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Review

Advances in instrumentation in liquid chromatography–mass spectrometry and related liquid-introduction techniques

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Abstract

In the past few years, the instrumental developments in the field of combined liquid chromatography–mass spectrometry (LC–MS) and related liquid-introduction techniques has been extremely fast. Soon after the demonstration of the ability to obtain multiply-charged ions from proteins by electrospray ionization, a major impetus was given to the field. Numerous LC–MS systems based on atmospheric-pressure ionization sources have now been described. This paper reviews these instrumental developments with reference to currently available commercial LC–MS systems. Not only low and high flow-rate electrospray on quadrupole instrument is discussed, but also electrospray on ion-trap, double-focusing sector, time-of-flight, and Fourier-transform ion-cyclotron resonance instruments are reviewed. © 1998 Elsevier Science B.V.

Keywords: Liquid-introduction techniques; Electrospray ionization; Interfaces, LC–MS; Atmospheric pressure ionization; Instrumentation, LC–MS; Reviews

Contents

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1. Introduction

Despite elaborate research efforts in LC–MS interfacing in the past 25 years, it is only in the last few years that LC–MS has become a technique which can be used routinely in a wide variety of analytical laboratories and application areas. Some LC–MS instrumentation is now actually being sold as an integrated detector for liquid chromatography, i.e. to enter the chromatography rather than the mass spectrometry laboratory. This can be attributed to the major progress that has been achieved in the development of reliable and userfriendly instrumentation for LC–MS and related liquid-introduction techniques for MS, which in turn is attributable to the vast advent of atmospheric-pressure ionization (API) devices.

The current API technology in fact comprises of two different interfaces for LC–MS, based on electrospray ionization and atmospheric-pressure chemical ionization (APCI). The history of electrospray as a sample introduction method for MS goes back to the experiments of Dole et al. [1–3] in the late 1960s, while APCI for LC–MS was founded by Horning et al. [4,5] in the early 1970s. However, it was not until the late 1980s that an actual breakthrough in API technology took place. As indicated in more detail below, this breakthrough can be attributed to the demonstrated ability to analyze large proteins on a quadrupole instrument by multiple charging of the proteins in electrospray ionization. Electrospray ionization, and especially its application in characterizing peptides and proteins, has been extensively reviewed in the past few years [6–12]. More general reviews on the application of API technology for LC–MS have also been published [13–15]. Finally, some reviews primarily pay attention to instrumental aspects of API technology [16–18]. The current review also concentrates on instrumental aspects. The confusing period of extremely rapid instrumental developments appears to come to an ease. Therefore, it was considered appropriate to review the current state-of-the-art in API instrumentation.

2. General outline of API instrumentation

A general schematic diagram of an API source is shown in Fig. 1. An API interface/source consists of five parts: (1) the liquid introduction device, (2) the actual atmospheric-pressure ion source region, where the ions are generated by means of electrospray ionization, APCI, or by other means, (3) an ion sampling aperture, (4) an atmospheric-pressure to vacuum interface, and (5) an ion optical system, where the ions are subsequently transported into the mass analyzer. The proper design of the last two parts is of utmost importance, as it determines

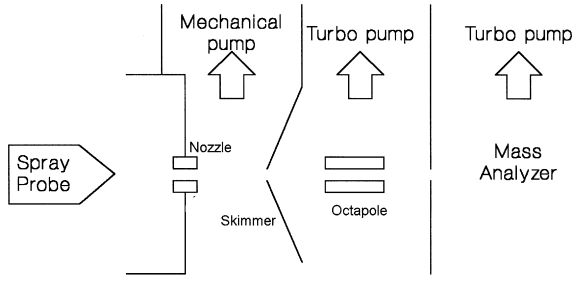

Fig. 1. General schematic diagram of an ion source for atmospheric-pressure ionization.

whether the very high ionization efficiency that can be achieved in API, is actually available for solving analytical problems. Ion losses are inevitable in the transfer region, but must be kept to a minimum.

The operational principle of the most widely applied API interface and ion source design is as follows. The column effluent from the LC (or any other liquid) is nebulized into an atmospheric-pressure ion source region. Nebulization is either performed pneumatically, i.e. in APCI, by means of the action of a strong electrical field, i.e. in electrospray, or by a combination of both, i.e. in ionspray or pneumatically assisted electrospray. Ions are produced from the evaporating droplets, either by gas-phase ion-molecule reactions initiated by electrons from a corona discharge, i.e. in APCI, or by the formation of microdroplets by solvent evaporation and repetitive electrohydrodynamic explosions and the desorption, evaporation or soft desolvation of ions from these droplets into the gas phase. The ions generated, together with solvent vapour and nitrogen bath gas, are sampled by an ion sampling device into a first pumping stage. The mixture of gas, solvent vapour and ions is supersonically expanding into this low-pressure region. The core of the expansion is sampled by a skimmer into a second pumping stage, containing an ion focusing and transfer device to optimally transport the ions in a suitable manner to the mass analyzer. From the vacuum point-of-view, it is not important whether a high flow-rate or a low flow-rate of liquid is nebulized, because the sampling orifice actually acts as the restrictor between the atmospheric-pressure region and the first pumping stage. From the ionization point-of-view, it is also not important whether the ions are generated by electrospray or APCI, although (slightly) different tuning of voltages in the ion optics might be needed due to some differences in the ion kinetic energies.

A major difference between the various designs is related to the number of pumping stages available. As discussed by Bruins [16] and by Niessen and Tinke [18], the possible pumping system designs are: (1) a one-stage pumping system with a high-efficiency (cryogenic) pump, (2) a two-stage differentially pumped system with an ion transfer/optics region and a mass analyzer region, both evacuated by (separate) high-vacuum pumps, (3) a two-stage differentially pumped system consisting of a molecular-beam stage pumped by a rotary pump, and a mass analyzer region pumped by a high-vacuum pump, (4) a three-stage differentially pumped system consisting of a molecular-beam stage, pumped by a rotary pump, an ion optics region and a mass analyzer region, both pumped by turbomolecular pumps. These possibilities are drawn schematically in Fig. 2.

It must be emphasized, that API technology is not only used for LC–MS applications. Unlike APCI, which is primarily used in LC–MS applications, electrospray interfacing is also applied for coupling capillary electrophoresis (CE) and MS and, more importantly, in a continuous sample infusion mode in order to characterize biomacromolecules. In fact, it can be estimated that in less than 20% of the electrospray-related papers on-line separation by either LC or CE is applied. In discussing the instrumentation, hardly any discrimination is made as to whether the device described is applied in combination with LC or CE or not. In principle, a system developed for continuous infusion can be used in LC–MS, and vice versa. Furthermore, most of the developments in API were actually performed

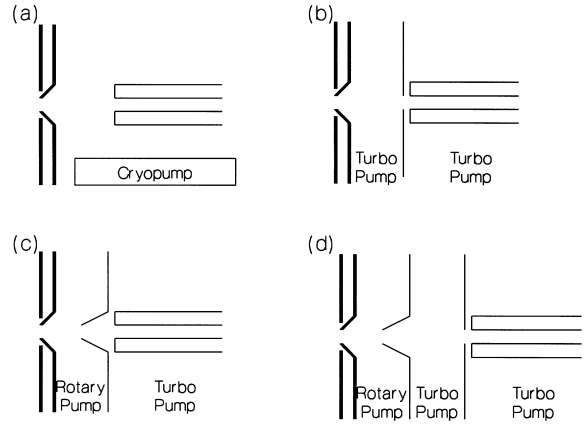

Fig. 2. Various vacuum systems for atmospheric-pressure ionization sources: (a) a one-stage pumping system with a high-efficiency (cryogenic) pump, (b) a two-stage differentially pumped system with an ion transfer/optics region and a mass analyzer region, both evacuated by (separate) high-vacuum pumps, (c) a two-stage differentially pumped system consisting of a molecular-beam stage pumped by a rotary pump, and a mass analyzer region pumped by a high-vacuum pump, (d) a three-stage differentially pumped system consisting of a molecular-beam stage, pumped by a rotary pump, an ion optics region and a mass analyzer region, both pumped by turbomolecular pumps.

for electrospray. However, all the devices described should also be applicable in combination with an APCI sample introduction device, provided a corona discharge needle is installed.

3. The impact of electrospray ionisation

3.1. Initial efforts on API sources

API sources for mass spectrometry were first described by Knewstubb and Sugden [19,20] in 1958. Subsequently, the work of the research group of Horning and Carroll [21,22] in the late 1960s resulted in a commercially available system (from Franklin GNO Corp.). This research led in 1974 to an atmospheric-pressure corona discharge ion source for LC–MS [4,5]. In this system, the LC effluent is introduced into a heated (250°C) glass evaporator via an inlet capillary by means of preheated gas. A corona discharge needle with a sharpened point is positioned approximately 3 mm in front of the 25 μm I.D. sampling aperture to the high vacuum of the mass spectrometer with a single-stage pumping system. Alternatively, electrons emitted by a ^{63}Ni source are used for primary ionization.

Since that time, several other API instruments were described. The most successful commercial instrument was the TAGA (trace atmospheric gas analysis) spectrometer, built by Sciex in Canada [23]. An important feature of this system is the use of a 20 m^3/s cryogenic pumping system, which allowed the use of a 100–200 μm I.D. sampling aperture. Other API instruments have been available from Extranuclear [24] and Hitachi [25]. The latter system contains a differentially pumped region between a 100 μm I.D. and a 200 μm I.D. aperture. An electric field is applied in this region to achieve collision-induced dissociation of the ions.

3.2. Development of API sources for LC–MS

In 1982, Henion et al. [26] demonstrated the analysis of a series of sulfa drugs using a direct liquid introduction interface and a Sciex TAGA API instrument. In those years, Yamashita and Fenn [27,28] tried in vain to reproduce the results of Dole et al. [1–3]. Their work resulted in an electrospray LC–MS interface, described by Whitehouse et al. [29] in 1985. In 1986, Covey et al. [30] demonstrated high-speed LC–MS–MS with a heated nebulizer APCI interface using the Sciex TAGA. And one year later, Bruins et al. [31] reported the ionspray (pneumatically-assisted electrospray) interface, which largely solved a major limitation of the Whitehouse electrospray interface, as it permitted flow-rates up to 200 μl/min instead of 10 μl/min. In 1988, a major and apparently sufficiently convincing impetus was given to the API field by Fenn et al. [32], who demonstrated at that year's ASMS Conference on Mass Spectrometry and Allied Topics, the possibility to achieve multiple charging of proteins by electrospray ionization. This observation widely opened a highly important and almost completely new application area for mass spectrometry, i.e. related to the characterization of biomacromolecules by MS. While in former years, the analysis of proteins by MS was a specialized area for either fast-atom bombardment (FAB) ionization on expensive sector instruments or not widely available ^{252}Californium plasma desorption time-of-flight instruments, electrospray ionization brought the analysis of proteins within the reach of a simple quadrupole instrument. Furthermore, the sensitivity and ease-of-operation in electrospray is considerably better than in FAB. Obviously, a further impetus to the protein field was also given by the discovery of matrix-assisted laser desorption/ionization (MALDI) by Hillenkamp and Karas [33].

It is this observation which led to the production and commercialization of electrospray ionization devices by all major MS instrument manufacturers as well as to the widespread installation of these instruments. APCI is often delivered as a supplemental and to some extent complementary tool.

