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

I. PERFORMANCE OF THE VACUUM EQUIPMENT

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SUMMARY

The direct introduction of liquid solutions into the vacuum space of a mass spectrometer for liquid chromatography–mass spectrometry (LC–MS) purposes requires that the vacuum pumping equipment for the apparatus evacuates very high throughputs of gas resulting from the vaporization of the solvent. Updating and testing of the vacuum equipment are then the first operations to be carried out before adapting a direct liquid introduction (DLI) interface to a chemical ionization mass spectrometer. The requirements of a successful DLI/LC–MS experiment are examined. The need for a cryopump with a large active surface area chilled at 80°K is emphasized.

INTRODUCTION

The on-line combination of a high-performance liquid chromatograph (HPLC) to a mass spectrometer (MS) is possible today by utilizing different techniques[1]. One of the methods is the direct introduction of a small constant fraction of the output solution from the liquid chromatographic (LC) column into the ion source of a chemical ionization (CI) mass spectrometer[2]. This method is fairly easy to achieve in practice, and it has already proved to be very effective for on-line liquid chromatography–mass spectrometry (LC–MS) coupling. In general, a high sensitivity of detection and informative CI spectra from series of non-volatile solute samples, including oligopeptides[3], underivatized nucleosides[4] and synthetic drugs[5] are obtained by this technique.

Preliminary results obtained when choosing this LC–MS method have prompted us to investigate different possible designs of an interface for direct liquid introduction (DLI), and the performances and the limits of the method have been evaluated. The results have a direct bearing on future LC–MS developments.

In a DLI interface, the solvent serves as the reagent gas for chemical ionization inside the ion source. The use of polar or non-polar solvents is permitted, although it is often advantageous to restrict the range of solvent systems to polar eluents such as those frequently used for reversed-phase liquid chromatography (RPLC), *i.e.*, methanol, acetonitrile or water. Slightly acidic solutions and buffered solutions containing inorganic volatile salts (*e.g.*, ammonium acetate) have been found to be suitable in some instances[4].

Most of the previous reports on DLI systems were focused on practical examples of applications in order to demonstrate the feasibility of the method. However, details of the operating parameters were not given or not discussed. This paper is the first in a series that will cover the different aspects of a DLI interface and its associated equipment. Design considerations and operating characteristics are considered for the vacuum equipment necessary for handling and evacuating the solvent molecules that result from the vaporization of the LC solution inside the MS.

EXPERIMENTAL

Two prototype instruments, called Lucie and Carole, were assembled and tested separately. The results presented here and in subsequent papers will include data obtained from both instruments. Their dimensions and their general characteristics are roughly similar, although different models of vacuum equipment or HPLC pumps from different manufacturers were sometimes used. Details of the different designs of DLI interfaces will be given in the next paper in the series.

Lucie

In the first LC–MS prototype, designed and assembled at the École Polytechnique, the mass spectrometer is a Nermag Model SQ 156 quadrupole filter, equipped with a standard CI ion source, electron multiplier, ion current amplifier and electronic control units. The vacuum envelope provides differential vacuum pumping between the source housing and the analyser housing by way of a small isolating circular slit of 1 cm^2 area.

The vacuum equipment consists of two separate pumping lines. To the source housing are connected a Varian-NRC Model VHS-1200 oil diffusion pump (Varian, Orsay, France), backed by an Alcatel Model ZM 2030 rotary forepump (Alcatel, Montrouge, France). A Varian Model NRC-1200W Cryotrap is installed on top of the diffusion pump, and two Vat isolating gate valves, Models 13117P and 13112KF, (Vat, Haag, Switzerland) are mounted in the gas connecting lines. To the analyser housing is attached an Edwards Model 63/150M oil diffusion pump (Zivy, Paris, France), backed by an Alcatel Model ZM 2015 rotary forepump.

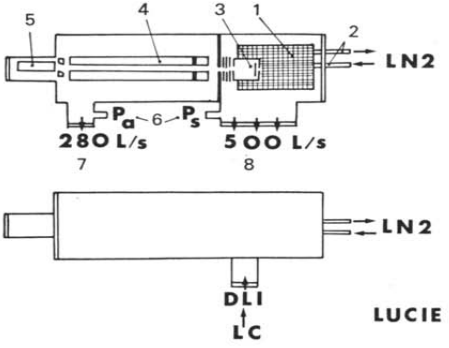
Gas pressures in the source housing and the analyser housing are measured by two hot-cathode ionization gauges and the foreline pressures are monitored by two thermocouple gauges. Each set of one ionization gauge and one thermocouple gauge is connected to a CVC Model GIC-300A controller (CVC, Plaisir, France).

A special cryopump was designed that consists of a hollow brass half-cylinder, attached to a high-vacuum flange and chilled with liquid nitrogen. It is installed horizontally inside the source housing and surrounds the CI ion source within a distance of 2–3 cm. The trap is 8 cm long and 13 cm in diameter; both the inner side

of the trap, which is in direct line of sight with the ion source, and the outer side were extensively machined to increase the active area. The total active surface area of the cryopump is about 1000 cm^2, including 300 cm^2 for the inner side. Liquid nitrogen is supplied to the trap by an Air Liquide assembly (Air Liquide, Le Plessis Robinson, France) including a Model TC 100 100-l tank, a Model DS30 output delivery assembly and a Model RNT liquid nitrogen level controller.

The walls of the vacuum envelope are heated to about 100°C by heating tapes (Heraeus, Orsay, France).

A schematic diagram of the prototype Lucie is shown in Fig. 1, and the special cryopump is shown in Fig. 2.



