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OPEN-TUBULAR LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY WITH A CAPILLARY-INLET INTERFACE

W. M. A. NIESSEN*,[*] and H. POPPE

Laboratory of Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam (The Netherlands)

SUMMARY

The capillary-inlet interface is a very simple and inexpensive interface for the coupling of open-tubular liquid chromatography (OTLC) and mass spectrometry (MS). It shows some attractive features from both the mass spectrometric and the chromatographic point of view. Due to the low flow-rates applied with 5–10 μm I.D. open-tubular columns, it is possible to obtain on-line electron-impact spectra with the OTLC–MS system. However, the range of applicability of this interface appears to be limited to volatile compounds. Various aspects concerning the coupling of OTLC and MS are discussed: the experimental set-up, chromatographic performance, mode of ionization, range of applicability and detection limits.

INTRODUCTION

In a capillary-inlet interface, the column effluent flows through a narrow capillary tube, evaporates near the end of that tube and can then be analyzed by mass spectrometry (MS). The capillary-inlet system is the first on-line liquid chromatography (LC)–MS interface reported[1,2]. Although it has never become commercially available, it is frequently described[3–21], probably because of its simple and relatively cheap design.

In the earliest experiments, such a system was used to transfer about 0.1% (1 μl/min) of the effluent from an open-bed LC column into a mass spectrometer, operated in the electron-impact (EI) mode[1,2]. Later, conventional high-performance liquid chromatographic (HPLC) columns were used. About 1% (10 μl/min) of the methanol–water effluents from those columns was fed into a mass spectrometer, operated in the chemical ionization (CI) mode[3–7]. In more recent experiments, small-bore LC columns were used. The complete effluent from such a column can be introduced into a CI-MS vacuum system[8–17]. Recently, the capillary inlet was also used to interface packed capillary columns with EI-MS[18,19].

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* Present address: Center of Bio-Pharmaceutical Sciences, Section Analytical Chemistry, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands.

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We selected the capillary-inlet interface for coupling open-tubular (OT)LC and MS, because of its simplicity and the absence of external peak broadening due to the interface. Such an interface has previously been used in OTLC–MS coupling by Ishii and Takeuchi[20] and by Tijssen *et al.*[21]. However, the former system is not very well documented, while in the latter system irregular peak shapes and fluctuating ion-source pressures were observed for concentrated samples or high-molecular-weight ($M > 250$) solutes. In both systems CI-MS was used.

It is the aim of this paper to show that a capillary-inlet system can be used to interface OTLC and EI-MS. Since the effluent has to be evaporated at the end of the column, the interface is expected to have a limited range of applicability. As little is known about this some appropriate experiments were made. The system was also used to study external peak-broadening effects in OTLC–MS.

Evaporation of solvents

The introduction of liquids through capillary tubes into a high-vacuum region has been extensively studied by Tal'roze *et al.*[1,2,22], Arpino *et al.*[23,24] and Bruins and Drenth[15]. In a capillary-inlet interface the mass flow of liquid introduced relative to the evaporation rate determines the position of the liquid–vapour interface: either inside the tube, at the end of the tube or even outside the tube. It can be shown that, in all cases relevant to capillary-inlet LC–MS, the complete evaporation of the solvent takes place inside the tube[15,23,24]. Under these conditions, the solvent is not evaporating into a vacuum, but into a vapour column inside the tube. The evaporation process is counteracted by the pressure build-up due to the solvent vapour flow in the capillary tube. To calculate the rate of evaporation, a set of two equations, given by Bruins and Drenth[15], must be solved. The pressure build-up due to the solvent vapour flow, P_g, can be calculated from the equation

$$P_g = \frac{256\, \eta_g\, lRTG}{\pi d_c^4 M} \tag{1}$$

in which η_g is the viscosity of the vapour, M is the molecular mass (in kg/mol), R is the gas constant, T is the absolute temperature, d_c is the column inner diameter and l is the length of the vapour column. The rate of evaporation, G, in kg/s, of the solvent obeys the equation[15]

$$G = (P_o - P_g)\, A \left(\frac{M}{2\pi\, RT}\right)^{\frac{1}{2}} \tag{2}$$

in which A is the evaporation surface area and P_0 is the vapour pressure of the liquid. These two equations can be used to calculate the distance, l, between the liquid meniscus and the end of the tube, *i.e.*, the length of the vapour column, as a function of flow-rate, column diameter and temperature. In Fig. 1, the length of the vapour column is given as a function of the temperature and flow-rate in a 10-μm I.D. column. Similar plots can be made for other column diameters. It can be concluded that the evaporation of the solvent takes place at moderate temperatures, and that the length of the vapour column will increase with increasing temperature and decreasing flow-rates.

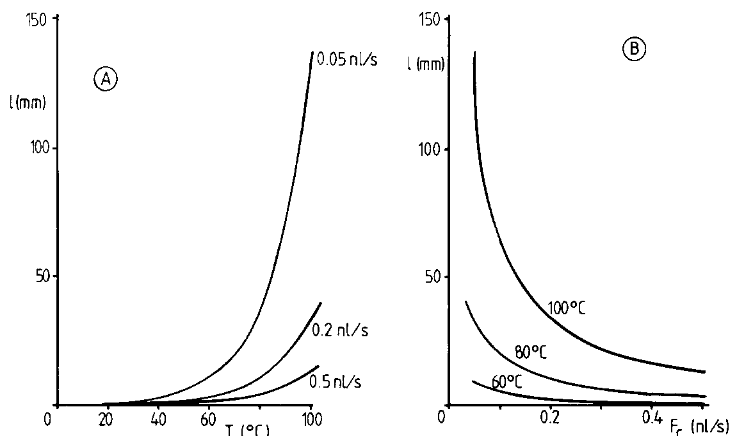

Fig. 1. Evaporation of methanol in a capillary-inlet interface (eqns. 1 and 2). (A) Length of the vapour column, l (mm), as a function of the temperature, T (°C), for several flow-rates in a 10-μm I.D. column. (B) Length of the vapour column, as a function of the flow-rate, F_c (nl/s), in a 10-μm I.D. column at several temperatures.

