



MS –

The Practical Art

The spread of liquid chromatography–mass spectrometry as an analytical tool during the past decade has promoted efforts to better accommodate applications. The physical realm of the atmospheric ionization source — materials, gas, and heat dynamics — and manipulating the ionization modes themselves perhaps are the current forefront of technological innovation.

Source Design and the Utility of Multimode Ionization

Because mass spectrometry (MS), and specifically liquid chromatography (LC) coupled with MS, has become such a mainstay in laboratories, the publishers of *LCGC* feel that a column addressing topical MS interests with a focus on the practical aspects is timely. This column is the first in a series that will deal with a variety of topics oriented toward the practice of MS.

In 1998, I had the opportunity to write an article to mark the 10-year anniversary of LC–MS (1). In that article, I reviewed the rapid growth of LC–MS as an analytical tool, and described some of the technologies that made it happen. Arguably, LC–MS had grown in that 10-year period from its academic roots to a commercially viable phenomenon. Researching and writing the article was a formidable task; however, looking back on it five years later, it was a worthwhile undertaking. The fundamental tool analysts have come to use in various routine tasks on a daily basis has created an increasing demand for improvements in how they can best put the technique to use. Practitioners are looking to forums and information sources not only for answers but also for a venue of increased understanding.

The American Society for Mass Spectrometry (ASMS), which celebrated its 50th anniversary in 2002, has been one of the central sources for LC–MS knowledge during the past 25 years. One of the true pioneers in the field and longtime member of the community, John Fenn, formerly of Yale University (New Haven, Connecticut) but currently at Virginia Commonwealth University (Richmond, Virginia), was honored at the annual meeting this past June in Montreal. Fenn had just received the Nobel Prize in Chemistry for his work originally published in 1988 (2) that characterized the fundamental properties of atmospheric-pressure ionization — what we think of as electrospray ionization — which opened the doors to what many analysts use to great advantage today.

The one- and two-day LC–MS short courses held during ASMS and other conferences have been well attended each year. The practitioners using LC–MS have come increasingly from among the ranks of those not trained specifically in MS. Chromatographers and other separations scientists routinely take advantage of the more mature robust hardware and software that MS provides. The increase in ASMS membership is well documented — from approximately 1200 members in the mid-1980s to 4500 this past year. The conference topics have reflected the change in membership interest as well. I noted in an article published as an ASMS preview last year that although discussions of MS physics and fundamentals dominated the literature in the 1950s, today's topics cover a wide range of interests from cutting-edge applications in biopolymers to discussions of enhancing routine analysis (3). Some conference sessions such as the LC–MS workshop drew as many attendees as the conference itself did in the 1950s.

Beginning in February 2004, I will present MS topics of interest to *LCGC* readers in a bimonthly column titled “MS — The Practical Art.” As the name implies, I will not limit my focus to on-line LC–MS techniques and practices; instead I will look at whatever could affect the practice of MS. Sample preparation and the related advances in materials certainly will be among the deserving topics as will be alternative techniques such as matrix-assisted laser-desorption ionization (MALDI) and desorption-ionization on silicon (DIOS). I encourage readers to let me know what areas they think should be discussed in this column. In addition to contacting me through *LCGC* by mail or e-mail, readers can reach me through the ASMS web site at <http://www.asms.org>. This web site has discussion groups for a number of topical interests, including LC–MS, which I currently moderate. Often, the conversations I encounter on the ASMS web site will affect

the content for the coming year at ASMS in oral session topics as well as the LC–MS workshop, so please feel free to comment.

Source Design: The Utility of Multimode Ionization

When laboratory workers think of LC–MS, they could make assumptions based upon practice rather than theory. For example, as a practical matter electrospray is a technique separate from other forms of ionization. Although related, atmospheric-pressure chemical ionization is unique and requires high heat and high LC flow rates, correct? So, the two techniques are inimical (see Table I).

In many pursuits, chemical diversity presents a thorny analytical problem. Although researchers have known from the earliest investigations of atmospheric ionization that several modes of ionization can and do occur, sometimes simultaneously, electrospray ionization has become the default mechanism for approximately 80% of the work. Therefore, electrospray ionization also has received the bulk of the research and development resources, and the adopted software and hardware conventions reflect its utility. What about the remaining 20% (or in some cases in which weakly polar analytes predominate the practice, it might be more like 100%)?

