Review

Liquid chromatography–mass spectrometry
General principles and instrumentation

W.M.A. Niessen[a],[*], A.P. Tinke[b]

[a]Division of Analytical Chemistry, Leiden/Amsterdam Center for Drug Research, P.O. Box 9502, 2300 RA Leiden,
Netherlands
[b]PBR Laboratories, P.O. Box 200, 9470 AE Zuidlaren, Netherlands

Abstract

In the past 20 years the on-line combination of liquid chromatography (LC) and mass spectrometry (MS) has become a robust and routinely applicable analytical tool. Principles and developments in the instrumentation for LC–MS are reviewed, with special attention for those interfaces that are likely to be the most important in the coming years: electrospray, atmospheric-pressure chemical ionization and particle beam. Further, attention is paid to some recent developments, which may result in important future LC–MS instrumentation.

Contents

1. Introduction

The on-line combination of liquid chromatography and mass spectrometry (LC–MS) is under

investigation for over 20 years. Pioneering research groups in the field were those of Tal'rose [1], Horning [2,3] and Scott [4], while significant instrumental developments were achieved in the laboratories of Arpino [5–8], Ishii [9], Henion [10–12], Vestal [13–15], Caprioli [16,17], Fenn

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* Corresponding author.

[18,19] and Browner [20]. Important contributions to the developments in the field were also achieved by many groups applying the early LC–MS technology and at the R & D laboratories of various instrument manufacturers, who finally made LC–MS to a robust, routinely applicable and wide-spread analytical technique.

LC–MS has been extensively reviewed over the past years, paying attention to instrumental aspects and/or applications, e.g., [21–27]. This is yet another review on LC–MS, in which the prime attention is focused at the instrumental aspects related to the most recent developments in the field. Biochemical and biomedical applications of LC–MS are reviewed elsewhere in this issue by Gelpi [28], while environmental applications of LC–MS are reviewed by Slobodnik and Van Baar [29]. Special attention is paid to LC–MS–MS by Busch [30]. Related topics in interfacing supercritical fluid chromatography and capillary zone electrophoresis to MS are reviewed by Arpino and Hass [31] and Henion and Cai [32], respectively.

Three major difficulties are met in combining the two powerful analytical techniques, LC and MS: (i) the apparent flow-rate incompatibility as expressed in the need to introduce 1 ml/min of a liquid effluent from a conventional LC column into the high vacuum of the mass spectrometer, (ii) the solvent composition incompatibility as result of the frequent use of non-volatile mobile phase additives in LC separation development, and (iii) the ionization of non-volatile and/or thermally labile analytes.

The ionization problem certainly was important in the early years of LC–MS research when only electron impact (EI), chemical ionization (CI) and field desorption/ionization (FD) were available for the ionization of organic compounds. FD is not applicable in on-line LC–MS. EI and CI essentially require that the sample is offered in a gaseous state, while LC and LC–MS are especially interesting for the analysis of non-volatile analytes. However, meanwhile considerable progress has been made in the development of other soft ionization methods, partly in conjunction with the LC–MS research. This led to the introduction of powerful soft ionization techniques such as fast-atom bombardment (FAB), thermospray and electrospray ionization, and more recently matrix-assisted laser desorption ionization (MALDI). As a result, the ionization of analytes is no longer considered a problem in LC–MS research.

In order to solve the flow-rate incompatibility a wide variety of technological solutions were developed: the LC–MS interfaces. These are discussed in detail below. Subsequently, some attention is paid to the problems related to the incompatibilities in mobile phase composition.

2. LC–MS interfaces

In this section, the various commercially available interfaces are briefly discussed. The interfaces presently most important are discussed in further detail in subsequent sections.

The *moving-belt* interface [33] consists of an endless continuously moving Kapton ribbon which transports the column effluent from the LC column outlet towards the MS ion source. During the transport, the mobile phase is removed via gentle heating and evaporation under the reduced pressure in two differentially pumped vacuum chambers. Desorption of the analyte into the ion source by flash evaporation at the tip of the moving-belt interface provides the analyte in a gaseous state, susceptible to conventional EI or CI. On the way back, cleaning of the belt is performed by heating and washing. Although quite successful for some years, the moving-belt interface nowadays is hardly used. The complex mechanical device is in its specific field of application (on-line EI mass spectra in LC–MS) replaced by the particle-beam interface.

In a *direct liquid introduction* interface [34,35] nebulization of (part of) the column effluent is achieved by liquid jet formation at a small diaphragm and the subsequent disintegration into small droplets. The nebulization takes place in a reduced-pressure desolvation chamber connected to the CI ion source. The desolvation of the droplets in the desolvation chamber provides a gentle means of transferring non-volatile and/

or thermally labile compounds from the liquid to the gas state. Solvent-mediated CI of the desolvated analyte molecules is achieved using the reversed-phase solvents as reagent gas. Primarily because the allowable liquid flow-rate is limited to 100 μl/min and due to the frequent clogging of the 4-μm-I.D. diaphragms, the direct liquid introduction interface has disappeared and is replaced by thermospray and subsequently atmospheric-pressure LC–MS interfaces.

In a *thermospray* interface [15,36,37] a jet of vapour and small droplets is generated out of a heated vaporizer tube. A schematic diagram of a thermospray interface is given in Fig. 1. Nebulization takes place as a result of the disruption of the liquid by the expanding vapour that is formed at the tube wall upon evaporation of part of the liquid in the tube. Prior to the onset of the partial evaporation inside the tube a considerable amount of heat is transferred to the solvent. This heat later on assists in the desolvation of the droplets in the low-pressure region. By applying efficient pumping by means of a high-throughput mechanical pump attached directly to the ion source up to 2 ml/min of aqueous solvents can be introduced into the MS vacuum system. The ionization of the analytes takes place by means of solvent-mediated CI reactions and ion evaporation processes. The reagent gas for solvent-

mediated CI can be generated either in a conventional way using energetic electrons from a filament or discharge electrode, or in a process called thermospray ionization, where the volatile buffer dissolved in the eluent is involved. The thermospray interface for many years has been the interface of choice. As a result of the advent of more sensitive and robust approaches based on atmospheric-pressure ionization, the thermospray interface is slowly disappearing from the scene.

In a *continuous-flow or dynamic fast-atom bombardment* (CF-FAB) interface [17] a small liquid stream, typically 5–15 μl/min, mixed with an appropriate FAB matrix solvent, e.g., glycerol, flows through a narrow-bore fused-silica capillary towards either a stainless-steel frit (*frit-FAB*) or gold-plated FAB target. At the frit or target a subtle balance between the solvent evaporation and the formation of a uniform liquid film is reached. Ions are generated by bombardment of the liquid film by fast atoms or ions, i.e., the common ionization process in FAB or liquid secondary ion mass spectrometry (LSI-MS). The dynamic nature of the processes occurring at the target provides several advantages of dynamic FAB over conventional static FAB. The renewal of the surface layer in CF-FAB is a more dynamic process, not only determined by analyte diffusion. As a result, the differences in response between the hydrophobic and hydrophilic analytes in a sample mixture, which are observed in static FAB, are significantly reduced in dynamic FAB. Furthermore, the background signal due to matrix-related ions is greatly reduced, resulting in considerably improved absolute detection limits. The use of CF-FAB is decreasing due to the introduction of especially the electrospray interface. However, owing to the ease of implementation of CF-FAB interfaces, especially at magnetic sector instruments, continued application of CF-FAB in the near future can be expected.