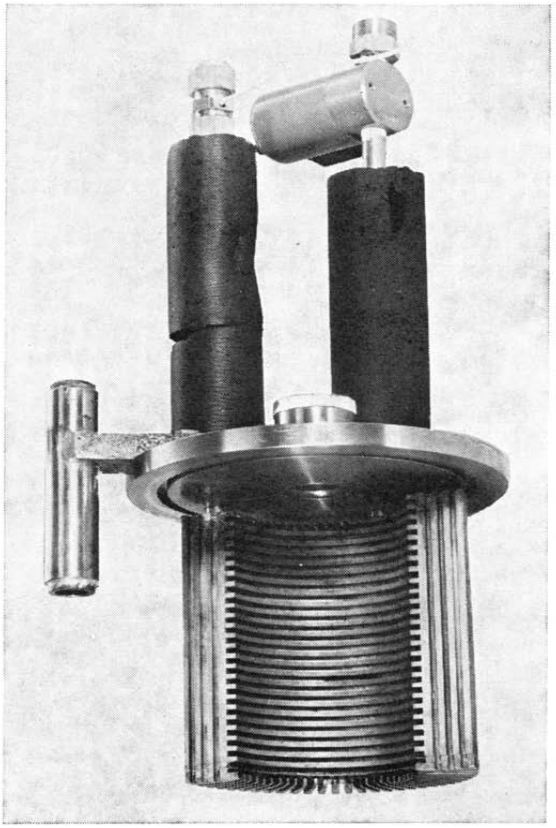


Fig. 1. Schematic diagram of the prototype Lucie. Top: section of side view. Bottom: top view of the instrument showing the LC–MS entrance port. 1 = Cryopump (see also Fig. 2); 2 = liquid nitrogen feed lines; 3 = ion source block; 4 = quadrupole rods; 5 = electron multiplier mounted off-axis; 6 = high-vacuum gauges; 7 = analyser DP; 8 = source DP.

Fig. 2. Special cryopump used in the prototype Lucie.

Carole

Assembly of the second LC–MS prototype (Carole) was executed at Nermag Inc. It is a modified version of a standard GC–MS apparatus, Model R-10-10-B, equipped with a SIDAR data acquisition and processing system. Details of the in-

strument can be found elsewhere[6]. The major modification consists of a cryopump of large capacity and large surface area mounted vertically on the top flange above the ion source. The cryopump consists of a liquid nitrogen reservoir to which are attached thin copper fins closely surrounding the ion source. The trap is not bolted to the source housing, but sits on the top flange and is sealed by a Viton O-ring. The entire cryopump is easily removed or installed when the source is at atmospheric pressure.

A schematic diagram of the prototype Carole is shown in Fig. 3.

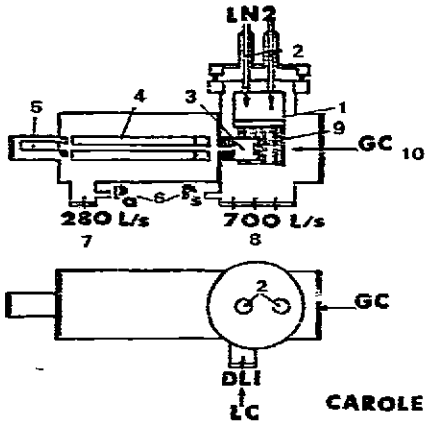


Fig. 3. Schematic diagram of the prototype Carole. Components 1–8 as in Fig. 1; 9 = thin copper fins attached to the liquid nitrogen reservoir; 10 = connecting line to a gas chromatograph.

RESULTS AND DISCUSSION

The maximum flow rate, Q_l, of liquid solution that can be introduced directly inside the MS is an important parameter as it is related to the maximum amount of solute sample that is utilized by the MS for detection and identification. Most analytical HPLC systems utilize LC columns of I.D. 2–4 mm packed with 5–10-μm particles. The flow-rate of the output solution is usually 0.25–2 ml/min. The entire output cannot be introduced into the MS and Q_l is only a small fraction, θ, of the LC output, and consequently the amount of solute used by the MS is θ times the total amount injected into the LC column.

The liquid introduced into the MS is vaporized and produces a throughput of gas, Q_g, which is eliminated by the vacuum system. At standard pressure and temperature, Q_g is simply related to Q_l by the equation

$$Q_g\,(\mathrm{STP}) = Q_l d \cdot \frac{22400}{M} \tag{1}$$

where d is the density of the liquid and M its molecular weight. If Q_l is given in ml/min, then Q_g is in atm·ml/min.

The maximum value of Q_g is determined by the total effective pumping speed of the vacuum equipment, S_{eff}, and by the maximum working gas pressure:

$$Q_g = PS_{eff} \tag{2}$$

Thus, the highest possible value of S_{eff} should be obtained, although the cost and the physical dimensions of the required equipment set a limit.

A quadrupole MS requires that the gas pressures in critical parts of the instrument remain within some given operating ranges[7]. Of interest to us are the pressure, P_a, in the analyser housing which contains the quadrupole rods and the electron multiplier, the pressure, P_s, in the source housing which contains the ion source block and the DLI interface, and the pressure, P_{is}, inside the CI source block. Typical operating values are as follows (pressures expressed in torr):

$$1 \cdot 10^{-6} < P_a < 5 \cdot 10^{-5};$$
$$1 \cdot 10^{-5} < P_s < 5 \cdot 10^{-4};$$
$$1 \cdot 10^{-1} < P_{is} < 1.$$

The flow of solvent molecules is assumed to be molecular inside the source and the analyser housing, viscous inside the CI ion source and intermediate in the vicinity of the cryotraps when P_s approaches $5 \cdot 10^{-4}$ torr. The contribution of the solute to the total throughput of gas is negligible.

So far only two types of vacuum pumps have been used for DLI/LC–MS: oil diffusion pumps (DP) backed by rotary pumps (RP), and cryopumps (CP). Turbomolecular pumps could be avantageous for replacing oil DPs, but high-speed turbomolecular pumps are still expensive, which may prevent their use in LC–MS design. The performances of DPs and CPs under different solvent conditions are examined below.

Evacuation of solvent vapour by oil diffusion pumps

The maximum pumping speed, S_{max}, for a vacuum above a DP is proportional to the area of the intake aperture and to a factor H which depends on the nature of the gas being evacuated and on the pump design[8]. The value of H is generally not known for different substance vapours, and S_{max} is usually given for air. To a first approximation we shall assume that the maximum speed is of the same order of magnitude for the different solvent vapours which are usually pumped during LC–MS. In reality, H decreases slightly as the molecular weight of the molecules increases.

When the DP is connected to the vacuum envelope, the conductances of the connections, U, reduce S_{max} to an effective pumping speed S_{eff}, so that

$$\frac{1}{S_{eff}} = \frac{1}{S_{max}} + \frac{1}{U} \tag{3}$$

Thus we would like U to be as large as possible.