The practice has been to physically remove analytes that failed to perform under electrospray ionization and either repeat samples (which also could include sample preparation such as removing the fraction) and subject them to atmospheric-pressure chemical ionization or use another technique such as electron ionization. Placing an analyte on a solids probe, which would require additional sample preparation, is common practice only for the more recalcitrant analytes such as the reagent monomers that can appear as synthetic impurities in early drug discovery. This manual intervention is costly to an industry that relies on quick and accurate assessments of novel chemistry to determine feasibility as a drug candidate.

In other words, practitioners first would have to identify the failed analytes by electrospray and then remove them to a system capable of atmospheric-pressure chemical ionization, which would involve the usual time-consuming steps of optimization. Often analysts who are most impeded by this type of shortcoming are not overly concerned with absolute sensitivity; utility or productivity is far more important to them.

Following several discussions about these topics and questions such as “Why can’t we do atmospheric-pressure chemical ionization at electrospray ionization flow rates?” I had the opportunity to pursue a modification of the ionization source used by single quadrupole and triple quadrupole instruments as well as time-of-flight (TOF) instruments to address the problem.

The technology envisioned to solve this problem was a consequence of some benefits already inherent in the electrospray source design. Some work performed by others helped point the way. Marshall Siegel (Wyeth Ayerst, Pearl River, New York) had published work about various means of obtaining both electrospray and atmospheric-pressure chemical ionization from the same instrument by improving the conditions required for desolvation using preheated nitrogen nebulizing gas flow and external switching to apply voltage to either the electrospray probe or the atmospheric-pressure chemical ionization pin used to generate the plasma (4).

The ESCi multimode ionization technology is the result of these elements and the inherent design features of the electrospray source (5). The rather small volume of the enclosed source housing at typical operating temperatures provided a secondary desolvation of the electrospray aerosol as it encountered the plasma on the atmospheric-pressure chemical ionization pin. The solvent, throughout a surprisingly wide range of LC flow rates, was dry enough at this point to enable true atmospheric-pressure chemical ionization charge transfer. Designing high-speed elec-

tronics created a same-source device capable of acquiring positive and negative electrospray and atmospheric-pressure chemical ionization at millisecond intervals (see Figure 1).

A Quick Review of the Processes Involved

Gas and heat dynamics are key elements in creating droplets, which are amenable to ion production, in an aerosol. When using typically useful LC flow rates of 50 $\mu\text{L}/\text{min}$ –2 mL/min, analysts must use sufficient heat in association with flowing nitrogen gas. The electrospray phenomenon occurs through a concentration of charge on the surface of droplets formed by imposing a high current, which exceeds the Rayleigh limits of the liquid, on the flowing stream as it emanates from a conductive spray tip. As the droplets desolvate to approximately 10 μm in diameter, the ions are liberated from the surface if the analytes are sufficiently polar or can be protonated in some way from the solution. If the analytes are weakly polar, they might not protonate directly but could be amenable to chemical ionization or atmospheric-pressure chemical ionization. This alternate form of ionization typically has been performed at much greater linear velocities than those provided by the LC flow rates associated with electrospray (closer to 1 mL/min as opposed to decreasing the flow to improve electrospray performance). The ionizing potential is applied, not through the liquid as in electrospray ionization, but at the tip of a needle as a plasma or corona through which the droplets pass. In effect, the mobile phase acts as an intermediary to transfer the charge to the analyte. An excellent review of the current understanding of these mechanisms can be found in a recent

Table I: Some differences between the two primary techniques

Atmospheric-Pressure Chemical Ionization	Electrospray Ionization
High LC flow rates provide better linear velocity	Much better efficiency at low LC flow rates
High heat input required	Low (or no) heat input required
Intended for analyzing weakly polar or neutral analytes	Intended for analyzing polar analytes

