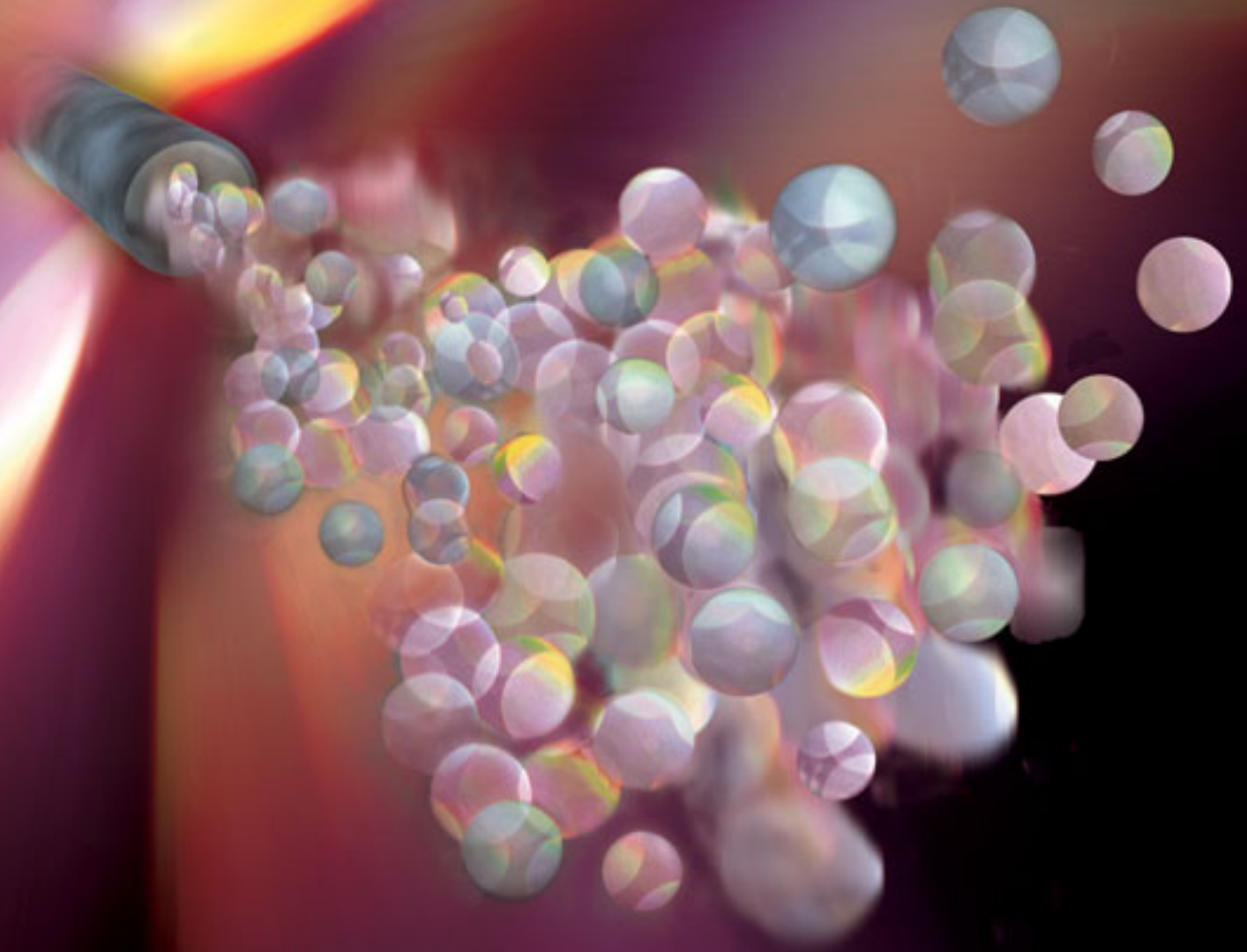


ELECTRON



IONIZATION

LC/EI MS may extend the simplicity of GC/MS to many HPLC-amenable compounds without compromising the full potential of LC.

FOR LC/MS

The widespread, multilevel development of MS has embraced a variety of expertise and a lot of human and economic resources, with palpable results. MS is responsible for many scientific advancements and has a primary role in determining the structure of unknown compounds, unveiling the sequence of proteins, locating posttranslational modifications, characterizing DNA chains, and detecting harmful substances and contaminants. This huge combination of endeavors demands sophisticated, sensitive, selective, and informative analytical instrumentation integrated with other powerful techniques such as GC, LC, and CE.

The key to fine-tuning MS instrumentation for a specific application is to first choose the correct ionization method. Any substance can be converted into its charged form by subtracting electrons (forming a positive radical ion, $M^{\dot{+}}$), adding or subtracting protons (generating pseudomolecular ions of given polarity, $(M+H)^+$ and $(M-H)^-$), forming adducts, or transferring a charged species. Under specific conditions, electrons can be also captured by receptive elements in the molecule, thus forming negative molecular ions, $M^{\bar{-}}$.