In a *particle-beam* interface [20,38,39] the column effluent is nebulized either pneumatically or by thermospray nebulization, into a near atmospheric-pressure desolvation chamber, which is connected to a momentum separator,
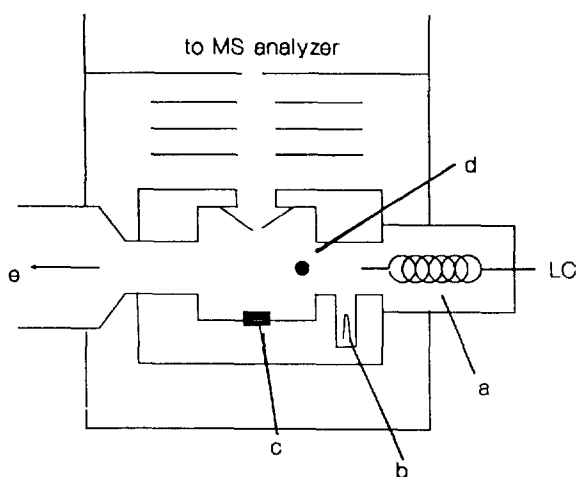

Fig. 1. Schematic diagram of a thermospray LC–MS interface. a = Vaporizer; b = filament; c = repeller electrode; d = discharge electrode; e = exhaust pump.
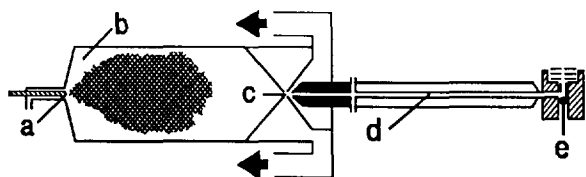
Fig. 2. Schematic diagram of a particle-beam interface. a = (Pneumatic) nebulizer; b = heated desolvation chamber; c = two-stage momentum separator; d = transfer tube; e = ion source.

where the high-mass analytes are preferentially transferred to the MS ion source while the low-mass solvent molecules are efficiently pumped away. The analyte molecules are transferred as small particles to a conventional ion source, where they disintegrate upon collisions at the heated source walls. The released gaseous molecules are ionized by EI or CI. A schematic diagram of the particle-beam interface is given in Fig. 2. The ability to achieve EI mass spectra is the most important feature of the particle-beam interface. The instrumental setup of the interface is discussed in more detail below.

Two different sample-introduction approaches are used in combination with *atmospheric pressure ionization* (API) devices. They primarily differ in the nebulization principle and in the application range they cover. In a *heated nebulizer* or *APCI* interface [40], the column effluent is pneumatically nebulized into a heated (quartz or stainless-steel) tube, where the solvent evaporation is almost completed. Atmospheric-pressure chemical ionization (APCI), initiated by

electrons from a corona discharge needle, is achieved in the same region. Subsequently, the ions generated are sampled into the high vacuum of the mass spectrometer for mass analysis. In an *electrospray* interface [18,19], the column effluent is nebulized into the atmospheric-pressure region as a result of the action of a high electric field resulting from a 3 kV potential difference between the narrow-bore spray capillary and a surrounding counter electrode. The solvent emerging from the capillary breaks into fine threads which subsequently disintegrate in small droplets. In some designs, the electrospray nebulization is assisted by pneumatic nebulization. Such an approach is called an *ionspray* interface [11]. These systems are discussed in considerable detail below.

Typical operating parameters of the various LC–MS interfaces discussed above, especially in terms of LC conditions, e.g., with respect to flow-rate and solvent composition, are summarized in Table 1. A schematic diagram, indicating the application areas of the various LC–MS interfaces in terms of analyte polarity and molecular mass, is given in Fig. 3.

3. Strategies in LC–MS interfacing

The developments in LC–MS interfacing reflect important changes in the strategic view on coupling LC and MS [26]. Initially, an LC–MS setup was pursued that closely resembled the successful GC–MS combination. The moving-

Table 1
Comparison of LC–MS interfaces in terms of allowable flow-rate and mobile phase composition

| Interface	Maximum flow-rate (ml/min)	Mobile phase composition
Moving-belt	2	Organic solvent
	0.5	Reversed-phase solvents, no buffers
Direct liquid introduction	0.05	Reversed-phase solvents, no buffers
Thermospray	2	Reversed-phase solvents with volatile buffers
CF-FAB	0.015	Reversed-phase solvents with volatile buffers
Particle-beam	0.5	Reversed-phase solvent (not too aqueous) with volatile buffers
Electrospray/Ionspray	0.5	Reversed-phase solvents with volatile buffers
Heated nebulizer/APCI	2	Reversed-phase solvents with volatile buffers
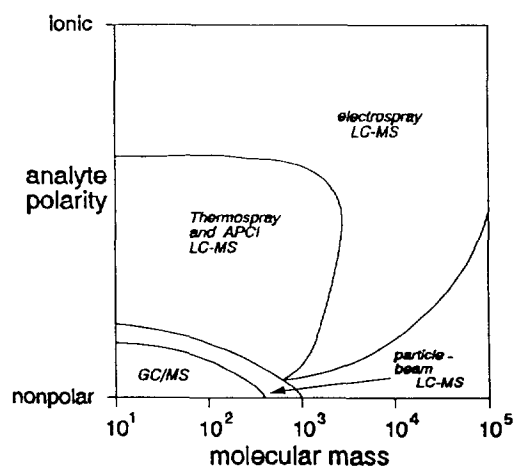
Fig. 3. Representation of the application ranges of LC–MS interfaces.

belt interface most clearly demonstrates this initial strategy: the solvent is removed prior to the introduction of the column effluent in the MS, and the analytes are vaporized prior to (EI) ionization. Subsequently, it was realized that the vaporization of analytes in fact limits the application range of LC–MS. In this respect, gentle desolvation of analytes by solvent vaporization from small droplets can be considered as a major breakthrough in LC–MS developments [7]. Simultaneously, the importance of EI ionization in LC–MS was placed in proper perspective: for many analytes amenable to LC–MS EI does not provide useful information. Both direct liquid introduction and thermospray are examples of this approach. Research in thermospray more or less accidentally led to ionization strategies other than solvent-mediated chemical ionization. The principles of thermospray ionization and ion evaporation further opened the view on LC–MS. The application of these new strategies in electrospray ionization finally opened important LC application areas, e.g., related to peptide and protein analysis, to LC–MS.

The role of the LC mobile phase has thus changed in time: from an active carrier in the LC process, which should be removed as quickly and thoroughly as possible, via a transfer medium for non-volatile and/or thermally labile analytes from the liquid to the gas state, to a constituent

essential in analyte ionization. Nevertheless, the LC mobile phase continues to put high demands and restrictions on the instrumentation, especially in terms of vacuum equipment and solvent compatibility problems. Gas-phase analyte-enrichment devices based on molecular beam technology and atmospheric-pressure sample introduction are nowadays routinely used to handle the vacuum problems. These are discussed in more detail in the next sections.

4. Atmospheric-pressure sample introduction

Despite the fact that a particle-beam interface has a different application area from the API interfaces, these systems have much in common, as they both introduce liquid effluents in a desolvation chamber, which is held at (nearly) atmospheric pressure. The principles of analyte introduction from an atmospheric-pressure chamber into a vacuum region are discussed in this section. Some of these topics have also been discussed in a review paper by Bruins [41].

In a quadrupole mass spectrometer, equipped with a turbomolecular or oil diffusion pumping system with an effective pumping speed S_b of 0.3 m³/s, the background pressure P_b is generally kept at ca. 1 mPa. The maximum throughput Q, i.e., the amount of gas that can be introduced directly into the instrument, e.g., via an artificial leak, is then given by:

$$Q = S_b P_b \qquad (1)$$

For the system described, the maximum throughput corresponds to a gas flow of ca. 200 μl/min from an atmospheric-pressure region ($P_0 = 10^5$ Pa). The conductance c of the artificial leak is defined as:

$$c = \frac{P_b S_b}{P_0} \qquad (2)$$

In principle, the conductances of openings with various sizes and dimensions can be theoretically calculated [42].