The DPs of the two instruments are connected to the vacuum envelope by short tubes of length l and an I.D. d_t which is roughly equal to the diameter of the intake aperture of the DP.

The conductances of the aperture of the connecting tube, U_a, and of the tube itself, U_t, are calculated from the following equations:

$$U_a = \frac{\Pi d_t^2}{4} \sqrt{\frac{RT}{2\Pi M}} \tag{4}$$

$$U_t = \frac{1}{6}\sqrt{\frac{2\Pi RT}{M}} \cdot \frac{d_t^2}{l} \tag{5}$$

where T is the absolute temperature of the gas and R the gas constant. If U is in l/sec, T in °K and d_t and l in cm, we obtain

$$U_a = 2.867 \cdot d_t^2 \sqrt{\frac{T}{M}} \tag{6}$$

and

$$U_t = 3.809 \cdot \frac{d_t^3}{l} \sqrt{\frac{T}{M}} \tag{7}$$

The combined conductance, U, is given by the following equations:

$$U = \frac{U_a \cdot U_t}{U_a + U_t} \tag{8}$$

$$U = \frac{3.809 \sqrt{\frac{T}{M}} \cdot d_t^3}{l + 1.33\, d_t} \tag{9}$$

The walls of the vacuum envelope must be maintained at about 100–150°C during LC–MS acquisition in order to prevent the condensation of an excessive amount of solvent molecules on the walls and on the ion optics of the MS, which results in a loss of MS resolution. The solvent is vaporized inside the heated CI ion source, leaves the ion source, and is pumped away. The temperature of the ion source is usually 100–250°C. In the following calculations we shall assume that T is 100°C (373°K): variations in T of a few tens of degrees do not modify significantly the conductances and the speeds of pumping, as eqns. 4–9 involve the square root of the absolute temperature. Thus, in practice, eqn. 10 was used for plotting the curves shown in Fig. 4:

$$U = \frac{73.56\, d_t^3}{\sqrt{M}\,(l + 1.33\, d_t)} \tag{10}$$

The difficulties posed by the solvent vapour are different in the source housing and in the analyser housing, and the two will be treated separately.

The Varian DP used in Lucie has rated values of S_{max} of 1200 l/sec for air and 1500 l/sec for helium. Attempts to use the DP alone were unsuccessful, despite the use of a low vapour pressure pump fluid (CVC Convalex 10), resulting in intolerable contaminations of the instrument by back-migration of pump fluid after only a few days of continuous LC–MS. The problem disappeared after installing the NRC cryotrap. The pumping speed on top of the gate valve above the trap is then 500 l/sec for air. The DP used in the source of Carole includes an integral high-vacuum isolation valve and a water-cooled baffle, and is rated at 700 l/sec for air with no back-streaming of pump oil being observed after near daily LC–MS runs for several months. In both prototypes, d_t and l are 15 cm, and the values of the conduc-

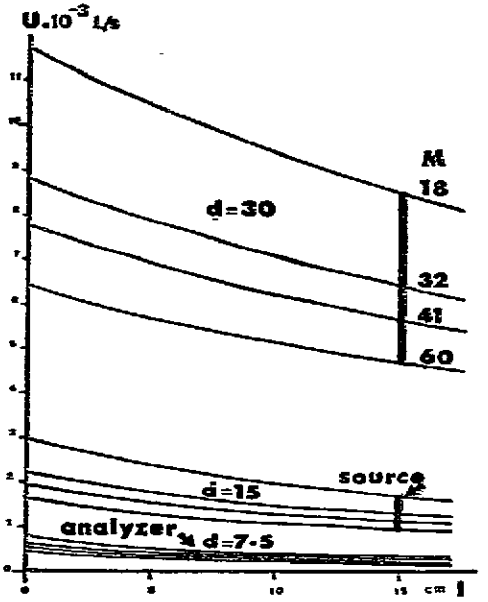


Fig. 4. Plots of the conductance, U, of tubes of diameter $d = 7.5$, 15 and 30 cm and of length l for gas molecules of four LC–MS solvents of molecular weights $M = 18$, 32, 41 and 60. Vertical heavy lines refer to our present instruments ($l = 7.5$, $d = 7.5$ cm on the analyser side; $l = 15$, $d = 15$ cm on the source side) and to the case of a possible instrument equipped with a larger DP attached to its source ($l = 15$, $d = 30$ cm).

tance U can be calculated by using eqn. 10. If we intend to maintain a source pressure P_S of $1 \cdot 10^{-4}$ torr in the source housing when introducing different solvents, then we must limit Q_1 to the values listed in Table I which range between 1 and 10 μl/min of liquid.

If we had installed a larger DP in Lucie, for instance a Varian Model VHS-3500 pump, with matched cryotrap and gate valve, S_{max} would have been 1300 l/sec for air and d_t 30 cm, and we could have kept l at 15 cm. The corresponding values

TABLE I

MAXIMUM FLOW-RATES OF SOLVENT VAPOUR WHICH CAN BE ELIMINATED BY THE DIFFUSION PUMP ATTACHED TO THE SOURCE HOUSING OF THE LC–MS

$P_s = 1 \cdot 10^{-4}$ torr; (1) $S_{max} = 500$ l/sec (air), $d_t = 15$ cm, $l = 15$ cm; (2) $S_{max} = 700$ l/sec (air) $d_t = 15$ cm, $l = 15$ cm; (3) $S_{max} = 1300$ l/sec (air) $d_t = 30$ cm, $l = 15$ cm.

Parameter	*Water (m = 18)*			*Methanol (m = 32)*			*Acetonitrile (m = 41)*			*Propanol (m = 60)*		
	1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
U (l/sec)	1674	1674	8508	1256	1256	6381	1109	1109	5637	917	917	4660
S_{eff} (l/sec)	384	493	1127	357	448	1079	344	428	1056	323	396	1016
Q_g (ml/min) (STP)	2.4	3.1	7	2.2	2.8	6.7	2.1	2.7	6.6	2.0	2.5	6.30
Q_1 (μl/min)	1.9	2.5	5.6	4.0	5	12	5.0	6.2	15.3	6.7	8.2	21

of Q_l are also listed in Table I. The maximum input flow-rate of solvent would be two to three times higher, but the cost would be trebled, apart from an increase in the size of the instrument. It will be shown below that a cryopump may easily achieve 10 times the vacuum pumping speed of the DP used in both types of apparatus. Consequently, the use of a large oil DP or of a high-speed turbomolecular pump is not recommended.