The amount of energy involved in the ionization process and the form in which it is transferred to the molecule can also be adapted to accommodate the needs of different molecular species and regulate chemical stability. In this approach, soft ionization techniques preserve molecular structure for molecular weight determination, whereas more energetic transitions promote fragmentation for enhanced structure elucidation. Ionization can be performed in the gas, liquid, or solid phases. Electron ionization (EI) and chemical ionization (CI) are suitable only for gas-phase ionization, and their use is limited to compounds with sufficient volatility and thermal stability. However, experimental conditions may be critical for preserving stability during gas-phase conversion for both techniques.

In thermospray and electrospray ionization (ESI), ionization takes place in a liquid phase through low-energy chemical processes. Ions result either from removal of solvents or by electrostatic expulsion. MALDI, secondary ion MS, fast atom bombardment, field desorption, and plasma desorption are ionization techniques used in a solid phase or a nonvolatile liquid phase. Ions are desorbed by energetic atoms or ions or by an intense electric field. All soft ionization techniques are particularly suitable for large molecules and require additional stages of fragmentation for structure characterization. Atmospheric pressure ionization (API) sources are also appropriate for small molecules, but because of

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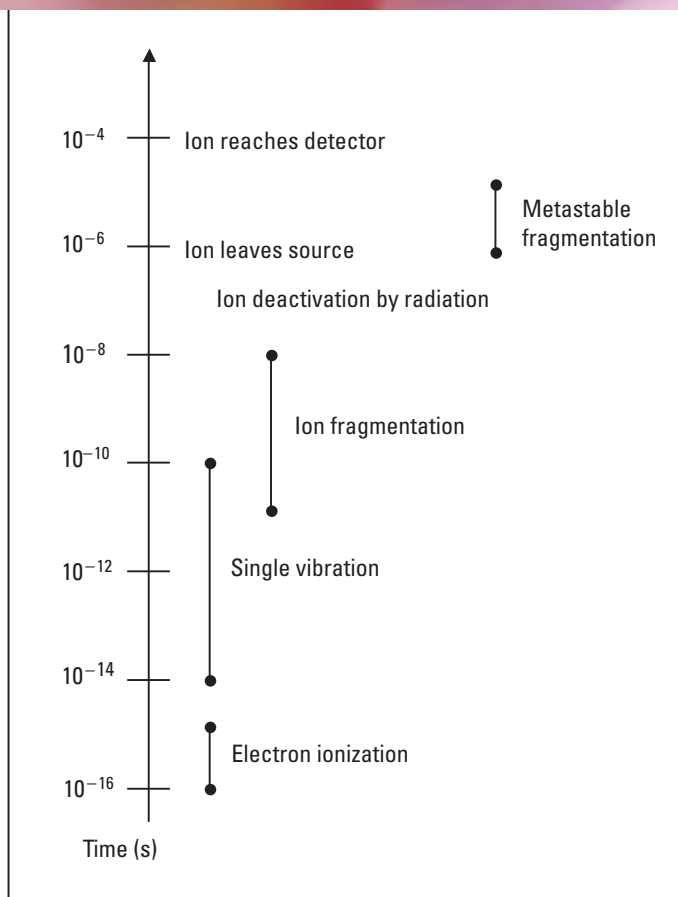


FIGURE 1. Time sequence during EI.

The energy is redistributed through a single vibration of the ion. After 10^{-8} s, the ion deactivates to the ground state if it did not fragment. This occurs well before the ion leaves the source and explains why EI is reproducible from one spectrometer to another. (Adapted with permission from Ref. 33.)

limited fragmentation, only partial information is obtained and multistage analyzers are required.

EI's impact

EI, formerly called electron impact ionization, was developed by Dempster at the beginning of the last century and improved by Bleakney and Nier many years later (1, 2). Continuous, although less significant, improvements over the years have made it standard equipment for GC/MS. Analytes are ionized exclusively in the gas phase through interaction with high-energy electrons. The energy surplus, not directly involved in the ionization process, is responsible for extensive fragmentation of the molecule, so the molecular ion is not always detected. However, because the fragmentation pattern is highly reproducible and characteristic of each molecular species, a direct comparison can be made with thousands of reference spectra for easier compound identification.

Electron energy is the key for achieving ionization and modulating fragmentation. Electrons are usually emitted by an electrically heated filament under high-vacuum conditions and accelerated by an electric field. Before being captured by the anode, electrons may intercept gas-phase molecules during their short journey in the ion source, producing an excited, positive radical ion, $M^{+\bullet}$. The ionization process is described by quantum mechanics because each moving electron is associated with an

electromagnetic wave whose wavelength λ is given by h/mv , in which m is the mass of the electron, v is its velocity, and h is Planck's constant. The wavelengths are 2.7 \AA for a kinetic energy of 20 eV and 1.4 \AA for 70 eV. When the electron travels close to a gas-phase molecule, the electron's electromagnetic wave is distorted and becomes complex.