Vaporization of a liquid flow of 1 ml/min, as used in conventional LC, results in an atmos-

pheric gas flow of more than 1 l/min. Consequently, at the most 0.01% of the liquid effluent, i.e., less than 0.1 μl/min of liquid, can be directly sampled into the mass spectrometer.

Liquid flow-rates in excess of 0.1 μl/min can only be introduced into the MS vacuum, if additional pumping stages are provided. For both particle-beam and API interfacing, this has been achieved by sampling the vaporized effluent from a (nearly) atmospheric-pressure desolvation chamber into the vacuum of the mass spectrometer via a differentially pumped expansion chamber system. A one-stage expansion chamber system consists of a nozzle and a skimmer. The latter samples only a part of the vaporized liquid from the expansion region into the mass spectrometer. If the various effluent constituents are sampled with comparable efficiency, the additional pumping step acts as a split: the ratio of the net analyte mass flow and the net vapour flow into the mass spectrometer remains unaffected. However, as a gas is expanding through a nozzle into a low pressure region, high-mass particles have a lower momentum perpendicular to the axis of expansion than the low-mass species. As a result, the low-mass particles will diffuse more out of the core of the expansion, and enrichment of high-mass species occurs. As in both GC–MS and LC–MS most analyte molecules have a considerable higher molecular mass than the mobile phase constituents, the skimming of the gas mixture after expansion will lead to the gas-phase enrichment of analyte molecules. This phenomenon was routinely applied in the coupling of GC and MS via a jet separator. If, in addition, the ionization of the analyte molecules is also achieved under atmospheric-pressure conditions, like in API devices, an even more pronounced sample enrichment may be achieved owing to the possibilities of electrical focusing of the analyte ions during expansion.

Assuming a hypothetical background pressure in the expansion chamber of 1000 Pa, which is achieved by means of a mechanical fore pump with an effective pumping speed S_1 of 20 m^3/h, a gas flow of ca. 3 l/min can be sampled from an atmospheric-pressure region. This in effect is a

higher gas flow than the gas flow corresponding to a 1 ml/min liquid flow. In conclusion, a single additional mechanical pumping step should theoretically be sufficient for the introduction of the 1 ml/min LC mobile phase into the mass spectrometer. This even holds when it is taken in account, that the vaporized liquid is generally diluted with either a nebulization gas, or a (heated) drying gas.

In practice, however, there are two reasons for the use of a second pumping stage: the incomplete liquid evaporation and the analyte transmission to the vacuum. The droplet desolvation in an atmospheric-pressure chamber is generally incomplete, resulting in a (substantially) larger fraction of the liquid entering the mass spectrometer. The low momentum of clusters or small droplets leads to a far better sampling efficiency than that achieved for a mixture of isolated molecules. Upon collision of the droplets and clusters on a hot surface inside the mass spectrometer, efficient heat transfer leads to immediate evaporation, and hence the effective gas flow into the mass spectrometer may exceed the pumping capacity, i.e., the pressure in the instrument increases. In order to prevent these types of problems, generally a two-stage differentially pumped expansion system is applied. Furthermore, the two-stage setup allows the use of somewhat larger skimmer openings, which is favourable in terms of analyte transmission to the high vacuum. A schematic diagram of a two-stage differentially pumped expansion system, consisting of a nozzle, a skimmer and a collimator, is depicted in Fig. 4. Since in this device gas-phase enrichment of heavy species is achieved, the two-stage expansion system is also called a momentum separator.

In a device like the one drawn in Fig. 4, the throughput of the nozzle can be written as a mass balance: it is equal to the summed throughputs of each individual pumping stage:

$$c_n P_0 = S_1 P_1 + S_2 P_2 + S_b P_b \qquad (3)$$

where c_n is the conductance of the nozzle. Other parameters are explained in Fig. 4. Calculation of the conductances of nozzle and skimmer
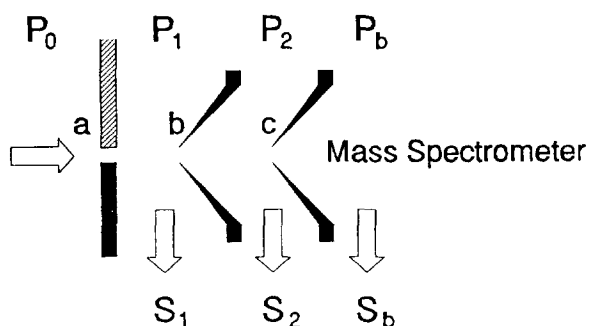
Fig. 4. Schematic diagram of a three-stage momentum separator or expansion system. a = Nozzle; b = skimmer; c = collimator. Three pumped regions with pressures P_1, P_2 and P_b are pumped by three pumping systems with effective pumping speeds S_1, S_2 and S_b, respectively. P_0 is the (nearly) atmospheric pressure in the desolvation chamber.

cannot readily be made, because part of the analyte is transferred as small particles and/or droplets, yielding a much higher mass flow than expected from a gas. Under proper conditions the term $S_b P_b$ can be neglected, as it is very small compared to the other terms. The conductance of the skimmer c_s is related to the conditions in the skimmer–collimator region and can be written as:

$$c_s P_1 = S_2 P_2 + S_b P_b \tag{4}$$

where $S_b P_b \ll S_2 P_2$. Therefore,

$$c_n P_0 = S_2 P_2 \cdot \left(\frac{S_1}{c_s} + 1 \right) \tag{5}$$

As c_c is the conductance of the collimator, $c_c P_2 = S_b P_b$, and Eq. 5 can be converted into:

$$c_n P_0 = S_b P_b \cdot \frac{S_2}{c_c} \cdot \left(\frac{S_1}{c_s} + 1 \right) \tag{6}$$

As a result, the throughput of the nozzle orifice of the LC–MS interface can thus be written as a function of the background pressure in the mass spectrometer, the various effective pumping speeds, and the conductances of the skimmer and the collimator.

In practice, a number of different designs of the expansion region are applied in API interfaces. The major differences between the various

approaches are shown in the schematic diagrams in Fig. 5.

In the system in Fig. 5A, surprisingly a single pumping stage is applied. This is possible due to the use of a cryogenic pump with an extremely high pumping efficiency, i.e., 180 m^3/s as opposed to the 20 m^3/h of the high-throughput mechanical pump discussed earlier. This setup is the earliest commercially available API LC–MS system [40]. A major drawback of this setup is the need for the regular regeneration of the cryogenic pump, which prohibits the system to be used for 24 h per day.

As indicated above, the formation of solvent clusters and related solvent cluster ions resulting from incomplete solvent evaporation is one of the problems in API interfaces. Various procedures have been developed for the breakdown of these clusters and cluster ions prior to the introduction into the expansion region. In the system shown in Fig. 5A, a counter-current nitrogen curtain gas is applied to dissociate the ion–solvent clusters by means of cluster–nitrogen collisions, prior to entering the sampling orifice of the mass spectrometer [40]. Obviously, additional heating can also be used to improve droplet desolvation. A system containing a heated spray chamber and ion source is described by Allen and Vestal [43]; this system is commercially available. Another approach to the heat-induced desolvation of cluster ions is the use of a heated stainless-steel sampling capillary, as described by Chowdhury et al. [44]. The commercial version of this system is schematically depicted in Fig. 5B. With this setup, flow-rates up to 1000 $\mu l/min$ can be electrosprayed without the need of a counter-current (heated) drying gas. In other systems either a counter-current drying gas or a specially designed counter electrode, which stimulates efficient cluster breakdown, is applied. An example of the latter approach is schematically depicted in Fig. 5C [45].