The importance and significance of electrospray ionization for the LC–MS field can be deduced from the bar graph in Fig. 3, where the annual number of contributions per interface at the annual ASMS Conference on Mass Spectrometry and Allied Topics is plotted as a function of the year. The increase, starting in 1992, in the number of contributions where electrospray ionization is used, is readily noticeable. Only ca. 20% of these contributions deal with on-line coupling of LC or capillary electrophoresis (CE). Most of the research effort is put into

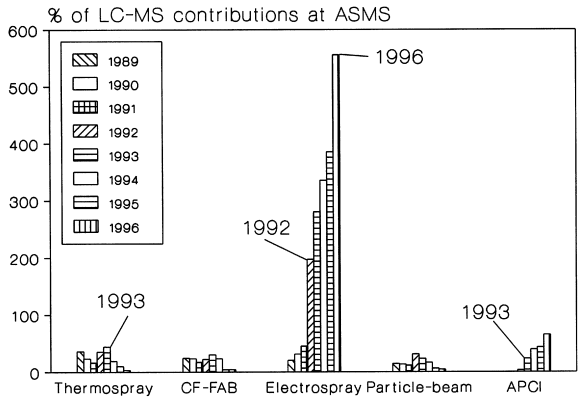

Fig. 3. Annual number of contributions per interface at the ASMS Conference on Mass Spectrometry and Allied Topics.

the characterization of peptides, proteins, nucleotides and other bio(macro)molecules, which are introduced by constant infusion, after off-line isolation and sample preparation.

3.3. Impact of electrospray in biochemical applications

The impact of electrospray in biochemical applications is difficult to review, as over 300 papers per annum appear in the refereed literature. Further progress in this field is achieved by the vast amount of papers, where matrix-assisted laser desorption/ionization (MALDI) is applied. The developments in this area can be considered as a continuous exploration of the potential of electrospray. Some landmarks are indicated below, although it is impossible to properly give credit to all individual researchers who have made contributions.

The research group of Fenn first demonstrated the ability to perform accurate determination (generally within 0.1%) of the molecular mass of proteins up to 30 kDa [34]. Because of the low sample consumption, the excellent sensitivity, and the ease of operation, this method was rapidly implemented as a tool for biochemistry and biotechnology [9–12]. New software tools were developed to rapidly deconvolute the mass spectra containing peaks due to the multiply-charged ions and to calculate the protein molecular mass [32,34,35]. Specialized software tools, for instance based on maximum entropy algorithms, were described [36,37]. These software tools allow the deconvolution of multiply-charged ion envelopes resulting from mixtures of proteins, e.g., mixtures of various haemoglobin variants [36–38]. The upper mass range of proteins for which electrospray was applicable was shifted to ca. 200 kDa, e.g., in the work of Feng and Konishi [39,40].

When determination of molecular mass is readily available, the question of primary structure arises. In a recombinant protein, for instance, the accurately determined molecular mass clearly indicates possible variations in the protein primary sequence. However, finding the modified amino acid is often more demanding. Using a combination of enzymatic digestion and electrospray MS or LC–electrospray-MS, peptide mapping can be performed, giving a series of peaks related to various peptide fragments resulting from the enzymatic cleavage. When such an approach is combined with MS–MS, sequencing of these peptides is possible as well, as was already demonstrated with FAB [41]. As the peptide sequencing based on collision-induced dissociation (CID) in principle is not hindered by the presence of (posttranslational) modifications of amino acids, its applicability apparently is wider than that of conventional Edman degradation. MS–MS often allows the identification of the modified amino acids as well. While posttranslational modification in itself is readily detected by determining the molecular mass of the protein, MS–MS can be used to determine the nature and position of the modification. This approach is nowadays routinely and widely applied in protein sequencing. Software tools have been developed to rapidly interpret the complex product-ion mass spectra of (often doubly-charged) peptides. The combination of partial sequence information from electrospray MS–MS and large protein sequence databases leads to highly effective tools in determining protein primary structures. While some years ago, elucidating the primary structure of a protein was a project of years, the time span is now sometimes reduced to weeks. The most time-consuming step is still the isolation of sufficient amounts of protein from the biological system.

Next to an approach based on enzymatic cleavage of the protein and amino-acid sequencing of the resulting peptides by MS–MS, an alternative approach was also investigated. Given the multiply-charged protein peaks in the mass spectra, much

higher effective collision energies can be achieved in CID of the intact proteins. The MS–MS analysis of multiply-charged proteins was pioneered by Smith et al. [42,43]. However, the interpretation of the MS–MS product-ion mass spectra acquired on triple-quadrupole instruments is greatly hampered by the lack of knowledge on the charge state of the product ions. This in turn led to the implementation of electrospray on instruments capable of efficient CID in combination with high-resolution measurement, i.e. Fourier-transform ion-cyclotron resonance instruments [44].

The ability to determine molecular masses of proteins and the need to often optimize (by trial and error or in a systematic way) the solvent composition for the electrospray MS analysis, resulted in the observation of shifts in the ion-envelopes. These effects were attributed to changes in the protein conformation and subsequently investigated more systematically. It is often not easy to correlate the observed changes in the mass spectra to three-dimensional conformational changes of the protein, especially because little is known about protein conformation in the isolated state, i.e. the gas phase. Nevertheless, significant progress is made in this field.

While some protein conformation aspects are related to covalent bonds, e.g., S–S bridges, most conformation is due to much weaker noncovalent interactions. This in turn led to investigations aiming at the observation of noncovalent protein complexes, e.g., biologically active multimeric structures, proteins with noncovalently bound cofactors, but also enzyme–substrate interaction, drug–protein interaction, peptide–nucleotide interaction and antigen–antibody interactions. This fascinating field certainly is not at the end of its development (it might even be used to find the most strongly binding and/or most effective drug of a combinatorial drug library [45]). As such, the introduction of commercially available electrospray devices in the early 1990s initiated a rapidly developing research, which currently developed from rapid molecular-mass determination via primary sequence elucidation to investigations into the quaternary structure of proteins.

Next to these developments in the biochemical and biotechnological applications of MS, electrospray and APCI LC–MS significantly contributed to the routine and robust application of LC–MS in, for instance, various (quantitative) pharmaceutical and environmental areas. Nowadays, interfaces based on electrospray ionization or APCI are the LC–MS interface techniques of choice to such an extent that hardly any other interfaces are used.

4. Development of atmospheric-pressure ion sources

In this section, the various API source designs are described, which led to the current commercially available API sources. Additionally, some other laboratory-built devices and more recent modifications are discussed. API sources, based on the features of these API source designs, are now available from all major MS instrument manufacturers. An overview of the various commercial systems, evaluating their possibilities and limitations, has been published by Voress [17] in 1994.

4.1. Fenn–Whitehouse molecular-beam source

A schematic diagram of the electrospray interface for LC–MS, as developed by Whitehouse et al. [29], is shown in Fig. 4. Sample solutions enter the spray chamber through a 100 μm I.D. stainless-steel hypodermic needle at a flow-rate of 5–20 μl/min. The needle is kept at ground potential, while for positive-ion detection the cylindrical electrode is set at a potential of −3.5 kV. The API source is sampled

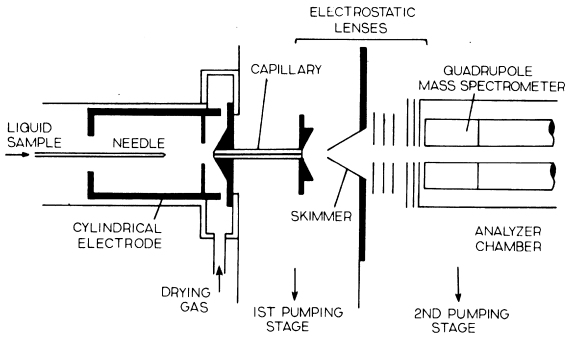

Fig. 4. Schematic diagram of the first electrospray interface for LC–MS (from Ref. [29], ©1985, American Chemical Society).

by a glass capillary with an internal diameter of 0.5 mm. The metallized inlet and outlet ends of the glass capillary are set at −4.5 kV and +40 V, respectively. The liquid is electrosprayed from the tip of the hypodermic needle and the droplets formed are further dispersed by means of a countercurrent stream of heated nitrogen gas with a flow-rate of ca. 150 ml/min. The solvent vapour from the rapidly evaporating droplets is swept away by the bath gas, while the ions that come near the inlet of the glass capillary, are entrained in dry bath gas and transported into the first vacuum chamber, forming a supersonic molecular beam. Thus, the outlet of the sampling capillary can be considered as a nozzle. The first vacuum chamber is evacuated down to ca. 0.05 Pa by means of a 1 m^3/s diffusion pump. The core of the supersonic jet is sampled by a 2 mm I.D. skimmer, kept at −20 V, and transported into the quadrupole analyzer region for mass analysis [24]. The major drawback of the electrospray interface for application in LC–MS is that the maximum allowable flow-rate is limited to ca. 20 μl/min.

This source design has subsequently been commercialized by Analytica of Branford and made available for retrofitting on existing instruments from various instrument manufacturers, e.g., Finnigan MAT, Hewlett Packard, Nermag, and Jeol. Collaborating with the group of Fenn, Green of VG (Micromass) also built an electrospray ion source, based on the Fenn–Whitehouse design [46]. These systems have been implemented on their successful BioQ instruments, and later on other commercial products from VG/Micromass as well. These systems mainly differ from the Analytica of Branford systems in the geometry of the ion sampling aperture (see below).

4.2. Bruins–Sciex ionspray source

The ionspray interface, developed by Bruins et al. [31] in 1987, was originally introduced in order to combine the principles of ion evaporation, as demonstrated by Iribarne and Thomson [47–50], and electrospray. Meanwhile, however, it became clear that the ionization mechanisms of both approaches are similar. The ion-evaporation mechanism appears to be effective irrespective of the nebulization method as long as droplets of typically 1 μm (or smaller) are produced. The main advantage of the ionspray or pneumatically-assisted electrospray interface over the conventional electrospray interface is the higher liquid flow-rates, that can be accommodated by ionspray, i.e. up to 200 μl/min. This is possible as a result of the combined action of electrospray and pneumatic nebulization.

A schematic diagram of the ionspray interface is shown in Fig. 5. The ionspray needle, described in more detail below, is positioned 5–10 mm off-axis of the sampling orifice in a 4-l ion source (120 mm×200 mm I.D.). As a result of the 20 000 l/s pumping efficiency of the cryogenic pump in the analyzer region, a 100 μm I.D. sampling orifice can be used without additional pumping stages. A nitrogen curtain gas flows around the orifice in order to prevent clogging of the orifice by nonvolatile material, to assist as a countercurrent gas in droplet

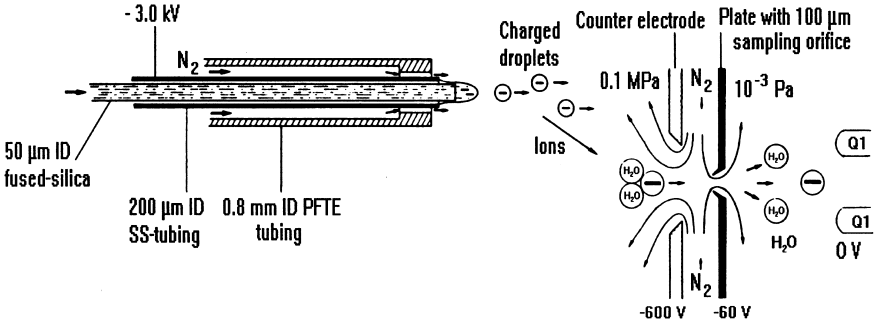

Fig. 5. Schematic diagram of the ionspray interface (from Ref. [31], ©1987, American Chemical Society).

evaporation, and to decluster the ion–solvent clusters by droplet–gas collisions. Unlike all other API source designs, this Sciex system is a single-stage pumping system.

This source design in combination with the ion-spray and heated-nebulizer APCI interfaces is commercially available in API-III from Perkin–Elmer Sciex. It is widely used in LC–MS, especially in pharmaceutical applications. Recently, Perkin–Elmer Sciex introduced a new series of instruments, i.e. API 100/300, where the cryogenic pump is replaced by turbomolecular pumps. In these instruments, a three-stage pumping system is applied.