The new wave can be described by the interference of a multiwave pattern of various frequencies ν . If one of the frequencies has an energy $h\nu$ equal to a specific electronic transition in the molecule, the corresponding energy is absorbed. The transition usually exceeds the typical ionization potential so that a thermal electron is expelled, thus leading to molecular ionization. Electron energies of 10–70 eV are sufficient to induce ionization in all organic species. Ionization does not occur at ≤ 10 eV, because this limit is the least amount of energy required for primary ionization and varies depending on the molecular structure.

A compound with all σ bonds in its structure requires a higher energy for ionization because σ bonds are the strongest; π bonds and nonbonding electrons have a lower energy limit for primary ionization. The probability for a successful ionization event increases with electron energy up to ~ 70 eV. Over this value, the impact section of the ionization process starts retreating, and electron–molecule interactions become weaker and unproductive. The number of ions produced (I) plotted against an increasing electron energy shows a characteristic curve with a wide maximum at 70 eV and an appearance potential at ~ 10 eV. For a given energy of the electrons, I is directly proportional to the analyte pressure p in the ion source such that $I = NpiV$, in which i is the flying electron current, V is the source volume, and N is a constant proportionality coefficient. This direct proportionality makes EI very suitable for quantitative measurements.

The secret of EI for generating classic, nicely matching spectra is in the very rapid sequence of events leading to fragmentation. For a 100-u molecular ion accelerated by a 1000-V potential toward the analyzer, the average time spent in the ion source depends on its speed and ranges between 10^{-6} and 10^{-7} s. The interaction between an electron and a gas-phase molecule is a very high-speed process; a 10-eV electron reaches a speed of 1.88×10^8 cm/s before being captured by the anode. The electron can cover a distance of 1.88 \AA across a molecule in 10^{-16} s, during which the interaction can be established and ionization may occur. For a 70-eV electron, the excess energy released to the molecule can be several electron volts. (Remember that the ionization process takes place under high-vacuum conditions, so any molecular collision is very unlikely.)

Under these conditions, the energy cannot be released by vibrational relaxation through the collision with other molecules, leaving open the only other possibility—photon emission. UV or visible-range radiation typically occurs in 10^{-8} s. If energy is distributed among the different parts of the molecule before fluorescence occurs, fragmentation is observed. Recombination is impossible because of the lack of collisions, so these unimolecular reactions depend only on the structure and energy content within the isolated molecule. After 10^{-8} s, the whole process is complete, but the ions are still within the ion source; they have not entered the mass analyzer (Figure 1). This process illustrates why—even

with different instruments and different times of flight through changes in the accelerating potential (a few volts for a quadrupole, kilovolts for a sector instrument)—the spectrum profile is not substantially modified. EI spectra are the result of intramolecular reactions only and are not influenced by other complex analytical parameters used in other forms of ionization. EI spectra furnish data that are the most easily compared.

EI and LC

Solvent and sample restrictions cause difficulties in creating any LC/MS interface. Sample components pass through the detector with the HPLC mobile phase, a solvent. Because a high vacuum is required at some point during MS operations, as little solvent as possible should enter the mass spectrometer when it functions as a detector. If the HPLC flow rate is 1 mL/min and the mobile phase is water, the gas volume generated in the spectrometer is ~1200 mL/min! LC separates samples that can be dissolved in solvents. The analytes may vary dramatically in weight, polarity, and chemical stability, which can severely restrict the hardware requirements for both interface and mass spectrometer.

HPLC is very useful for a large array of delicate, thermally sensitive, or high-molecular-weight compounds of various origins, leaving GC with a relatively easier job. So then, why use a gas-phase, GC-style ionization for compounds that are amenable to HPLC?

API techniques, namely ESI and atmospheric pressure chemical ionization (APCI), can handle a broad range of different substances (3–6). Under ESI conditions, the ionization of the sample is obtained at atmospheric pressure in the liquid phase, and only the ionic species are admitted into the high-vacuum analyzer region. This particular ionization process is considered “soft” in terms of energy, and normally, little or no fragmentation is observed.

However, for API techniques to be successful, certain conditions must be met. Polarity is a key factor for obtaining a satisfactory response in this type of interface. API interfaces often rely on simple acidic or basic chemical processes to ionize the sample in the liquid phase. Despite API’s straightforward simplicity, even a slight variation in the mobile-phase composition may greatly influence the response of the analytes, thereby altering the chemical equilibrium. In addition, all the species involved in the process may compete for the charges available, sometimes unexpectedly suppressing some other chemical species’ signal response. This situation is particularly true for samples of unknown composition when ionization cannot be optimized for all the components.

The ideal conditions for sample ionization by ESI or APCI are often in conflict with the composition of the mobile phase chosen for optimizing the separation. Postcolumn addition of a suitable modifier can sometimes improve ionization and enhance signal response. Fragmentation will be limited and not sufficient for a thorough characterization, however, so analysis must be done on a multistage instrument.

