Journal of Chromatography, 271 (1983) 71–82
Chromatographic Reviews
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM 15,497

EXPERIMENTS WITH THE COMBINATION OF A MICRO LIQUID CHROMATOGRAPH AND A CHEMICAL IONIZATION QUADRUPOLE MASS SPECTROMETER, USING A CAPILLARY INTERFACE FOR DIRECT LIQUID INTRODUCTION

SOME THEORETICAL CONSIDERATIONS CONCERNING THE EVAPORATION OF LIQUIDS FROM CAPILLARIES INTO VACUUM

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SUMMARY

The total effluent from a micro liquid chromatograph, operating at a flow-rate of 8 μl/min, has been introduced into the ion source of a quadrupole mass spectrometer, which is used in the chemical ionization mode. The solvent vapour serves as the reactant gas. The interface is constructed from flexible fused-silica capillary tubing. The capillary extends into the ion source and the tip of the capillary is in thermal contact with the source via a copper block. Transfer of heat is necessary to support evaporation of the liquid. Calculations on the rate of evaporation indicate that the liquid vaporizes inside the capillary. It is shown that the rate of evaporation and the distance between the liquid front and the end of the capillary are strongly dependent on temperature.

INTRODUCTION

When we investigated the possibility of on-line combined liquid chromatography–mass spectrometry (LC–MS), the Finnigan transport system[1] was ruled out by its price, which could not be accommodated in our budget. Because other interface systems are not commercially available for the Finnigan 3300, our attention was directed to home-made interfaces.

Direct liquid introduction systems for quadrupole mass spectrometers using a glass capillary and a splitter have been published[2,3]. The prime advantage is the very low cost of the interface. The major disadvantage lies in the use of only 1–2% of the eluate for mass spectrometric identification.

If the standard liquid chromatograph is replaced by micro high-performance liquid chromatographic (HPLC) equipment operating at a much lower flow-rate (5–15 μl/min)[4] the total effluent can be fed into the chemical ionization (CI) source, giving a dramatically increased sensitivity. This approach has been pioneered by

0378-4355/83/$03.60

Rottschaefer *et al.*[5] and Henion and Maylin[6], and has also been adopted by Schäfer and Levsen[7].

The use of a pneumatic nebulizer[8] was not considered because of the constraints imposed by the small diameter of the solids probe introduction port of the Finnigan 3300 mass spectrometer.

The various types of small-bore HPLC columns may be classified as open-tubular (capillary), packed capillary and microbore columns[9]. At present, the last category is beyond the development stage. Columns, pumps and UV detectors can be purchased from an increasing number of suppliers.

This paper describes the development of a direct liquid introduction interface for the connection of an unmodified micro-HPLC instrument with a standard quadrupole mass spectrometer, operating in the CI mode.

EXPERIMENTAL

A Finnigan 3300 GC–MS instrument equipped with the standard CI source was used. Data were processed with a Finnigan 6110 computer system. Full-scan spectra were recorded under the following conditions: mass range, 150–350, integration time, 12 msec; seconds per scan, 3 As will be shown below, it is imperative that the source temperature be kept constant, which was effected with a CRL 405 digital temperature controller (CRL, Worthing, Great Britain), using the original Finnigan thermocouple as sensor The thermocouple was repositioned and clamped inside a hole drilled in the source block close to the solids probe inlet port. The temperature readout was calibrated against a platinum resistance thermometer, inserted into the source via the solids probe inlet The calibrated source temperature was varied between 200 and 265°C.