As it is not practical to have an effective chromatographic column length which depends on the flow-rate and temperature, and as non-volatile compounds in the effluent stream tend to precipitate in the capillary tube when the solvent evaporates, it was decided to build an interface which allows heating of only a small part of the column and effective cooling of the remaining part of the column in the interface. Then the evaporation (or precipitation) takes place in the last few centimetres of the capillary column.

It must be pointed out that the capillary-inlet interface differs significantly from other direct introduction type interfaces, such as the thermospray interface. In the former system complete evaporation takes place inside the interface and a stream of vapour is introduced into the ion source, while in the latter evaporation is essentially partial, forming a beam of vapour and droplets. The capillary-inlet interface will become a thermospray-like interface when it is used at very high linear liquid velocities (> 0.5 m/s). However, such conditions are not practical in open-tubular columns.

EXPERIMENTAL

Chromatography

The solvent-delivery system used in our experiments was as described previously[25]. The mobile phase was delivered by one of the heads of a dual-head pump (Model DMP 1515 or Model AE 10-4.4; Orlita, Giessen, F.R.G.), operated as a constant pressure pump using appropriate fused-silica (SGE, Ringwood, Australia) capillary restrictions. A schematic diagram of the system is given in Fig. 2.

The flow system was built from stainless-steel capillaries and standard Swagelock fittings. Connections to fused-silica capillaries were made with PTFE, Vespel, graphitized Vespel or graphite ferrules (Chrompack, Middelburg, The Netherlands; SGE). Such connections can be made leak-tight up to at least 30 MPa.

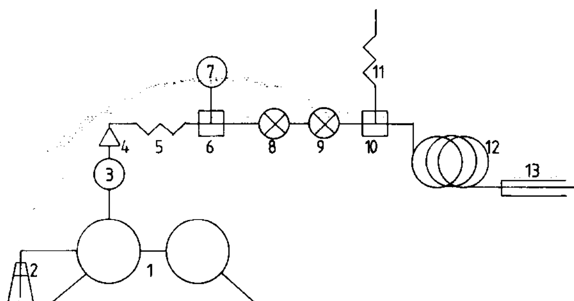

Fig. 2. Solvent-delivery system used in the OTLC–MS experiments. 1 = Dual-head pump; 2 = solvent reservoir; 3 = flow-through manometer; 4 = line filter; 5 = 0.12 m × 50 μm I.D. capillary restriction; 6 = T-piece; 7 = precision manometer; 8 = optional injection valve with a 1.6-ml loop; 9 = injection valve with a 10-μl loop; 10 = split-injection T-piece; 11 = 0.20 m × 100 μm I.D. capillary restriction; 12 = fused-silica open-tubular column; 13 = capillary-inlet interface probe.

Two manometers were used in the OTLC pumping system: a 0–40 MPa Bourdon-type flow-through pressure gauge, for monitoring the pressure at the pump head, and a 0–6 MPa Bourdon-type precision pressure gauge, for monitoring the column pressure. The latter manometer was connected by means of a Swagelock 1/16-in. T-piece. A short, fused-silica capillary restriction was inserted between these manometers in order to create a back pressure of at least 5 MPa to ensure good performance of the Orlita pump. A line filter (0.5 μm) (Nupro, Willoughby, OH, U.S.A.) was included in the pumping line. In most experiments, a Model 7020 (or Model 7120) injection valve with a 10-μl loop (Rheodyne, Berkeley, CA, U.S.A.) was used. The total flow-rate through this system was between 20 and 60 μl/s.

The injection valves were used in combination with a splitting device, assembled as follows. The injection valve was connected to a Swagelock 1/16-in. T-piece by a short, narrow-bore, stainless-steel capillary. The fused-silica open-tubular column was inserted through the T-piece and the stainless-steel capillary to a position as near to the valve rotor as possible, in order to make the dead volume at the top of the column as small as possible. The open end of the T-piece was connected to a short, fused-silica capillary restriction, which defines the splitting ratio in the split-injection system. Similar split-injection devices have been described[26,27].

The split injection system, described here, was found to be a convenient method both of injecting small volumes and controlling small flow-rates. The relative standard deviation in measured peak heights and in the peak residence times in series of injections can be less than 4 and 0.2%, respectively.

Unmodified, fused-silica tubings with various internal diameters were used as dummy columns in the experiments. It appeared that the column diameters, as stated by the manufacturers (SGE; Chrompack; Polymicro Technologies, Phoenix, AZ, U.S.A.) were not always correct. It was decided to calculate the column diameter from our own experimental data on the pressure drop and peak residence time. According to Poiseuille's equation, the column diameter, d_c, is given by

$$d_c = \left(\frac{32\,\eta\,L^2}{\Delta P\,t_0}\right)^{\frac{1}{2}} \qquad (3)$$

in which η is the solvent viscosity, L is the column length, ΔP is the pressure drop over the column and t_0 is the peak residence time. The measured pressure drop is the difference between the pressure at the top of the column and the atmospheric pressure. Since in the capillary inlet the column effluent flows directly into the high-vacuum region of the mass spectrometer, the actual pressure drop over the column is 0.1 MPa larger than the measured inlet pressure. The calculation was made with the peak residence times at five different values of the pressure drop.