4.3. Smith electrospray CE–MS source

The Smith electrospray interface is developed for the coupling of CE and MS [51–53]. In principle, the system should be readily applicable to LC–MS operation as well, although at limited flow-rate. A schematic diagram of the system is shown in Fig. 6. The API source is somewhat based on the Sciex TAGA source design in that a hot 2.5 l/min nitrogen curtain gas is used to clean the sampling aperture or nozzle. However, the instrument contains three differential pumping stages, i.e. a mechanically pumped region between the 0.5 mm I.D. nozzle and the 1.2 mm I.D. skimmer (100 Pa), a high-vacuum region containing an RF only quadrupole, and the quadrupole analyzer region.

This system has not been commercialized, although the needle design, i.e. the concept of concentric tubes for analyte and sheath flows, is widely used in many commercial instruments (see below). The recently introduced API 100/300 from Perkin–Elmer Sciex contains a three-stage pumping system, thus closely resembling the design of Smith.

4.4. Chait heated-capillary source

A modified API source for a quadrupole MS was described by the group of Chait [54]. The system is based on the Fenn source design. The main difference is that the transport and desolvation of the ion–solvent clusters is affected by a 203 mm×0.5 mm I.D. heated stainless-steel transfer capillary. Flow-rates in the range of 0.5–2 μl/min are applied. The vacuum system is a three-stage vacuum system. This system has been commercialized by Finnigan MAT and can be used for both electrospray and APCI.

A 0.5 mm I.D. heated ion sampling capillary is also used in the API system described by Duffin et al. [55]. It is part of a laboratory-built interface to perform API on a Hewlett–Packard mass-selective detector benchtop mass spectrometer. A modified

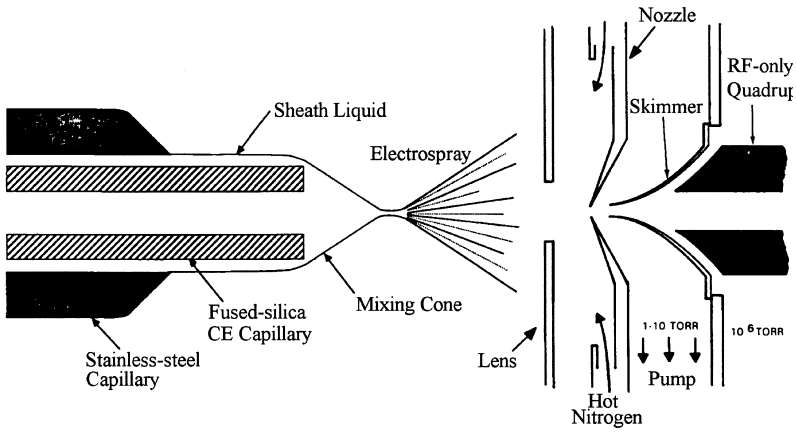

Fig. 6. Schematic diagram of the coaxial sheath-flow electrospray interface for capillary electrophoresis (adapted from Ref. [53], ©1988, American Chemical Society).

version of this system, featuring three-stage differential pumping, is described by Basic et al. [56].

4.5. Hitachi API source

A variety of API sources have been described by Kambara and Sakairi [57–59] of Hitachi. They are primarily used in APCI as well as with other sample introduction methods such as atmospheric-pressure spray [58–60] and sonic spray [59,61,62]. The system is three-stage differentially pumped. A drift voltage is applied to the intermediate region between two skimmer-shaped electrodes in order to improve the ion transmission efficiency and to dissociate cluster ions. The system is described in more detail elsewhere [59].

4.6. Vestec heated API source

The electrospray API system, designed and sold by Vestec, differs from the other systems, because no countercurrent or curtain gas is used [63,64]. A schematic diagram of the system is shown in Fig. 7.

The electrospray-generated droplets are transported through a nozzle by ambient gas. The system consists of a three-stage differentially pumped system, containing a 0.4 mm I.D. nozzle, a 0.6 mm I.D. skimmer and a 1.0 mm I.D. collimator. It can be considered as a retrofit to the Vestec model 201 instrument, manufactured for thermospray. The skimmer is mounted onto the heated ion source block (temperature typically 200–250°C). The ions transported through the collimator are focused by means of an Einzel lens into the quadrupole mass analyzer [63,64].

4.7. Hewlett–Packard orthogonal-sprayer source

In all API sources described sofar, the spray device is in axial position, or only slightly off-axis, relative to the sampling orifice or capillary. Hiraoka et al. [65] recently described an electrospray ion source, where the sprayer is orthogonally positioned relative to the sampling orifice. This design allows higher flow-rates to be used.

An API system containing an orthogonally-positioned spray device has recently been introduced by Hewlett–Packard [66]. A schematic diagram of the system is shown in Fig. 8. The system can be used to both electrospray and APCI. The orthogonal sprayer position significantly reduces the contamination of the sampling orifice. It can be used for high flow-rate electrospray operation. An important additional feature of the system is that the control, data acquisition and data handling in LC–MS operation is fully integrated in the software of the Hewlett–Packard LC and UV diode-array detector. As such, the LC–MS system can be considered as a powerful additional detector for LC, the operation of which does not significantly differ from UV detection.

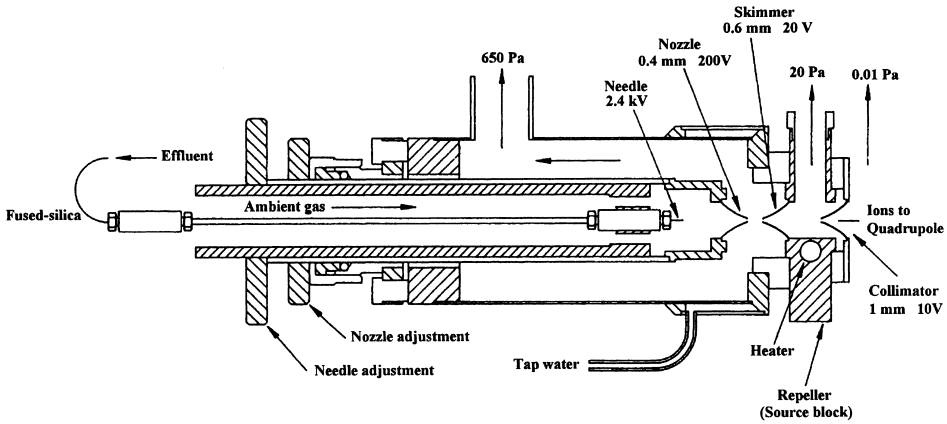

Fig. 7. Schematic diagram of the Vestec electrospray source (from Ref. [63], ©1992, Elsevier Science).

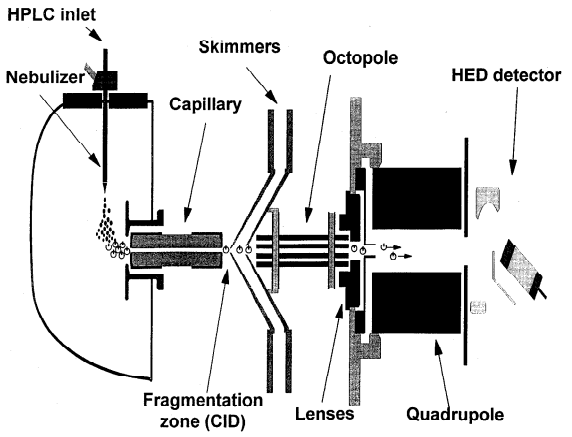

Fig. 8. Schematic diagram of the Hewlett–Packard orthogonal electrospray system (Source: Hewlett–Packard).

4.8. Other electrospray ion sources

Next to the systems described above, several laboratory-built API systems or modifications have been reported.

For studies on fundamental aspects of electrospray ionization, Hiraoka and Kudaka [67] described a laboratory-built electrospray ion source. A combined electrospray-LSIMS source was described by Papac et al. [68] allowing the use of both ionization methods without the need to reconfigure the instrument. A probe-housed electrospray source, which fits into a standard 13-mm vacuum lock, was described by Sproch and Kruger [69]. The system was used in combination with a standard EI/CI lens assembly.

API sources to be fitted onto thermospray ion sources have also been described. While the API source described by Jackett and Moini [70] is positioned axial to the quadrupole analyzer, the system described by Van der Hoeven et al. [71,72] is positioned orthogonally to the quadrupole mass analyzer, thus replacing the thermospray vaporizer probe. A heated transfer capillary is applied in the latter design [71,72].

Various API source modifications have been described to study gas-phase reactions of multiply-charged ions. Thermally-induced dissociation of multiply-charged ions in a 100 mm×0.5 mm I.D. resistively-heated stainless-steel capillary was studied by Rockwood et al. [73]. A capillary interface/reactor to be positioned between the electrospray needle and the ion sampling aperture has been described by the group of Smith [74,75]. The system is used to study gas-phase proton transfer reactions between water [74] or diethylamine [75] and electrospray-generated multiply-charged protein ions. A Y-shaped capillary interface/reactor was also described [76]. The systems allows investigations of ion–ion reactions. Gas-phase ions are produced at the two spatially separated ion sources by means of electrospray or APCI, introduced into the system, and finally mixed in the reaction chamber. The reaction products are subsequently analyzed by MS. Reaction between positive and negative ions as well as between positive and positive ions have been studied using this device [76].

An ion source where polyprotonated proteins generated by electrospray ionization could be subjected to ion–molecule reactions in the intermediate-pressure region, was described by Ikonomou and Kebarle [77].

4.9. Commercial API sources for quadrupole instruments

Three-stage differentially pumped vacuum systems are nowadays applied in most instruments. Table 1 contains an overview of some features of the most widely used API systems. Some features are briefly discussed below.

Various designs of the sampling apertures have been described, e.g., capillary restrictors or (conical) orifices. The size of the ion sampling aperture depends on the pumping capacity of the vacuum system and the design of the sampling device. Generally, ca. 100 μm I.D. sampling apertures are used.

The API sources available from Micromass initially contained a so-called ‘‘pepperpot’’ device prior to the ion sampling orifice for ion sampling and ion declustering [78]. The pepperpot lens allows for sampling of the ions from the spray plume, while desolvation is promoted and nonvolatile material is trapped in order to reduce contamination of the sampling cone. Subsequently, a modified device was introduced, the crossflow device. This ion-source insert improves the tolerance to nonvolatile material in the mobile phase or sample. The crossflow device

Table 1
Some features of the most widely-used commercially available API sources.

	High-pressure side	Sampling aperture	Intermediate region	Ion optics
Finnigan MAT		Heated stainless-steel capillary	Tube lens In-source CID	Octapole
Hewlett– Packard	Orthogonal sprayer	Dielectric platinum Plated capillary	In-source CID	Octapole
Micromass	Pepperpot, crossflow or Z-spray	Conical orifice	In-source CID	Hexapole
Perkin–Elmer Sciex	Droplet shield	Conical orifice	In-source CID	Quadrupole

(see Fig. 9a) contains a deep well, where the nonvolatile material is collected. The periphery of the spray plume is sampled and blown across the sampling cone, i.e. orthogonal to the ion-optical direction. In the latest modification of this device, the so-called Z-spray (see Fig. 9b), ions are orthogonally extracted from the API source into a pumped chamber, and subsequently drawn into the high-vacuum chamber for mass analysis. The latter system showed greater versatility than the pepperpot and better sensitivity than the crossflow. This system

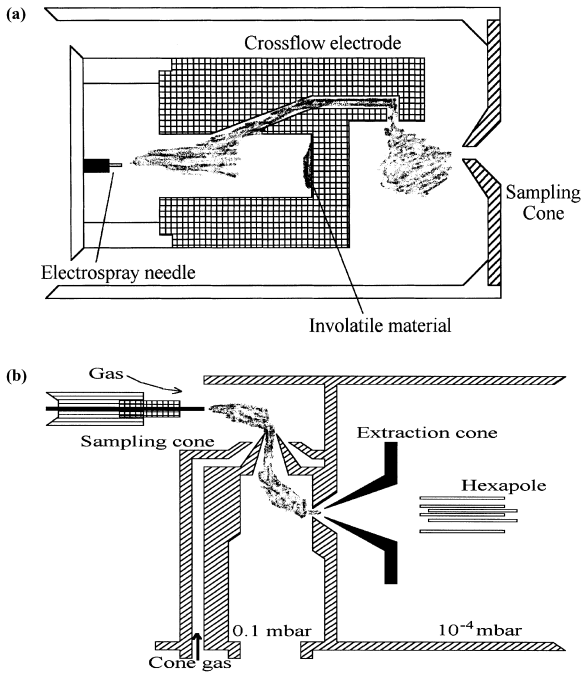

Fig. 9. Schematic diagram of the Micromass (a) crossflow, and (b) Z-spray electrospray source.

should allow the use of phosphate buffers in the mobile phase. Since these developments are very recent, proper evaluation of the performance and claims is not yet possible.