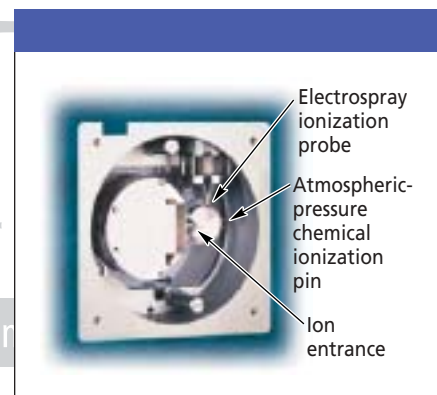


Figure 1: The ESCi multimode ionization source. (Courtesy of Waters Corp., Milford, Massachusetts.)

article by one of the foremost authorities on the subject, Paul Kebarle (6).

With everything researchers know currently, it still is the transfer of the ions generated at atmospheric pressure into the vacuum system that impedes the next major developmental step. These losses can range from 100 to 5000 molecules in the original solution to yield a single ion entering the vacuum chamber, and the efficiency is highly dependent upon the flow rate of the original solution as well as the chemical characteristics of the molecules themselves. According to Tom Covey of MDS Sciex (Concord, Ontario, Canada), who is one of the individuals largely responsible for our ability to use electrospray at utilitarian flow rates,

“Dispersion of the aerosol droplet to regions remote from the entrance aperture and incomplete droplet desolvation play a major role in ion losses. Both of these are controlled by fundamental gas dynamic principles which can be modeled both analytically and with computational fluid dynamic models which reveal that, with sonic nozzles such as are used to nebulize the chromatographic eluent into the electrospray ion source, the physical processes of gas entrainment and recirculation dominate the trajectory of the aerosols. The motivation behind the design of this [Sciex 4000] ion source was to control and manipulate the gas entrainment in the nebulized aerosol with strategically located high temperature gas injectors.”

Recent developments have attempted to use these characteristics as added instrument capability. The Turbo V source (MDS Sciex) used with some MDS Sciex instruments allows electrospray and atmospheric-pressure chemical ionization to be optimized in the same source through the use of specifically designed

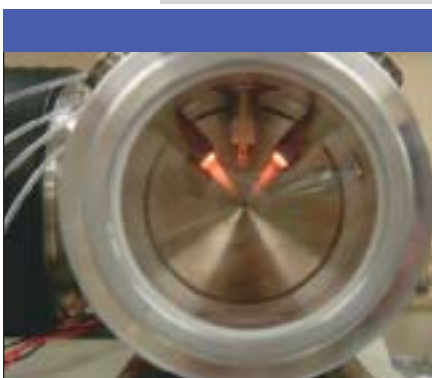


Figure 2: The MDS Sciex Turbo V source. (Courtesy of MDS Sciex, Toronto, Ontario, Canada.)

materials and temperature control (see Figure 2). In this case, the design intent takes advantage of naturally occurring hydrophilic differences to enable time to heat or cool the source and provide optimum performance. That is, the weakly polar analytes will not be eluted until later in the run so the time in minutes between electrospray ionization acquisitions and atmospheric-pressure chemical ionization acquisitions is used to bring the environment to atmospheric-pressure chemical ionization–amenable conditions (7).

The control of heat and gas dynamics obviously is important when transitioning reproducibly between the conditions amenable to electrospray ionization and the drier vapor environs necessary for atmospheric-pressure chemical ionization. Ceramic surfaces, which supposedly have low adsorptivity (reduced sample memory or persistence), and attempts to draft exhaust vapors after the aerosol is formed are critical.

Thermo Finnigan (San Jose, California) instruments follow similar physical constraints, of course, but this company has its own solution. A recent characterization by Rohan Thakur of Thermo Electron Corp. points out that the company’s Ion Max source relies upon controlling heating and gas dynamics as well but focuses the aerosol into “a contoured ion-entrance heated tube. The exhaust or drain port is positioned directly across from the aerosol to minimize additional gas interaction.”