An additional improvement in these interface designs over earlier designs is the use of an radio frequency (RF)-only hexapole or octapole filter at the low-pressure end of the interface. Compared to the conventional set of flat lenses, the
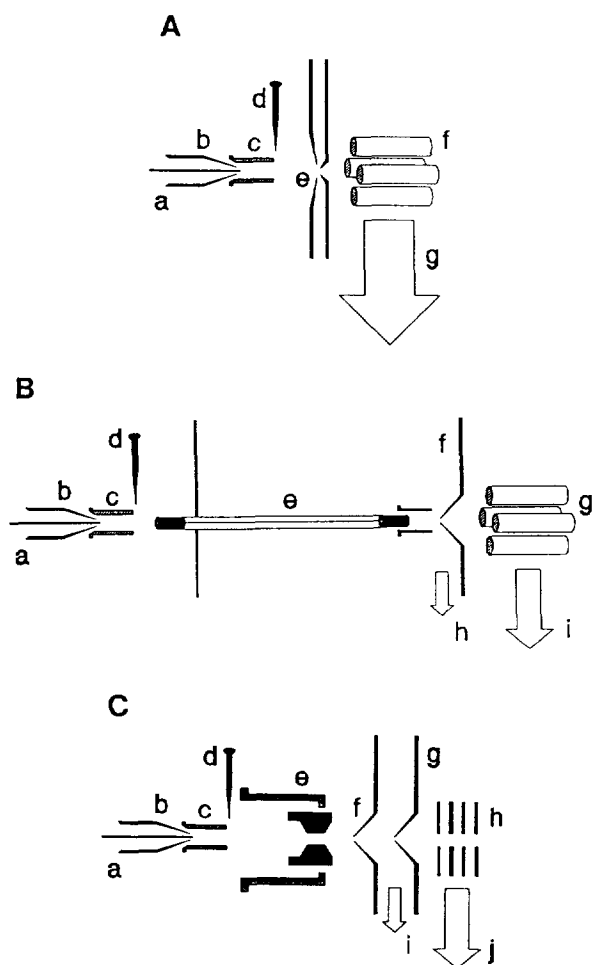
A

B

C

Fig. 5. Three different designs of an API expansion region. (A) API system consisting of a nozzle, counter-current drying gas and a high-efficiency cryopump: a = LC capillary; b = nebulization gas capillary; c = heated quartz tube for APCI; d = corona discharge electrode for APCI; e = counter-current drying gas; f = quadrupole ion focussing device; g = cryogenic pump. (B) API system consisting of a two stage-expansion system, comprising a heated sampling capillary and an octapole lens: a = LC capillary; b = nebulization gas capillary; c = heated stainless-steel tube for APCI; d = corona discharge electrode for APCI; e = heated sampling capillary; f = skimmer; g = octapole ion focussing device; h = mechanical pumping stage; i = turbomolecular pumping stage. (C) API system consisting a two-stage expansion system, comprising a special design nozzle to promote droplet desolvation: a = LC capillary; b = nebulization gas capillary; c = heated tube for APCI; d = corona discharge electrode for APCI; e = heated desolvation chamber with special nozzle; f = skimmer; g = collimator; h = flat lens system for ion focussing; i = mechanical pumping stage; j = turbomolecular pumping stage.

hexapole or octapole filter provides substantial improvements in signal-to-noise ratios due to the better ion focusing and transmission characteristics [46].

5. Interfaces for atmospheric-pressure chemical ionization

APCI is the chemical ionization of compounds in an ion source operated at atmospheric-pressure conditions [3,47,48]. In APCI, the reagent ions for the ionization of analyte species are generally created by means of a corona discharge. The ionization mechanisms in APCI are identical to those in conventional medium-pressure CI. Positive-ion formation can be achieved by proton transfer, adduct formation, or charge exchange reactions, while in negative-ion mode ions are formed due to proton transfer (abstraction), anion attachment, or electron-capture reactions. Differences in mass spectra between APCI and conventional CI can be explained from the fact that in APCI the ion products reflect the equilibrium conditions rather than the reaction rates while the latter dominate the ion products in medium-pressure CI. In theory, significantly better detection limits may be achieved in APCI than in medium-pressure CI as a result of the higher ion–molecule reaction efficiencies achieved under APCI conditions. However, owing to the difficulties in ion transmission from the atmospheric-pressure ionization chamber into the high vacuum mass analyzer, the gain in detection limits is generally less than expected from the 10^3–10^4 times higher ionization efficiencies. The actual gain observed in current instrumentation is difficult to quantify. Some conclusions about the gain achieved in practice, i.e., in terms of improved detection limits, may be drawn from a comparison of detection limits between the various interfaces for some model compounds (see Section 8).

For the coupling of LC to the APCI system, a heated nebulizer inlet system has been developed [40,49]. The heated-nebulizer inlet probe basically consists of a concentric

pneumatic nebulizer and a large-diameter heated quartz tube. The nebulized liquid effluent is swept through the heated tube by an additional gas flow, which circumvents the nebulizer. The heated mixture of solvent and vapour is then introduced in the API source, where a corona discharge initiates APCI. High tube temperatures may induce thermal degradation of analyte molecules, but no serious thermal degradation of analyte species is reported. The heated nebulizer interface is easy to operate. It can be used in combination with aqueous liquid flow-rates up to 2 ml/min. In contrast to the thermospray interface, no extensive temperature optimization is needed. For systems providing a counter-current drying gas, it is claimed that volatile as well as non-volatile buffers can be used. Uncharged (non-)volatile material is swept away from the nozzle by the counter-current drying gas and non-volatile contamination deposited in the atmospheric-pressure ion source chamber can readily be wiped away without the need to switch off the vacuum system.

An early demonstration of the potential of a heated nebulizer interface in combination with APCI is the high-speed LC–MS–MS analysis of phenylbutazone and two of its metabolites in plasma and urine [50]. In subsequent years, the power of this approach in quantitative bioanalysis was further demonstrated in the analysis of steroids in equine and human urine and plasma by the group of Henion [51–53] and of various other drugs by other authors [54–56]. With the wider commercial availability of APCI system from several manufacturers, i.e., from 1992 onwards, a growing number of applications of APCI in LC–MS is reported and a significant increase in applications of heated-nebulizer APCI in LC–MS is expected for the near future.

It is interesting to note that a more or less independent development of APCI interfacing for LC–MS took place in Japan. An APCI interface used in combination with a thermospray nebulizer was described by Sakairi and Kambara [57,58]. This system, which is commercially available from Hitachi, is frequently applied in bioanalytical LC–MS by Japanese authors.

6. Electrospray interfacing

The technology of electrospray interfacing and ionization has been developed by Fenn and co-workers [18,19,59]. Their experiments were based on both earlier electrospray experiments of Dole and co-workers [60,61] and the work of Iribarne and Thomson [62,63].

The process of electrospray ionization includes both the nebulization of a liquid into an aerosol of highly charged droplets and the ionization of solvated analyte species after desolvation of the charged droplets. The electrospray process is initiated by applying an electrical potential of several kV to a liquid in a narrow-bore capillary or electrospray needle. By increasing the potential difference between the needle and the counter electrode, a series of processes takes place. The transitions between these processes can be monitored by measuring the current between needle and counter electrode (cf. Fig. 6). The four stages of electrospray nebulization are [19]:

(i) At low electric fields the liquid drops almost vertically out of the needle, because the field effect is insufficient. Subsequently, at higher fields a nearly horizontally liquid column is formed, extending beyond the end of the capillary.