The conditions for vacuum pumping in the analyser are less demanding than those in the source, although the gas pressure should be one order of magnitude lower ($1 \cdot 10^{-5}$ torr).

If we ignore the leaks of air into the analyser, the throughput of gas, Q_g, to be evacuated by the DP is then equal to the throughput of gas, Q_{sg}, passing through the differential pumping slit which isolates the analyser from the source housing. The value of Q_{sg} depends only on the area, A_{sl}, of the slit and on the difference in pressure across the slit, according to the equation

$$Q_{sg} = A_{sl} \sqrt{\frac{RT}{2\Pi M}} \cdot (P_s - P_a) \tag{11}$$

and

$$A_{sl} = \frac{Q_{sg} \sqrt{M}}{70.253 (P_s - P_a)} \tag{12}$$

Lucie and Carole use the same model of DP to evacuate their analysers, with S_{max} rated at 280 l/sec for air, $d_t = 7.5$ cm and $l = 7$ cm. Assuming that the maximum value of P_a is $1 \cdot 10^{-5}$ torr, values of S_{eff}, U, Q_g and Q_l are then calculated using eqns. 3 and 10. The results are given in Table II. Assuming that $Q_g = Q_{sg}$ and using eqn. 12, we calculated the values of A_{sl} that would allow the penetration into the analyser of the throughput Q_{sg}, taking $P_a = 1 \cdot 10^{-5}$ torr and $P_s = 1 \cdot 10^{-4}$ torr. The resulting values are also given in Table II.

TABLE II

MAXIMUM FLOW-RATES OF SOLVENT VAPOUR WHICH CAN BE ELIMINATED BY THE DIFFUSION PUMP ATTACHED TO THE ANALYSER HOUSING

$P_a = 1 \cdot 10^{-5}$ torr; $S_{max} = 280$ l/sec (air); $d_t = 7.5$ cm; $l = 7$ cm.

Parameter	*Water*	*Methanol*	*Acetonitrile*	*Propanol*
U (l/sec)	430	322	285	235
S_{eff} (l/sec)	170	150	141	128
Q_g (ml/min) (STP)	0.105	0.093	0.087	0.079
Q_l (μl/min)	$8.4 \cdot 10^{-2}$	$16.8 \cdot 10^{-2}$	$20.3 \cdot 10^{-2}$	$26.3 \cdot 10^{-2}$
A_{sl} (cm^2)	1.03	1.21	1.29	1.41

We observe that in practice a slit area of 1 cm^2, which is large enough to transmit the ion beam from the ion source, impedes the introduction of an excess of solvent vapour into the analyser. Small sized vacuum equipment is then satisfactory for evacuating the analyser of the LC–MS.

The use of an oil DP alone for DLI/LC–MS calls for different remarks. With no modification to the vacuum system, about 1–10 μl/min of liquid solution can be

introduced directly into most modern quadrupole mass spectrometers designed for GC–MS. Fig. 5 shows plots of Q_g(STP) and Q_l *versus* M at different S_{max}. Although this may represent values of θ from 0.1 to 1% when connecting a conventional 4 mm I.D. LC column, successful DLI/LC–MS analyses have been obtained in this manner[5]. It is also possible to connect directly narrow-bore LC columns with output flow-rates in this range[9].

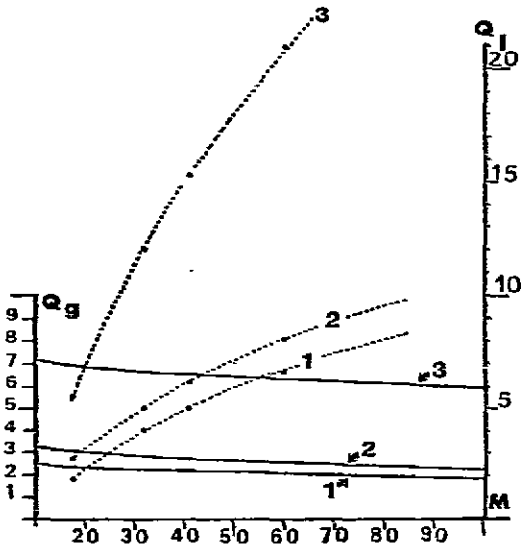


Fig. 5. Plots of Q_g(STP) and Q_l *versus* molecular weight (M) of solvent vapour evacuated by different oil DPs at $P_s = 1 \cdot 10^{-4}$ torr and $T = 373$°K. Curves 1, 2 and 3 refer to DP with $S_{max} =$ 500, 700 and 1300 l/sec, respectively, and U calculated in Fig. 4.

A characteristic feature of oil DPs is that their vacuum pumping speed is constant for gas pressures at the intake aperture in the range 10^{-9}–$5 \cdot 10^{-3}$ torr, but decreases rapidly to zero for pressures above $5 \cdot 10^{-3}$ torr, as shown in Fig. 6. The uncontrolled introduction of an excess of solution shuts off the action of the DP. Similarly, the DP may also stop if the value of the critical back-pressure at the DP outlet port is exceeded. The critical back-pressure depends on the throughput of gas and on the pumping speed of the forepump, and is 0.3–0.5 torr for most diffusion pumps. In each of the two cases, the interruption of the DP does not stop the introduction of additional amounts of liquid, because one side of the DLI is always under liquid pressures of 1–10 bar (see Part II) and the other side is still under vacuum. Consequently, both P_s and P_a jump immediately to high values, and some damage to the MS may result unless safety switches are triggered by pressure-sensing devices. When such a failure occurs, the DLI must be completely removed from the MS, the DPs isolated as they are still hot, and the excess of liquid solution in the vacuum space must be eliminated by the forepumps before the DPs may resume their action. The return to normal operating conditions may take many minutes. Similar problems are better overcome by using cryopumps (see below).