For high flow-rate operation with ionspray (see below), the use of a conically shaped liquid droplet shield in front of the ion sampling orifice of a Sciex API-III was described by Hopfgartner et al. [79,80]. Such a device is implemented in the sources available on the API-100/300 series from Perkin–Elmer Sciex.

Basically, two types of ion sampling devices are used in the API source, i.e. a glass or stainless-steel capillary or a (conical) orifice. An important advantage of the glass capillary with metallized ends is that it electrically insulates the atmospheric-pressure ion source from the ion optical device. The latter may be at high voltages, e.g., in a magnetic sector instrument (see below). The gas flow through the capillary drags the ions towards the vacuum system, even against an electric field along the capillary. It must be realized that in positive-ion mode a potential of -3 kV is applied at the entrance end of the glass capillary and typically $+40$ V at the exit end. As a result of the transport of ions over a longer distance, the desolvation of ions is assisted. The latter is more efficient if a heated capillary is applied. The transport of ions by means of a viscous gas flow through a capillary sampling device has been fundamentally studied by Lin and Sunner [81]. It is concluded that significant ion losses at the capillary walls occur, initially due to space–charge expansion, and later due to diffusion. This may result in discrimination against low mass ions.

Except in the source with the heated transfer capillary and in the heated API source, a countercur-

rent heated nitrogen flow is used to assist in droplet evaporation. In the case of the Perkin–Elmer Sciex API source, the nitrogen curtain gas flow is assisting in the prevention of aperture clogging as well.

An important part of the instrumentation is the ion optical device, which should transfer as many ions entering the intermediate pressure region though the nozzle as possible towards the mass analyzer. In an API source available from Finnigan MAT a so-called ''tube'' lens is positioned at the outlet side of the capillary in order to focus the ions towards the skimmer [82].

For the transfer of ions from the intermediate-pressure region to the mass analyzer, a variety of ion optical devices have been described, e.g., (1) a series of three flat lenses, as commonly used in EI/CI sources, (2) a series of three lenses where the first lens is conically shaped, while the other two are flat, (3) RF-only quadrupole prefilters, and (4) RF-only hexapole or octapole ion guide devices. RF-only multipole devices are used in most commercial systems, nowadays (cf. Table 1).

5. In-source CID

The intermediate-pressure region of the ion source has two main functions, i.e. to facilitate the analyte transition from atmospheric pressure to high vacuum, and decluster solvated ions by ion–molecule collisions. The efficiency of the ion–molecule collisions can be enhanced by applying a small potential difference between the two ion sampling plates or ion–optical elements, i.e. between the nozzle and the skimmer or between the skimmer and an octapole ion guide. This effect has already been studied in early API source designs [58,83].

It was demonstrated by the group of Smith [84–87] that by further increasing the potential difference between the nozzle and the skimmer the excess internal energy that is gained by the ions can be further increased and that fragmentation of the multiply-charged ions may be induced. The fragmentation is due to collisional activation of the ions and can be considered as collision-induced dissociation (CID). These effects are denoted as in-source CID in this text.

Initially, it was observed that the charge distribution in the ion envelope of a multiply-charged protein shifts to the more highly-charged ions, if the potential difference is decreased [84]. These results indicate that the multiply-charged ions may be susceptible to collisional activation. In subsequent papers [85–87], it was demonstrated that the internal energy of the multiply-charged ions can be manipulated by means of the potential difference between nozzle and skimmer. The mass spectra of mellitin, shown in Fig. 10, were obtained with low and high potential difference. Considerable structural information can be obtained at the high potential difference between nozzle and skimmer. Obviously, the interpretation of such spectra may be difficult for unknowns, because the various fragment ions may have different charge states. Subsequently, the fragment ions generated by in-source CID were compared to the fragment ions generated by means of CID in the collision cell of a triple-quadrupole instrument [86,87]. Similar effects have been described by others, using other experimental setups [88,89].

Subsequently, Voyksner and Pack [90] as well as several others [91–93] demonstrated that in-source CID can also be achieved for small molecules and may be used in structure elucidation. They found that mass spectra for compounds like aldicarb, propoxur, carbofuran and cloxacillin obtained with a 30–50-V potential difference between nozzle and skimmer closely resemble those obtained in conventional CID at 30-eV collision energy. Good product ion yields were achieved with minimum losses in the overall ion current. Obviously, the abundance of the intact protonated molecule decreases upon in-source CID.

A nice application of in-source CID is described by Bitsch et al. [93] in the structural analysis of taxol-related compounds. Taxoid side chain fragments were generated by means of in-source CID. The fragment ions were further structurally characterized by means of CID in the collision cell of a triple-quadrupole instrument.

This approach of two-step fragmentation for structure elucidation is also applied by others. As an example the in-source CID and the combined in-source CID and MS–MS spectra of the adduct of malondialdehyde and 2-deoxyguanosine are shown in Fig. 11 [94]. In MS–MS, the adduct fragments with the loss of the deoxyribose group, which thus

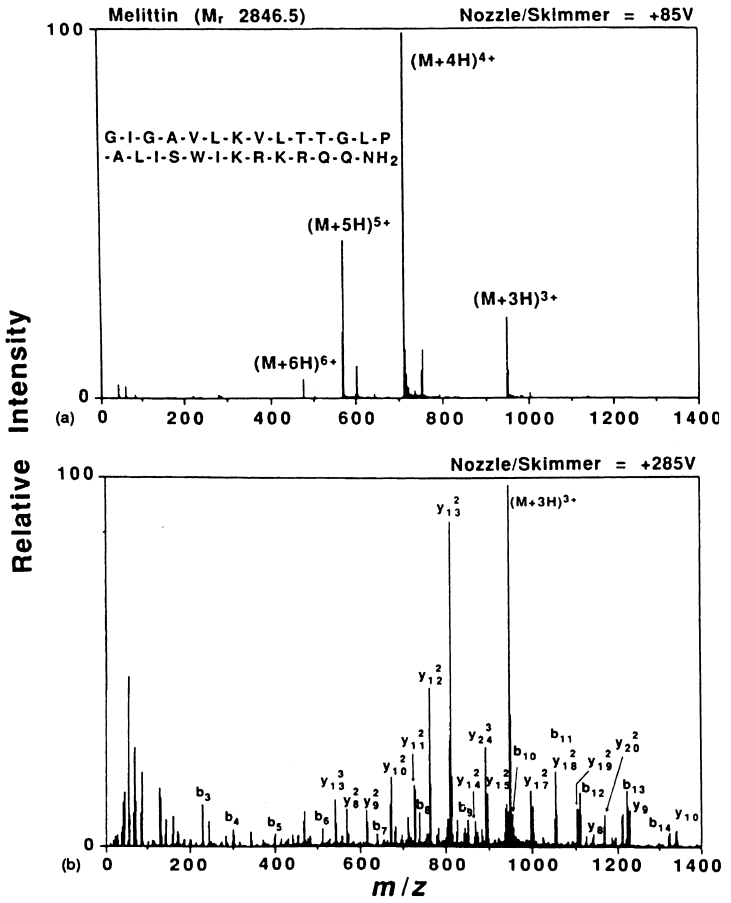

Fig. 10. Electrospray mass spectra of mellitin at 85 V (upper) and 285 V (lower) nozzle–skimmer potential difference (from Ref. [85], ©1990, Elsevier Science).

does not provide information on the structure of the adduct, except that the modification did not take place at the ribose. The same type of fragmentation is observed in in-source CID. The fragment at m/z 188 due to the protonated base $[BH_2]^+$ is then fragmented in CID in MS–MS, which allows structure elucidation of the modified base [94].

While in most systems the in-source CID is performed in the region between nozzle and skimmer, it can also be performed between the skimmer and (one of) the octapoles. The advantage of this is an improved collection and refocusing of the product-ions generated.

Fragmentation by in-source CID is sometimes denoted as poor-men's MS–MS. Although in-source CID certainly is a useful technique, especially when used in combination with pure compounds or highly efficient separation methods, the method also has distinct limitations. By means of in-source CID all ions present are subjected to CID. In CID in the collision cell of an MS–MS instrument, on the other hand, a selection of a particular precursor ion m/z ratio is performed first, thus leading to significant improvements in signal-to-noise ratios, which are not achievable by in-source CID.

Little systematic investigations were reported on the comparison of in-source CID and CID in MS–MS, especially with respect to the type of fragmentation and energetics involved. Most authors simply state that the fragmentation observed in in-source CID is similar (or identical) to that obtained in MS–MS, although significant differences in relative

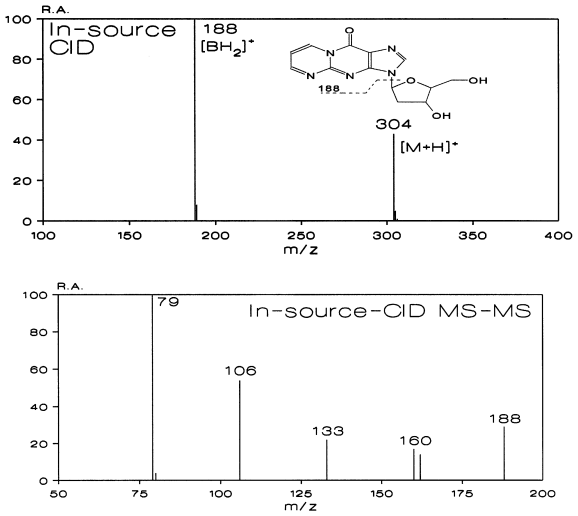

Fig. 11. In-source CID electrospray mass spectrum of the adduct of malondialdehyde and guanosine and product-ion mass spectrum of m/z 188, selected after in-source CID.

abundance of the various fragment ions are observed as well. Obviously, because of the selection of the target ion, i.e. the precursor ion, in MS–MS, the CID process can be performed in a more controlled way. In in-source CID, the collision activation applies to all ions and charged complexes and clusters, which depending on the experimental conditions may lead to differences in the internal energy uptake by the analyte ions.

6. Electrospray needle designs

In the first electrospray systems, a stainless-steel hypodermic needle is used for sample introduction [27–29]. In most systems, 100–200 μm I.D. capillaries are used for sample introduction. The system is restricted to liquid flow-rates in the range of 1–10 μl/min. Subsequently, modified sample introduction capillaries have been described. The needle modifications aim at optimization of the flow-rate introduced and at improving the versatility of the system, especially with respect to solvent composition. Remarkably, the developments with respect to sample flow-rate direct both towards needle designs, which allow a further reduction of the flow-rate introduced, and towards needle designs which allow higher flow-rates to be introduced. While the former developments are important in protein characterization, where the sample amount is generally limited, the latter developments are especially important with respect to LC–MS.