The original Varian thermocouple vacuum gauge is not well suited for recording the source pressure. It is located upstream in the flow of vapours and gases towards the ion volume and it has a fairly slow response. We have used an IM 10 high-pressure ion gauge (Leybold-Hereaus, Cologn, G.F R.), which has a measuring range of $1 \cdot 10^{-6}$ to 1.0 mbar, to monitor the pressure in the pumping line to the source diffussion pump. A value of approximately 2 10^{-3} mbar (1 mbar = 0.75 Torr) was measured if acetonitrile–water (70:30) was introduced at 10 μl/min The magnitude of the pressure fluctuations was recorded by connecting the output of the IM 10 to a pen recorder. The reactant ion spectrum was also recorded, using the hardware ion current integrator of the Quadrupole Electronics Module (mass range, 10–100; response, fast, electron multiplier at 1000 V) connected to the second channel of the two-pen recorder. When ammonia gas was added to modify the reactant ion spectrum[10] it was admitted via the solids probe solenoid valve, to ensure that solvent vapour is swept efficiently towards the ion volume.

The micro liquid chromatograph was a Jasco Familic 100N (Jasco, Hachioji, Japan), equipped with a Jasco ML 422 micro loop injector (0.3 μl internal loop), a Jasco pressure monitor with changeover valve and a Jasco Uvidec 100-III spectrophotometer detector (cell volume 0.3 μl). The home-made PTFE columns (about 150 mm long × 0 5 mm I.D × 1.8 mm O.D) were packed with Nucleosil C_{18} (5 μm particles) (Machery, Nagel & Co., Düren, G.F.R) using a tetrabromoethane–*n*-butanol (1.1) slurry. The columns had a plate count of 2000–2500 for the compounds

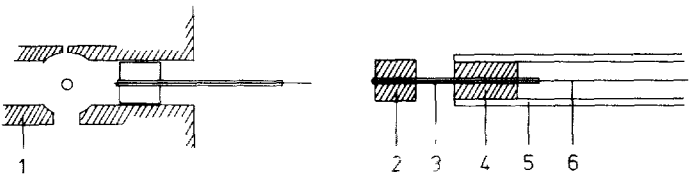

Fig 1 Schematic diagram of the LC–MS interface probe 1 = Source block, 2 = copper cylinder, 4 9 mm O D × 5 mm long, 3 = stainless-steel tube, 0 50 mm O D × 0 25 mm I D ; 4 = PTFE insulator, 5 = stainless-steel tube, 6 4 mm O D × 4 6 mm I D , 6 = 50 μm I.D flexible fused-silica capillary

shown below. It is expected that better columns can be made by following the directions given by Westwood *et al.*[11].

The LC–MS interface probe is shown in Fig. 1. The essential part is an approximately 70 cm–1 m long × 50 μm I.D. flexible fused-silica capillary tube (SGE, Melbourne, Australia). One end of the capillary protrudes into the source and is heated by heat transfer from the source via a copper block (4.9 mm O.D.) located approximately half way inside the inlet port of the source block (5.1 mm I.D.). The fused-silica capillary and the copper cylinder are mounted in a 1/4 in. O.D. stainless-steel tube. The entire system can be inserted and removed via the solids probe insertion lock in the same manner as the standard solids probe. The other end of the capillary is connected to the outlet of the UV detector.

RESULTS

During preliminary experiments with standard HPLC equipment only a very small portion of the eluate (1–2%) was actually used for mass spectrometry The interface was a 0.1 mm I D. × 4.0 mm O D. glass capillary tube with a narrow restriction on the side which protrudes into the ion source[12,13]. Attempts to create a jet of droplets[10,14] were successful but a fairly high liquid pressure was required A stable source pressure could not be maintained, however. To investigate the cause of this problem, we observed the jet inside a glass envelope. It appeared that a jet which was straight in air started to bend when a vacuum was applied. Our explanation is that both large and small droplets are formed and the small droplets evaporate more rapidly than the larger droplets. If the small droplets are predominantly formed on one side of the jet, owing to the irregular shape of the orifice, the expanding vapours from the small droplets push the larger droplets away from the axis of the jet when a vacuum is applied. This phenomenon is not unique to the simple capillary system: the occasional deviation of the jet emitted from a pinhole orifice has been reported[10] Because we had no rigid control over the shape and diameter of the restriction in the glass capillary, we abandoned the principle of jetting the liquid into the source.