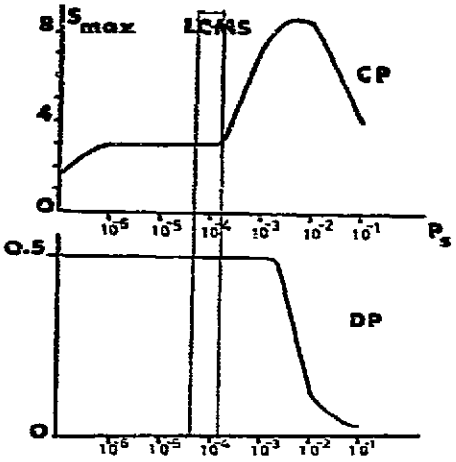


Fig. 6. Comparison of the maximum pumping speed of a cryopump (top) and a diffusion pump (bottom) for increasing gas pressures. The top trace is taken from ref. 11 and the bottom trace is from the manufacturer's literature (VHS-1200 reference manual, Varian NRC).

No serious degradation of the DP fluid has been observed after periods of use of more than 1 year. Only the rotary pump oil needs to be purged frequently and changed after 1 month of daily LC–MS use.

Evacuation of solvent vapour by cryopumps

A cryopump can achieve very high pumping speeds, at the cost of the refrigerant[10,11]. Another major feature for LC–MS applications is that it is a clean pump which does not contaminate the vacuum space. The pumping speed of a cryopump in the free molecular flow region may be calculated[8,10,11] using the equation

$$S_{max} = A_T \sqrt{\frac{RT}{2\Pi M}} \cdot \alpha \tag{13}$$

where $\alpha = 1 - (P_m/P_s)$, A_T is the active surface area of the trap, P_s the vapour pressure of the gas in the vacuum chamber and P_m the vapour pressure of the solid solvent condensed on the trap; α is the sticking coefficient and is a slowly varying function of the solvent molecule temperature in the vacuum space and of the cryopump temperature. It should be determined experimentally. We shall assume that α is close to unity for the solvents investigated here, at $T = 373$°K and with the cryopump cooled at 80°K with liquid nitrogen; this is only a first approximation, as for instance $\alpha = 0.8$ at $T = 290$°K for water vapours trapped at 80°K[11]. The values of S_{max} achieved by the cryopump in Lucie, considering only the inner side of the trap of 300 cm², are given in Table III. As the trap is located within a few centimetres of the ion source, it may be assumed that S_{max} is practically equal to S_{eff} as the conductance of the space between the ion source and the trap is very large. Table III shows that the cryopump traps 10 times more solvent vapour in the source housing than does the oil DP.

TABLE III

FLOW-RATES OF SOLVENT VAPOUR TRAPPED BY A 300-cm² CRYOPUMP COOLED AT 80 °K, ASSUMING $\alpha \approx 1$

Parameter	*Water*	*Methanol*	*Acetonitrile*	*Propanol*
$S_{eff} \approx S_{max}$ (l/sec)	4970	3728	3293	2722
Q_g (ml/min) (STP)	30.8	23.1	20.4	16.9
Q_l (μl/min)	24.8	41.7	47.6	56.3

In fact, the pumping speed is even greater as the outer side of the cryopump also traps solvent molecules, and LC–MS equipment has been run with flow-rates of acetonitrile of up to 100 μl/min.

Another interesting feature of CPs for LC–MS is that they show a self-regulating action against over-pressures in the source housing. Eqn. 13 remains valid for source pressures in the range $10^{-5} < P_s < 10^{-4}$ torr; under these conditions the pumping speed does not depend on the pressure of the gas. For $P_s > 5 \cdot 10^{-4}$ torr eqn. 13 no longer holds as intermolecular collisions occur with increasing frequency and S_{max} increases above the value that can be obtained in the free-molecular flow regime, as discussed by Davey[11]. Fig. 6 compares the pumping speeds of an oil DP and of a cryopump for increasing gas pressures. Data acquisition during LC–MS runs are obtained with P_s between $5 \cdot 10^{-5}$ and $2 \cdot 10^{-4}$ torr, where the S_{max} values of both pumps are constant. The uncontrolled introduction of an excess of liquid may temporarily shut the DP, but it will also increase the speed of the CP, and consequently the pressure in the vacuum space will rapidly return to normal conditions. In no instance is the CP turned off by the excess of solvent vapour.

There are only a few drawbacks to the use of cryopumps for LC–MS. Firstly, they cannot be used alone, and a DP or a small turbomolecular pump must remove non-condensable molecules (mainly air). Secondly, a cryopump cannot be run continuously: the accumulation of solid solvents reduces both the pressure in the vacuum space and the pumping speed. At pressures of the order of 10^{-4} torr, the build-up of condensate is still slow and the pump can be operated for several hours (5–8 h for most solvents) with no significant deterioration in performance.

The trap can be regenerated in many ways. The least satisfactory method is to disconnect the liquid nitrogen supply, and to allow the trap to warm to ambient temperature during an overnight partial shut-down of the instrument. The DP should be pneumatically isolated or turned off, because as soon as the cryopump surface becomes warm the solvent evaporates very rapidly. As about 10–15 ml of liquid have been trapped, rapid evaporation causes a sharp and high surge of pressure which remains high (10^{-2}–10^{-1} torr) for 20–40 min. The solvent vapour is not completely evacuated by the forepumps before 1–2 h, and accumulates in the pump oil. Hence the fluid must be purged daily by admitting air through the gas ballast of the rotary pump; in any case, the exhaust port of the forepumps should be routed to a hood and not to the atmosphere of the laboratory. Although this method is not recommended, it could be possible to automate the different steps of the regeneration to save operator time.

Another procedure has been used regularly and satisfactorily by us during 6 months, although the cryopump must be easily removable, as is the case for Carole. In the procedure, the source and the analyser housings are isolated from the DPs,

which are kept turned on. Next, a stream of pure nitrogen is introduced into the analyser, then into the source through the differential pumping slit. When the pressure is atmospheric, the trap is simply removed while still cold. The condensed solvent appears as solid white crystals on the surface, and they are evaporated under a hood, using a heat-gun. The trap is then reinstalled. The complete cycle of regeneration between two consecutive LC–MS runs can be as short as 15 min. No degradation of DP or RP pump fluid has been observed, and only occasionally we purged the RP oil using the gas ballast. The introduction of nitrogen protects effectively the quadrupole optics and the electron multiplier. Thus the daily interruption of the vacuum was not found to be detrimental to the MS.