6.1. Ionspray needles

The first needle design modification described is the ionspray or pneumatically assisted electrospray interface [31], commercially available from Perkin–Elmer Sciex. A schematic diagram of the ionspray interface is shown in Fig. 5. The column effluent flows through a 50 μm I.D. fused-silica (or stainless-steel) capillary, which tightly fits inside a 200 μm I.D. stainless-steel capillary, which is kept at ±3 kV, depending on the polarity of ionization. A 0.8 mm I.D. PFTE tube with a narrower PFTE insert at the tip surrounds the stainless-steel capillary; nitrogen gas flows through the PFTE tube. The dimensions of the PFTE insert are chosen to provide for linear gas velocities exceeding 200 m/s at the tip, which are needed for successful pneumatic nebulization. The relative position of the three concentric tubes must be adjusted to give a fine symmetric spray plume. In practice, the fused-silica capillary protrudes from the stainless-steel capillary, which again protrudes from the PFTE tube [31]. Obviously, the commercial ionspray needles have a slightly different design.

A modification of this type of needle is described by Siu et al. [95]. A 50 μm I.D. fused-silica sample introduction capillary is connected in a low dead volume connection coupled to a 100 μm I.D. (33 gauge) stainless-steel capillary. This tube is inserted into a ca. 400 μm I.D. (22 gauge) stainless-steel capillary and protrudes ca. 0.5 mm from the latter. Nitrogen nebulization gas flows through the outer stainless-steel tube. This system is used in a series of fundamental and mechanistic studies by the group of Siu.

6.2. Coaxial electrospray needles

For use in CE–MS coupling via electrospray interfacing, a coaxial needle design was developed by Smith et al. [51–53]. Although initially single needle electrospray systems were developed [51,52], the coaxial design showed greater versatility and

robustness. With the single fused-silica needle designs, difficulties were experienced in establishing the electric connection. In the coaxial design, the inner tube is the 100 μm I.D. fused-silica capillary where the electrophoretic separation is performed, while the outer tube is a 0.25 mm I.D. stainless-steel sheath-liquid capillary, which also serves as an electric connection, i.e. an electrode required in the CE process. The fused-silica capillary protrudes 0.2–0.4 mm from the stainless-steel capillary. By using a sheath liquid, the CE separation can be performed with the optimum solvent composition, e.g., in a pure aqueous buffer, while the sheath liquid ensures suitable solvent composition for successful electrospray.

After demonstration of the capabilities of electrospray ionization in protein characterization [9,12], the coaxial interface was also extensively used by the group of Smith in their protein characterization studies. Generally, the aqueous protein solution was introduced at a flow-rate of ca. 0.5 μl/min through the inner 100 μm I.D. fused-silica tube, while methanol or 2-propanol was introduced as a sheath liquid at a flow-rate of ca. 3 μl/min through the outer 0.2 mm I.D. stainless-steel tube.

In many commercial systems, such as the ones built by Analytica of Branford and later on by other manufacturers as well, a coaxial electrospray needle is used. The needle assembly consists of three coaxial capillaries, the outer two generally made of stainless-steel, while the inner capillary is made of either stainless-steel or fused-silica. The inner capillary is used to introduce the sample, while the two outer capillaries may be used to introduce a sheath liquid and/or a gas. In early designs, the dimensions of the assembly were such, that no efficient pneumatically-assisted electrospray could be achieved. The gas primarily functioned in sustaining the spray. In later designs, pneumatically assisted electrospray or ionspray is possible.

6.3. Towards higher flow-rates

A conventional electrospray interface is restricted to liquid flow-rates in the range of 1–10 μl/min. This requires split ratios of 0.01 when the interface is used to couple conventional 4.6 mm I.D. LC columns. The low flow-rates stimulated the use of 1 mm I.D. microbore and especially 0.32 mm I.D. packed microcapillary columns [96]. However, various means to introduce higher flow-rates were investigated as well. However, the higher flow-rate does in most cases not lead to improved concentration detection limits. It is generally observed that the performance of the electrospray interface is better at lower flow-rates.

The first modified electrospray interface capable of the introduction of higher flow-rates is the ionspray interface. While with conventional electrospray interfaces a significant reduction of response is observed if the flow-rate is increased from 5 to 50 μl/min, this is not true for the ionspray interface, which performs about equally well at a flow-rate of 50 μl/min.

A variety of names are used by the various manufacturers to describe the high flow-rate electrospray interfaces based on pneumatically-assisted electrospray, e.g., high-flow and megaflow electrospray. This nomenclature, dictated by patents, distracts attention from and obscures the actual fundamental similarities between the various approaches. In this text, the term ‘‘electrospray’’ is used throughout, eventually with pneumatically assisted or ultrasonic as adjectives, while in some instances the term ‘‘ionspray’’ is kept for historical reasons.

In a so-called turbo-ionspray device, shown schematically in Fig. 12, the desolvation of the sprayed droplets is stimulated by the application of a

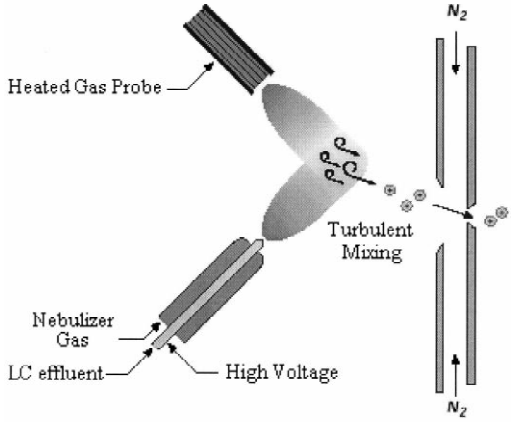

Fig. 12. Schematic diagram of a Perkin–Elmer Sciex turbo-ionspray interface.

heated gas beam. Flow-rates of up to 1 ml/min can be introduced with this system.

Thermally assisted electrospray allowing flow-rates up to 500 μl/min is described by Lee and Henion [97]. A 100 mm×100 μm I.D. stainless-steel electrospray needle is sleeved with a 70 mm×250 μm I.D. resistively heated stainless-steel tubing. The electrospray needle protrudes 0.5 mm from the heater. The temperatures used are in the range of 150 and 240°C. By turning off the high-voltage, the device can be used as a thermospray nebulizer for API–MS. It was also found that thermolabile compounds, which undergo thermal decomposition under conventional thermospray can be successfully analyzed by thermally assisted electrospray without decomposition.

A high-flow option to be used in combination with pneumatically-assisted electrospray is described by Hopfgartner et al. [79,80]. A conically shaped liquid shield is used to catch the larger liquid droplets in the spray. Flow-rates up to 1 ml/min can be introduced, while without the liquid shield the maximum flow-rate is 0.2 ml/min.

An ultrasonically assisted electrospray device is described by Banks [98,99]. The ultrasonic nebulizer consists of a 125 μm I.D. stainless-steel capillary with a sharp point, locked to a two-part stainless-steel body. Between these two parts a pair of piezoelectric crystals are positioned. The resonant frequency of the device was determined to be 186.8 kHz, which is a sharp optimum. A deviation as little as 0.1 kHz can dramatically reduce the signal. The ultrasonic nebulization produces larger droplets, i.e. 8–11 μm, than conventional electrospray (2–3 μm). Both countercurrent drying gas and a concurrent focusing gas, at flow-rates of 4 l/min and 400 ml/min, respectively, are used. It was demonstrated that the response was hardly dependent on the content of organic modifier in the solution introduced (10–90% methanol or acetonitrile tested) and on the flow-rate introduced (0–100 μl/min tested). At higher flow-rates reoptimization of the gas flows is required.

The use of multiple sprayers in the pneumatically assisted electrospray has been investigated by Kostiainen and Bruins [100] in order to study the dynamic range and the flow-rate limitations of an API system. The device allows flow-rates of up to 1 ml/min to be introduced and provides improved signal stability at higher flow-rates. A multichannel electrospray device was also described by Shia and Wang [101]. This system could be used for flow-rates up to 0.6 ml/min.

An electrospray needle device with a heated tip was described by Ikonomou and Kebarle [102]. Heated air is also guided around the capillary tip to sustain the spray. Flow-rates up to 100 μl/min of liquids with up to 100% water could be efficiently introduced, resulting in a signal with good sensitivity and stability.

A high-flow electrospray interface was also described by Hiraoka et al. [65]. A pneumatically assisted electrospray needle is positioned parallel to the ion sampling orifice, i.e. orthogonal to the ion-optic direction. Signals obtained from flow-rates as high as 4 ml/min were reported. The response for the doubly charged ion of the test component, tubocurarine chloride, was found to be essentially independent of the flow-rate in the range of 0.01 to 2 ml/min. The orthogonal spray setup shows various advantages, such as more intense signals, reduction of ion source contamination, flow-rates up to 4.4 ml/min without experimental difficulties and non-critical needle positioning in the source. A similar orthogonally positioned spray device used for high flow-rate introduction in an API system was recently introduced by Hewlett–Packard [66] (see Fig. 8).

6.4. Towards lower flow-rates

The main objective in designing electrospray needles capable of flow-rates lower than 1 μl/min is the reduction of the sample consumption during protein characterization by electrospray MS.

While Chowdhury et al. [54] initially applied a 152 μm I.D. stainless-steel syringe needle, which could not be used to introduce highly conductive aqueous solutions, they subsequently demonstrated that by electropolish sharpening of the tip of a 150 μm I.D. needle a stable electrospray of 1.0 μl/min of water could be achieved [103]. A heatable electrospray needle (25–96°C) based on the latter design was constructed to study heat-induced conformational changes in proteins [104].

A nonsheath low-flow electrospray needle was described by Gale and Smith [105]. The sample

solution is introduced through a 60 mm×5–20 μm I.D. etched fused-silica capillary. The possibility of coaxial introduction of a sheath gas, i.e. SF_6 to prevent electrical discharge in the negative-ion mode [106], is implemented as well. The needle can be used to introduce flow-rates smaller than 0.25 μl/min. A lower needle potential can be used for the low-flow needle, probably due to the higher local electric field gradient at the smaller diameter tip. The signal for the 20+-myoglobin ion is ca. 8 times more intense with a 2.5 times less sample consumption, while the ion envelopes are identical. The long-term (minutes) stability also improved.

A so-called microelectrospray needle is described by Emmett and Caprioli [107,108]. The device was built as a modification of the Vestec electrospray source [63]. Fused-silica needles with inner diameters ranging from 5 to 250 μm were used. The polyimide coating was burnt off the tip, the tip was etched with hydrofluoric acid until a tapered needle tip was achieved. The flow-rates used are in the range of 0.3 to 6.4 μl/min. Stable electrospray performance could not be achieved for the 5 and 10 μm I.D. needles, while the 20 μm I.D. capillaries were prone to clogging. In contrast to the observations of Gale and Smith [105], an increase in needle potential was required with decreasing needle diameter. The sensitivity, i.e. the absolute amount of peptide needed for a spectrum with a signal-to-noise ratio of 2.5, increased with decreasing internal diameter of the needle. A response with a signal-to-noise ratio of 8 at the triply protonated neurotensin was reported for a 0.82 μl/min constant infusion of a 320 zeptomoles/μl solution (0.32×10^{-18} mol/μl) [108].

A microelectrospray needle device for use in the LC–MS analysis of complex peptide mixtures at a flow-rate below 1 μl/min was described by Davis et al. [109]. The needle consists of a flame-drawn, uncoated, fused-silica tip with an outer diameter of 15–20 μm and an inner diameter of less than 5 μm. Clogging of the tip is prevented by the use of a hydrophilic PVDF membrane filter, which is integrated into the needle assembly. The spray potential of only 0.5–1 kV was applied to the sample stream via a capillary union connecting the needle and the liquid transfer line. In order to avoid the use of a sheath flow in combination with microelectrospray needles, required to establish the electric contact, special gold-coated fused-silica capillaries were described by Kriger et al. [110].