LC–MS interface

The first experiments in our laboratory with a capillary-inlet system were performed by inserting the open-tubular column through the gas chromatographic (GC) transfer line into the MS vacuum system. The column end was near the EI source. In this configuration, heat transfer between the transfer line and the column was poor, resulting in severe pressure fluctuations in the source envelope when flow-rates over 20 nl/s of methanol were used. The pressure fluctuations are probably due to freezing of the solvent at the end or in the capillary tube. Furthermore, this system is not very convenient to operate: in order to replace or unplug a column, the complete vacuum system must be turned off. Therefore, it was decided to build an inlet probe to fit the laboratory-built direct insertion inlet of the mass spectrometer.

In a second set of experiments, the top of a 10-mm O.D. laboratory-built capillary-inlet probe was heated by a Macor (a machinable ceramic glass; Corning Glass Works, New York, NY, U.S.A.) block, fitted close to the CI source (Fig. 3). In this way, the heat transfer was improved: up to 100 nl/s of methanol could be introduced without severe pressure fluctuations. Almost all experiments described in this paper were actually performed with this interface probe.

Mass spectrometry

The mass spectrometer used was a Model 3100 GC–MS system (Finnigan, San Jose, CA, U.S.A.). The vacuum system was modified to allow differential pumping between the analyzer and the ion-source housing[25]. The analyzer and ion-source regions were separated by a baffle.

The analyzer and multiplier region was evacuated with the standard 0.3-m^3/s oil-diffusion pump (Model NRC HS2; Varian, Lexington, MA, U.S.A.).

The ion-source housing was evacuated using a 0.3-m^3/s oil-diffusion pump (Model MK2-100/300; Edwards, Crawley, U.K.), backed by a 9.5-m^3/h rotary fore pump (Edwards, Model E2M8). In some applications a 0.035-m^2 active-surface-area cryogenic pump was used. It consisted of a 60-mm diameter cylindrical stainless-steel tube, which could be filled with liquid nitrogen which was added regularly from a

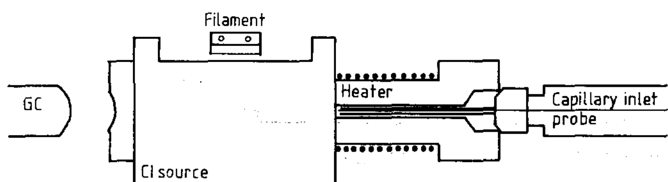

Fig. 3. Schematic diagram of the capillary-inlet interface probe with external-heater assembly.

Dewar flask. The liquid-nitrogen consumption in the system is about 2–4 l per day. By installing butterfly valves, cleaning of the cryopump was possible without turning off the vacuum system. The pressure in the ion-source housing was monitored with a Penning ionization gauge (Edwards, Model 8).

The LC–MS interface probes fitted a laboratory-built direct insertion inlet[25]. This inlet system was evacuated by a 4.5-m^3/h mechanical pump[28] (Alcatel, Model 2004A), equipped with an Edwards Model FL20K foreline trap.

Range of applicability

Chromatographic peaks from short, 5–10-μm I.D. open-tubular columns have peak standard deviations less than 1 s. In studying the range of applicability of the capillary-inlet interface, it is desirable to have more time to study the spectrum and to tune the instrument, especially in the absence of a data system. By modifying the solvent-delivery system, a versatile method for the continuous introduction of samples over a relatively long period was created. A second injection valve (Model 7020, Rheodyne) equipped with a 1.6-ml loop was added between the pressure gauge monitoring the column pressure and the small-volume injection valve, *cf.*, Fig. 2. The injection loop was filled with a 1–5 mg/ml methanolic solution of a particular solute, using a peristaltic pump (Model Mini-Micro 2/6; Instamatic, Zurich, Switzerland) and an in-line filter. A 1–1.5 m long, *ca.* 8-μm I.D., fused-silica capillary was inserted as a dummy column in these experiments. The temperatures of both the ion source and the capillary-inlet heater were about 170–200°C.

After injection, the appearance of the solute was monitored, using one of the channels of the programmable multiple-ion monitoring (PROMIM), set at the *m/z* ratio of one of the ions expected. Some examples of the selected-ion tracings observed are shown in Fig. 4. As the flow-rate through the loop was about 3.6 μl/s in these experiments, a plateau, indicating constant delivery of sample to the mass spectrometer, was observed for about 7 min. During this period the mass spectrometer and the PROMIM channels were tuned and the spectra were obtained.

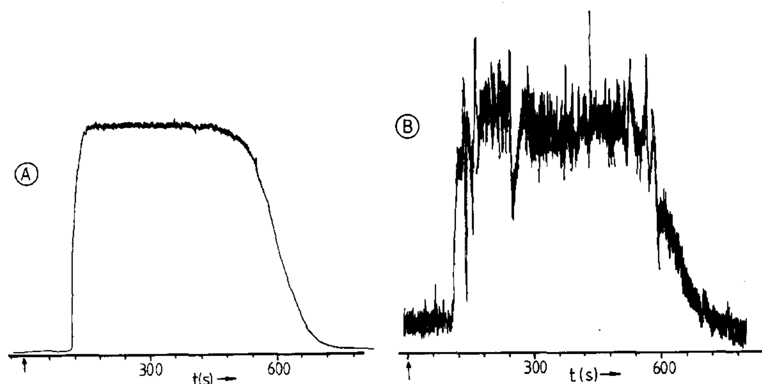

Fig. 4. Selected-ion tracings for the "continuous" introduction of (A) toluene (*m/z* 91, 5 mg/ml) and (B) diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea, *m/z* 284, 1.8 mg/ml]. Conditions: 1.50 × *ca.* 8 μm I.D. untreated, fused-silica column; 0.5 nl/s methanol; capillary inlet under EI conditions.

