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TEN YEARS OF LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

A review is given of the evolution over the last decade of major interfacing methods for liquid chromatography–mass spectrometry (LC–MS), *i.e.*, the moving belt, direct liquid introduction and the thermospray. Considerations of the present status of LC–MS and on future trends are based on a brief survey of reports from users. Emphasis is placed on the research of improved moving-belt interfaces and on the rapid development of analytical investigations utilizing thermospray interfaces. The transfer of interest from direct liquid introduction to thermospray appears to have developed in several laboratories. Active research programs on LC–MS are now conducted in several directions, although thermospray appears to be predominant. Commercial equipment in customer's laboratories have not been used extensively during the last decade. The situation should change with the advent of improved systems.

INTRODUCTION

Defining the origin of an analytical technique poses great difficulties, and may even be considered to be irrelevant, as nowadays a new method seldom arises from a single person or a research group but often emerges from the competitive efforts, over long periods of time, of several teams of scientists. In the case of liquid chromatography–mass spectrometry (LC–MS) the situation is even more complex as, even today, several different instrumental techniques are advocated for achieving the best results in routine application. Each technique has a different history and has gone through different stages of development.

The analytical potential of LC–MS was easy to predict but the technical difficulties were equally obvious. Yet 1973–1974 may indeed appear as the beginning of LC–MS, since a few groups launched research programs at nearly the same time. Some of their results have led to many of the present day interfaces, while others were abandoned a few years later. At any rate, there seemed to be a general consensus that the time had come to investigate the design of LC–MS equipment for general use.

For example, direct liquid introduction (DLI) under chemical ionization was studied by McLafferty and co-workers[1]; Scott's moving-wire interface[2] was soon followed by the first commercial moving-belt interface from Finnigan Instrument[3].

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A modification of the atmospheric pressure ionization (API) source for LC–MS work was investigated by Horning *et al.*[4], and while this did not result in a commercial LC–MS interface, it did possess features which were incorporated in the atmospheric pressure nebulizer used by Thomson *et al.*[5] for their ion-evaporation method. Finally, Vestal and co-workers[6] indicated recently that 1974 was the year when they first applied for a grant for the development of a LC–MS machine which after several stages of evolution became the thermospray (TS) method 6 years later[6,7].

It should be emphasized that pioneers had already investigated the LC–MS method during the 60's, and that the specific and difficult problems, which are still being faced today, were rapidly recognized. These early works have been referred to in a previous review[8] and will not be discussed here. Ingenious devices based on moving belts, direct injection of liquid effluents or spraying of charged droplets were suggested. However, they did not find widespread acceptance among the scientific community and did not lead to commercial instruments. A noticeable exception is the 1968 work of Dole and co-workers[9,10] on the generation of macro-ions from charged droplets using an electrospray, which is currently being reinvestigated by Yamashita and Fenn[11] at Yale University. Dole's work can also be linked to many recent LC–MS methods including Thomson's ion evaporation[5], the liquid ionization of Tsuchiya *et al.*[12] or even to thermospray (TS) ionization[7,13].

I sought to answer three questions concerning LC–MS:

Has LC–MS in general made a significant impact and is it now an established technique?

Did users adopt individual LC–MS techniques, especially those which were commercially available?

Are there clear trends for future LC–MS research?

A source of data for this survey was the annual proceedings of the American Society for Mass Spectrometry and Allied Topics (ASMS). Although data from such a source could be biased and may not represent the general situation, the following facts point to their usefulness:

(1) This annual meeting of ASMS is attended by a large audience, *ca.* 1000 participants, coming not only from the U.S.A. but from an increasing number of countries. It has become an international event. Data from the triannual European conference are sometimes added in the present report, but they generally follow trends similar to those reported at the ASMS meeting.

(2) Nearly all the various aspects of mass spectrometry are equally covered at this meeting, making the relative development of an individual MS technique easy to follow.

Other data have been taken from my own bibliographic search based on the Chemical Abstract Service, a large collection of previous LC–MS reviews and the exhaustive literature survey compiled by Edmonds *et al.*[14] which covers LC–MS from its origins up to 1982.