By an appropriate design, the consumption of refrigerant can be kept low. The cryopump in Carole is a pool cryopump with a well isolated feed and exhaust lines requiring about 5–10 l of liquid nitrogen per day. The use of a colder but much more expensive refrigerant, such as liquid helium, offers no advantages for LC–MS.

Consequently, the drawbacks of a cryopump for DLI/LC–MS can be limited by careful design of the pump, and they do not outweigh the two outstanding advantages of such a device. Firstly, a large pumping speed is obtained at moderate cost and with no increase in the instrument size. The gain in effective pumping speed is illustrated in Fig. 7. Secondly, it shows a self-protective effect against liquid introduction failures. For these reasons, we conclude that a cryopump is a necessary component in a DLI/LC–MS.

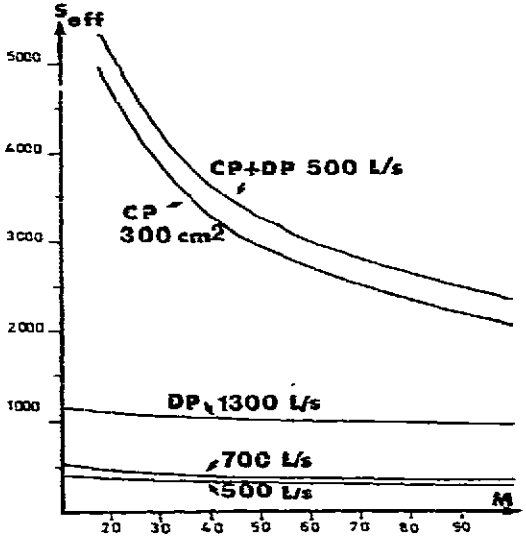


Fig. 7. Plots of S_{eff} *versus* M for DP with $S_{max} = 500$, 700 and 1300 l/sec and conductance U as in Fig. 4, and for a CP with $A_T = 300$ cm² cooled at 80°K. $T = 375$°K.

Evacuation of solvent vapour by the combined action of a diffusion pump and a cryopump

A DLI/LC–MS apparatus equipped with both a 300-cm² cryopump and a 500 l/sec DP accepts the direct coupling of about 30–70 μl/min of most solvents used

for RPLC, as shown in Fig. 8, with the CP taking nearly all of the solvent load. For conventional LC columns of 4 mm I.D. run at 1 ml/min, this represents 3–7% of the LC column output. By reducing the column inner diameter to 2 mm, with a column flow-rate of about 0.2 ml/min, the splitting ratio would be 15–35%. A 1 mm I.D. packed LC column[12] can be directly coupled to the DLI with no loss of solute[9].

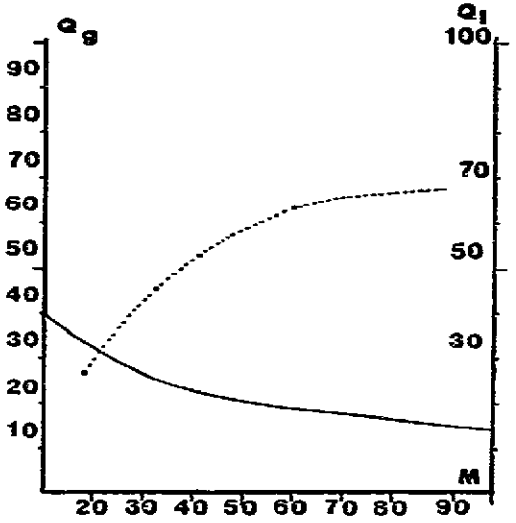


Fig. 8. Plots of Q_g (STP) and Q_l *versus* molecular weight (M) of solvent vapour evacuated by a 500 l/sec DP and a 300-cm² at $P_s = 1 \cdot 10^{-4}$ torr and $T = 373$°K.

Under normal LC–MS operations, at $P_s = 10^{-4}$ torr, both the DP and the CP achieve constant pumping speeds (see Fig. 6), so that fairly stable gas pressures are obtained in the ion source and in the source housing for introduction of liquid at a constant flow-rate through the DLI.

The experimental values of Q_l for different solvents can be measured directly by using the simple method illustrated in Fig. 9. The micro-pipette is filled with 0.05–0.1 ml of a given solvent and the liquid is then forced into the vacuum system by the pressure of gas required to obtain the desired reading on the high-vacuum hot-cathode ionization gauge attached to the vacuum envelope. A correction factor must be applied as the reading depends on the nature of the solvent vapour; for instance, the reading is half the correct value for water vapour and about correct for acetonitrile. The input flow-rate of liquid into the MS is calculated from the rate of movement of the meniscus at the rear of the liquid column in the micro-pipette.

Alternatively, one can use the LC–MS signal of a solute injected directly in front of the DLI, assuming that the volume of liquid between the point of injection and the ion source is known accurately; for DLI systems using long capillary tubes this is easily achieved. The flow-rate is obtained from the value of the retention time of the solute in the interface.

Experimental values for water, methanol and acetonitrile were found to be in agreement with calculated values to within 10–20%, which is sufficient in vacuum equipment design.

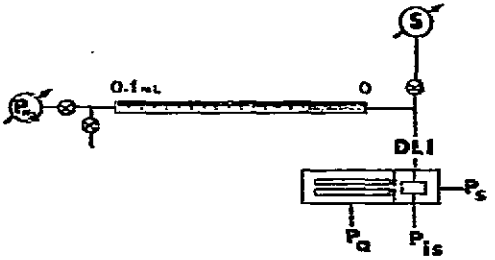


Fig. 9. Schematic diagram of the experimental set-up for measuring the performances of the vacuum equipment of the LC–MS for introduction of different organic solvents. S is a source of solvent, P_{N_2} is a regulated pressure of nitrogen, ⊗ are shut-off valves. The micro-pipette is first filled using S, and is then emptied using P_{N_2}.

Pressure in the CI ion source

A CI ion source is a small, closed box with at least two necessary openings: the ion exit slit and the electron beam entrance. Let A_{so} be the total area of the different openings. If we assume that the flow of solvent vapour is viscous inside the ion source, then P_{1s} is related to Q_g by the equation[8]

$$P_{1s} = \frac{Q_g}{A_{so} K \sqrt{\frac{RT}{M}}} \tag{14}$$

If r is the ratio P_s/P_{1s}, then for values of $r_0 < r < 1$, with $r_0 \left(\frac{2}{\gamma+1}\right)^{\frac{\gamma}{\gamma-1}}$, K is an expression which is given by

$$K = \sqrt{\frac{2\gamma}{\gamma-1}} \cdot r^{\frac{1}{\gamma}} \sqrt{1 - r^{\frac{\gamma-1}{\gamma}}} \tag{15}$$

where γ is the heat capacity ratio.