RESULTS AND DISCUSSION

Ionization mode

Solvent spectra. Operating open-tubular columns near the kinetic optimum velocity of a few mm/s will give flow-rates of between 0.05 and 10 nl/s. Upon evaporation, this will result in gas flows of between $6 \cdot 10^{-6}$ and $1.2 \cdot 10^{-3}$ Pa · m^3/s for water and of between $2.8 \cdot 10^{-6}$ and $5.5 \cdot 10^{-4}$ Pa · m^3/s for methanol. Introduction of 10 nl/s of water (or methanol) into a high-vacuum chamber while maintaining a pressure of 1 mPa in that chamber requires an effective pumping speed of about 0.9 m^3/s (0.4 m^3/s for methanol). Therefore, the total effluent of a 5–25 μm I.D. open-tubular column can easily be introduced into a modern, differentially pumped, quadrupole mass spectrometer. The pressure in the ion source will be low enough to obtain EI spectra. Pressures in the ion-source housing of about 1.5 mPa were obtained when 14 nl/s of methanol were introduced into the differentially pumped vacuum system described. Higher flow-rates can be tolerated, or lower pressures can be obtained, by using the liquid nitrogen trap installed in the system.

Methanol mass spectra were obtained using the EI source in both the modified and the unmodified vacuum system, and using the CI source in the modified vacuum system. The spectra obtained using the "open" EI source closely resemble methanol EI spectra reported in the literature, with m/z 31 as the base peak. With the "more gastight" CI source, this resemblance is obtained only when less than 2 nl/s of methanol are introduced. Some low-intensity solvent cluster ions can be observed at m/z 45 and 63. At higher flow-rates, mixed EI–CI spectra are obtained. When the relative ion intensity at various m/z ratios in the methanol spectrum is studied as a function of the flow-rate, a shift from EI to CI spectra can be observed. When the flow-rate is increased, the intensities of the ions at m/z 31, 45 and 63 decrease, while the intensities of those at m/z 33, 47 and 65 increase[29].

Solute spectra. The capillary-inlet interface has found frequent application in LC–MS studies. However, chemical ionization is the ionization method used in almost all studies. The ability to obtain EI spectra in OTLC–MS is certainly very attractive. When the applicability range of the capillary-inlet interface was studied, spectra of several solutes were obtained. Extensive fragmentation profiles were characteristic of these spectra. In Table I, data for some of the solutes measured are compared with EI reference spectra[30,31]. Some of the spectra obtained are shown in Fig. 5. The locations of the various peaks in the spectra are correct in most cases. Deviations in relative abundance between the measured and reference spectra may be due to various causes: *e.g.*, some thermal decomposition of the solutes in the interface, pyrolysis on the hot surfaces of the ion source, insufficient tuning of the ion source and discrimination of quadrupole mass filters against ions having higher m/z ratios.

It can be concluded that EI-like mass spectra can be obtained using the capillary-inlet system as an interface for OTLC–MS. Of course, it must be admitted that at the low-mass end of the spectrum, peaks due to solvent ions will interfere with the solute spectrum. The use of the open EI source and 5–10 μm I.D. columns, which can be used at low flow-rates, is most advantageous in this respect. It is generally agreed that such columns have the best prospects for OTLC applications, because of the combination of high efficiency with a favourable phase ratio and mass loadability.

TABLE I

EI-LIKE SPECTRA, OBTAINED WITH OTLC–MS AND THE CAPILLARY-INLET INTERFACE. COMPARISON WITH REFERENCE SPECTRA[30,31]

The presence of the mass peak at m/z 149 is due to a phthalate impurity.

Naphthalene			*2-Aminobenzoic acid*			*2,4,6-Trichlorophenol*			*Caffeine*			*Nicotinamide*			*Diazepam*		
m/z	*Ref.*	*Exptl.*	m/z	*Ref.*	*Exptl.*	m/z	*Ref.*	*Exptl.*	m/z	*Ref.*	*Exptl.*	m/z	*Ref.*	*Exptl.*	m/z	*Ref.*	*Exptl.*
128	100	100	119	100	84	196	100	37	194	100	45	122	100	53	256	100	100
51	12	*	93	79	100	198	97	36	67	66	71	104	80	47	283	87	47
129	27	19	137	59	40	200	31	10	109	66	86	78	80	100	284	69	43
64	11	*	92	59	50	97	20	100	55	44	*	106	60	14	255	42	33
127	10	13	44	38	*	131	18	51	82	39	100	51	60	*	257	41	54
			65	35	*	133	12	34	42	28	*	77	50	21	285	38	35
			66	27	*	99	10	41	40	18	*	50	40	*	258	35	40
			39	21	*	132	8	?	41	16	*	52	25	*	286	24	21
									149	0	63	149	0	33	220	0	39

* No peaks with m/z less than 70 available.