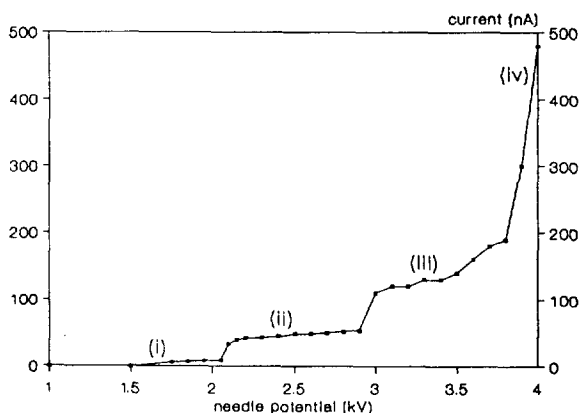(ii) The liquid column elongates, resulting in the formation of a fairly sharp point at its tip, the

Fig. 6. Electrospray needle current as a function of electric potential, demonstrating the four stages of electrospray nebulization. For explanation, see text.

so-called Zeleny cone. A fine mist of small droplets is produced from the jet at the cone tip as a result of electrically affected Rayleigh instabilities at the surface of the liquid jet. This is the onset of the electrospray process.

(iii) At still higher potentials, this axial spray mode transforms in another spray appearance: the liquid cone vanishes and the fine mist of small droplets is now generated from a number of points at the sharp edge of the capillary tip. This is the so-called rim emission mode.

(iv) Finally, at still higher potentials, a stable discharge is formed in the ion source. In practice, electrospray ionization MS is performed in the axial and rim emission modes of spraying.

The mechanisms of the electrospray ionization process is a matter of considerable debate in the past few years. Important contributions in this field are Refs. [18,19,59,62–72]. It is beyond the scope of the present paper to review this discussion. According to the most generally accepted mechanism is electrospray ionization the result of the decrease of the droplet radius by the evaporation of neutral solvent molecules and the accompanying increase of the electrical field strength at the droplet surface. When the field strength at the droplet approaches the Rayleigh instability limit, field-induced surface instability leads to the formation of a liquid jet at the droplet surface, from which on its turn small microdroplets are emitted. These processes of solvent evaporation and field-induced Rayleigh instabilities proceed. At a certain critical field strength at the microdroplet surface, direct emission of desolvated ions from the microdroplets is believed to occur. The droplet evaporation is stimulated by the use of a counter-current (heated) gas and/or a heated sampling capillary (cf. Fig. 5). In practice, electrospray ionization of analytes occurs in a more complex mechanism, since besides ion evaporation of preformed ions from the microdroplets also gas-phase ion–molecule reactions play an important role in the ionization process.

An important breakthrough in the field of electrospray LC–MS, which gave a huge impetus to the interest in the electrospray interface development, was the observation in the late 1980s of the multiply charging of peptides and proteins by protonation at the amino terminus as well as at the basic amino groups in the molecule, i.e., lysine, arginine and histidine [73,74]. As a result, a statistical charge distribution is observed as an ion envelope in the region of $m/z = 500$–1500. From the mass spectrum, an accurate calculation (0.01% or better) of the mass of the macromolecule can be performed. The application of these phenomena resulted in new exiting fields of biochemical and biological MS [19,70,71,75].

A major drawback of the first generation commercial electrospray LC–MS interfaces was the limitation of the effluent flow-rate to ca. 10 μl/min. An alternative approach which allows the use of higher liquid flow-rates, is the ionspray interface, introduced by Bruins et al. [11]. In an ionspray interface, electrospray nebulization is combined with pneumatic nebulization. Initially, the ionspray LC–MS interface allowed liquid flow-rates up to 200 μl/min, but by placing a conical liquid shield between the nebulizer and the ion entrance pinhole the introduction of liquid flow-rates up to 2 ml/min is possible [76]. The ionspray LC–MS system can handle mobile phases with high water contents and can be readily applied under gradient elution conditions.

A second generation of commercial electrospray LC–MS interfaces was developed and became available in 1993. Improvements relative to the first generation were made by modifications of three parts of the system: (i) the spray needle device, e.g., by implementing pneumatic nebulization next to electrospray nebulization, (ii) the nozzle–skimmer system, e.g., by implementing a heated transfer capillary or other more efficient solvent cluster breakdown devices, and (iii) the ion collection and focusing devices, e.g., by replacement of conventional flat lens systems by RF-only quadrupole, hexapole or octapole devices behind the skimmer or the collimator. The second generation devices permit mobile phase flow-rates in electrospray as high as 1 ml/min, although the best sensitivity is still obtained at low flow-rates, e.g., 10 μl/min. The atmospheric-pressure LC–MS interfaces, in

which a rapid change-over between electrospray introduction and ionization and a pneumatic nebulizer with APCI is possible, are more robust, often provide better sensitivities, and require less optimization of experimental parameters than the thermospray LC–MS interface, which can be used in a similar flow-rate range. Therefore, the use of thermospray decreases.

Numerous applications of electrospray ionization have been reported in the past few years. In most of these papers the use of electrospray in the characterization of biomacromolecules, especially proteins, is described without the use of on-line LC. The potential of electrospray in such biochemical studies has been reviewed by several authors [70,71,75,77]. More recently, a number of LC–MS applications with electrospray has been reported [78–80]. Since with the second generation of electrospray interfaces the allowable flow-rates are more in agreement with flow-rates currently used in LC, a more extended use of electrospray in combination with LC–MS can be predicted for the near future.

Interestingly, next to the development of commercial electrospray interfaces which allow higher flow-rates to be used, a number of research groups is working to miniaturize the electrospray interface [81–83]. The rationale for this type of research lies in improvements of sensitivity, compatibility with low flow-rate separation techniques like capillary zone electrophoresis, and applications in sample-limited situations. Obviously, the sensitivity improvement only implies an improved absolute detection limit, while in fact the corresponding concentration detection limit decreases. The latter is due to the fact that the mass spectrometer is a mass-flow sensitive detector, as explained in more detail elsewhere [84].

7. Particle-beam interfacing

The particle-beam interface is a commercial implementation of the monodisperse aerosol generating interface for chromatography (MAGIC) introduced by Willoughby and Browner [20] in 1984. An important feature of

the particle-beam interface is the ability to acquire EI and solvent-independent CI mass spectra. As indicated in Table 1, the particle-beam interface is compatible with 0.1–0.5 ml/min flow-rates, when a pneumatic nebulizer is used. Higher flow-rates, i.e., up to 1 ml/min can be introduced when a thermospray nebulizer is applied, as is the case in the thermabeam and the universal interface [85]. The universal interface consists of a thermospray nebulizer, a desolvation chamber, a membrane separator for additional gas-phase analyte enrichment, and a two-stage momentum separator [85].

Similar to the LC–API-MS systems described above, a gentle evaporation of liquids from small droplets is performed in the desolvation chamber of the particle-beam LC–MS, which is held at nearly atmospheric pressure (ca. $2.5 \cdot 10^4$ Pa). However, in the particle-beam interface the analyte is sampled from a closed desolvation chamber and the analyte is transferred to the vacuum of the mass spectrometer prior to ionization. A closed desolvation chamber demands that the total input to the desolvation chamber, i.e., the vapours from 0.1–0.5 ml/min of liquid and the 1–2 l/min helium used as nebulizer gas, is introduced into the mass spectrometer. From the equations given above, it follows that at least a two-stage differentially pumped expansion system is required. A schematic diagram of a two-stage particle-beam interface is depicted in Fig. 2. Partial droplet desolvation is affected by heat transfer by the helium gas from the heated desolvation chamber walls to the droplets. Since many organic modifiers evaporate much easier than water, generally better results are obtained for not-too-aqueous solvent mixtures.

