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INFLUENCE OF THE PACKING MATERIAL AND THE COLUMN FILTERS ON THE RELIABILITY OF A HIGH-PERFORMANCE LIQUID CHROMATOGRAPH–MASS SPECTROMETER INTERFACE BASED ON THE DIRECT LIQUID INLET PRINCIPLE

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SUMMARY

The direct liquid introduction interface for coupled high-performance liquid chromatography (HPLC)–mass spectrometry consists of a 1–3-μm pinhole in a nickel diaphragm. This interface produces an axial jet of HPLC eluent into the ionization source. Variations in the jet affect the mass fragmentation pattern. Physical modifications of the pinhole also induce substantial changes in the jet. The influence of the packing material and the column filters has been examined and solutions are given for preventing plugging of the pinhole.

INTRODUCTION

The use of a mass spectrometer as a detector for liquid chromatography is now well established. Combined high-performance liquid chromatography–mass spectrometry (HPLC–MS) is considered to be the best tool for the analysis of polar, non-volatile or thermolabile compound that are not amenable to gas chromatography–MS.

The interface that we used has been described elsewhere[1,2] and is based on the direct liquid introduction (DLI) principle developed by McLafferty and co-workers[3,4], in which a fraction of the liquid coming out of the HPLC column is introduced directly into the ion source of a mass spectrometer through a fine pinhole. Chemical ionization of the solute is obtained by using the solvent vapour as a reactant gas.

Although the DLI technique is intrinsically simple, a lack of reproducibility of results can be observed under certain conditions. The cause of this effect has been attributed to changes in the jet direction[1].

The analysis of several thermolabile molecules using the Nermag LC–MS interface has shown that the probability of observing the molecular or pseudo-molecular ion is low when the liquid jet impacts on the walls of the ion source. When no

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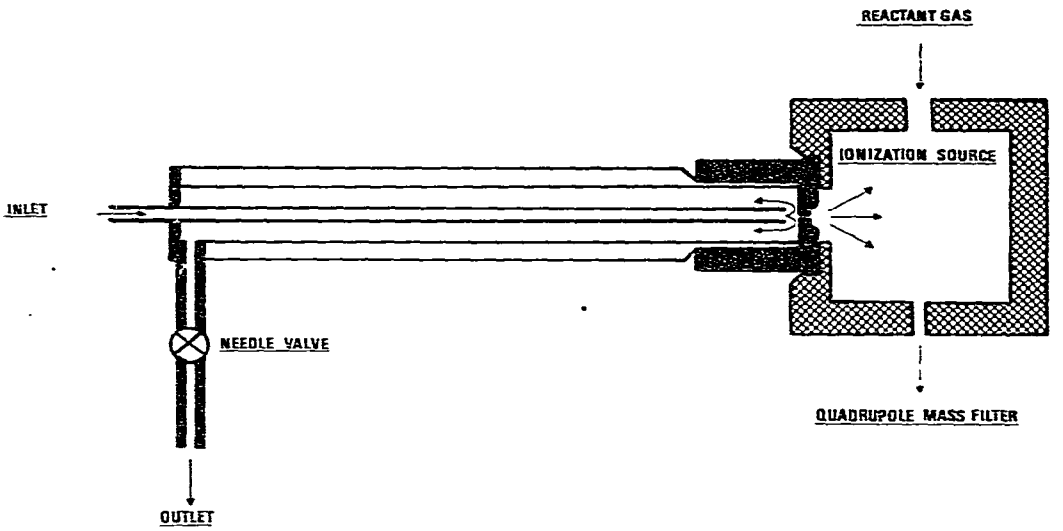

Fig. 1. Schematic diagram of the HPLC–MS interface allowing direct liquid introduction.

impact occurs, chemical ionization without fragmentation can be observed. Thus, for optimal HPLC–MS analysis, the jet must be axial for several hours. In practice, we have observed under certain experimental conditions substantial changes in the jet direction.

The purpose of this paper is to demonstrate that it is material from the HPLC column which is responsible for these changes.

EXPERIMENTAL

We observed the pinhole diaphragm after several HPLC–MS runs with a Cameca MEB 07 scanning electron microscope; the diaphragms were glued to an aluminium stub with silver lac and metallized by gold–palladium evaporation. Colloidal silica was detected on a Jeol JSM 35C stereoscan, with an Ortex X analyser. The HPLC pump (6000 A; Waters Assoc., Milford, MA, U.S.A.), the injector (U6K Waters Assoc.), tubing and HPLC–MS probe were the same throughout the experiments; only the HPLC column and solvents were varied. The HPLC–MS interface is shown in Fig. 1, and has been described elsewhere[1,2].