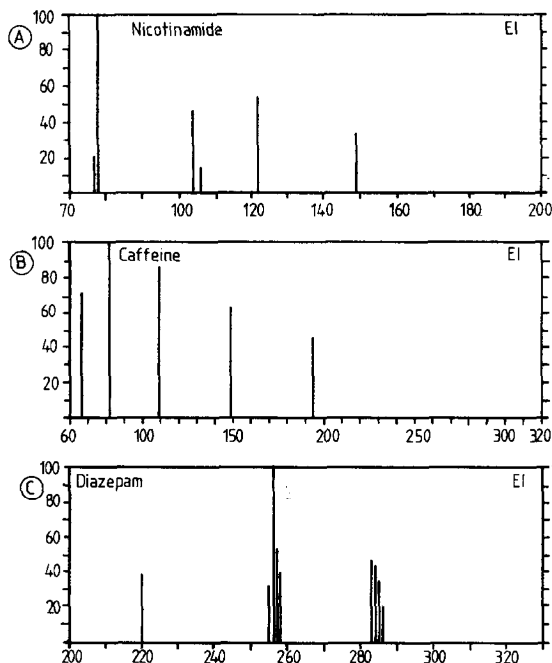

Fig. 5. Some mass spectra obtained with OTLC–MS by using the capillary-inlet interface. Conditions: continuous introduction of 1–5 mg/ml solution in methanol (flow-rate, 0.5 nl/s); probe and source temperatures, 170–200°C. (A) Nicotinamide (3-pyridinecarboxamide); (B) caffeine (1,3,7-trimethyl-2,6-purinedione); (C) diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one).

Range of applicability

In the theoretical section the evaporation of the LC mobile phase from a capillary inlet has been discussed. That discussion does not answer questions concerning the evaporation of solutes, which are less volatile than the solvent. Since in a capillary inlet the sample evaporates in the column, the system is expected not to be applicable to the analysis of solutes with low volatility. Problems of capillary blocking due to the collection of solvent impurities and non-volatile solutes at the end of the capillary tube have frequently been described, *cf. e.g.*, refs. 23 and 24. No systematic studies have yet been made to determine the limits.

Either the chromatographic peaks from reconstructed total- or selected-ion monitoring, or complete mass spectra, have been reported for over 150 compounds. Chemical ionization has been used in the majority of these experiments[2–21]. Among the compounds studied are polycyclic aromatic hydrocarbons, pesticides, phenol derivatives, steroids and various drugs. The molecular masses of these compounds range from 18 to 638 g/mol, while the majority of the compounds investigated have molecular masses between 100 and 300 g/mol. For about 80 of these compounds, boiling points are available from general handbooks[31–33], ranging from 35 to 495°C (at atmospheric pressure). Several of the other compounds are expected to decompose upon heating. Melting points, when available, are also collected, especially for those compounds for which no boiling points are available. About 15 of the latter com-

pounds have a melting point between 250 and 300°C. It can be concluded that a capillary-inlet interface can be applied to the analysis of rather polar compounds, having high boiling or melting points. Some interesting applications have been reported for compounds, best analysed by LC[2-21].

In the present work, over 50 compounds have been studied. Either spectra or chromatographic peaks have been obtained. Among the compounds studied are phenol and benzoic acid derivatives, polycyclic aromatic hydrocarbons, pesticides, *e.g.*, diuron and aldrin, and drugs, *e.g.*, caffeine, phenacetin and diazepam.

Fig. 4 shows the difference in the selected-ion tracing when either a volatile solute, such as toluene, or a rather involatile solute, such as diuron, was introduced. In the latter case, the ion tracing is considerably more noisy.

Some of the compounds investigated did not give any spectrum at all, or even blocked the capillary tube. Ascorbic acid gave peaks of low intensity at the low-mass end of the spectrum only; its PROMIM signal was weak and very irregular. Theophylline hydrochloride, phenylalanine and glucose blocked the capillary tube. Glucose decompostion products were observed in the capillary tube by microscopic inspection. Caffeine gave rise to irregular pressures in the ion-source housing, although chromatographic peaks were detectable in this case.

The molecular masses of the compounds studied in our experiments range from 90 to 360 g/mol. For most compounds, either the boiling point or the melting point or both are available from general handbooks[31-33]. Spectra or chromatographic peaks were observed for compounds with boiling points up to 340°C and/or melting points up to 250°C. No blocking of the capillary tube was observed when these compounds were analyzed.

Fig. 6 shows the chromatographic peak shapes resulting from *ca.* 50-pl injections of toluene and phenacetin onto the 8-μm I.D. dummy column. Although this system is certainly not optimized for the peak shape, as seen from the shape of the toluene peak, the shape of the phenacetin peak shows that less volatile solutes may give rise to irregular peaks. This observation is in agreement with that of Tijssen *et al.*[21]. The reason may be either that the heat transfer is too slow at the probe tip, or that the temperature of the probe-tip heater and/or the ion source is too low. The importance of fast and efficient heat transfer to the open-tubular column has been

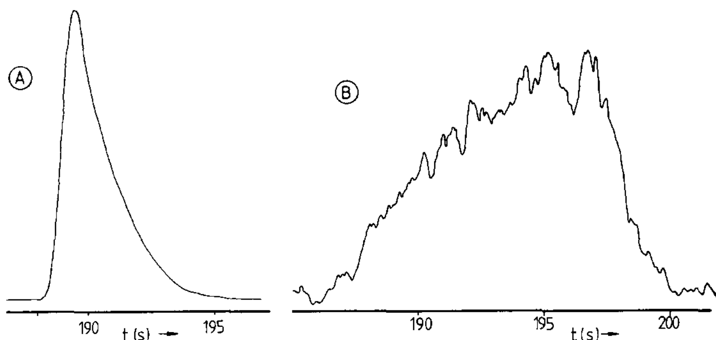

Fig. 6. Chromatographic peak shapes of (A) toluene (*m/z* 91, 5 mg/ml) and (B) phenacetin [N-(4-ethoxyphenyl)acetamide, *m/z* 108, 3 mg/ml] from 50-pl injections. Conditions as in Fig. 4.

recognized. Therefore, a capillary-inlet probe, ensuring better heat transfer, was built, but could not be used in practice due to a design fault.