Optimization of experimental parameters in particle-beam interfaces has been investigated by several authors [39,40,86–89]. In a particle-beam interface, one deliberately aims at incomplete droplet evaporation. The degree of desolvation of the droplets depends on the heat capacity of the solvent, the desolvation chamber temperature, the extent of heat transfer, and on the size of the droplets and the droplet size distribution. In their original design, Willoughby and Browner [20] applied cross-flow nebulization for the crea-

tion of aerosols with a well defined and narrow droplet size distribution. However, most of the current particle-beam interfaces are equipped with concurrent pneumatic nebulizers, which yield a broader droplet size distribution.

In the two-stage expansion system, consisting of nozzle, skimmer and collimator, gas-phase enrichment of the partly desolvated spray droplets/particles occurs as the mixture of gas and particles expands from the desolvation chamber into the nozzle–skimmer region kept at ca. 10^3 Pa by a mechanical pump. The enriched fraction of heavy particles is subsequently sampled from the nozzle–skimmer region into the skimmer–collimator region, which is kept at 100 Pa. The expansion in the skimmer–collimator region induces further enrichment of the heavy species. The enriched fraction is finally sampled into a transfer tube, which leads the particles to the ion source of the mass spectrometer. After flash evaporation of the particles on the hot surface of the ion source walls, filament-on ionization of the gas-phase molecules is performed, either in EI or in CI mode.

The extent of droplet desolvation achieved in the desolvation chamber is an important, but difficult to control parameter in particle-beam interfacing. Complete droplet desolvation generally leads to loss in analyte transmission. The final particle/droplet size appears to depend on the analyte concentration as well. The decrease in particle size with decreasing analyte concentration can be held responsible for non-linearity effects at low concentrations. Improved analyte transmission and linearity has been achieved by the addition of coeluting compounds or low-volatility buffers, which may act as carrier [90–95].

Despite the non-linearity effects, the limited sensitivity and the limited applicability range, particle-beam LC–MS is a powerful tool, especially in environmental analysis of medium-polarity low-mass compounds or in other areas where on-line LC–EI mass spectra are of importance. Further improvement of the performance of the particle-beam interface, especially with respect to analyte transmission and ionization efficiency, is needed. This may be achieved by obtaining a better understanding of the sampling of molecular and particle beams in the two-stage momentum separator and by investigating alternative, possibly more efficient ionization techniques [96].

8. Comparison of LC–MS interfaces

As illustrated in Fig. 3, the choice of an LC–MS interface for a particular application depends on polarity and molecular mass of the analyte. In practice, the availability in the laboratory plays a major role, although nowadays in laboratories which deal with LC–MS more than one interface is present.

A useful comparison of LC–MS interfaces can also be based on their figures of merit, i.e., on analytical data on minimum detectable quantities or concentration and on information content. With respect to information content it must be realized is that the information one wants in a mass spectrum depends on the type of applications: in a qualitative analysis extensive structure-informative fragmentation is highly desirable, while in quantitative analysis the presence of 1–3 selective peaks in the mass spectrum is sufficient. Therefore, the use of the particle-beam interface with its ability to generate EI mass spectra is most attractive in qualitative analysis, although many compounds to be analyzed in LC–MS may not give useful fragmentation in EI. The importance of MS–MS in both qualitative and quantitative analysis in combination with soft ionization methods must be stressed (see also [30]).

With respect to minimum detectable concentration of the various LC–MS interfaces it appears to be quite difficult to extract comparable data from various papers in literature. Furthermore, it must be realized that such a comparison can only be made for compounds that can be and have been analyzed by a variety of LC–MS interfaces. The presently more difficult and challenging problems for LC–MS can generally be solved only using one or two LC–MS interfaces, while with other LC–MS approaches no results are obtained. In such an application, the ability

to generate mass spectral data is initially of more importance than a low detection limit. Detection limits of various LC–MS interfaces are compared for three compound classes below, i.e., N-methyl carbamate, clenbuterol and three enzyme inhibitors.

Detection limits for some N-methyl carbamate pesticides in LC–MS analysis using thermospray, ionspray and heated-nebulizer APCI interfaces were compared by Pleasance et al. [97]. The data are summarized in Table 2. It may be concluded that the detection limits obtained in APCI are superior to those obtained by other systems. LC–MS detection limits with another APCI interface were reported by Doerge and Bajic [45]: 0.15 ng for carbofuran and 0.5 ng for carbaryl.

LC–MS data of the β-agonist clenbuterol was reported from three interfaces: thermospray, electrospray and APCI. For thermospray LC–MS concentration detection limits for clenbuterol of 100 ng/ml and 250 ng/ml were reported by Blanchflower and Kennedy [98] and Debrauwer et al. [99], respectively. The detection limit in constant infusion of a clenbuterol solution to an electrospray interface was 0.5 μg/ml [99]. The fragmentation of the β-agonists that can be induced by applying a voltage differences between the nozzle and the skimmer in the electrospray interface, was also extensively studied by

Debrauwer and Bories [100]. For APCI, concentration detection limits of 10 ng/ml were reported by Doerge et al. [101]. Again, APCI provides the lowest detection limits.

A comparison of the performance of thermospray, APCI and electrospray LC–MS in a drug metabolism study was reported by Iwabuchi et al. [102]. Three hydroxymethylglutaryl–coenzyme A reductase inhibitors, i.e., pravastatin sodium and its metabolites, lovastatin and simvastatin, were analyzed. The responses in both positive-ion and negative-ion mode in thermospray, APCI and electrospray of the 13 model compounds were compared and correlated to the analyte polarity, measured as log P values using n-octanol and a phosphate buffer. For the 13 model compounds the log P values ranged from -2.49 for the hydrophilic compounds to $+4.40$ for the hydrophobic compounds. In both APCI and electrospray the hydrophilic compounds show (somewhat) lower response in positive-ion mode and better response in negative-ion mode, while in thermospray hydrophilic compounds show lower responses in both ionization modes. In APCI the favourable hydrophobic compounds were detected at 5–10 ng level, while hydrophilic compounds were hardly detected at 1 μg. In thermospray favourable compounds were detected at 20–30 ng, unfavourable ones at 100 ng. In electrospray all compounds were detected at

Table 2
Comparison of single-ion detection limits in the LC–MS analysis of N-methyl carbamates with various interfaces

Interface	Detection limit (ng)				Flow-rate (ml/min)
	Methomyl	Aldicarb	Carbofuran	Carbaryl	
Moving-belt (methane CI)	–	6	25	3	0.5
Direct liquid introduction	–	–	50	40	0.02
Thermospray	2.8	0.9	0.8	0.8	1.0
Ionspray	0.4	1.5	1.5	1.0	0.05 after 20:1 split
APCI	0.06	0.07	0.05	0.05	1.0
Particle-beam with EI	250	500	55	10	0.4 after 3:2 split

Data from Pleasance et al. [97]. Additional data for the moving-belt and the direct liquid introduction interfaces from Ref. 27, p. 401.

15 ng or less. For the hydrophilic compounds, electrospray in the negative-ion mode showed 10 × better detection limits than thermospray and 100 × better than APCI. Different instrumentation was used for APCI than for electrospray and thermospray. Furthermore, column-bypass injections were performed in thermospray and APCI, while constant infusion at 1 μl/min was applied in electrospray.

From the data provided in this section conclusions may be drawn on trends in detection limits for various LC–MS interfaces, e.g., APCI provides superior detection limits, although for some applications the situation may be different from this general trend. It is important to realize that a proper comparison of detection limits is often difficult because different experimental conditions, especially in terms of flow-rate, must be used. From the second-generation electrospray interfaces described in detail above not many analytical results are presently available.