RESULTS

The best conditions of analysis are obtained when the solvent jet is axial (Fig. 2). Fig. 3 shows a new diaphragm before use. The internal diameter of the pinhole is about 2 μm. Fig. 4 shows a diaphragm after several hours of use. The pinhole appears partially plugged by packing particles and by another material. Small cracks are visible on the surface of the diaphragm, looking like dry mud, which are due to the packing material itself and not to fissures in the diaphragm. It can be concluded that at least two phenomena are able to modify the geometry of the pinhole and, consequently, the characteristics of the jet.

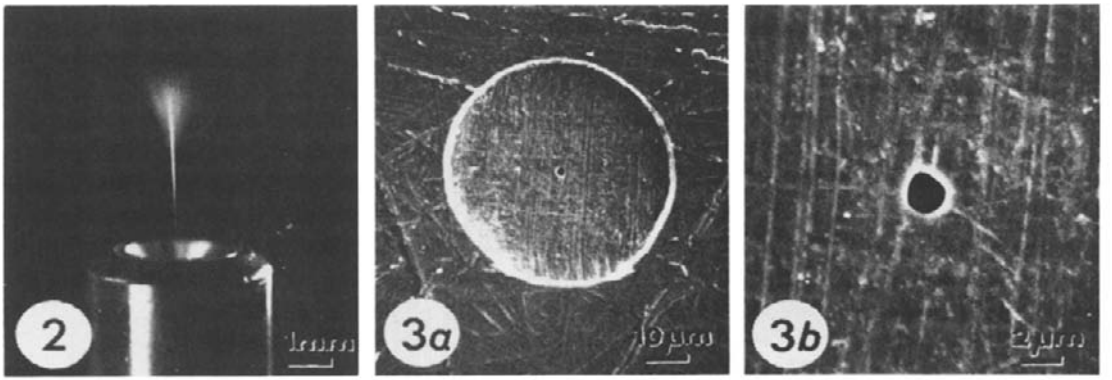

Fig. 2. Axial liquid jet on coming out of interface, expanding into a droplet in the ionization source, observed under atmospheric conditions.

Fig. 3. A clean pinhole before use. (a) Primary groove in 3 mm diameter diaphragm in which pinhole was bored; (b) pinhole observed at high magnification.

Loss of stationary phase from the column through the frit

The first phenomenon is due to the deposition or fixation of packing particles on the walls of the pinhole. These particles leave the column through the frit. As an example, Fig. 5 shows the particles collected on a diaphragm when using a Hibar HPLC column packed with LiChrosorb RP-18 (5 μm) stationary phase. This packing is shown in Fig. 6 with a factor magnification of 1600. The shape and dimensions of the collected particles are clearly representative of the packing material. Similar results can be obtained when using spherical particles as packing material (Figs. 7 and 8). The dimensions of these collected particles are in the micron range and generally below 2 μm, which corresponds to the diameter of the pinhole. This value also corresponds to the limit of efficiency of the classical filters (2 μm porosity).

Fig. 4. The same diaphragm observed after several hours of use. Solvent, acetonitrile–water; stationary phase, LiChrosorb RP-18 (5 μm).

Fig. 5. Pinhole observed after use of a Hibar Column (Merck) packed with LiChrosorb RP-18 (5 μm): 2 μm porosity outlet and filter.

Fig. 6. Reversed-phase LiChrosorb RP-18 (5 μm) (Merck).

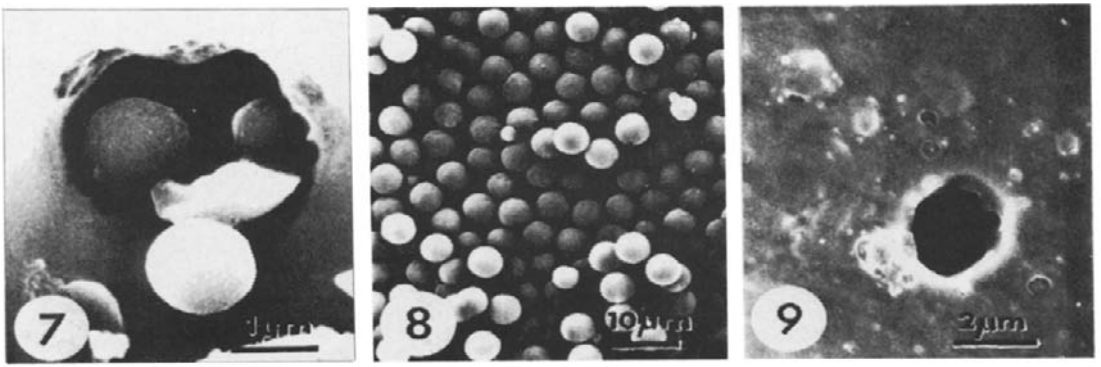

Fig. 7. Pinhole observed after use with a column packed with Zorbax-Sil as stationary phase.