On the other hand, API techniques excel in high-molecular-weight applications or when sensitivity is demanded. High ionization efficiency allows low-picogram detection for many ana-

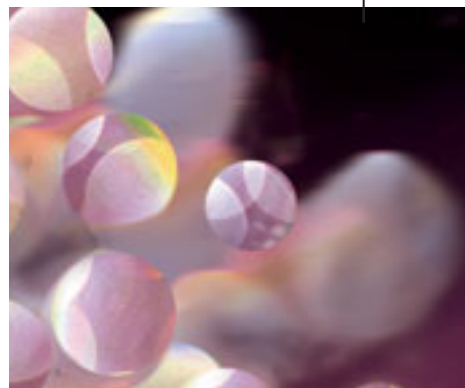
lytes. In addition, EI produces a large number of ionic fragments in a peculiar combination of abundance and m/z , a real chemical fingerprint that characterizes each compound. EI is influenced neither by polarity nor by mobile-phase composition, especially once all the solvents have been removed from the ion source. Although the unbeatable identification capability of EI is precious for detecting unknowns or for confirmation, its accuracy can be compromised by solvent vapor residue from the mobile phase (7, 8). EI does suffer from limited ionization efficiency; however, picogram-level sensitivity is commonly obtained. Library sample identification can be automated with advanced deconvolution software that can even resolve coeluting compounds, thereby enabling high-throughput, fast LC/MS.

The first successful on-line combination of LC and EIMS, called the moving belt interface, dates back more than 20 years and was based on a mechanical connection between the eluate and the ion source. It relied on a rotating belt to evaporate the solvents and transport the solute into the high-vacuum region for vaporization. Although some good EI spectra were recorded, the technique had many drawbacks and was soon replaced by a new concept called particle beam (PB) interfacing.

The PB concept was based on the conversion of the liquid effluent into an aerosol, which, after evaporation of the aerosol droplets and elimination of the solvent vapors through a multistage momentum separator, was transformed into a beam of solute particles. This first attempt, initially known as a monodisperse aerosol generator-based interface, was later renamed PB to emphasize the conversion of the solute into a beam of small particles (9).

Instead of relying on the few available ionization techniques, researchers designed the PB interface to accommodate EI processes. At that time, EI was not a choice; it was a necessity. GC/MS was everywhere, and LC/MS was taking its first steps. As an alternative to the moving belt interface, the PB interface offered a new approach for delivering analytes from a liquid phase to the more energetic gas phase required by EI. In fact, as later confirmed by ESI, the fractionation of the eluate into thousands of aerosol droplets turned out to be the best choice for rapid and al-

**Electron energy
is key for
achieving
ionization and
modulating
fragmentation.**



most complete solvent removal. Instead of freeing charged molecules, as happens in ESI, PB aerosol evaporation creates a beam of solute particles that can be vaporized and ionized in the ion source. Only the most volatile fraction will be lost from the beam, but then, LC/MS is not for low-boiling compounds.

At this point, thermally sensitive or barely volatile compounds that are efficiently transmitted at near-ambient temperature through the interface could be halted in the hot EI ion source by a combination of poor vaporization and chemical decomposition. The key parameter responsible for determining the extent of solute transferred into the gas phase is the surface-to-mass ratio of the particles facing the exposure to heat. The higher the value, the faster the analyte is vaporized, reducing thermal decomposition and allowing for a larger number of substances to be analyzed by EI.

Of course, such results are acceptable only for those HPLC-amenable substances with a minimum of heat resistance and vapor pressure. For a classic solid-probe introduction, the surface-to-mass ratio is $\sim 10 \text{ cm}^2/\text{g}$, where-

as for particle sample introduction, this value jumps to $>10,000$ depending on the particle size and its distribution. When solute is vaporized in the form of particles, heat conduction is the favored phenomenon. The heat flow through a solid is described by $q = -K(dT/dy)$, in which q is the amount of heat transferred per unit of time and surface, K is the conductivity coefficient, and dT/dy represents the thermal gradient in the solid. A 1000-fold increase in the surface-to-mass ratio has a positive impact on the heat flow because of the simultaneous increase in the total exposed surface and the thermal gradient. However, because PB was designed for high-flow-rate HPLC columns and very small droplets, small particles were rarely obtained. In addition, when water was present in the mobile phase, it evaporated slowly; many wet solute particles were lost when they hit the interface walls, which reduced sensitivity—especially when reversed-phase HPLC was used.

The smaller, the better

The impact of a liquid phase on an almost vapor-free environment containing only solute is always dramatic. The unavoidable long chain of interface mechanisms used to remove the solvent while saving the solute for ionization requires a compromise in performance: limits on sensitivity, range of linearity, tolerance for water in the mobile phase, and tolerance for nonvolatile, thermally sensitive compounds.