Mixed and Multiple Ionization Modes

One of the earliest practical forms of ionization used in LC–MS was called thermospray (8). The technique, which now has been superseded by electrospray ionization, produced ions through desolvation of the liquid stream using the shear forces associated with largely aqueous mixtures at high flow rates (linear velocities) by adding heat. Unfortunately, cumbersome pumping and trapping systems were necessary to remove the roughly 1-L/min amount of resulting vapor into a vacuum system at the optimal 1-mL/min mobile phase flow rates. This technique worked better with highly aqueous mobile phases.

Bear in mind it is common to see thermally induced ions with some compounds akin to those found in early thermospray work, but with today’s techniques they are produced at atmospheric pressure rather than under reduced pressure as with thermospray. It also is common to find that

adjusting solvent characteristics — increasing or decreasing the pH to shift protonation tendencies or changing the surfactant properties of the liquid to promote the liberation of ions from the surface once formed — can reveal electrospray ions from an analyte previously thought to be intractable.

The development of a high-speed electrospray–atmospheric-pressure chemical ionization switching source made differentiating the ionization mechanisms in the early efforts of paramount interest. Questions naturally arose such as “How much time must be allowed when changing between electrospray ionization and atmospheric-pressure chemical ionization?” and “How can we be sure we are performing true atmospheric-pressure chemical ionization?”

Several applications of corona (or plasma)-assisted electrospray ionization have been used for large molecules (9) as well as a variety of interesting mixed-mode behaviors (10).

My co-workers and I used a simple set of circumstances to prove the boundary and fidelity of the ionization state that occur in our multimode ionization functions. Compounds that easily protonate in the electrospray mode can be added with those that yield characteristic signatures such as phenones, which produce radical cations only under atmospheric-pressure chemical ionization. Figure 3 provides an example of how multimode ionization can switch at high speeds between electrospray and atmospheric-pressure chemical ionization to capture analytes that might not be acquired easily, such as weakly polar or neutral species that do not protonate. Ultimately as many as four modes — positive and negative in electrospray ionization and atmospheric-pressure chemical ionization — are acquired and mapped by the UV signature for each analyte. At each mode switch, we were able to detect any indication of carryover in the assigned channels.

The requirement to provide the utility for this type of application required designing suitable software and a power supply. The power supplies in many quadrupole instruments required internal scan delays of 300–500 ms to stabilize changes before acquisition. That time was wasted because no data points were acquired while a chromatographic peak was in transition. The power supply initially developed for the multimode ionization technology could switch between positive and negative and between voltage (for electrospray ioniza-

tion) and current (for atmospheric-pressure chemical ionization) in less than 100 ms. The next-generation power supplies work in less than 10 ms.

When users attempt to switch at very high speed between two heretofore inimical modes of ionization, they also need to optimize the behaviors in the presence of each other. In a reversed-phase separation, weakly polar analytes that require atmospheric-pressure chemical ionization

undoubtedly will be more likely to appear later in the run, so a method can be set to maximize the duty cycle by invoking the mode later in the run. The tune page is a real-time monitor that allows operators to view the effects of changes to gas, heat, and various voltages on ion propagation. As Figure 4 shows, the tune page software permits the optimization of electrospray and atmospheric-pressure chemical ionization compounds as they are infused.