Fig. 8. Zorbax-Sil stationary phase.

Fig. 9. Influence of a 0.5 µm porosity frit on plugging of the pinhole. The filter was introduced between the column and the diaphragm.

Different packing materials are shown in Figs. 6 and 8. However, numerous observations were made with other commercial packings. It can be concluded that, when the particles are not spherical, the particle size distribution can be broad, *e.g.*, from 0.5 to 25 µm. In that event, the frit is not effective in preventing a loss of the column packing. Such a phenomenon may also cause the formation of a dead volume in the column itself and, consequently, a decrease in efficiency.

The second consequence of these particles passing through the frit is that they may cause partial or a total plugging of the pinhole during HPLC–MS runs. Hence changes in the jet direction can be explained by the adhesion of one or more particles on the walls of the pinhole (Fig. 7).

To prevent such an effect, we inserted a stainless-steel frit of 0.5 µm porosity, 1/16 in. O.D. and 1 mm thickness (Chrompack, Middelburg, The Netherlands) between the column and the HPLC–MS probe. The influence of this filter is shown in Fig. 9, where the packing material in the column was LiChrosorb RP-18 (5 µm). Comparison of Figs. 5 and 9 clearly indicates the importance of using a 0.5 µm instead of a 2 µm porosity frit; the shape of the pinhole remains unchanged after several days of use.

The porosity of the filter was satisfactory, and the back-pressure of the system was not significantly increased.

The probability a filter becoming plugged increases as its porosity decreases. However, we used a 0.5 µm porosity stainless-steel frit inserted in a microbore column (stationary phase Partisil 10 µm) for 3 months without observing any plugging of the filter.

The solvents used were *n*-hexane, dichloromethane and isopropanol–*n*-hexane mixture.

Dissolution of silica

Even when using an efficient frit, a pinhole can become plugged after several

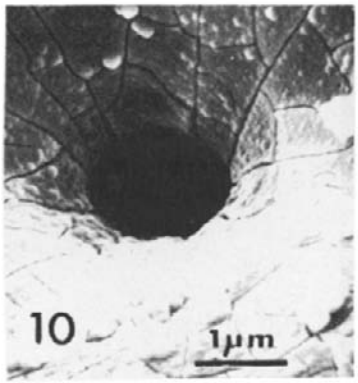

Fig. 10. Pinhole after several days of use: structureless material is observed on the wall of the pinhole.

days of use. This effect appears as due to the deposition of a structureless material on the walls of the pinhole (Fig. 10).

This material was analysed with an Ortex X analyser. The results obtained are shown in Fig. 11. The small amounts of silica are detected with an energy of 1.74 keV. The other main peaks correspond to the gold and palladium used for metallization, which were detected at different energy levels.

Analyses have shown that this type of plugging effect was due to dissolution of silica in the mobile phase and its deposition on the wall of the pinhole.

Silica deposition can be observed when using either reversed- or normal-phase conditions (Fig. 12). This result is not surprising when we consider that the silica in the stationary phase can dissolve in the mobile phase and consequently be deposited in the diaphragm region. The deposition of this colloidal silica is favoured by the presence of stationary phase particles on the pinhole wall. These two phenomena are

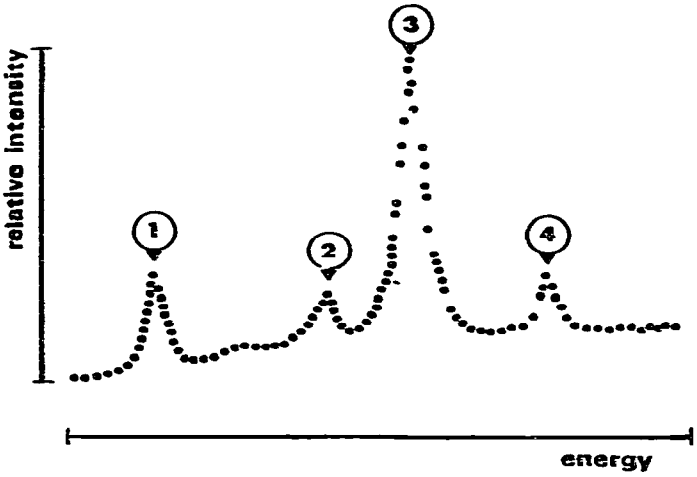

Fig. 11. Determination of compounds observed by scanning electron microscopy with an Ortex X analyser. Each compound is characterized by its energy level: 1, 0.93 keV (copper); 2, 1.74 keV (silica); 3, 2.12 keV (gold); 4, 2.89 keV (palladium).