Several of the problems encountered in our studies had been anticipated when we started this investigation. These problems result from the conditions prevailing in OTLC–MS which are quite different from those in most of the experiments previously reported[2–21]. The inner diameter of the inlet tube in the OTLC–MS experiments is 5–30 μm, while inlet tubes of 50–100 μm I.D. were used in most earlier experiments. A capillary tube of *ca.* 8 μm I.D. is used in the present work. Flow-rates of 0.1–0.3 μl/s have been reported, resulting in linear velocities between 15 and 40 mm/s for a 100-μm I.D. inlet tube, and over 60 mm/s for a 50-μm I.D. inlet tube. In the present OTLC–MS experiments, linear velocities of 5–10 mm/s were used; the flow-rate was *ca.* 0.5 nl/s. The 200-fold reduction in flow-rate resulted in an equal reduction in the mass flow-rate of the solute. In most experiments reported previously[2–21] CI was used, while in the OTLC–MS experiments described here EI was used, resulting in considerably more fragmentation and a less "clean" spectrum.

Summarizing, it may be concluded that some interesting compounds can be studied when the capillary-inlet interface is used in OTLC–MS. However, our experiments clearly demonstrate three problems: the clogging of the capillary tube, the heat transfer to the latter and the detection limit.

Chromatographic performance

Operating open-tubular columns near the kinetic optimum results in extreme requirements for reduction of the external peak broadening. Knox and Gilbert[34] have shown that it is necessary to reduce external peak broadening to values as low as 1 nl. Since in a capillary-inlet interface the total column effluent evaporates into the high-vacuum ion source, the interface is not expected to contribute to peak variance. However, Fig. 6 shows that slow evaporation processes may result in significant peak broadening and irregular peak shapes. This problem probably can be avoided to some extent by enhancing the heat transfer to the open-tubular column.

A volatile solute (toluene) was used to study the external peak broadening in our complete chromatographic system. Untreated, fused-silica tubings of various inner diameters, giving unretained peaks, were utilized as dummy columns. The measured plate heights were compared with those calculated according to the Golay equation for unretained peaks

$$H = \frac{2\,D_m}{u} + \frac{d_c^2\,u}{96\,D_m} \tag{4}$$

in which H is the plate height, d_c is the column diameter, u is the linear velocity and D_m is the diffusion coefficient of the solute in the mobile phase. The latter is calculated from the Wilke-Chang equation[35] to be $1.67 \cdot 10^{-9}$ m²/s for toluene in methanol. The measured plate heights were also compared with those corrected for external broadening, calculated by using the equations derived by Anderson and Walters[36] for the peak deconvolution of exponentially modified Gaussian peaks, *cf.*, refs. 29 and 37.

Plots of plate height *vs.* linear velocity for various columns are shown in Fig. 7. When discussing these plots, it must be remembered that significant inaccuracies may be inherent in the values of the diffusion coefficient of the solute in the mobile

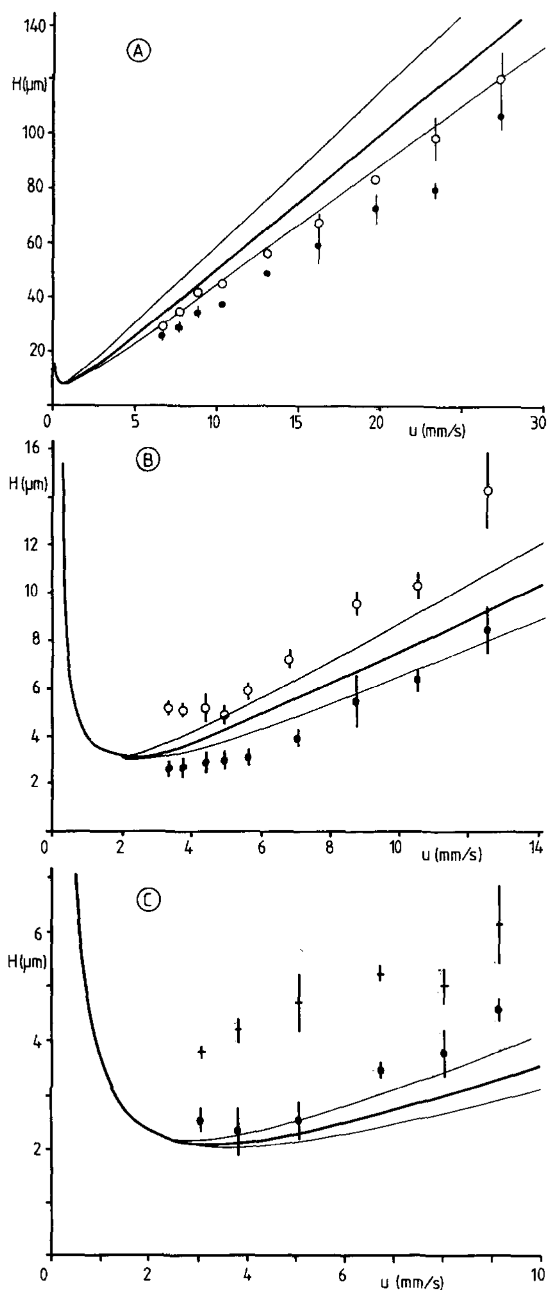

Fig. 7. Plots of plate height, H (μm), *vs.* linear velocity, u (mm/s), for untreated, fused-silica columns (mobile phase, methanol; solute, toluene). ○, Measured plate height; ●, corrected plate height; heavy line, theoretical line for the diffusion coefficient, $D_m = 1.67 \cdot 10^{-9}$ m^2/s and column diameter, d_c, as stated below; fine line, theoretical lines for either d_c − S.D. and D_m + 10% or d_c + S.D. and D_m − 10% (for explanation see text). Columns: (A) 6.82 m × 28.0 μm I.D.; (B) 2.03 m × 10.7 μm I.D.; (C) 1.81 m × 7.1 μm I.D.