The way to improve EI of an HPLC eluate is straightforward—reduce the flow rate (10). A few years ago, in collaboration with Waters, we developed a new LC/MS interface called capillary-EI (Cap-EI), which is based on a typical PB interface with a radically new nebulizer and simplified hardware (11). The optimal operating liquid flow rate in the interface is 1–5 $\mu\text{L}/\text{min}$. Because the impact of the liquid is drastically reduced, the interface can be simplified without altering the solute transport efficiency. To accommodate the lower flow rate, we redesigned the nebulizer. Old instruments equipped with a PB interface used a pure pneumatic nebulizer (Figure 2a).

In a pure pneumatic nebulizer, the liquid effluent released by the chromatographic column is forced into a capillary surrounded by a helium flow. The liquid stream increases its linear velocity and breaks up into small aerosol droplets while exiting the tubing. The droplets are created because of the natural instability of the high-velocity cylindrical liquid jet in open space. The size of the droplets depends essentially on the nature of the liquid phase and the diameter of the capillary. Temperature can also influence droplet size. The surrounding helium mixes with the incoming aerosol. Helium does not promote aerosol formation but prevents droplets from coalescing or impacting on the interface walls. A mixture of only solute particles, solvent vapors, and helium should pass through the nozzle at the end of the desolvation chamber. As a matter of fact, only a 100% organic solvent mobile phase can be completely removed by using a pure pneumatic nebulizer. Because of its low volatility, any water in the mobile phase slows down the process, thereby reducing the number of

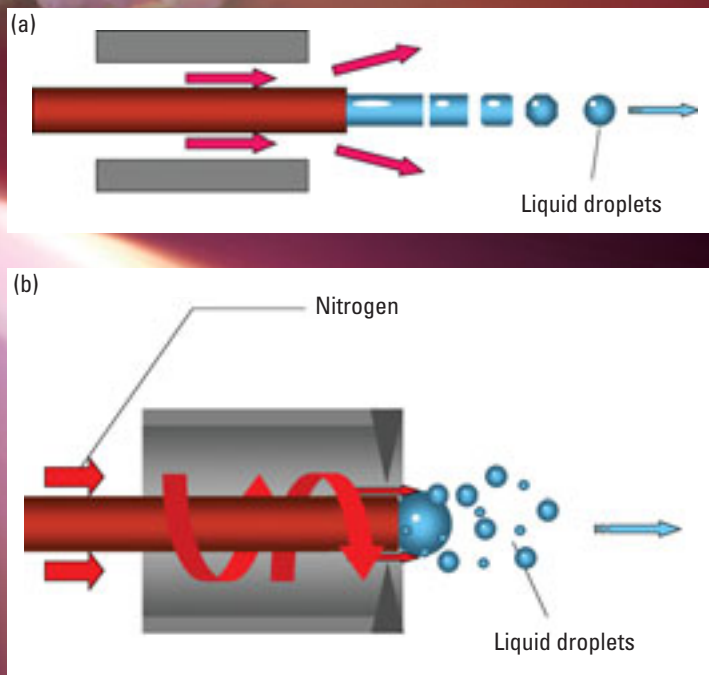


FIGURE 2. How they work.

(a) Scheme of a pure pneumatic nebulizer. The aerosol is formed by the natural instability of the liquid when it exits the tubing. Droplet dimension depends on the capillary diameter, liquid linear velocity, temperature, and cohesion forces within the liquid phase. Helium mixes with droplets to avoid aggregation. (b) In a Cap-EI interface, the gas is forced through a narrow passage around the capillary, thereby increasing its velocity. The gas jet breaks up the emerging liquid, producing a very fine aerosol scarcely influenced by other parameters such as temperature or mobile-phase composition. Cap-EI creates smaller solute particles with higher surface-to-mass ratios.

dry particles that can escape the nozzle gap. In reversed-phase conditions, early peaks eluted when the water concentration is higher are always discriminated, which negatively affects their detection limits.

The Cap-EI interface relies on a pneumatic nebulization mechanism based on a high-velocity gas jet coaxial with the liquid stream (Figure 2b). In this nebulizer, dispersion gas plays an active role in generating the aerosol. To do so, the gas orifice forms a sharp restriction just around the end of the capillary tubing where the mobile phase protrudes. The gas is thus forced through the ring-shaped passage, which sharply increases its speed. The high-velocity surrounding gas fractionates the emerging liquid into small liquid droplets and forms a very fine and homogeneous aerosol.

One of the major improvements is that the new nebulizer is fully compatible with nitrogen instead of helium, and the highest aerosol production is obtained at a lower flow rate (0.1–0.2 mL/min), thereby reducing gas costs. Because of the drastic reduction of the liquid intake, a capillary-scale nebulizer is no longer influenced by the volatility of the solvents used in the mobile phase. The new nebulizer operates at ambient temperature and requires neither capillary tubing position nor gas-pressure adjustments. The initial setup procedure is made during instrument installation and is valid for any analyte and any chromatographic condition. Because tuning procedures are not needed, start-up is shortened considerably and the overall instrument throughput is optimized. Sensitivity is significantly improved compared to PB, whatever the mobile phase or other chromatographic conditions might be. Despite these improvements, momentum separation of solute and solvents prior to solute particle vaporization and ionization is still required, and thus additional pumping stages are needed in which some sample can accidentally sink.