For the next series of experiments the standard HPLC equipment was replaced by the Jasco micro-LC system[5–7], and the restriction made to the glass capillary was not as narrow. The solvent and solute now have to evaporate at the restricted end of the capillary. This, of course, limits the LC–MS system to samples that can be run off the normal direct insertion probe at reasonable temperatures (probably $< 300°C$). Nevertheless, there are enough problems that can be solved in this way[2,3,5–7]. In our hands success was variable, but the time spent on this approach was rather short.

Severe source pressure fluctuations were sometimes observed Making connections to the glass capillary was also inconvenient

A major step forward was the advent of narrow-bore flexible fused-silica capillary tubing. A length of 70 cm is sufficient to achieve transfer from the UV detector into the ion source without making further connections, and still allows adequate flexibility in the positioning of the chromatograph relative to the mass spectrometer. Capillaries with inner diameters of 25 and 50 μm have been tried so far. The 25-μm capillary required a pressure drop of 20 bar at a flow-rate of 10 μl/min (acetonitrile–water, 70:30), whereas the 50-μm capillary showed a pressure drop of only a few bars. All further experiments were carried out with the 50-μm I.D. capillary, which was considered more suitable in view of the pressure that can be delivered by the syringe pump of the Jasco micro-LC system

The main problem with a simple capillary direct liquid introduction system is to obtain a stable ion source pressure. Because no restriction is made to the side of the capillary that is located in the source, the liquid may be under reduced pressure. Moreover, the last 20 cm of the capillary, located inside the solids probe insertion lock, is at a slightly elevated temperature (30–35°C) by the convection of warm air circulating around the diffusion pumps and rotary pumps Gases dissolved in the mobile phase may easily form bubbles that expand and make the liquid being transferred into the source in the form of a series of short plugs. Severe pressure fluctuations are the result. This problem has been overcome by thoroughly degassing the mobile phase using an ultrasonic bath, followed by extensive flushing of all connecting tubing, the column and the detector cell.

A second problem is that the mobile phase mixture may freeze during the evaporation process if insufficient heat is transferred to the tip of the fused-silica capillary. This phenomenon can easily be recognized if the fused-silica capillary is connected directly to the micro loop injector valve, omitting the column and UV detector. As soon as a plug of ice is formed inside the capillary, the Jasco pressure monitor indicates a pressure build-up, which is accompanied by a pressure drop in the source and source pumping line. After some time the plug of ice is forced out of the capillary and the source pressure shows a peak, while the liquid pressure drops. A nude fused-silica capillary is showed this effect very markedlyu. The thermal mass of the tip and the transfer of heat to the tip are insufficient to support steady evaporation. The situation was improved by sliding a 50 mm long piece of 0.5 mm O.D. × 0.25 mm I.D. stainless-steel tubing over the fused-silica capillary. To have better control over the temperature the interface was further modified as shown in Fig. 1. The fused-silica capillary is fed through so far that its end is just observed under a magnifying glass. All further experiments were performed with this interface.

The Finnigan 3300 CI source has been designed to accept 20 ml (STP) methane per minute from a packed GC column, and reach a pressure reading of 1 0 Torr at this flow-rate (0.5 Torr on a McLeod gauge). A flow of 10 μl/min of water will produce 12 ml (STP) of water vapour per minute A 10-μl volume of liquid acetonitrile will produce 4.2 ml of vapour, so the CI source can easily accept the total effluent from the micro-LC system at 10 μl/min, and there is enough pumping capacity to modify the reactant ion spectrum by bleeding ammonia gas into the source[10].