9. Future perspectives in LC–MS interfacing

Twenty years of LC–MS research have learned that even when exiting new developments have opened new application areas, have improved detection limits, have facilitated use, or have improved robustness, further investigations aiming at still further improvements and innovations immediately continue. Most of this innovative research is presently directed at the application of other types of mass analyzer systems, thus replacing the most widely used quadrupole mass filters.

On-line LC–MS on double-focusing sector instruments has been possible for a long time. Moving-belt interfaces were ideally suited to be combined with sector instruments. However, with the advent of LC–MS technology with higher gas throughputs and higher ion source pressures, the combination with sector instruments is becoming more difficult due to vacuum restrictions in the analyzer as well as the risks of electric discharges owing to the high ion source voltage. Thermospray LC–MS in combination with sector instruments has found only limited

application [103,104]. These studies also showed problems related to collisionally induced dissociation of protonated analytes by ion–molecule collisions as they move from the high-pressure ion source through the lens system of the source towards the ion entrance slit of the analyzer. In this respect, the combination of a particle-beam interface with a sector instrument is less difficult [105,106].

Combination of API systems, and especially electrospray ionization, with sector instruments is highly attractive. Higher resolution in m/z determination may lead to more accurate molecular mass determination and provides direct means to determine the charge state of a particular peak in the ion envelope, which is especially important in structure elucidation and peptide sequencing, either by in-source generated collisionally induced fragmentation or in tandem mass spectrometry. Research in this area has been reported by various groups [107–112].

Considerable research effort has also been put in the combination with electrospray and Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Again, the possibilities for high-resolution measurements to obtain accurate molecular masses and improvements in absolute detection limits by signal averaging and other techniques are the motive in this research, although the advent of electrospray coincides with a more analytical instead of fundamental presentation of FT-ICR-MS by instrument manufacturers. It seems that FT-ICR-MS is moving from the field of highly specialized instrument development and operation towards a more general and routinely applicable instrument with several interesting analytical features. A variety of external atmospheric-pressure ion sources for FT-ICR-MS have been developed and described [113–117]. On-line reversed-phase LC–electrospray-FT-ICR-MS for some peptides has recently been reported as well [118].

Another exiting field is the combination of electrospray with quadrupole ion trap mass spectrometers. In ion traps, the mass analysis is based on m/z-related instabilities which result in emission of ions out of the quadrupole field generated inside the ion trap and subsequent ion

detection. To some extent, ion traps combine advantages of quadrupole filters, e.g., low costs, ease of operation, unit-mass resolution, and not too severe vacuum restrictions, with some advantages of FT-ICR-MS instruments, e.g., trapping and selection of ions, multi-stage MS–MS, and high-resolution measurements [119]. Furthermore, ion traps are shown to provide excellent sensitivities. Ion traps can be produced much cheaper than conventional quadrupole filters, because the design tolerances and the assembly of the quadrupole field generating parts, i.e., the ring and end-cap electrodes in the ion trap as opposed to the hyperbolic rods in a quadrupole filter, are less severe and difficult in ion traps than in quadrupole filters. It can be envisaged that bench-top ion-trap instruments for LC–MS–MS will be commercially available in the near future at a much lower cost than current triple-quadrupole instruments. On-line LC–MS with quadrupole ion traps has been described for thermospray [120], particle-beam [121,122] and electrospray and ionspray interfaces [123–125].

Another area of great interest is the use of time-of-flight (TOF) mass spectrometers. TOF instruments combine a great ease of operation, a relatively low cost, an excellent ion transmission, and a practically unlimited mass range. Perhaps, the only drawback of TOF instruments relative to the other mass analyzers is the limited mass resolution. The interest in TOF instrument for chromatographic applications has recently enlivened [126–128]. Furthermore, a huge effort in development and performance optimization of TOF instruments is stimulated by the introduction of MALDI as a powerful ionization technique for high-mass biomacromolecules [129].

A variety of approaches towards LC–MS on a TOF instrument have been described. Sin et al. [130] describes an instrument for APCI in combination with TOF. Ions generated in the API source enter the high vacuum in an expansion chamber. The molecular beam generated enters perpendicular to the direction of acceleration. Pulsed ion introduction into the flight tube is achieved by an electric pulse applied to the repeller plate opposite to the accelerating grid.

Several groups described electrospray ionization in combination with TOF instruments [131–134]. Initially, on-axis electrospray and TOF-MS was demonstrated [131]. Subsequently, the ions are introduced perpendicular to the TOF axis, again with the use of a pulsed repeller lens [132,133]. A more complex instrument is described by Michael et al. [134]. Ions generated in the electrospray ionization source are focused by means of an Einzel lens into a quadrupole ion trap followed by pulsed ion ejection from the ion trap into the reflectron TOF instrument. The combination of ion-trap and reflectron TOF provides enhances sensitivity and resolution [134].

Considering the potential of MALDI in terms of versatility and sensitivity, on-line LC–MALDI-TOF-MS would be a highly attractive possibility. Given the experiences with the direct introduction of small matrix-containing liquid streams into high-vacuum instruments, as obtained in CF-FAB interfacing, it took surprisingly long before a device for liquid introduction to MALDI was described. A continuous-flow MALDI device was first described by Li et al. [135], based on the frit-FAB interface design. MALDI from aerosols generated by a pneumatic nebulizer was described by Murray and Russell [136,137]. These techniques are still in an early stage of development but, when becoming mature, may further widen the applicability range of LC–MS.

Finally, some attention must be paid to improvements and developments in particle-beam interfacing. A modified pneumatic nebulizer for the introduction of 1–5 μl/min of liquid into a conventional particle-beam interface was described by Cappiello and Bruner [138], providing improvements in the response at high water contents and in the absolute detection limits. Other developments aim at improvements in overall performance by introducing new ionization strategies. In this respect, FAB or (L)SIMS in combination with particle-beam interfacing has been described [139,140], although the detection limits achieved are rather poor.

The implementation of surface ionization in particle-beam LC–MS has been one of the

research topics of the authors of this paper [96,141,142]. Since collisions with hot surfaces in the ion source is an essential step in the performance of the particle-beam interface, we investigate to combine these surface collisions with analyte ionization. In principle, various surface ionization modes can be distinguished, some of which appear to be highly efficient. In a thermoionic emitter, ions are generated out of a surface, i.e., ions from the surface material or from included impurities. This is, for instance, successfully applied in the caesium gun used in LSIMS and in alkali-CI, as recently reviewed by Teesch and Adams [143]. The emission of sodium ions from a heated surface may used in combination with a particle-beam interface to achieve Na^+-CI, as demonstrated in the particle-beam LC–MS analysis of polyethylene glycols [96].

Interaction of neutrals and a heated surface may result in the formation of radical cations when the work function of the surface material exceeds the ionization energy of the neutral. The efficiency of this so-called thermal surface ionization process can further be enhanced by delivering the neutrals to the surface as a highly energetic, i.e., supersonic molecular beam. The latter approach is entitled hyperthermal surface ionization [144] and has been successfully applied in GC–MS analysis. The column effluent is expanded in a two-stage momentum separator prior to collision with the heated surface. Our results, aiming at the application of hyperthermal surface ionization in LC–MS, indicate that during expansion in the momentum separator of the particle-beam interface the molecular beam gains insufficient kinetic energy to provide efficient hyperthermal surface ionization [96]. Subsequently, the modification of the molecular beam expansion chamber led to on-line LC–MS with hyperthermal surface ionization [142]. Further optimization of the setup may lead to a more robust and more generally applicable LC–MS interface system.