Solvent vapours used in LC–MS at 373°K in the source have values of γ in the range 1.33–1.05, and therefore $r_0 = 0.54$–0.59.

For $r < r_0$, K is a constant given by

$$K = \sqrt{\gamma\left(\frac{2}{\gamma+1}\right)^{\frac{\gamma+1}{\gamma-1}}} \tag{16}$$

This is the condition which applies to LC–MS as we want $r \approx 10^{-3}$. The smallest molecule used as a possible solvent for LC–MS is water, with $\gamma = 1.33$. Other solvents used have more than four atoms and show smaller values of γ, in the range 1.33–1.05. Corresponding values of K are nearly constant: $K = 0.67$ for $\gamma = 1.33$ and $K = 0.62$ for $\gamma = 1.05$. Therefore, we can ignore the variation of K for different solvent vapours and use $K = 0.65$ in practical calculations. The following equation assumes that $T = 373$°K:

$$P_{1s} = \frac{Q_g \sqrt{M}}{114.5 A_{so}} \tag{17}$$

For CI, the pressure in the ion source should remain in the range 0.1–1 torr. However, it is not advisable to operate at too high a source pressure, as this would require the energy of the electrons that ionize the solvent vapour to be increased above 100 eV. A second reason is that at high source pressures polar solvents tend to cluster extensively, yielding ions with molecular weights above 120. Consequently, we operate the ion source at $P_{is} = 0.3$ torr, which was found to be satisfactory for LC–MS, using 100-eV ionizing electrons. We can calculate the area, A_{so}, of the openings into the ion source block by using eqn. 17, assuming that $P_{is} = 0.3$ torr and that the throughput of gas, Q_g, equals the values listed in Table III. We find that $A_{so} = 0.067$ cm^2.

The ion source block in both prototypes is the same: it includes a circular opening of diameter 1.2 mm for the ion exit slit and a circular opening of diameter 0.5 mm for the entrance of the ionizing electron beam. The total combined area is 0.013 cm^2. The source is then too tight, and at the maximum tolerable flow-rate the pressure P_{is} would be 1.5 torr. A new opening was made by disconnecting the feed line which is normally attached to a gas chromatograph for GC–MS operations. This leaves open a circular hole about 3 mm in diameter, and the total area A_{so} is then 0.089 cm^2. Consequently, the pressure inside the ion source is 0.2–0.3 torr when 30–70 μl/min of liquid solution are sampled inside the ion source.

The source block is fairly open in comparison with conventional CI sources in commercial CI–MS apparatus. If only a DP is used for evacuating the solvent vapour and 1–10 μl/min of liquid are sampled into the ion source, the ion source block can be the same as that normally used for GC–MS. On the other hand, if the vacuum pumping speed is increased by adding a CP so that 10 times more solvent is sampled, some reworking of the ion source block may be necessary in order to avoid too high an inside source block pressure.

The value of P_{is} calculated by using eqn. 17 closely approaches the experimental value obtained when connecting tightly to the ion source block either a thermocouple gauge (correcting the reading for the different gases being measured), or a McLeod gauge. However, in some instances this does not give the value of the local pressure at which CI reactions occur. In some DLI interface models, the liquid solution evaporates before penetrating into the ion source, so that P_{is} should be close to the actual pressure of neutral solvent molecules in the ionizing plasma. Other models of interface have been found to be more effective for the successful transmission of involatile solutes. They spray droplets of liquid solution into the vacuum of the MS, so that the solute flows through the interface and leaves it while in solution. Plugging problems are then virtually eliminated. The solvent evaporates from the liquid droplet during its flight from the tip of the DLI interface to the ion source block. Optimal conditions are met when the last molecules of solvent evaporate shortly before the solute molecules are ionized by the electron beam. In such a dynamic system, the effective solvent and solute partial pressures at the intersection between the solute molecular beam and the ionizing electron beam are much higher than the average gas pressure into the ion source. The value of P_{is} calculated from eqn. 17 gives an approximate estimate of the source pressure which is useful for adjusting the ion source openings to the pumping capacity for vacuum and to the flow-rate of input liquid solutions. It also shows that the pressure P_{is} is unchanged if the throughput of gas, Q_g, is varied so that $Q_g \sqrt{M}$ remains constant when sampling

different liquids. In practice, variations of P_{is} by a factor of 1.5–2 do not affect the LC–MS signal significantly, so that the accurate regulation of $Q_g\sqrt{M}$ is not too critical.

Another modification to the ion source was found to be useful, although it is not directly related to vacuum performances. A thin metallic plate with a circular hole of diameter 2 mm was placed between the heated rhenium ribbon, which emits ionizing electrons, and the ion source. The plate is at the same potential as one end of the rhenium ribbon. The device prevents excess of solvent vapour leaving the ion source from reaching the ribbon and focuses the electron beam into the ion source. Consequently, the lifetime of the filament is significantly extended and is usually 3 months for daily LC–MS runs using acetonitrile and water as solvents.

Influence of the molecular weight of the solvent

The chemical nature of the solvent used in a DLI system affects to some extent the nature of the CI reaction products. This will be examined in a future paper. The consequences of sampling different liquids into the vacuum of the MS are considered below.