ESTABLISHMENT OF LC–MS AS AN ANALYTICAL TECHNIQUE

The question of the impact of LC–MS can be addressed by considering the total number of papers (oral and poster presentations) and the number of papers covering LC–MS subjects in each of the past 7 years (Fig. 1). Even if relevant papers

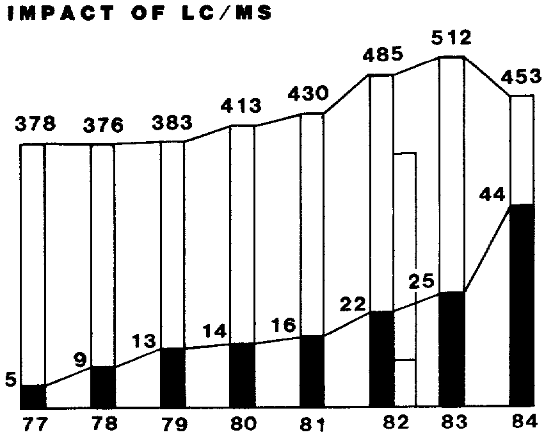

Fig. 1. Number of LC–MS papers (oral presentations and posters) and total number of communications given at the ASMS meeting during the years 1977–1984 (extra data for 1982 are from the MS Vienna conference).

were published prior to 1977, they were from less than ten groups throughout the world. In fact LC–MS remained marginal until 1980, when research activity started to increase and has become spectacular in 1984. Note that the total number of papers presented at the ASMS meeting did not decrease in 1984, but contrary to previous years, only the extended abstracts were included in the proceedings. Thus *ca.* 60 papers were not accounted for, making the sharp increase in the number of LC–MS papers even more significant. The number of participants at meetings dedicated only to LC–MS, such as the Montreux Workshop, follows similar trends. Thus there is no doubt that there is a growing interest in LC–MS which in general has become an established analytical technique.

The evaluation of the impact of major LC–MS techniques, *i.e.*, the moving belt, DLI and TS (Figs. 2–4), was based on the total number of identified laboratories using a given type of interface rather than the total number of papers presented at the ASMS meeting or in the literature. A few laboratories have been at the forefront of the development of some models and have published many papers. Although of great value for LC–MS research, these many articles could obscure the evaluation of the real range of application of this technique among users. Another limitation of this method of evaluation arises when several groups collaborate and jointly publish their results. In this report, only the laboratory where MS data had been obtained was considered. This may have led to some omissions in a small number of cases. Estimates obtained from the ASMS proceedings and from the bibliographic search were compiled separately. ASMS data offer the possibility to consider laboratories which do not publish in the literature but do participate in symposia, as is the case for some industrial laboratories and instrument companies.

Finally, the number of interface users was compared to the estimated number of interfaces sold by manufacturers. Of the six major LC–MS equipment manufacturers contacted, four communicated the number of interfaces they had sold (Finnigan-Mat, Nermag, V.G., Vestec), one could not say exactly (Hewlett-Packard) and the last one considered the question to be strictly confidential (Vacumetrics). Thus figures for installed DLI interfaces may not be accurate. This survey did not include other known LC–MS equipment manufacturers, *i.e.*, Kratos, Extranuclear, Sciex, Hositrad and Phrasor, because the corresponding number of users was estimated to be small at the present time. This should not be considered to be a criticism of the quality of their equipment.

TRANSPORT INTERFACES

Moving-belt interfaces were introduced first and were commercially available by the end of the 70s. The Finnigan model of 1976 was soon followed by that from VG, the major improvements being the penetration of the belt into the ion-source block and a high voltage insulator making the interface adaptable to a sector instrument. These features are now found in the recent Finnigan-MAT model. A comparison between commercial belt systems was published recently[15]. The total number of belt interfaces in laboratories is *ca.* 90: at least 85 are commercial equipment with the others being laboratory-made machines. A moving-belt interface requires precise machining and is difficult to design; this is why only a few groups have attempted to build their own systems.

The rapid increase in the use of early commercial equipment is seen in Fig. 2. A maximum in the ASMS data in 1979 is followed by a maximum in the number of publications during 1980–1982. The decrease during 1982–1983 can be explained by the difficulties faced by users when utilizing aqueous solvents, conventional 4 mm I.D. colums for high-performance liquid chromatography (HPLC) and thermal desorption from the plastic belt used for vaporizing samples into the ion source. These problems may have discouraged scientists from using their LC–MS equipment. However, a renewed interest in transport interfaces occurred in 1984. This is probably due to improvements in conventional systems brought about by adopting pneumatic nebulization of the liquid solution for spray deposition onto the belt, and micro-HPLC equipment[16]. In addition, new ionization methods are now applied directly to non-volatile samples on the belt. These are laser desorption[17], fast atom bombardment (FAB) to a glycerol matrix[18,19] or secondary ion mass spectrometry (SIMS) from a metallic ribbon[20,21].