The third surface ionization mode observed in our experiments is not yet very well understood. Upon expansion and surface collision of ionic compounds, e.g., quaternary ammonium salts, in the particle-beam interface, intense signals from molecular cations without any fragmentation are observed, while no signal is observed for the corresponding negative counterions. In this case, efficient transfer to the gas phase of preformed ions is achieved. The minimum detectable concentration as low as 100 pg/ml, as obtained in these studies [96,142], compete well with other interface approaches that are more suitable for the ionization of ionic compounds, e.g., electrospray and ionspray.

In conclusion, it can be stated that the field of LC–MS interfacing research is still moving.

10. Mobile phase incompatibility

From the previous sections it may be concluded that LC–MS nowadays is a reliable and robust technique that can be routinely applied in many areas of analytical chemistry and its fields of application. Two of the three problems in LC–MS interfacing, indicated in the introduction, can be considered to be solved. Obviously, further improvements in these areas are possible and likely to be made in the years to come. For one of the three problems, no technical and general solution is available: the problem of incompatibility of mobile phase composition. A routine, long-term use of non-volatile mobile phase constituents, such as phosphate buffers and ion-pairing agents, is prohibited by all current LC–MS interface techniques, although some interfaces, e.g., the APCI system with counter-current drying gas, show a higher tolerance than others. In this respect, current developments in the LC field, e.g., with the introduction of new stationary phase materials, such as mixed-mode, restricted-access and perfusion materials, are promising for future LC–MS developments.

The current state-of-the-art in solving mobile phase incompatibilities can be summarized as follows. The only general solution is a change of the phase system in the LC separation, i.e., by the removal of all non-volatile additives from the mobile phase. However, this solution is dictated purely from the MS point of view and sometimes

extremely difficult to achieve in LC. There are some technological solutions, which may be used to solve the problem. However, all solutions briefly reviewed below are tailor-made solutions, which must be carefully adapted to the conditions and requirements of one's particular application. Very elegant results may be achieved, but only at the cost of significant investment in terms of method development. Obviously, the latter is only worthwhile in developing an analytical procedure to be used for a prolonged period of time and/or for many samples.

On-line continuous-flow liquid–liquid extraction was demonstrated as a possible solution already in the early days of LC–MS developments by the group of Karger and co-workers [145,146]. The approach is later on applied by others [147,148].

A second approach is based on coupled-column and valve-switching techniques, e.g., as demonstrated by Edlund et al. [149]. Subsequently, the coupled-column approach was simplified by Van der Greef and co-workers [150–152] to the phase-system switching technique, which was also implemented by others, e.g., [153].

A third approach is based on the application of micromembrane suppressors, initially developed for conductometric detection in ion chromatography. On-line ion-exchange chromatography MS was demonstrated by Simpson et al. [154] for thermospray, Conboy et al. [155] for ionspray, as well as in combination with particle-beam [156,157]. This approach was subsequently extended to the on-line LC–MS analysis of oligosaccharides using mobile phases containing a sodium acetate concentration as high as 0.4 M [158]. The use of micromembrane suppressors in on-line combination of ion-pair LC and MS has also been described [159].

Finally, the (pseudo)electrochromatography approach [160,161] must be mentioned, in which chromatographic separation is combined with electromigration phenomena. In this way, the use of ion-pairing agents in the analysis of ionic compounds can be avoided. Electromigration methods, such as capillary zone electrophoresis,

are frequently advocated as a powerful alternative for the separation of ionic compounds prior to MS detection. In practice, however, considerable difficulties are experiences in the application of this approach [84].

11. Conclusions

LC–MS has developed into a mature technique, which can be routinely applied in a large number of application areas, e.g., environmental and bioanalysis, analysis of natural products and biochemistry-related applications.

A large number of interfaces for LC–MS have been developed over the past 20 years. At present, the most powerful and promising interfaces are the particle-beam interface, which provides on-line EI mass spectra, and the interfaces that can be used in combination with atmospheric-pressure ionization, i.e., electrospray and a pneumatic nebulizer APCI system. A relatively reliable indicator of future developments in the use of LC–MS interfaces in our experience is the number of contributions with each interface at the Annual Conference on Mass Spectrometry and Allied Topics, organized by the American Society of Mass Spectrometry (ASMS). The total number of contributions, in which particular LC–MS interfaces are used, at the 1994 conference in Chicago was 413, comprising 35% of the total number of contributions. The distribution of these contributions over the various interfaces is shown in a pie chart in Fig. 7A. Obviously, the electrospray interface is responsible for by far the largest part. However, many of the electrospray-related contributions are due to biochemical studies in which no on-line LC–MS is applied; the electrospray interface is only applied as a sample-introduction device. Interestingly, in a growing number of contributions to the 1994 conference various LC–MS interfaces are applied, demonstrating the fact that solving real analytical problems requires a multidimensional approach. The developments in interface use in the past seven years (1988–1994) is depicted in Fig. 7B. Again, the use of electrospray is marked, but also to increase in
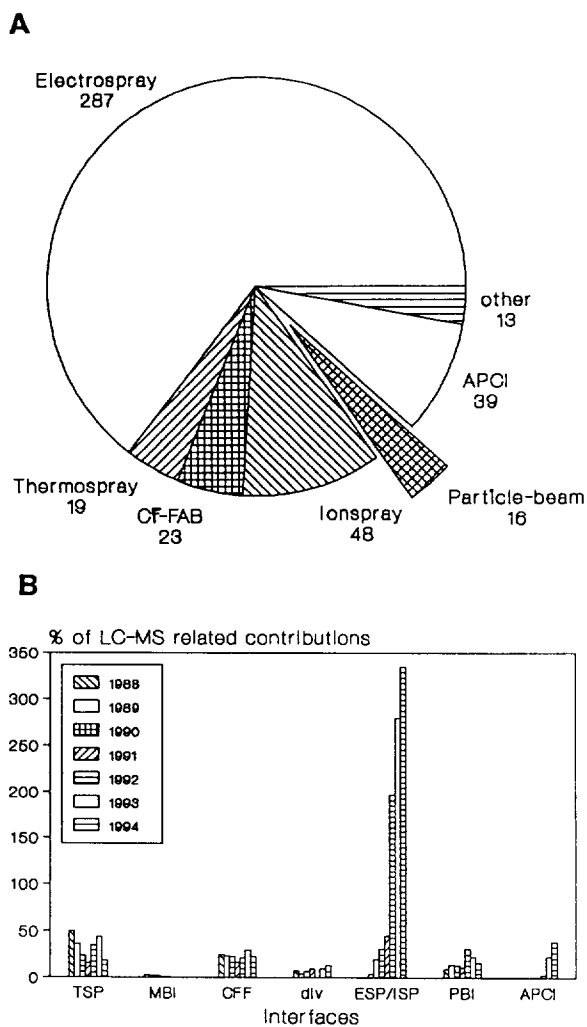
A

B

Fig. 7. LC–MS-related contributions at *Annual ASMS Conferences on Mass Spectrometry and Allied Topics.* (A) Pie chart of the 1994 contributions on various LC–MS interfaces. (B) Bar graph showing a seven-year development in interface use in contributions at ASMS Conferences. TSP = Thermospray; MBI = moving belt interface; CFF = continuous-flow fast-atom bombardment; div = other interfaces; ESP = electrospray; ISP = ionspray; APCI = atmospheric-pressure chemical ionization.

APCI-related papers. No proper explanation can be given for the apparent decrease in thermospray-related contributions.

Interesting future developments are primarily in the combination of known interface strategies with alternative mass analyzers. Especially, the combination of electrospray interfacing and

either TOF or FT-ICR instruments appear to grow in importance. Other new developments, which are still in an early stage, are the on-line LC–MALDI-TOF-MS and the (hyperthermal) surface ionization strategies. Whether these type of approaches will actually become mature and will be used widely, remains to be seen.

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