phase (10%; *cf.*, ref. 35) and in the values of the column diameter. Theoretical lines for two extremes, *i.e.*, column diameter −1 standard deviation (S.D.), diffusion coefficient +10% on the one hand, and column diameter +1 S.D., diffusion coefficient −10% on the other, are also shown in Fig. 7. It is concluded that plate heights close to the theoretical values may be found for the 6.82 m × 28.0 μm I.D. and the 2.03 m × 10.7 μm I.D. columns, while for the 1.81 m × 7.1 μm I.D. column, the plate heights are somewhat higher than expected. The deviation between the measured plate heights and the ones corrected for external broadening increases with decreasing column diameter.

It is important to discuss the consequences of these figures. The measured peak variance is the sum of the peak variance due to the open-tubular column and that due to the various external peak-broadening processes (if these processes are linear). An important conclusion is that external peak broadening considerably less than 1 nl is easily obtained with the OTLC–MS system described here. Values well below 100 pl were measured in the experiments with the 1.81 m × 7.1 μm I.D. column. Thus, it seems easy to achieve the 1-nl limit for external peak broadening, which is one of the prerequisites for the successful operation of open-tubular columns.

Short columns were used in these experiments, especially with the 10.7- and 7.1-μm I.D. capillaries. At a given linear velocity, the peak variance due to the open-tubular column is directly proportional to the column length. Therefore, increasing the column length would, in effect, reduce the percentage contribution of the external broadening. In the case of the 10.7- and 7.1-μm I.D. columns, lengths of 3–4 m should be sufficient to reduce the contribution of the external peak broadening to below 5%. As we worked at column pressures less than 6 MPa, the column length can indeed be increased.

Retention will also lead to larger peak variances, and thereby reduce the influence of the external peak broadening. We have calculated that retained peaks with capacity factors of about 0.2–0.5 can be measured without distortion with short 10.7- or 7.1-μm I.D. columns. For example, separations requiring 280000 plates can be performed in about 900 s ($k' = 0.5$) with a 1.81 m × 7.1 μm I.D. column having a film thickness of 0.09 μm and a linear velocity of 3.1 mm/s. Under the same conditions, 530000 plates can be achieved for an unretained peak with the given external broadening, while 880000 plates would be possible without any external peak broadening.

In Fig. 8 a peak from a 4.45 m × 8.36 μm I.D. column is shown, giving 1.8 million plates in only 16 min. This is an unretained peak. Retention produces a dramatic decrease in the plate number compared with the plate number of unretained peaks. A retained peak with a capacity factor of 2 would still show 200000 plates in 50 min. These results show that very high plate numbers are attainable in a short time in open-tubular liquid chromatography.

Minimum detectable quantities

For volatile solutes, such as toluene, we measured the minimum detectable quantities (MDQ) (based on a signal-to-noise ratio of 3) as 1–10 pg. This corresponds to concentration detection limits of about 1–10 μg/ml for injections into the 28.0-μm I.D. column. For less volatile solutes, higher MDQs were found, *e.g.*, *ca.* 35 pg for phenacetin (detected with single-ion monitoring at the most abundant ion, *m/z*

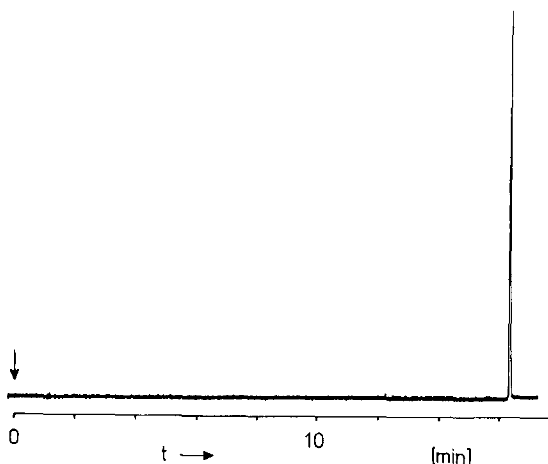

Fig. 8. Unretained peak of toluene from a 4.45 m × 8.36 μm I.D. open-tubular column, showing 1.8 million plates in 16 min. Mobile phase: methanol.

108) or about 70 pg for caffeine (*m/z* 194). Concentration detection limits at least three orders of magnitude lower are required in OTLC–MS detection. The problems of the detection limits will be discussed in more detail in our next paper[29].

CONCLUSIONS

The coupling of open-tubular liquid chromatography and mass spectrometry with a capillary-inlet interface has been studied. The capillary-inlet interface has two very attractive features in OTLC–MS. (1) Since the column effluents evaporates directly from the open-tubular column into the high-vacuum ion source, no contribution to peak variance due to the interface is expected. (2) Since the flow-rates in the near-to-kinetic-optimum operation of open-tubular columns are very low, the pressures in the ion source can be sufficiently low to attain EI ionization. EI-like mass spectra were indeed observed for several compounds. However, the range of applicability of the interface is limited to relatively volatile molecules. Compounds cannot be analyzed with the capillary-inlet system if their boiling point, polarity or thermal lability is too high, as they either block the capillary tube or produce broad and irregular peak shapes. Therefore, the prospects of the capillary inlet as an interface in OTLC–MS are not very promising. Some improvements, probably minor ones only, are to be expected from enhancing the heat transfer between the heater and the open-tubular column.