It has been stated[4] that a DP discriminates against the use of high-molecular-weight solvents, but a CP compensates for the action of the DP by trapping more effectively high-molecular-weight liquids. Therefore, the combination of a DP and a CP permits the sampling into the MS of roughly equal flow-rates of different pure or mixed liquids under constant CI–MS operating conditions. This is not strictly correct in LC–MS. As the molecular weight of the solvent increases, the DP factor H tends to decrease slowly, whereas the sticking coefficient, α, for the CP increases and approaches unity more closely, so that the performances of the two pumps then vary in opposite directions, as stated above. However, these variations are probably small and to a first approximation can be ignored, in addition, the CP traps nearly all of the solvent vapour. Eqns. 3, 4, 5, 10 and 13 show that the conductances and effective pumping speeds of DP and CP vary as $1/\sqrt{M}$, so that the total effective pumping speed for vacuum is reduced by $1/\sqrt{M}$. If we decide to keep P_s, P_a and P_{is} constant, we must adjust Q_g so that $Q_g\sqrt{M}$ is also constant. However, Q_g is related to Q_l by eqn. 1. For introduction at a constant flow-rate into the MS, Q_g is reduced by M, whereas S_{eff} is reduced by $\sqrt{M}$. The net effect is the possibility of introducing $\sqrt{M}$ times more liquid to keep identical values of the different gas pressures. This is exemplified in Fig. 8. It appears, then, that one should rather use a high-molecular-weight solvent for LC–MS because for the same liquid flow-rate, assuming that the densities of most organic solvents are roughly equal, it yields less vapour after evaporation.

However, M should not be too high. Firstly, the mass spectrum of the solvent must not overlap that of possible low-molecular-weight solutes. Before we examine the second reason, the significance of P_a and P_s should be reconsidered. The vacuum into the quadrupole MS is necessary in order to effect electrical insulation between the quadrupole rods and between the different lenses; however, this is always achieved for gas pressures up to 10^{-3} torr. The main reason is that the transmission of the ions from the source to the detector and their mass separation requires no interaction with neutral molecules during their trajectories. The cross-section for the reaction between a solute ion and a solvent neutral molecule probably increases with the

dimensions of the molecule, and thus with the mass of the solvent. This means that loss of transmission efficiency and resolution increases with the molecular weight of the solvent used for LC–MS. The higher operating values of P_1 and P_{1s} are then not the same and depend on the nature of the solvent.

In practice, a compromise situation is obtained by selecting solvents with molecular weights in the range 40–100 (except for water) and by operating the mass spectrometer at pressures 50% below the maximum calculated pressures. We then adjust the solvent flow-rate through the DLI to 30–70 μl/min. The pressure in the source housing is $8 \cdot 10^{-5}$–$1 \cdot 10^{-4}$ torr. These are the conditions which we now apply routinely during our LC–MS experiments. Two to three times higher flow-rates could be handled by our vacuum equipment. The input of solute would be higher but as the sensitivity of the MS would be reduced, the signal intensity for the solute would be about the same and the CP would need to be regenerated more often.

CONCLUSION

The parameters discussed in this paper are easily adjusted by simple calculations and tolerances of 10–20% are permitted in practical applications. They are the first parameters to understand and control when one wishes to use or to modify a mass spectrometer for DLI/LC–MS operation.

Most CI mass spectrometers tolerate the direct introduction of 1–10 μl/min of liquid with no changes to the vacuum equipment or the ion source, but there are some risks of vacuum failures by introducing too much solvent.

Whenever possible, a cryopump of large surface area should be introduced inside the source housing and be easily removable for rapid regeneration. The cryopump allows safer and more reliable operating conditions and the introduction of 10 times more liquid. However, higher flow-rates of liquid require that the conductance of the ion source block be enlarged and that the electron-emitting filament be protected.

The discussion was mainly oriented toward quadrupole mass spectrometers because they are the type available to us. The same discussion applies also to magnetic sector mass spectrometers although high voltages in the ion source pose insulation problems, as discussed in an other paper[13]. When they are overcome, a DLI interface can be adapted and operated satisfactorily[14].

Finally, the main question is: how high a flow-rate of liquid should we introduce?

On the one hand, the design of a mass spectrometer accepting 0.5–1 ml/min is feasible by enlarging the dimensions of the CP and to a lesser extent that of the source DP. Such an instrument would probably be more bulky than current instruments used for GC–MS and the handling of very high throughputs of gas may not be very simple. However, it could be made.

On the other hand, the best chromatographic performances have until recently been obtained on large-diameter LC columns, with output flow-rates around 1 ml/min. A splitter is then necessary to adapt them to a DLI/LC–MS similar to our prototypes, resulting in a loss of solute. The situation in some instances is not desirable. Early reports on the use of narrow-bore columns run at low flow-rates showed poor chromatographic performances[15], making them of limited value for practical applica-

tions. However, recent results have demonstrated that high performances can now be obtained by narrow-bore packed columns with output flow-rates matching exactly our tolerances[12].

Consequently, we see no reason for increasing the vacuum pumping capacity of our mass spectrometers. We may rather attempt to develop adequate coupling devices for the combined use of narrow-bore packed LC column and a DLI interface. The problems are severe, as will be discussed in a later paper, but they can be solved, and the detection of nanogram amounts of solutes injected on columns will be achieved, as already reported after preliminary experiments[9].

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SYMBOLS

Q_g Throughput of solvent gas to be evacuated by the vacuum equipment.
Q_{sg} Throughput of gas through the differential pumping slit.
Q_l Liquid flow-rate of solvent.
d Solvent density
M Solvent molecular weight.
P_a Gas pressure into the MS analyser housing.
P_s Gas pressure into the MS source housing.
P_{ls} Gas pressure into the ion source block.
P_m Vapour pressure of solvent condensed on the cryopump.
S_{eff} Effective vacuum pumping speed.
S_{max} Maximum vacuum pumping speed.
U Total conductance for vacuum.
U_t Conductance of a tube.
U_a Conductance of an aperture.
T Absolute temperature of solvent molecules.
l Length of connecting tubes between a DP and the evacuated vacuum space.
d_t Diameter of the tube.
A_T Total active surface area of a cryopump.
A_{so} Total area of openings into the ion source block.
A_{sl} Surface area of the differential pumping slit.
α Sticking coefficient on a cryopump.
γ Heat capacity ratio of the solvent gas.
θ Fraction of the HPLC column output introduced through the LC–MS interface.

ABBREVIATIONS

DLI Interface for direct liquid introduction.
LC–MS Combined liquid chromatography–mass spectrometry.
GC–MS Combined gas chromatography–mass spectrometry.

HPLC High-performance liquid chromatography.
RPLC Reversed-phase liquid chromatography.
STP Standard conditions of pressure and temperature.
MS Mass spectrometry.
LC Liquid chromatography.
CI Chemical ionization.
DP Oil diffusion pump.
CP Cryopump.
RP Rotatory pump.

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