The commercial availability of good, reliable nanoscale HPLC columns recently provided the opportunity to develop a very straightforward LC/EI MS interface called Direct-EI (12). The goal is to give the general GC/MS customer an LC option but still use the same mass detector and software with only a minimal adjustment in instrumentation. Direct-EI is an almost no-interface device through which the HPLC effluent directly enters the EI source.

Readers may remember that, more than 20 years ago, all interfaces were abandoned and MS became the common detection scheme of many gas chromatographs worldwide because of the rapid diffusion of fused-silica GC columns. The direct liquid introduction approach was developed at the very beginning of

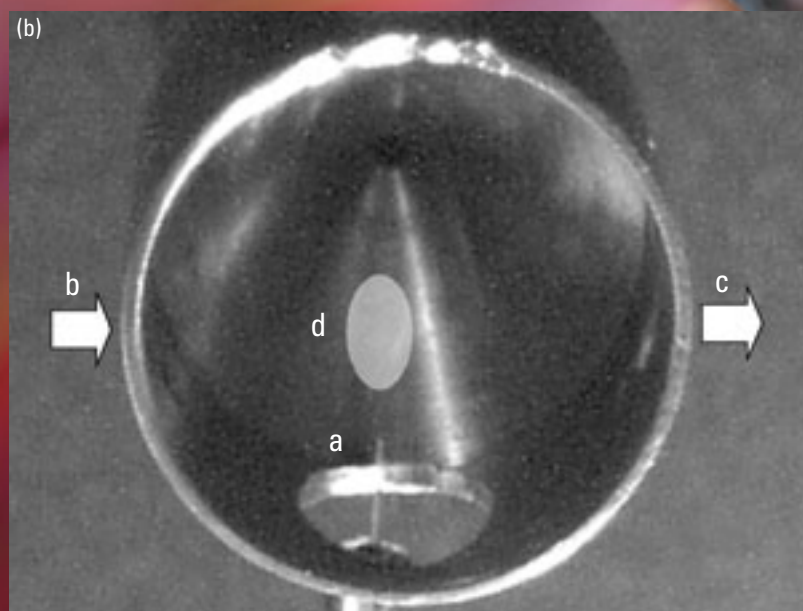
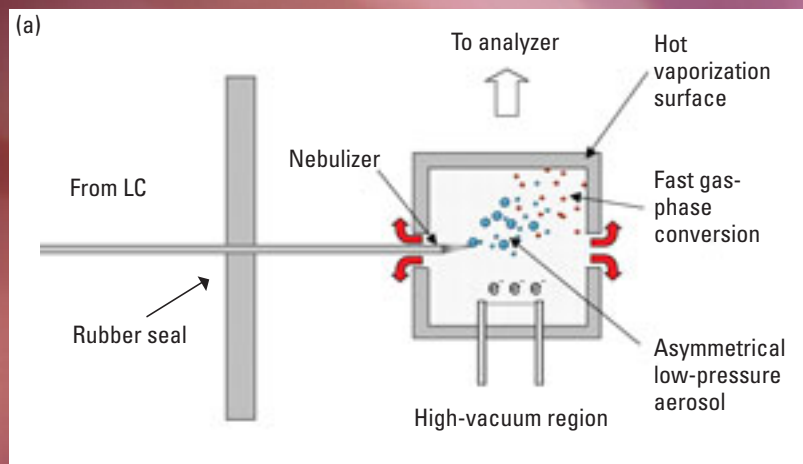


FIGURE 3. Direct-EI interface.

(a) A stream at a nanoscale flow rate is nebulized inside the ion source and proceeds toward a vaporization surface. During a very short journey, the droplets are converted into solute particles that are vaporized against the hot surface. Solvent vapors are removed from the source by an additional opening, thus reducing the risk of CI processes. (b) Microphotograph of the modified ion source in a Direct-EI interface. a, nebulizer tip; b and c, electron path; and d, solute vaporization spot.

LC/MS, but because it suffered from the lack of a reliable capillary LC instrument, it was prematurely discarded (13–17). We fully exploited this past experience and added fresh technology to avoid some of the weak points. The originality of our approach is to squeeze all the interfacing process into the small volume of the EI ion source of the mass spectrometer.