Fig. 2 shows the reactant ion spectrum Sample molecules are ionized by pro-

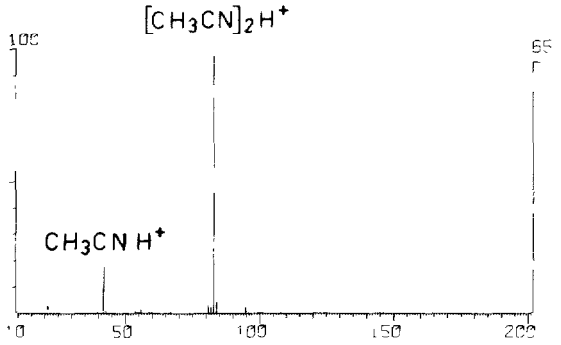

Fig. 2 Reactant ion spectrum of acetonitrile–water (70 30). Source temperature, 250°C.

ton transfer from the $[M + H]^+$ and $[2M + H]^+$ ions of acetonitrile at *m/z* 42 and 83. The reactant ion spectrum modified by ammonia is shown in Fig. 3. Ammonia has a higher proton affinity than acetonitrile so that the ions at *m/z* 42 and 83 have disappeared, while *m/z* 18 and solvated ammonium ions are generated instead. It may be advantageous to use a modified reactant gas because it will result in a softer proton transer to the sample, or in the formation of $[M + NH_4]^+$ ions[10]. Owing to our limited experience we cannot yet judge whether the performance of the interface is also influenced by the addition of ammonia gas.

Fig. 4 shows the liquid chromatogram of a mixture of four components. Full details regarding the analytical chemical aspects of the samples have been published elsewhere[15]. Fig. 5 presents the reconstructed (total ion current) liquid chromatogram for the same run, and indicates that 10 ng per component is just sufficient in the scanning mode (*m/z* 150–350). The signal-to-noise ratio of the total ion current profile can be improved by using only *m/z* 200–350 from the same data file for calculation of the reconstructed chromatogram. Injection of 50 ng per component of the same mixture resulted in the total ion current profile (*m/z* 150–350) in Fig. 6. The extracted ion current profile of *m/z* 266, corresponding to components 1 and 2, is also shown. The separation efficiency has deteriorated compared with the UV trace

During these experiments it appeared to be necessary to keep the source tem-

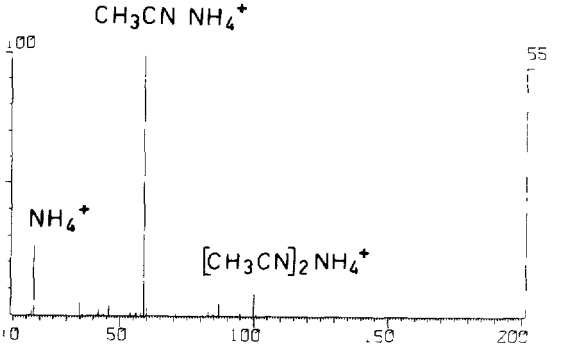

Fig 3 Reactant ion spectrum of acetonitrile water (70 30), modified with ammonia gas Source temperature, 250°C

