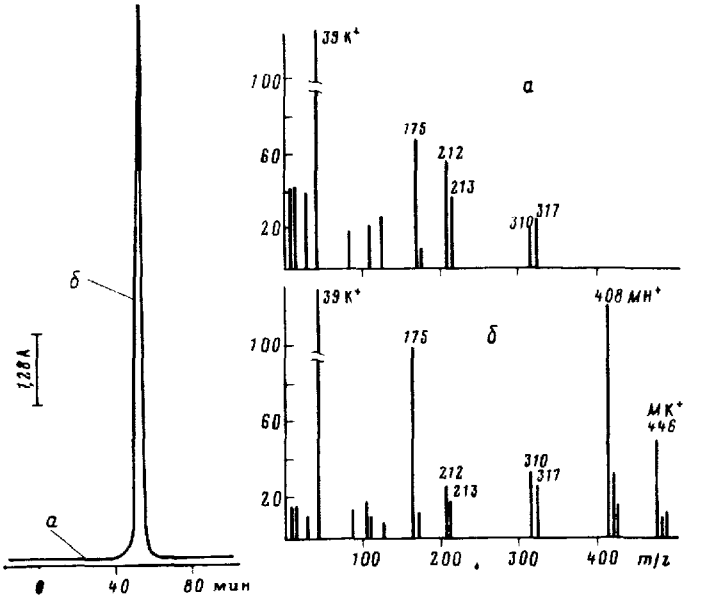

Fig. 12. Electrospray LC–MS of dansylarginine (MW 407). Left: UV detection at 210 nm; top right: background spectrum; bottom right: background plus sample spectrum. Column, 62 × 2 mm I.D.; flow-rate, 5 μl/min; eluent, 2-propanol–water (30:70), pH 3, 0.01 *M* KH_2PO_4. Reproduced from ref. 70 with permission.

stable; the system is not prone to fouling, as the counter-current flow of nitrogen sweeps away solvent vapour and other uncharged material.

Once incorporated in a commercial mass spectrometer, electrospray LC–MS should hold great promise for the future.

CONCLUSION

Microbore LC permits transfer of the full column effluent into a capillary vapourizer, a capillary nebulizer, a DLI interface and the electrospray interface. Micro LC extends the range of eluents that can be accommodated by a belt transport interface, and improves sensitivity.

Although the thermospray interface accepts the full effluent of a standard 4.6 mm I.D. column, it is likely that micro LC with post-column addition of a buffer solution will allow the greatest possible flexibility in selection of chromatographic conditions.

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