A nanoelectrospray needle device, produced by drawing heat softened 0.5 mm I.D. glass capillaries into 1–3 μm I.D. glass tips, was demonstrated by Wilm and Mann [111,112]. These tips can be used with flow-rates as low as 25–50 nl/min. In this setup, the needle is filled with ca. 1 μl of the protein solution to be investigated. The electrospray generated at the needle may be stable for a long as 1 h, allowing the performance of a large series of MS and MS–MS experiments. The nanoelectrospray device is commercially available from the Protein Analysis Company in Odense, Denmark.

Sheathless, low flow-rate electrospray needle devices for use in CE–MS coupling were described by Severs et al. [113,114] and Valaskovic and McLafferty [115]. While in the former system the electric contact, required for CE and electrospray is established using a microdialysis junction, a metallized tip is used in the latter system.

A commercial ''nanoflow'' electrospray with a 20 μm I.D. capillary tip, which can be used with flow-rates in the range of 100–1000 nl/min, is available from Micromass [116]. With the use of nitrogen-pressurized nanovials the system can even be used to introduce a liquid sample at 30 nl/min. Similar micro- or nanospray devices are available from other instrument manufacturers, e.g., Finnigan MAT and Perkin–Elmer Sciex.

Picoelectrospray needles with 2–3 μm I.D. tip diameters were fabricated by laser-heated pulling of 5–20 μm I.D. fused-silica capillaries, chemical etching and surface metallization [117]. These needles allow a further reduction of the flow-rate down to less than 1 nl/min. These needles are used in combination with a Fourier-transform ion-cyclotron resonance MS instrument.

Low flow-rate electrospray from a multichannel microchip device has also been described [118,119]. Electroosmotic pumping of liquid at 100–200 nl/min is applied.

7. Sample introduction devices for APCI

While for other LC–MS interfaces that rely on

mobile-phase nebulization the nebulizers have been described in great detail, this is not true for the nebulizers used in APCI interfaces. A variety of systems have been described.

In the nebulizer of the early system described by Horning et al. [4] the column effluent is mixed with preheated gas. The stainless-steel liquid introduction device is positioned in a glass solvent evaporation device, which is kept at 280–300°C by means of a cartridge heater. This system is primarily used for the introduction of up to 1 ml/min of normal-phase LC effluents, i.e. pure organic solvent systems.

The use of a Hewlett–Packard Direct Liquid Introduction probe, where the liquid nebulization is achieved as the result of the disintegration of the liquid jet formed at a small diaphragm, in combination with a heated desolvation chamber into the API source is described by Henion et al. [26]. Flow-rates up to 100 μl/min of water–acetonitrile mixtures were actually introduced.

The heated nebulizer interface used in the early quantitative work of Covey et al. [30] consists of a concentric pneumatic nebulizer and a quartz vaporizer tube. A schematic diagram is shown in Fig. 13. First, the liquid (typically 1.5 ml/min of a reversed-phase solvent) is pneumatically nebulized, using nitrogen as nebulizer gas (at a typical pressure of 0.8 MPa). Next, the aerosol is swept through the heated (typically 400–500°C) quartz vaporizer tube by means of a make-up gas (nitrogen at 1–3 l/min at 0.5 MPa). This results in a vapor temperature of ca. 100°C, by which nearly complete vaporization of the liquid is achieved. The corona discharge needle is positioned at the exit of the quartz tube. This system is commercially available in the Perkin–Elmer Sciex API systems.

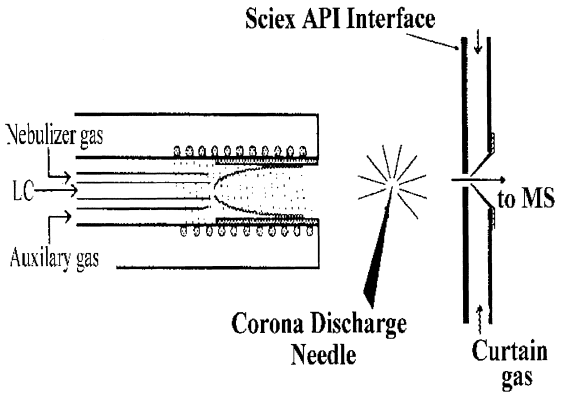

Fig. 13. Perkin–Elmer Sciex heated nebulizer APCI interface (Adapted from ''The API Book'', Sciex, 1989).

Two different nebulizer designs have been described for the Hitachi API system, e.g., a piezoelectric ultrasonic nebulizer [57], and a thermospray-type vaporizer [58,60]. The latter consists of a 0.1 mm I.D. stainless-steel capillary brazed into a copper block which can be heated up to 400°C. The vapor–droplet mixture is then introduced into a heated vaporizer region in order to achieve (nearly) complete vaporization of the liquid. The latter system is called the atmospheric-pressure spray device. Typical temperatures used are ca. 250°C at the nebulizer and ca. 400°C at the vaporizer region.

Pneumatic nebulizers, comprising of three concentric tubes, i.e. a liquid tube in the centre, a nebulizer gas tube, and an auxiliary gas tube, and used in combination of a heated vaporization zone, have also been described for use in combination with API sources from other instrument manufacturers, e.g., Micromass [120] and Finnigan MAT. A schematic diagram of such a device is shown in Fig. 14.

Recently, a sonic spray nebulizer device has been described by Hirabayashi et al. [61,62] for use in combination with a Hitachi API instrument. The device consisted of a 100 μm I.D.×200 μm I.D. fused-silica capillary fitted in and carefully centred in a 400 μm I.D. Duralumin orifice. The capillary protrudes 0.6 mm beyond the orifice. A flow of nitrogen through the space between the capillary and orifice is used to achieve nebulization. The sonic spray device can in principle be used in combination with a corona discharge needle, although as a result of the small droplets produced electrospray-like ionization can be achieved as well.

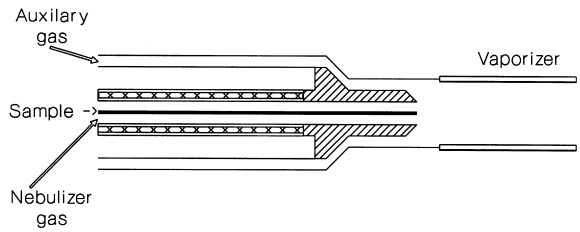

Fig. 14. Schematic diagram of a pneumatic nebulizer interface probe for APCI.

In general, little information is provided on the amounts of gas required in the APCI interface, i.e. gas for nebulization, auxiliary gas, and in some systems countercurrent gas. Depending on the solvent system and flow-rate as well as on the system used, the required gas flow can be as high as 600 l/h, requiring at least a bottle of pressurized nitrogen of high purity every 24 h of operation. In most systems, up to 0.7 MPa gas pressure must be available.

8. API sources for quadrupole ion trap instruments

While in previous sections, API devices were described which are primarily used on quadrupole instruments, API can also be performed on other types of mass analyzers, sometimes needing only minor modifications. In this and the next three sections, developments in API sources for ion trap, magnetic sector, Fourier-transform ion-cyclotron resonance, and time-of-flight type analyzers are reviewed.

An external API source for a quadrupole ion trap mass spectrometer was first described by Van Berkel et al. [121] in 1990. The system comprises of a two-stage differentially pumped device. The first pumping stage is kept at ca. 40 Pa by means of a rotary pump, while the second stage, containing the ion trap, is evacuated by a turbomolecular pump.

The ion trap requires a pulsed ion introduction, which is achieved by the application of a suitable voltage to one of the half-plates of one of the entrance lenses. While a conventional electrospray needle was applied in the first experiments, a pneumatically assisted electrospray device was used later [122].

Subsequently, electrospray ion trap systems have been described by others.

A similar two-stage differentially pumped system, based on a Varian Saturn-II ion-trap detector, was developed by Mordehai et al. [123] and used in both LC–MS and CE–MS experiments. The pulsed ion introduction is achieved by means of two deflector electrodes in the high-vacuum chamber. In the subsequently modified system [124], the vacuum system is redesigned, e.g., with a separate turbomolecular pump at the electron multiplier region. The application of storage waveform techniques for the manipulation of the ions in the quadrupole ion trap is studied. This approach allows the selective accumulation of ions in a selected m/z window 100u wide as well as the reduction of chemical noise [125].

On-line LC–electrospray-ion-trap MS is also described by Lin and Voyksner [126,127] for the determination of environmental contaminants and the analysis of neuropeptides using perfusion LC.

A major breakthrough in this field is the recent commercial introduction of two ion-trap based dedicated LC–MS instruments with an API source for electrospray and APCI, i.e. the Finnigan MAT LCQ and the Esquire, from a joint venture by Bruker and Hewlett–Packard. At present, it is too early to fully evaluate the claims made by the instrument manufacturers on the performance of these systems, because of the limited number of publications from independent laboratories available. Given the significantly reduced cost of an ion-trap MS–MS system compared to a triple quadrupole system for MS–MS, elaborate use of ion trap instruments for LC–MS may be envisaged. The stepwise breakdown of precursor ions in multiple stages of MS–MS facilitates the structure elucidation of complex compounds. Therefore, an important field of application of the ion-trap system will be in qualitative analysis. Preliminary results in quantitative analysis indicate that 5–10 times higher detection limits in selective reaction monitoring are achieved using an ion-trap system compared to a triple-quadrupole [128].

Finally, ion traps are also used as storage devices in electrospray experiments with reflectron time-of-flight instruments (see below). Another hybrid system, consisting of a double focusing sector instrument as front end mass analyzer and an ion-trap back end, has recently been introduced by Finnigan MAT. Such a system combines high resolution mass determination and multiple stages of MS–MS with superior collision efficiency.

9. API sources for magnetic sector instruments

The major difficulties in coupling an API source to a sector instrument are related to the high acceleration voltage and the large pressure difference be-

tween ion source and analyzer, which requires extensive pumping in order to avoid discharges, electrical breakdown, as well as extensive collision activation of the ions. In general, an additional pumping stage is used, i.e. a total number of four stages instead of three. Additional precautions for electrical breakdown are sometimes incorporated.

Electrospray ionization on a magnetic sector instrument was first demonstrated by Allen and Lewis [129], using a laboratory-built electrospray source contained in a retractable probe.

Larsen et al. [130–133] described the use of an electrospray source from Analytica of Branford on a VG ZAB double focusing sector instrument. A schematic diagram of the system is shown in Fig. 15. Initially, a discharge-suppressing baffle system was used, surrounding the glass inlet capillary (cf. Fig. 15, [130]). Later on, this baffle system was removed as it severely restricted the pumping capacity in the nozzle-skimmer region. While the first prototype contained three differential pumping stages prior to the ion entrance slit, i.e. between the nozzle and plate in front of the skimmer, between the plate and the skimmer, and between the skimmer and the ion entrance slit [130,131], the plate was removed in later studies [132,133]. This resulted in a substantial improvement of the signal-to-noise ratio as well as a shift in the ion envelopes towards higher charge states. For ubiquitin the ion envelope maximizes at 6+ to 4+ ions in the original source design and at 9+ in the later design. The latter spectrum is more in agreement with those obtained by means of quadrupole instruments. After establishing the performance of the system [130,131], accurate-mass and high-resolution measurements for carbonic anhydrase and ovalbumin were demonstrated [132,133]. This allows the direct determination of the charge state of a peak by determining the number of ^{13}C isotope peaks per mass unit.