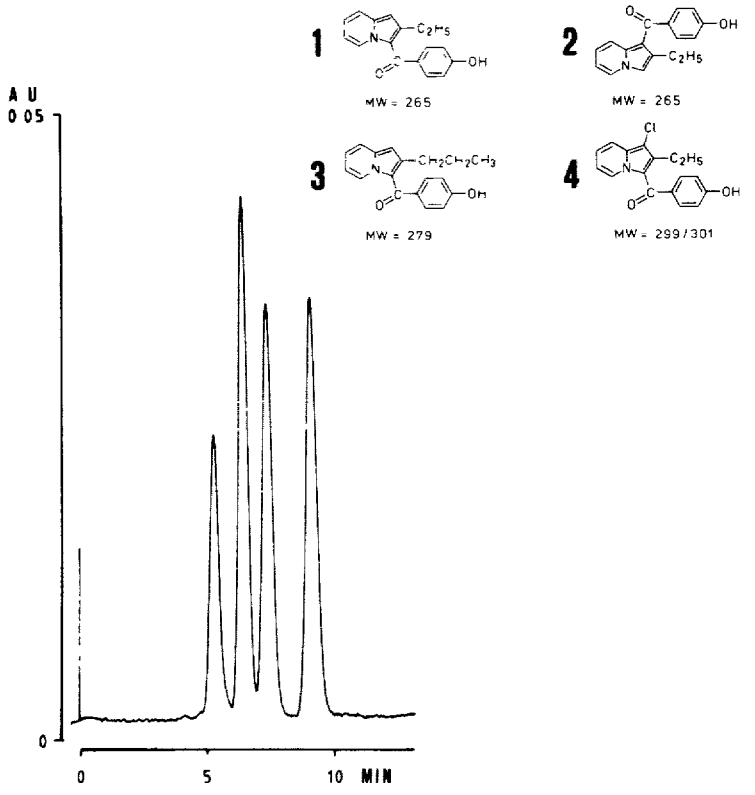

Fig 4 Liquid chromatogram of a mixture of four components, 10 ng per component The numbers indicate the order of elution Mobile phase, acetonitrile-water (70 30), flow-rate, 8 μl/min, column, 150 × 0 5 mm I D Nucleosil $5C_{18}$, UV detection at 390 nm

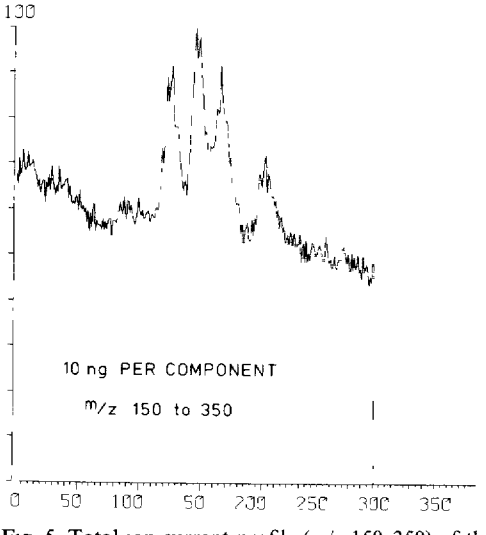

Fig 5 Total ion current profile (m/z 150–350) of the liquid chromatogram shown in Fig 4 Source temperature, 250°C

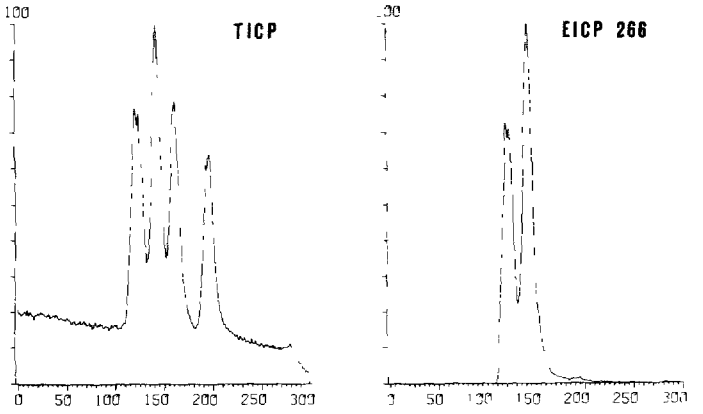

Fig 6 Left. total ion current profile of the same mixture as in Figs 4 and 5 (m/z 150–350), 50 ng per component injected Right extracted ion current profile (mass chromatogram) of m/z 266, corresponding to components 1 and 2 Ammonia gas added, source temperature, 250°C

perature at at least 250°C, which is in accordance with the observations made by Schäfer and Levsen[7]. Ammonia gas was added to the source in the experiment shown in Fig. 6. It did not cause a change in the observed mass spectra of the compounds under investigation. Fig. 7 presents the mass spectrum of component 4.

DISCUSSION

The results show how we arrived at a reasonable system in a purely empirical way. The diameter of the fused-silica capillary was dictated by the problems that we expected to occur when the syringe pump had to deliver a high pressure. The length of the copper block which transfers heat to the tip of the interface was arbitrarily chosen as 5 mm.