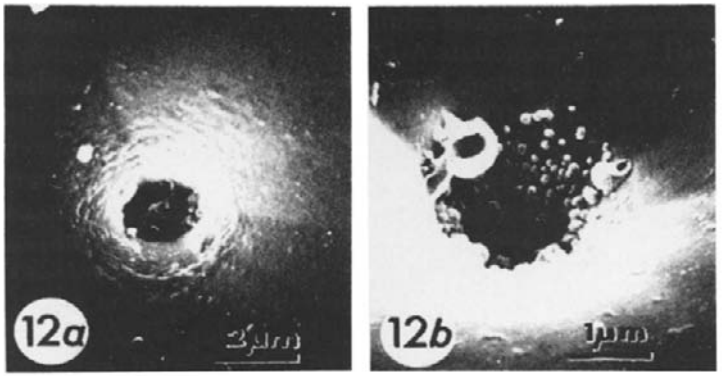

Fig. 12. Diaphragms observed after several days of use: (a) reversed phase; (b) normal phase.

linked. In fact, dissolution of silica leads to the presence of very small stationary phase particles in the column that consequently are carried through the frit. Examination of the stationary phases used confirmed this increase in the smallest particles relative to a fresh packing.

DISCUSSION

The shape and size distribution of a column packing material are critical parameters with respect to the reliability of HPLC–MS coupling when using a DLI interface with a diaphragm. However, this method of column degradation should also not be neglected when working with the usual detectors as a loss of material in the column itself can produce either an increased dead volume in the HPLC column itself with an apparent decrease in the efficiency of the analysis and a reduction in the lifetime of the column, or plugging of the frit.

From a technical point of view, it would be easier to obtain a uniform distribution with spherical particles rather than with irregular shaped particles. It can be concluded that totally spherical particles are the better choice for HPLC–MS purposes. However, we have observed that initially spherical particles can be modified in shape during column packing owing to breakage. Hence, the probability to finding particles with dimensions smaller than 2 μm cannot be neglected. In addition, the specific surface area is higher with particles of irregular shape than with those of spherical shape.

The difficulties increase when using smaller particles, *e.g.*, 3 μm[5,6], although a higher efficiency, lower solvent consumption, increased sensitivity and higher rate of separation can be obtained[7,8]. With such a packing material and a 2 μm porosity column outlet frit, we can predict very rapid plugging of a pinhole of 2–5 μm diameter. The simplest solution would be to replace the usual 2 μm frit with a 0.5 μm porosity frit. The latter is commercially available but should be chosen carefully to minimize the dead volume.

An alternative solution consists in inserting a 0.5 μm porosity filter between the column and the detector, but this does not prevent the loss of packing material in the column itself. The advantage of this solution is the possibility of removing and clean-

ing the filter without removing the column outlet frit, thus avoiding possible disturbance of the column bed.

Another cause of the loss of packing material in the column is dissolution of silica, which has long been recognized[9]. The rate of dissolution depends on the solvent mixture, the pH and the salt concentration in solution[2,10]. For this reason, many experiments are carried out in the pH range 2–8. At pH > 7, dissolution of the silica destroys the backbone support of the organic-modified packing. At pH < 2, acid cleavage of the Si–O–Si bonds can occur[11]. Silica dissolution can occur even in neutral mobile phase, as indicated by the experimental results we have obtained.

To prevent a loss of silica in the column, two approaches are possible. The first is to use silica pre-columns, which saturate the mobile phase with silica[11,12], to reduce the dissolution of particles in the analytical column. The second, more efficient method, is to use a non-siliceous phase such as a synthetic macroporous copolymer[13].

Only with the latter method can the deposition of silica on the wall of the pinhole be completely avoided. In practice, the effect of silica on the dimensions of the pinhole is small, and is negligible in comparison with the action of micron-sized particles. When only silica deposition from solution occurs, the lifetime of diaphragm is at least 1 week. This means that dissolution of silica in the mobile phase is not critical for HPLC–MS purposes, except that it shortens the lifetime of the column.

It is also advisable to immerse the mounted interface in solvent to prevent the deposition and desiccation of silica on the diaphragms when the apparatus is not being used.

CONCLUSION

With conventional HPLC detectors (UV, RI, etc.) a loss of material from the column has negligible influence on the system. The only consequence is a slow decrease in the efficiency of the column due to the creation of a dead volume.

With a mass spectrometer as a detector and especially with an interface based on the DLI principle (even with a diaphragm or a capillary tubing), the consequences can be more dramatic and a substantial decrease in the reliability of the system can be observed under certain conditions. This means that for HPLC–MS purposes the HPLC column itself is an important parameter. The packing material and column outlet frit have to be chosen carefully in order to avoid plugging of the pinhole whith particles from the column.

In practice, the use of a 0.5 μm porosity filter instead of the usual 2 μm porosity filter gives a reliable system. However, it is necessary, for HPLC–MS purposes, to bear in mind that the reliability of the system mainly depends on the reliability of the HPLC column.

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