The design and performance of a Kratos electrospray source for double focusing magnetic sector instruments is described in two papers [134,135]. The system contains three source pumping stages, i.e. a rotary pump for the region between the sampling orifice and the first skimmer, a turbomolecular pump at the region between the first and second skimmer, and a turbomolecular pump at the region between the second skimmer and the ion entrance slit (source housing region). An ion acceleration potential of 4 kV was applied to the first

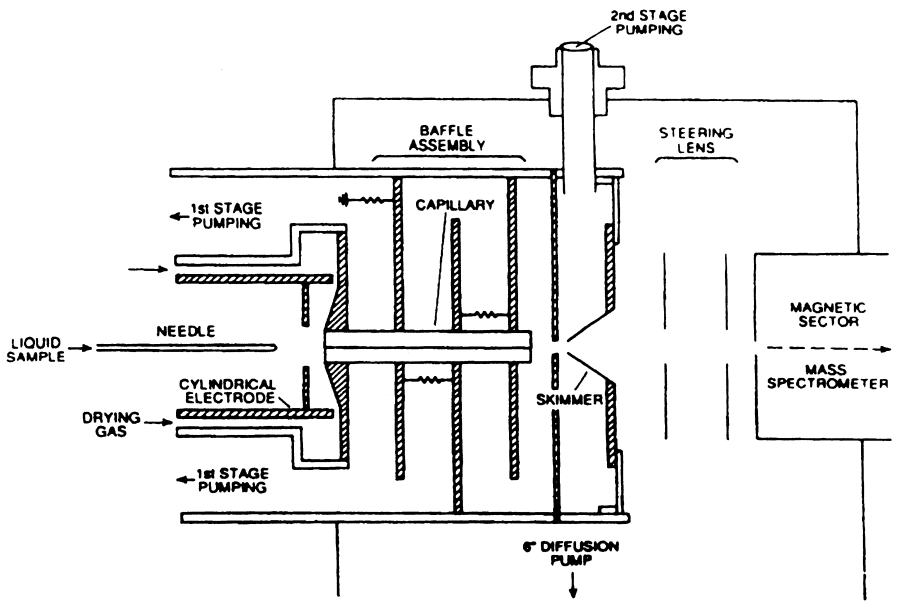

Fig. 15. Schematic diagram of the prototype electrospray source for a magnetic sector instrument (from Ref. [130], ©1990, Wiley and Sons Ltd.).

skimmer. Other voltages are: 8 kV at the needle, 5 kV at the endplate in front of the sampling orifice.

Subsequently, accurate mass determination and high resolution measurements (10 000 at 10% valley) was demonstrated on a double-focusing Jeol sector instrument equipped with an Analytica of Branford electrospray source [136,137]. Optimization of skimmer voltages in such a system is discussed by Murata et al. [138]. A comparison between point and array detection in electrospray was reported by Cody et al. [139]. Improvements in detection limits of a factor of at least 10 were achieved. For hen egg-white lysozyme the detection limit (signal-to-noise ratio of 2) is 500 amol/μl.

A modified electrospray source for Finnigan MAT magnetic sector instruments was described by Dobberstein and Schröder [140]. The design of the source is similar to the source designed for Finnigan MAT quadrupole and ion trap systems. It contains a heated transfer capillary, a tube lens, a skimmer, an octapole ion focusing device, and a conventional lens assembly prior to the ion entrance slit. Three differential pumping stages are provided prior to the ion entrance slit. The application of this system is described by various authors [141–143]. In the former two papers, the electrospray ion source is fitted onto a MAT 900 instrument, equipped with a PATRIC array detector.

The Mark-2 three-stage differentially pumped electrospray source developed by VG/Micromass contains elements also found in similar sources for quadrupole instruments, e.g., a pepperpot counter electrode and a hexapole lens for improved ion transmission. This type of system was for instance used by Eckart and Spiess [144] to study the biotin binding to streptavidin.

A laboratory-built electrospray source to be applied on a VG ZAB double-focusing sector instrument was described by Jiang and Moini [145]. The interface features a heated transfer capillary and allows optimization of the distance between the heated capillary tube outlet and the first 0.5 mm I.D. skimmer. The highest response was obtained at a distance of 7 mm. However, this results in a high source pressure, too high to resolve isotope clusters. Therefore, a 5-mm distance was applied, resulting in a 7 times lower pressure and a 3 times lower response.

An important drawback of these sector-based electrospray systems is that an 8-kV voltage is present at the electrospray needle, i.e. at the outside of the instrument.

10. API sources for fourier-transform ion-cyclotron resonance instruments

An electrospray ion source for an FT-ICR-MS instrument has first been described by Henry et al. [146–148]. The incentives in this research are the possibilities of (ultra)high-resolution measurements, the improvements of detection limits by signal averaging, and the efficient tandem mass spectrometry possibilities. On-line electrospray FT-ICR-MS resulted in a more extensive analytical use of FT-ICR-MS instruments. In fact, commercial electrospray interfaces for FT-ICR-MS instruments are available from Bruker Instruments, Extrel FT–MS, and Finnigan FT/MS. The FT-ICR-MS analysis of biomacromolecules is reviewed by Buchanan and Hettich [149].

In the first electrospray FT-ICR-MS experiments, an external ion source is used, initially on the instrument built in the group of Hunt and Shabanowitz at the University of Virginia [148], and next on a dual ion cell prototype Nicolet FTMS-2000 at Cornell University, operated at 2.8 T [147,148]. The ions are transmitted to the ion cell through a series of differentially pumped vacuum chambers by means of RF-only quadrupoles. A schematic diagram of such a system is shown in Fig. 16. Data are shown for various proteins, e.g., gramicidin S, RNase A, myoglobin, bovine albumin and cytochrome C, while MS–MS data are shown for gramicidin S, generated by means of photodissociation with a pulsed excimer laser [146–148]. As a result of the relatively high pressure in the FT-ICR cell, i.e. 10^{-5} Pa instead of the necessary 10^{-7} Pa, the achievable resolution is limited to ca. 5000. At this resolution, the isotope peaks of the 3+, 4+ and 5+ ions of mellitin can be resolved.

These early results clearly indicate some of the instrumental problems in electrospray ionization on a FT-ICR-MS system. Obviously, the most important problem is related to the pressure in the ion cell. A pressure in the 10^{-7}-Pa range is required for high

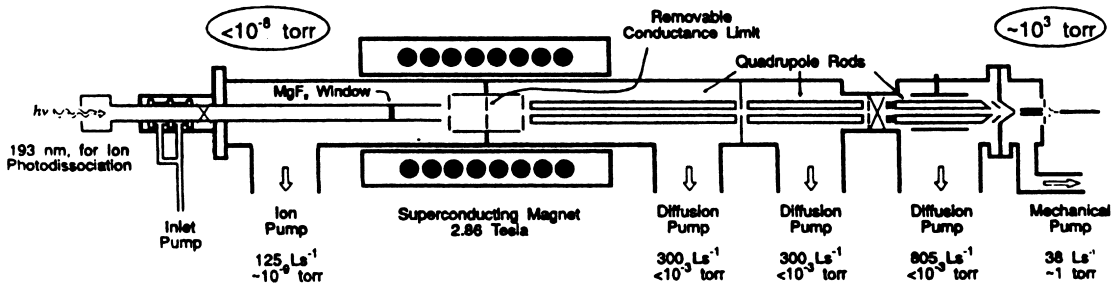

Fig. 16. Interface for electrospray ionisation at a Fourier-transform ion-cyclotron resonance instrument. Use of quadrupole ion guides to transport the ions into the FT-ICR cell and magnetic field (from Ref. [147], ©1990, Wiley and Sons Ltd.).

resolution. A second problem is related to the kinetic energy the ions gain in the nozzle-skimmer region and between the skimmer and the first quadrupole, which limits efficient trapping of ions in the cell. Furthermore, the sensitivity of the system should be further enhanced.

Subsequently, electrospray on a FT-ICR-MS instrument was demonstrated and further investigated by Laude et al. [150–155]. In their system, a probe-mounted electrospray interface is used, which extended into the high field region (1.5 T) of the magnet. In this respect, the design differs from that of the Cornell system [146–148] and of most other instruments developed later. In most cases, an external ion source with an ion guide to transfer the ions into the cell in the high magnetic field is applied. The interface is based on the Chait design [54], featuring a resistively heated ion transfer capillary. In the first prototype, mounted on a Extrel FTMS-2000, five concentric vacuum chambers are used. However, the pressures in the ion cell were still in the 6×10^{-3} Pa range, significantly limiting the achievable resolution [150]. Compared to the Cornell design [146], the ion transfer distance between source and FT-ICR cell is much shorter, i.e. 0.25 m instead of 1.25 m. This results in significantly higher electrospray currents, i.e. 350 pA instead of only a few pA in the Cornell design.

Further studies on electrospray FT-ICR-MS of the research group of Laude [151–155] comprise the optimization of ion injection and trapping as well as ion excitation and detection. By performing the electrospray ionization in a radially homogeneous magnetic field, a more efficient ion injection into the FT-ICR cell is achieved. However, this yields problems with efficient differential pumping due to limited accessibility of the magnet bore. Nevertheless, by minor modification in terms of distances and skimmer dimensions a reduction of the cell pressure down to 2×10^{-5} Pa was achieved, which results in a 10-fold improvement in signal-to-noise ratio and mass resolution. The nozzle–skimmer distance in the source is rather critical, both with respect to reduction of pressure and of ion kinetic energy. At a distance of 4.5 mm, the voltage applied to the skimmer cone controls the ion kinetic energy. The trapping potential to accumulate the ions in the FT-ICR is related to the skimmer potential, e.g., ca. 2 and 5 V at skimmer potentials of 5 and 15 V, respectively. A narrower energy distribution is obtained at lower skimmer voltages. The optimum ion accumulation time is found to depend on the charge state and charge distribution of the ion population: it is shorter for a broad and highly charged distribution (32+ to 54+ for bovine albumin) than for a narrow distribution (12+ to 16+ for cytochrome C). The excitation parameters (sweep rate, energy) were also found to depend on analyte mass, mass-to-charge ratio and background pressure [151]. These aspects are studied later on in greater detail and extended to experiments with the selection of different conformational states of proteins [152]. Further improvements are achieved by remeasurement techniques, e.g., a 7-fold improvement in the signal-to-noise ratio for horse myoglobin by applying 50 remeasurements [153], and postionization separation by kinetic energy filtering in the analysis of protein mixtures [154]. A redesigned vacuum system is also reported, enabling further pressure reduction in the TF-ICR cell down to 2×10^{-6} Pa. Mass resolution in excess

of 20 000 for the 4+-ion of mellitin is reported for this system [155].

Significant progress in resolving power is reported by Beu et al. [156,157] using the Cornell design by replacing the 2.8-T magnet by a 6.2-T magnet, improving the effective pump speeds, and installing an all-copper open FT-ICR cell and a resistively heated metal capillary. Mass resolution in excess of 10^5 for carbonic anhydrase (29 kDa) is achieved. An example of the available performance is shown in Fig. 17 where a single-scan electrospray spectrum of a mixture of cytochrome C (13+ ions) and myoglobin (18+ ions) is shown. From the indicated mass-to-charge differences the charge state of the ions can be determined. The theoretical values for cytochrome C and myoglobin are 0.07718 and 0.05574, respectively. A mass accuracy of 2.5 mDa can be obtained. MS–MS by means of FT-ICR was also investigated. Both collisional activation and photodissociation were found to be ineffective with high mass ions. Useful structural information of the proteins could be obtained by performing in-source CID [156,157].