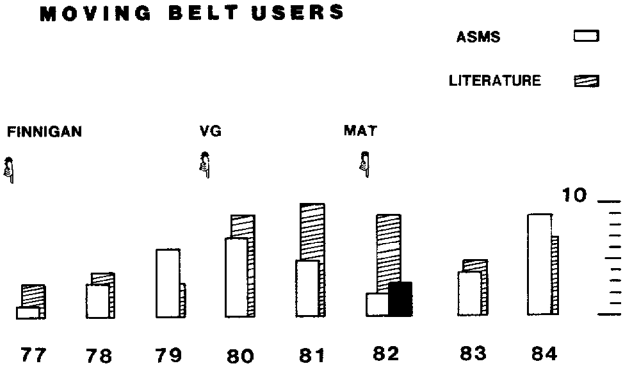

Fig. 2. Number of moving-belt users who have published reports in the literature (hatched area) or have given a talk at the ASMS annual meeting. The completely filled area for 1982 corresponds to groups who gave a talk at the Vienna conference.

The number of users of moving belts who have written publications is small considering the systems available, but this situation is not specific to moving-belt interfaces. However, if the increase in research activity in 1984 is maintained, the continued use of this method in the future seems assumed.

DIRECT LIQUID INTRODUCTION

The situation for DLI is shown in Fig. 3. Here too, the increase in the number of reports presented in 1980–1981 follows the marketing of commercial systems by Nermag[22] and Hewlett-Packard[23]. Both models are rather similar and use a metal diaphragm pierced by a 2–5 μm pinhole for vacuum nebulization of chromatographic effluents. Major modifications introduced later were the addition of a desolvation chamber for better droplet desolvation[24,25] and a cryotrap for a better vacuum pumping[26,27].

The particular model introduced by Extranuclear was based on work done at the National Bureau of Standards in Washington D.C.[28] but the number of models that were sold is probably small. The on-line concentrator of this interface was an interesting idea, but the liquid pressure of the concentrated solution in front of the DLI nebulizer was too low and could not be adjusted.

DLI probes are now available from mass spectrometer manufacturers and from accessory dealers, *e.g.*, Vacumetrics, Hositrad, and can be fitted to any model of mass spectrometer. Quadrupoles are generally used but work using sector instruments has also been reported[1,29,30]. A diaphragm is no longer the unique method of vacuum nebulization. Other devices including fused-silica capillary tubes[31,32] and coaxial pneumatic nebulizers[33,34] have been described, some of which are commercial products.

More groups have reported results from DLI experiments than with any other interface. However, the number of DLI interfaces which can be found in laboratories is probably higher than of any other system. More than 100 units have probably been obtained from commercial sources. In addition, DLI interfaces are cheap and simple to construct and detailed descriptions have been published[35,36]. Thus the total number including laboratory-built interfaces could be 130–150. As for the moving-

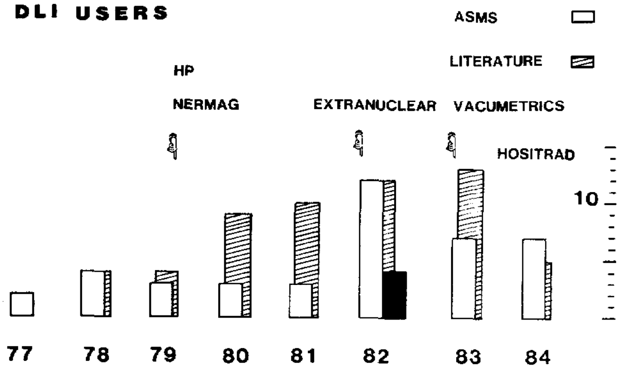

Fig. 3. Survey of DLI users, as in Fig. 2.

belt interface, the number of reports from DLI users is certainly low considering the number of interfaces available.

DLI interfaces are simple to install, rather easy to operate and have often produced good analytical results when applied to difficult samples[24,37]. Both filament-on (solvent chemical ionization) and filament-off modes of ionization[32] have been reported, the former being by far the most frequently selected. A cryotrap, which imposes practical constraints, is no longer necessary and can be replaced by a rough pump directly attached to the ion source in a manner first described for TS experiments.

Nevertheless, all DLI interfaces suffer more or less from severe plugging problems and irregular responses, especially those models which utilize a small diaphragm[38]. Another inherent limitation is the poor heat transfer to liquid droplets in a vacuum when attempting to vaporize the solvent, even when the droplets pass through a very hot desolvation chamber[39]. The situation can be improved by preheating the solution while it flows through the DLI probe, a method now called "hot DLI"[32]. The difference from TS is that a straight jet is still produced in the air from the nebulizer. Pneumatic nebulizers have also been claimed to improve nebulization and desolvation conditions[33,34]. A phase separator has been described for the exchange of polar solvents, which are difficult to vaporize, by apolar solvents[40].

These difficulties are responsible for the decrease in the number of reports published in 1984. In addition, DLI users have tended to shift to thermospray as the change from a DLI set-up requires little modification. Another interest in DLI systems could be the direct introduction of cold or medium temperature liquid solutions at low flow-rates, *ca.* 30–150 μl/min, as delivered by micro-HPLC equipment. For the moment, TS appears to accommodate a low liquid flow-rate input with some difficulties, although this may be a temporary situation.