However, the capillary inlet has enabled us to evaluate the total chromatographic system for volatile solutes. It was shown that the system contributes significantly to peak broadening. Nevertheless, the attainment of external broadening well below 1 nl was easier than expected. Further research on the various sources of external peak broadening pointed the way to optimization of the chromatographic system[37].

REFERENCES

1 V. L. Tal'roze, V. E. Skurat and G. V. Karpov, *Russ. J. Phys. Chem.*, 43 (1969) 241.
2 V. L. Tal'roze, V. E. Skurat, I. G. Gorodetskii and N. B. Zolotai, *Russ. J. Phys. Chem.*, 46 (1972) 456.
3 P. J. Arpino, M. A. Baldwin and F. W. McLafferty, *Biomed. Mass Spectrom.*, 1 (1974) 80.
4 P. J. Arpino, B. G. Dawkins and F. W. McLafferty, *J. Chromatogr. Sci.*, 12 (1974) 574.
5 F. W. McLafferty, R. Knutti, R. Venkataraghavan, P. J. Arpino and B. G. Dawkins, *Anal. Chem.*, 47 (1975) 1503.
6 J. J. Brophy, D. Nelson and M. K. Withers, *Int. J. Mass Spectrom. Ion Phys.*, 36 (1970) 205.
7 J. D. Henion, *Anal. Chem.*, 50 (1978) 1687.
8 J. D. Henion and G. A. Maylin, *Biomed. Mass Spectrom.*, 7 (1980) 115.
9 J. D. Henion, *J. Chromatogr. Sci.*, 19 (1981) 57.
10 N. Evans and J. E. Williamson, *Biomed. Mass Spectrom.*, 8 (1981) 316.
11 K. H. Schäfer and K. Levsen, *J. Chromatogr.*, 206 (1981) 316.
12 K. Levsen and K. H. Schäfer, *Int. J. Mass Spectrom. Ion Phys.*, 46 (1983) 209.
13 K. Levsen, K. H. Schäfer and J. Freudenthal, *J. Chromatogr.*, 271 (1983) 51.
14 A. P. Bruins and B. F. H. Drenth, *Int. J. Mass Spectrom. Ion Phys.*, 46 (1983) 213.
15 A. P. Bruins and B. F. H. Drenth, *J. Chromatogr.*, 271 (1983) 71.
16 R. Tiebach, W. Blaas, M. Kellert, S. Steinmeyer and R. Weber, *J. Chromatogr.*, 318 (1985) 103.
17 R. Tiebach, W. Blass and M. Kellert, *J. Chromatogr.*, 323 (1985) 121.
18 H. Alborn and G. Stenhagen, *J. Chromatogr.*, 323 (1985) 47.
19 T. Tsuda, G. Keller, and H.-J. Stan, *Anal. Chem.*, 57 (1985) 2280.
20 D. Ishii and T. Takeuchi, *J. Chromatogr. Sci.*, 18 (1980) 462.
21 R. Tijssen, J. P. A. Bleumer, A. L. C. Smit and M. E. Van Kreveld, *J. Chromatogr.*, 218 (1981) 137.
22 V. L. Tal'roze, I. G. Gorodetskii, N. B. Zolotai, G. V. Karpov, V. E. Skurat and V. Ya. Maslennikova, *Adv. Mass Spectrom.*, 7 (1978) 858.
23 P. J. Arpino, P. Krien, S. Vajta and G. Devant, *J. Chromatogr.*, 203 (1981) 117.
24 P. J. Arpino and C. Beaugrand, *Int. J. Mass Spectrom. Ion Proc.*, 64 (1985) 275.
25 W. M. A. Niessen and H. Poppe, *J. Chromatogr.*, 323 (1985) 37.
26 P. Kucera and G. Guiochon, *J. Chromatogr.*, 283 (1984) 1.
27 F. J. Yang, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 348.
28 C. E. Parker, C. A. Haney and J. R. Hass, *J. Chromatogr.*, 237 (1982) 233.
29 W. M. A. Niessen and H. Poppe, *J. Chromatogr.*, submitted for publication.
30 M. S. Middleditch, S. R. Missler and H. B. Hines, *Mass Spectra of Priority Pollutants*, Plenum, New York, 1981.
31 J. G. Grasseli and W. M. Ritchey, *CRC Atlas of Spectral Data and Physical Constants for Organic Compounds*, CRC Press, Cleveland, OH, 2nd ed., 1975.
32 R. C. Weast (Editor), *CRC Handbook of Chemistry and Physics*, CRC Press, Cleveland, OH, 56th ed., 1975–1976.
33 M. Windholz (Editor), *The Merck Index*, Merck & Co., Rahway, NJ, 9th ed., 1976.
34 J. H. Knox and M. T. Gilbert, *J. Chromatogr.*, 186 (1979) 405.
35 S. Bretsznajder, *Prediction of Transport and Other Physical Properties of Fluids*, (transl. J. Bandrowski), Pergamon, New York, 1971.
36 D. J. Anderson and R. R. Walters, *J. Chromatogr. Sci.*, 22 (1984) 353.
37 W. M. A. Niessen, H. P. M. van Vliet and H. Poppe, *Chromatographia.*, 20 (1985) 357.