The major theoretical question concerning a capillary system, where liquid jet formation does not occur, is whether evaporation of the mobile phase takes place

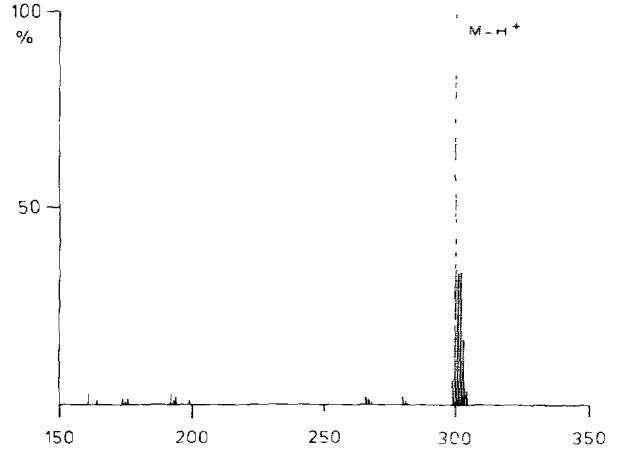

Fig 7 Mass spectrum of component 4 from Figs 4 and 6

inside or outside the capillary. Talroze *et al.*[16] have made calculations, which have been summarized by Arpino *et al.*[10]. Using the Poiseuille equations for flow of an incompressible liquid and a compressible, ideal gas, the lengths of the liquid column L and gas column l were calculated.

$$\frac{dn}{dt} = -\frac{\pi r^4 \rho \left(P_1 - P + \frac{2\sigma}{r}\right)}{8 \eta_l L M} \text{ mol/sec (liquid)} \tag{1}$$

$$\frac{dn}{dt} = \frac{\pi r^4 (P^2 - P_s^2)}{16 \eta_g l R T} \text{ mol/sec (gas)} \tag{2}$$

The liquid flow entering the capillary equals the gas flow leaving the capillary, so by elimination of $\frac{dn}{dt}$, the ratio

$$\frac{l}{L} = \frac{\text{length of gas column}}{\text{length of liquid column}}$$

can be calculated. It was concluded that evaporation always takes place inside the capillary.

This conclusion is correct, as long as the maximum possible speed of evaporation at the liquid–vapour phase transition is high compared with the mass flow-rates of liquid and gas in the capillary In a more recent paper, Zolotoi *et al* [17] have shown that if this condition is not fulfilled, *i.e.*, if on the contrary the evaporation rate is smaller than the flow-rate of the liquid, it is indeed possible to make the liquid flow out of the capillary into the vacuum Their calculations were confirmed by experiments. Fig. 8 gives a schematic representation of the evaporation of the liquid. Situation a corresponds to eqns. 1 and 2, and the maximum evaporation rate from the area of the meniscus is high compared with the liquid flow-rate. In case b the evaporation rate is equal to the liquid flow-rate. In case c, the liquid flow-rate is higher than the evaporation rate from the area of a meniscus just inside the capillary. The

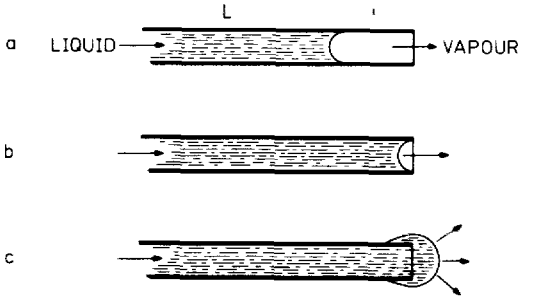

Fig 8 Three possible situations during evaporation of a liquid from a capillary into a vacuum

liquid now flows out of the capillary, the evaporation area is enlarged and equilibrium between liquid flow-rate and evaporation area is established.