THERMOSPRAY

Thermospray (TS) has evolved from large crossed beam machines assembled in Salt Lake City by Vestal and co-workers[41]. Data in Fig. 4 for the period 1977–1980 represent accounts of these early works and include a similar work by Futrell and co-workers[42], who used an ultrasonic oscillator instead of a laser for nebulizing liquid solutions.

Three facts have contributed to the rapid recent development of thermospray:

(i) The design of the interface was continuously simplified in order to achieve a simple device applicable to different models of mass spectrometers. For example, nebulization was achieved successively by a high power laser[41], a oxy-hydrogen torch[6], several electrical cartridges[43] and finally by a tube directly heated by an electric current[44]. The original cost was reduced by at least four orders of magnitude.

(ii) The presentation at the 1979 ASMS meeting on ions produced by simply spraying electrolytic solutions in the absence of other ionization devices, the so-called filament-off mode, received considerable attention. The result was confirmed during the following years and is still one of the most spectacular features of the method. It now appears that this effect applies only to polar compounds and preformed ions. Nevertheless these molecules are often separated exclusively by liquid chromatography and are difficult or impossible to analyze by conventional MS. For non-polar

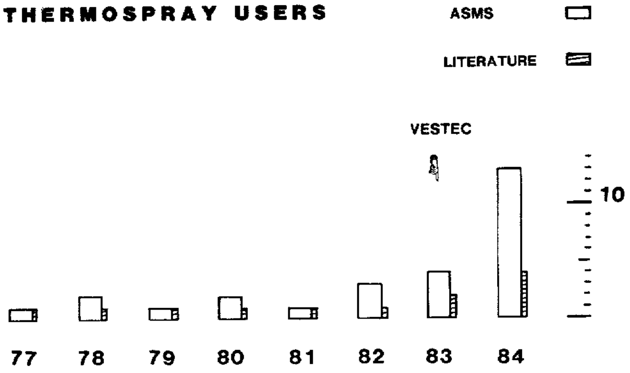

Fig. 4. Survey of thermospray users.

molecules and a large number of medium polarity samples, electrons from a heated filament are necessary for inducing the series of chemical ionization reactions that produce sample-derived ions. This "abnormal" mode is referred to as the filament-on mode or solvent chemical ionization. Which of these ionization modes should be applied to an unknown molecule cannot be predicted. An increasing number of molecules appear to need the filament-on condition. Nevertheless, filament-off operation is an interesting simplification when applicable. It also leads to interesting theoretical considerations of ionization mechanisms under the conditions of TS, and also under those of other MS techniques such as FAB and electrohydrodynamic ionization.

(iii) TS interfaces are more reliable with respect to plugging than previous DLI interfaces. This is why many DLI users have turned to thermospray. However, as for DLI, the sensitivity is compound dependent. The detection limit is comparable or even lower with TS. Applications to quadrupoles[43,44] and magnetic sector instruments[45] have been demonstrated.

All these reasons account for the spectacular increase in the number of presentations at the ASMS meeting in 1984, including many from instrument companies. According to Vestal, *ca.* 40 units are in service. More and more applications from new users are now being reported, but some of these also contain criticisms. Games[46] conducted comparisons and concluded that the optimization of experimental conditions can be more difficult with TS than with a moving-belt interface, and studies of samples containing unknowns can present difficulties. Thermospray is at a stage of exponentially increasing development, and more important, it attracts to LC–MS new teams of scientists who were previously worry of the practical difficulties involved in working with existing commercial interfaces.

CONCLUSIONS

It was not the purpose of this survey to cover all the recently described interfacing methods, despite the potential interest in some of them. Thus only a brief mention was made of ion evaporation[5]. An LC–MS interface based on this principle is available from Sciex, but its usage appears to be limited. The comparable method of electrospray is still under reinvestigation[11]. Both methods could lead to interesting developments and commercial models as they have a great potential for handling polar and high-molecular-weight molecules. The same could apply to the monodisperse aerosol interface for chromatography (MAGIC), although its scope should be limited to compounds of high or medium volatility[47].

The general trends in LC–MS research are quite clear from Figs. 2–4. One of the findings of this survey was that most LC–MS interfaces in laboratories appear to be unused. Often they were accessories bought for potential use at the same time as new mass spectrometers, but they were not subsequently used routinely. Hopefully, this situation should change with the advent of recent LC–MS interfaces that have reduced many of the operational difficulties of early models.

Looking back at the evolution of LC–MS since its origin in 1974, one is impressed by the tremendous efforts of academic institutions to solve LC–MS difficulties, and by the heavy investments made by instrument companies to offer commercial products rapidly. Future developments should be more oriented toward practical applications.

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