The flow rate accepted by the interface is a maximum of 0.8 $\mu\text{L}/\text{min}$ for most applications. The interfacing mechanism is based on the formation of the aerosol in high-vacuum conditions, followed by a quick droplet desolvation and final vaporization of the solute prior to ionization. The process requires <8 mm of space and only slight modifications to the original EI ion source (Figure 3a). At the core of the interface is a micronebulizer, which consists of a cone-shaped tip slightly bent sideways and an orifice of $\sim 5 \mu\text{m}$ (Figure 3b). The nebulizer tip protrudes 2 mm into the ion source. The emerging liquid phase is con-

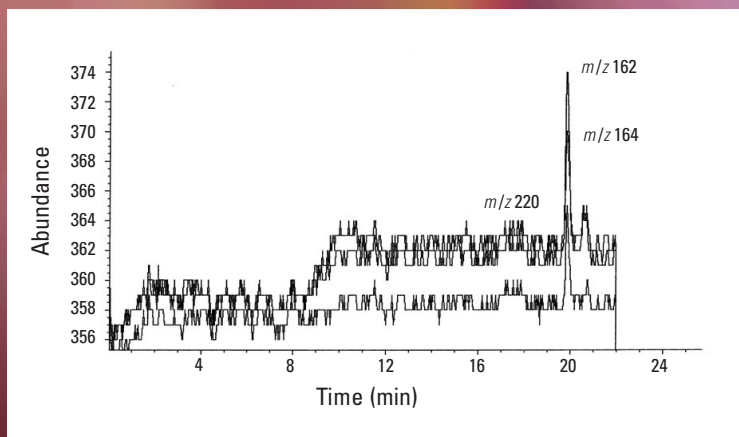


FIGURE 4. Ion profiles of the on-column detection of 100 µg of 2,4-D. Injection volume, 10 µL; flow rate, 2 µL/min. (Adapted from Ref. 27.)

ticular transport mechanism involved in the interfacing process reduces sample losses, enhances sensitivity, and extends the range of possible applications. In other words, the interfacing process has less influence on the sample transition, and the only limitation is the applicability of the EI. Clogging of the interface or CI was not observed. Direct-EI is even compatible with high concentrations of nonvolatile buffers in the mobile phase, opening the door to a wide range of challenging applications.

Other groups are heating up the battle for the best EI-based LC/MS interface. Amirav and co-workers are investigating a new apparatus for obtaining high-quality, library-searchable EI spectra based on supersonic molec-

ular beam (SMB) MS (18). In their system, the output of a liquid chromatograph (50–250 µL/min) is vaporized at atmospheric pressure and expands from a supersonic nozzle into the vacuum system as neutral molecules. The vibrationally cold molecules in the SMB are ionized by 70-eV electrons in a fly-through EI ion source and analyzed by a quadrupole mass analyzer. Sample vaporization is based on spray formation followed by fast, soft vaporization of thermally labile compounds behind or inside a channel supersonic nozzle. The short time spent by the compounds at the heated nozzle substantially reduced the degree of molecular dissociation. The ionization of vibrationally cold molecules in the SMB (cold EI) gives mass spectra that are a combination of the standard library-searchable EI fragments plus enhanced molecular ion that are almost always observed, a result indicative of accurate sample identification.

Performance and applications

Direct-EI and Cap-EI interfaces were tested with several compounds of environmental, forensic, pharmaceutical, and biological interest under very different analytical conditions (19–29). Pesticides, hormones, coumarins, explosives, and nitro-PAHs were determined at trace-level concentrations in various matrixes, demonstrating good versatility of the approach. Many compounds were chemically sensitive or difficult to vaporize by GC/MS, but thanks to the advantages of microflow nebulization, all compounds gave stable and intense signals.

One of the most challenging applications is enhancing the tolerance toward nonvolatile buffers added to the mobile phase. Nonvolatile ionic species, such as phosphate and sulfate buffers, in the ESI spray are deleterious; they cause salt deposits on the metal surfaces and a complete loss of ion transmission. In addition, if the anion and cation pair too strongly with the analyte, then the analyte ions may be prevented from carrying the excess charge on the droplet surface, and a very low ESI response may result. For these reasons, when ESI is interfaced with HPLC or CE, volatile buffers composed of weak acids and bases must replace nonvolatile modifiers (29).

In addition, strong acids, such as trifluoroacetic, heptafluorobutyric, and hydrochloric acids, which are used as ion-pairing agents, tend to mask the analyte signal (30–32). Under certain conditions, nonvolatile buffers do not interfere with the performance of the micronebulizer. Because of the extremely reduced liq-

verted into aerosol droplets and directed asymmetrically toward a specific target surface. Because of the reduced flow rate, droplets are quickly evaporated and converted into particles before reaching the surface. Particles from a 1-mg/mL phosphate buffer collected on the ion source surface that served as the particle target over several hours at a flow rate of 300 nL/min. This result translates into a deposition rate of 0.3 µg/min inside the ion source that did not affect the spray mechanism. Most particles were in the micrometer-diameter range, ~60,000 cm²/g of surface-to-mass ratio for a particle with an average size of 1 µm.

The mass spectrometer was tested intensely during method development for the pharmacokinetic evaluation of an anti-inflammatory drug. The spectra of dexamethasone, acquired before and after introduction of the buffer, show that instrument performance was stable. A 10-mM phosphate buffer was introduced into the system, and several parameters were continuously monitored for 60 h of total acquisition time. The repeller potential, a critical parameter indicative of variable instrument performance, was never influenced by the long-term buffer intake. An estimated total of 5 mg of phosphate salt introduced into the system was barely visible as a fine, even dispersion of white powder inside the ion source. The buffer deposit was mixed with other brownish, unvaporized material and was easily removed during cleaning.