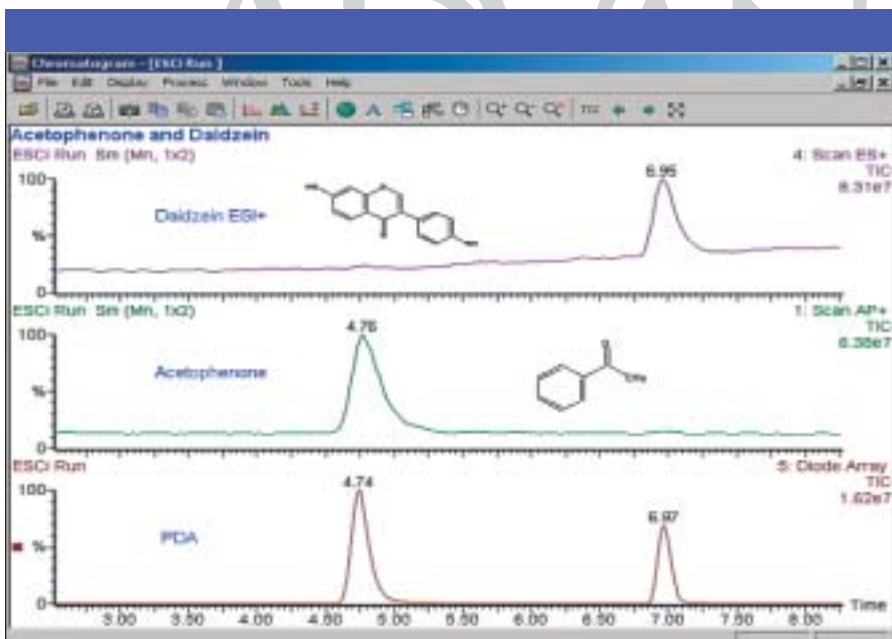


Figure 3: An example of multimode ionization and its ability to switch at high speeds between electrospray and atmospheric-pressure chemical ionization. (Courtesy of Waters Corp.)

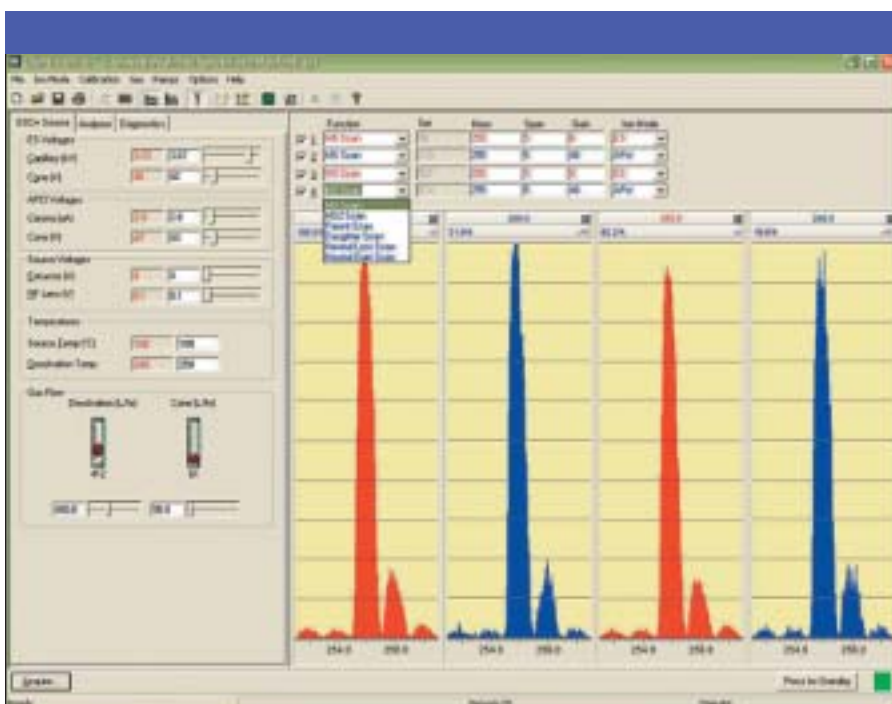


Figure 4: An example of the tune page real-time monitor and the optimization of electrospray and atmospheric-pressure chemical ionization compounds. (Courtesy of Waters Corp.)

One of the unique benefits of this technique is the ability to perform atmospheric-pressure chemical ionization at electrospray ionization flow rates; therefore analysts also can use nitrogen at the lower flow rates common to electrospray ionization operation. Solvent consumption can be reduced to 50 $\mu\text{L}/\text{min}$ flow rate with an appropriate reduction in nitrogen while maintaining the column loading associated with larger-diameter formats.

Among my earliest collaborators in this research were Richard Gallagher and co-workers from Astra Zeneca (Macclesfield, United Kingdom), who provided a perfectly suited test case. They faced the task of characterizing a 500,000-analyte library. Modest flow rates (0.7 mL/min) and sensitivity — typically no more than 100 ng — and otherwise typical constraints of this practice (80% of the compounds worked well by electrospray ionization) required removing the remaining 20% (or 100,000 analytes) as they were discovered to a second operation for characterization. The resulting benefit for Astra Zeneca when its workers compared the throughput of electrospray ionization alone, atmospheric-pressure chemical ionization alone, and multimode ionization was a 10% increase in efficiency (see Figure 5). They found 50,000 more compounds using the high-speed switching method the first time, which they otherwise would have needed to rerun (11).