The rate of evaporation, Z, of a liquid at a liquid–vacuum interface, from an area A at temperature T, can be calculated from the kinetic gas theory[18]:

$$Z = P_0 A \sqrt{\frac{1}{2\pi mkT}} \text{ molecules/sec} \tag{3}$$

or in SI units:

$$G = P_0 A \sqrt{\frac{10^{-3}M}{2\pi RT}} \text{ kg/sec} \tag{4}$$

The area A is assumed to be a hemisphere with radius r (radius of the capillary). P_0, the saturated vapour pressure of the liquid, can be taken from tables (water, methanol) or can be calculated (acetonitrile) from critical temperature and pressure using the Van der Waals expression[19].

$$\log \frac{P_0}{P_c} = \text{constant} \left(1 - \frac{T_c}{T}\right) \tag{5}$$

Fig. 9 shows the calculated evaporation rates from an area equal to a hemisphere of radius 25 μm as a function of temperature for water, methanol and acetonitrile. At a liquid flow-rate of 10 mg/min (10 μl/min for water, 12 μl/min for

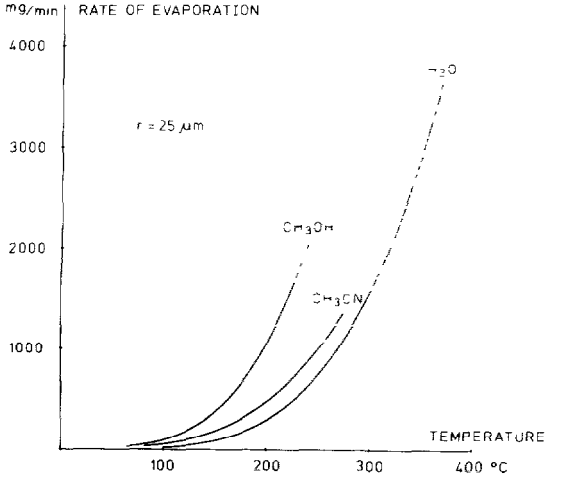

Fig 9 Rate of evaporation of three liquids from a capillary of radius 25 μm as a function of temperature between the boiling point and the critical point The evaporation area is assumed to be a hemisphere, see Fig 8b

methanol and acetonitrile), situation b in Fig 8 is met at 76, 36 and 44°C with water, methanol and acetonitrile, respectively A higher temperature results in situation a and a lower temperature gives situation c.

Experimentally, a stable source pressure is achieved above about 250°C, as demonstrated by our experiments and literature data[3,6,7]. Schäfer and Levsen[7] have reported that cooling the capillary interface results in strong source pressure fluctuations. In case c, the evaporation area is not fixed, but is variable, which explains the uneven evaporation of the liquid phase. The experimental data, combined with the theory presented above, suggest that it is necessary to work in situation a in Fig. 8.

Further calculations can be made to obtain the length, l, of the gas column in case a. When the meniscus has retracted inside the capillary, the vapour pressure above the liquid rises. The pressure required to force a gas flow, dn/dt, through a capillary is calculated from eqn 2. If the assumption is made that the source pressure P_s is very small compared with P, eqn. 2 is reduced to

$$G = \frac{\pi r^4 \cdot 10^{-3} MP^2}{16\ \eta_g lRT} \text{ kg/sec} \tag{6}$$

or

$$P = \sqrt{\frac{16\ \eta_g lRTG}{\pi r^4 \cdot 10^{-3} M}} \text{ Pa} \tag{7}$$

Using this equation, the pressure, P, necessary to force 10 mg/min of water vapour into the ion source through a capillary of length l was calculated and is shown in Fig. 10.