The high temperature of the ion source, 200–300 °C, has a double function: It compensates for the latent heat of vaporization during the droplet desolvation and converts the solute into the gas phase upon contact with the hot target surface. The nebulizer is connected to the analytical nanocolumn by 30-µm i.d. capillary tubing. The tubing is kept well insulated from the source heat to avoid premature mobile-phase vaporization and possible solute degradation. An additional opening, opposite the nebulizer tip, speeds up solvent vapor removal and avoids ion-molecule reactions. Neither the electron path nor the electric fields are influenced by the interface intrusion into the ion volume, and high-quality mass spectra are thus produced. The tip of the nebulizer can be replaced to accommodate different mobile-phase flow rates. The lowest flow rate required for generating a fine and homogeneous aerosol is 100 nL/min. The major advantages are extreme compactness and simplicity without the need for additional complex devices between the liquid chromatograph and the mass spectrometer. The lack of any par-

uid intake, the Cap-EI and Direct-EI slowly displace negligible salt deposition within the ion chamber with no appreciable interferences in ion generation. After many days of continuous operation, a whitish salt deposit is barely visible in the ion source and can be removed by routine maintenance before changes in performance are observed.

To demonstrate the feasibility of the new interfaces withstanding a continuous intake of nonvolatile buffers, hexylammonium phosphate, a non-volatile salt used as an ion interaction reagent (IIR), was added to the mobile phase for the simultaneous separation of hydrophilic and hydrophobic herbicides (27, 28). The IIR is the salt of a cation with a lipophilic chain that can be absorbed onto the surface of the stationary phase in a common reversed-phase column. The cation and its counterion give rise to an electrical double layer that retains and separates cationic and anionic species. In these conditions, acidic pesticides were eluted before the basic ones. All pesticides except bromoxynil and dichlorprop are fully separated. Completely different ion profiles allow MS separation of the two analytes. Without the modifier, this analysis would require two separate injections and two types of columns. The interface operated at a mobile-phase flow rate of only 1 $\mu\text{L}/\text{min}$, resulting in a salt deposition rate of $\sim 0.5 \mu\text{g}/\text{min}$ for a 5-mM reagent concentration in the mobile phase, which did not interfere with analyte ionization. Detection limits range from a few picograms to nanograms, depending on compound behavior in the EI source. Figure 4 shows the ion profiles recorded from an on-column injection of 2,4-D. For all tested substances, we always generated good-quality, library-matchable mass spectra and obtained a concentration linearity range of at least 2 orders of magnitude.

EI may offer a clear advantage over ESI in several applications, such as organophosphorus pesticides. In particular, parathion-ethyl and -methyl that give no signal with ESI generate an intense and very informative spectrum using EI (Figure 5). When this spectrum was compared to a reference spectrum, a matching quality of 68% allows appreciable identification in real sample applications. Better matching quality results ($>90\%$) were obtained for phorate, phoxim, diazinon, and other pesticides.

LC/EI MS methods and instrumentation are not a replacement but rather a complementary approach to ESI-MS methods. They may extend the simplicity of GC/MS over many HPLC-amenable compounds without compromising the full potential of LC.

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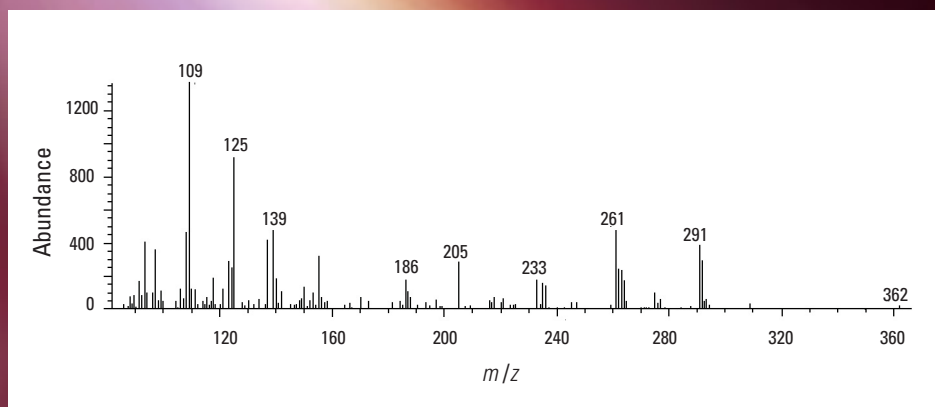


FIGURE 5. Mass spectrum of a 6-ng injection of parathion-ethyl.

new methods for preparing biological, pharmaceutical, and environmental samples with complex matrices. Address correspondence to Cappiello at Istituto di Scienze Chimiche "F. Bruner", Università di Urbino, Piazza Rinascimento 6, 61029 Urbino, Italy (achille@uniurb.it).

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