On the Horizon

The benefit of undertaking work such as this research is to open doors for future developments. By using reproducible observations about behaviors that analysts always associated with electrospray ionization, researchers now can match these behaviors with atmospheric-pressure chemical ionization mechanisms to obtain better insight into the gas and heat dynamics that occur in the source. This area is of fundamental importance, but unfortunately it is very difficult to model and observe effectively. Although the importance has not escaped most researchers, the difficulty of providing proof has made progress slow.

Other areas worth watching — in conjunction with and building on what scientists already have learned — are uses of novel ionization technology. At the ASMS meeting in Montreal, Karl Hanold of Syngen Technology (Tustin, California) presented work about using the electrospray aerosol to leverage atmospheric-pressure photoionization. Atmospheric-pressure photoionization typically is hosted using a

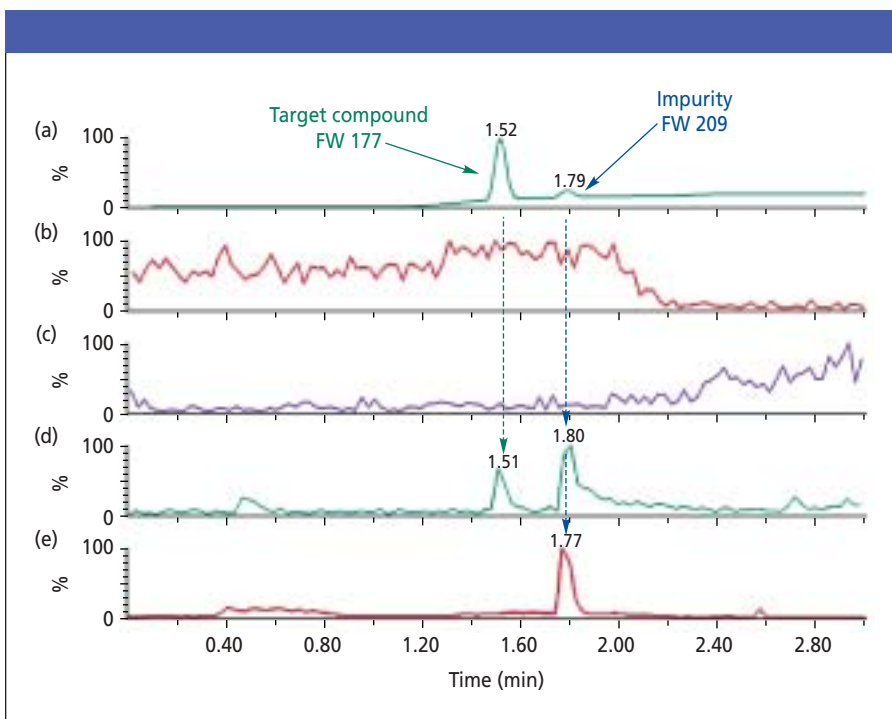


Figure 5: Astra Zeneca example in practice. Shown are results from (a) photodiode-array detection, (b) negative electrospray ionization, (c) negative atmospheric-pressure chemical ionization, (d) positive atmospheric-pressure chemical ionization, and (e) positive electrospray ionization. (Courtesy of Richard Gallagher, Astra Zeneca, Macclesfield, United Kingdom.)

traditional atmospheric-pressure chemical ionization aerosol, but the two techniques have a large overlapping range of analyte ionization performance. The more refined nature of the electrospray ionization spray seems to hint at a more utilitarian multi-mode device with a broader range of ionizable analytes, an ability to switch between modes without intervention, and sufficient independence between the two techniques to allow true optimization of both modes independently.

Source design is the remaining frontier for what started in the late 1980s and has provided us with such a powerful analytical tool. The motivating force behind such invention is what it always has been — the need to answer questions better and faster with tools that provide greater utility.

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