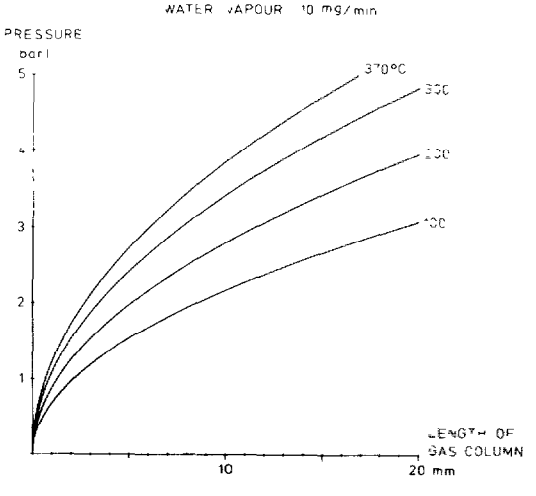

Fig 10 Pressure build-up inside a capillary (radius 25 μm) when water vapour is transferred to a vacuum at a flow-rate of 10 mg/min as a function of the distance from the outlet of the capillary

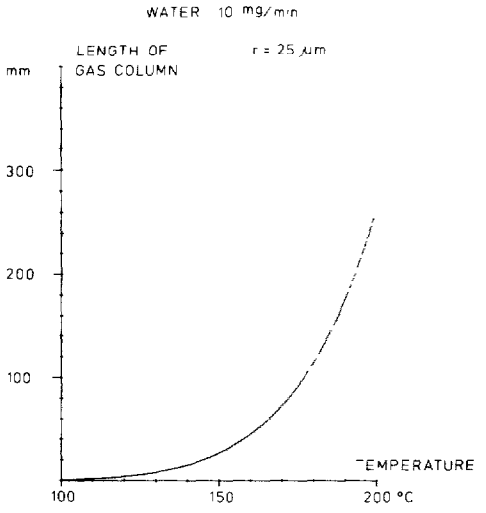

Fig 11 Distance between the liquid level and the outlet of a capillary (radius 25 μm) as a function of temperature for water at 10 mg/min

If evaporation does not take place into a vacuum, but into a vessel with pressure P, the evaporation rate has to be calculated from[18]

$$G = (P_0 - P)\, A \sqrt{\frac{10^{-3} M}{2\pi RT}} \text{ kg/sec} \tag{8}$$

After substitution of eqn. 7 into eqn 8 and choosing $G = 10$ mg/min, the relationship between l and T is obtained, which is shown in Fig. 11. It appears that at 122°C the liquid level of water has retracted 5 mm inside the capillary, which is the length of the copper block (Fig. 1)

The calculations show that evaporation inside the capillary takes place at moderate temperatures. The experimental data seem to indicate that the temperature of the liquid at the meniscus is much lower than the source temperature, in spite of the heat transferred by the copper block

CONCLUSION

A simple LC–MS interface can be constructed from fused-silica capillary tubing, provided that care is taken to transfer enough heat to the tip of the capillary inside the ion source. The experimental data and theoretical considerations show that evaporation of the liquid takes place inside the capillary. Temperature plays a very important role, so that a constant source temperature is required to achieve a stable evaporation rate and a stable source pressure.

A disadvantage of the present system, compared with direct liquid introduction systems using liquid jet formation, is that non-volatile samples and impurities will tend to block the capillary. On the other hand, fused silica tubing can be purchased by the metre, and is easily replaced, if necessary

SYMBOLS

STP	standard temperature and pressure
dn/dt	molar flow-rate (mol/sec);
r	radius of capillary tube (m);
σ	solvent surface tension,
P_1	liquid pressure at inlet of capillary (Pa);
P	vapour pressure at liquid–vapour phase transition (Pa),
P_0	saturated vapour pressure of liquid (Pa);
P_s	ion source pressure (Pa);
P_c	critical pressure (Pa);
η_l	viscosity of liquid (Pa sec),
η_g	viscosity of gas (Pa sec),
R	gas constant, 8.3 (Joule/°K mol);
M	molecular weight;
$10^{-3} M$	mass of 1 mol (kg);
l	length of gas column inside a capillary (m),
L	length of liquid column inside a capillary (m);
G	mass flow-rate of evaporation rate (kg/sec);
T	temperature (°K),
T_c	critical temperature (°K),
A	area of surface from which evaporation takes place (m^2);
m	mass of one molecule,
k	Bolzmann constant.

Conversion factors: 1 bar = 100 kPa; 1 mbar = 0.75 Torr

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