At this stage, a third research group entered the field of electrospray on FT-ICR, i.e. the group of Smith at the Pacific Northwest Laboratory [158–161]. Their system comprises of an external ion source containing a resistively heated transfer capillary, six differential pumping stages, quadrupole RF-only ion guides in the fourth and sixth stages (the latter is 1 m long), two high-speed electromechanical shutters, and a 7-T magnet. The shutters are only

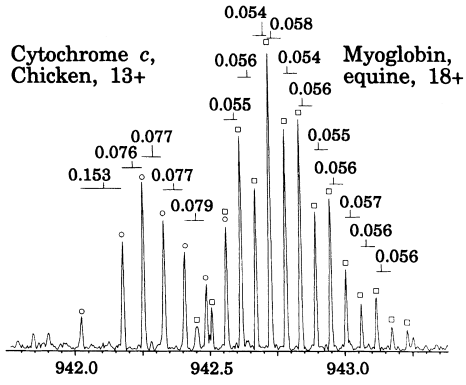

Fig. 17. Single-scan electrospray mass spectrum of a mixture of cytochrome C (13+ ions) and myoglobin (18+ ions) to demonstrate high resolution measurement on a FT-ICR-MS instrument (from Ref. [156], ©1993, Elsevier Science).

opened to inject ions from the electrospray source into the FT-ICR cell. The operating pressure in the FT-ICR cell is below 10^{-7} Pa. By comparing the total ion current exiting the source and that arriving at the cell, it was found that ca. 50% transmission of the ion packets was achieved. Typical ion currents in the ion cell are 10–30 pA. Using a 25-ms ion injection, spectra were obtained from 4 fmol of bovine insulin and ubiquitin with sufficient resolution to resolve isotopic envelopes and to determine charge states. Mass resolution as high as 700 000 was achieved for the 4+ ion of bovine insulin [158]. Subsequently, various improvements have been reported, e.g., the time-base modulation in order to correct the cyclotron frequency shifts in long-lived transients [159], the accumulation of ions with a selected m/z in the FT-ICR cell by single-frequency quadrupole cooling [160] and sustained off-resonance irradiation in order to dissociate ions at different mass-to-charge ratios [161].

As a result of the successes obtained in electrospray FT-ICR-MS and the growing importance of electrospray MS in many field, various other groups started research in electrospray FT-ICR-MS coupling. A low voltage ion transport system, based on the transfer of ions from the external source to the FT-ICR cell by means of conventional electrostatic lenses instead of quadrupoles, was described by Ijames and Markey [162]. By means of a gate valve, which is closed after ion injection, sufficiently low pressure for high resolution measurements is achieved, e.g., 410 000 at the 5+-ion of aprotinin (6512 Da) and 50 000 at the 8+ and 9+ ions of horse heart cytochrome C (12 360 Da).

Electrospray ionization on commercial FT-ICR-MS systems from Extrel and Bruker have been described [163,164]. In the latter case an ultrasonic-assisted electrospray source from Analytica of Branford was modified for a Bruker external ion source [164]. LC–MS analysis of peptide mixtures was demonstrated. Electrospray FT-ICR-MS is also commercially available from IonSpec Corporation, while Extrel systems are now part of ThermoQuest, which also contains Finnigan MAT.

At this stage of the research in electrospray FT-ICR-MS, the system is ready for applications and studies similar to the ones also performed with other electrospray systems. Charge-state shifting of in-

dividual multiply-charged ions of bovine albumin dimers due to gas-phase ion–molecule reactions with ammonia are studied by Cheng et al. [165]. High-resolution tandem mass spectrometry of carbonic anhydrase was reported by Senko et al. [166]. Dissociation of the tetrameric ions of noncovalent streptavidin complexes was described by Schwartz et al. [167]. The addition of copper(II) to an ubiquitin solution for electrospray has been studied by Jiao et al. [168].

Recent instrumental improvements to electrospray FT-ICR-MS systems are the use of a capacitively coupled open cell to improve the signal-to-noise ratio [169] and the use of an electrostatic ion guide replacing the quadrupole ion guides [170].

11. API sources for time-of-flight instruments

API sources, and especially electrospray, have also been coupled to time-of-flight (TOF) instruments. Three basic instrumental designs have been described: (1) an electrospray source on-axis with the (reflectron) TOF analyzer, (2) an electrospray source perpendicular (or orthogonal) to the direction of acceleration of ions into the TOF analyzer (cf. Fig. 18), and (3) an electrospray source coupled to the ion-trap storage device, positioned on-axis with a reflectron TOF analyzer.

An important feature of a TOF instrument is the necessity of pulsed ion introduction, which is readily achieved by the use of a pulsed voltage to a repeller plate positioned opposite the accelerating grid. However, this results in a reduced effective duty cycle: while ions are generated continuously, only part of these ions are sampled for mass analysis. In order to achieve higher duty cycles, in-line ion storage devices are used.

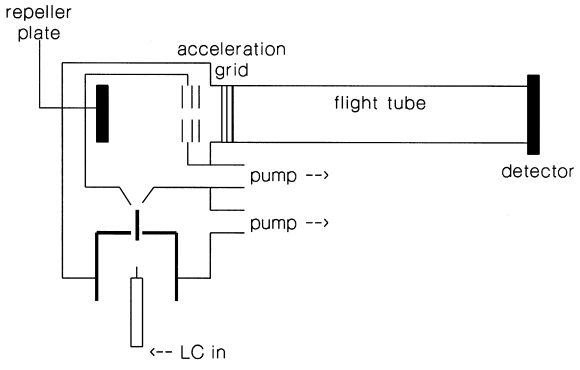

Fig. 18. Schematic diagram of an orthogonal ion introduction from an electrospray ion source into a time-of-flight mass analyzer.

APCI on a TOF instrument was first described by Lee and coworkers [171]. The systems consists of a two-stage pumping system, a perpendicular ion introduction, a pulsed repeller plate, and a linear TOF analyzer with a microchannel plate detector.

An on-axis electrospray source on a linear TOF analyzer has been described by Boyle et al. [172]. The source is an Analytica of Branford system. Ions generated by electrospray ionization in the API source pass through the transfer capillary, two skimmers in series and a grid extraction lens. Next, they are decelerated and stored between two grids. The stored ions are then extracted from the storage region and admitted into the flight tube. The sequence of storage and introduction is performed at a frequency of a few hundred Hertz. Next, the orientation of the source has been changed to perpendicular in order to reduce the longitudinal energy spread of the ion packets delivered to the TOF analyzer. A reflectron TOF instrument is now used. The ion storage device consisting of two grids is used. The system can be operated at a resolution of ca. 1000 [173].

Similar systems are subsequently described by Mirgorodskaya et al. [174], Verentchikov et al. [175] and Fang et al. [176]. These systems feature electrospray perpendicular to a reflectron TOF analyzer. Mirgorodskaya et al. [174] explain in greater detail the ion storage device (delayed extraction or time-lag focussing) also used by Boyle et al. [173]. Initially, the repeller and the first grid are at ground potential. Ions entering move to the ion collector. When the space between repeller and first grid is filled with ions, a push-out and a draw-out voltage is applied to the repeller and the second grid, respectively, and the ion packet is drawn to the acceleration region [174]. Mass resolution of these systems is typically 2000. The feature of unlimited mass range of a TOF analyzer is demonstrated by Verentchikov et al. [175] in the observation of a 20+ ion of ß-galactodidase (113 600 Da). This system is also used to investigate noncovalent protein–protein interactions in soybean agglutinin. Multimers up to dodecamers (~350 000 Da) have been observed in this study [177]. In a

more fundamental study it was found that the residual gas pressure in the system influenced both the sensitivity and the resolution in TOF mass analysis. To some extent, these effects can be attributed to collision-induced dissociation of protein ions in the drift region. Especially, proteins in their native conformational state and low charge state appear to be sensitive to high residual gas pressures [178]. On-line capillary electrophoresis electrospray TOF MS with a system of similar design is demonstrated by Fang et al. [176].

The combination of orthogonal ion introduction, delayed extraction and a reflectron TOF analyzer has been demonstrated to allow high resolution measurements, i.e. resolution as high as 6000. This allows determination of charged states of multiply-charged ions as well as accurate mass measurement of relatively small molecules. LC–electrospray-TOF systems have become commercially available from Analytica of Branford and Perseptive Biosystems, while a similar system from Micromass has been announced.

As an alternative to the lens-based ion-storage device described above, the use of a quadrupole ion trap for ion storage has been demonstrated by the group of Lubman [179–183]. The ion trap is an efficient ion storage device, which can be emptied in pulsed mode. Initially, a system for use in combination with APCI was built [179]. Subsequently, electrospray ionization on an ion-trap reflectron TOF instrument was demonstrated [180]. The system is capable of a nearly 100% duty cycle in converting the continuous electrospray ion beam into a pulsed source for TOF MS. This compares very well with duty cycles reported for systems with other ion-storage devices (~20%, [175]). The system consists of an API source, a heated transfer capillary, a first pumping region containing a tube lens, a 325 μm I.D. skimmer, an Einzel lens system for ion focusing and introduction into the ion trap, an ion trap in the second pumping region, an acceleration grid and focusing lenses in front of the reflectron TOF. The ion trap storage reflectron time-of-flight system is described in more detail in a separate contribution to this issue [183].

In 1996, Micromass introduced two special instruments containing a TOF analyzer for MS–MS, i.e. the Autospec-TOF, where the front end is a double focusing sector instrument [184], and the Q-TOF, where the front end is a quadrupole analyzer. Both systems provide very high product-ion transmission in MS–MS and are therefore ideally suited for high-sensitivity peptide sequencing and other protein characterization studies. Both systems are equipped with a (nano)electrospray source for analyte introduction. The first mass analyzer is used for precursor ion selection. The selected precursor is collisionally dissociated in a hexapole collision cell. The resulting product ions are orthogonally accelerated into a reflectron TOF analyzer. For the Q-TOF a 100-fold improvement in sensitivity compared to triple quadrupole systems is claimed.

12. LC–MS via MALDI–TOF

Next to approaches in which LC–MS on a TOF is achieved via an API interface, the possibilities of LC–MS via MALDI is also investigated. In this respect, there are two methods under investigation.

Li et al. [184–187] applies a continuous-flow type interface, similar to the systems used in continuous-flow fast-atom bombardment. This approach is described in a separate contribution to this issue [187]. A similar device is described by the group of Lubman [188], to be used in combination with an ion-trap reflectron TOF instrument (see above).

Aerosol MALDI, i.e. MALDI from small aerosol particles generated by the pneumatic nebulization of a MALDI matrix containing solution has been described by Murray and Russell [189–193]. The aerosol particles are dried in a heated skimmer tube prior to ionization by a pulsed UV laser.

13. Conclusions and perspectives

In the past few years, LC–MS has rapidly evolved to a technique which cannot only be used by the experienced mass spectrometrist, but also finds broader applicability. Numerous benchtop LC–MS instruments have become available, which are particularly built for use as a detector for LC. The robust systems are produced with software control, which makes these systems extremely easy to operate. Unattended automatic operation can be readily

achieved. In combination with the price reduction, such instruments will find their way into LC laboratories, where they will be used in combination with UV photodiode array detection, to obtain optimum information on the complex samples analyzed. On-line measurement of molecular mass, automated in-source CID to obtain structure information, assessment of peak purity based on molecular mass rather than differences in UV spectra will readily be available to liquid chromatographists. In that respect, LC–MS can be expected to have a much wider applicability than GC–MS. New instruments have been introduced almost on a monthly basis in the past year; other innovations are announced, such as a benchtop sector instrument with LC–MS by Jeol. New companies enter the field and start selling their LC–MS instruments.

As a mass spectrometrist, one is somewhat overwhelmed by the speed of developments. The newer instruments are introduced as if the mass spectrometry people always lied about the difficulties of their technology: everything is easy, simple and straightforward now, everything is automated. The way data appear to be obtainable does not agree with the hectic fights of the past. However, when the speed slows down and many instruments will have entered new laboratories, the new mass spectrometry researchers will certainly find out, that despite the ease with which the instrumentation can be operated, mass spectrometry still is a difficult technique, e.g., in the interpretation of complex MS–MS spectra, in the validation of a quantitative method at ultimate sensitivity. Futhermore, it should be dismissed that the chromatographist uses only a very minute amount of the mass spectrometric information that is obtained during an LC–MS run. The still high investment in an LC–MS instrument generally cannot be justified by molecular mass information alone.

New developments will continue to be important for the overall progress of the field. Some instrumentation still needs the skills of the experienced mass spectrometrist, e.g., the use of magnetic sector and FT-ICR instruments.

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