Mass Spectrometry Forum

Chemical Noise in Mass Spectrometry

Part II — Effects of Choices in Ionization Methods on Chemical Noise

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Kenneth L. Busch is in pursuit of the grand unified theory of mass spectrometry. Within the context of signal and chemical noise, this quest seeks to identify intrinsic elements of signal and chemical noise in a mass spectrum, and then to design basic approaches that amplify the elemental differences. Pondering such topics fills the strap-hanging commute time to his office at the National Science Foundation in Arlington, VA. As always, the opinions and views in this article are strictly his own and not those of the National Science Foundation. He can be reached at buschken@hotmail.com.

In the previous installment of "Mass Spectrometry Forum," we provided examples of procedures undertaken to reduce chemical noise in mass spectrometric measurements. These procedures could be initiated anywhere along the path of a sample into the mass spectrometer, or the transit of an ion through the mass spectrometer, from ion formation through mass analysis to ion detection. Reductions in chemical noise are profitably accomplished early on in an experimental sequence. Accordingly, within the mass spectrometer, the ionization process itself can be an effective tool to reduce chemical noise, through informed selection of experimental conditions and ionization method to maximize selectivity and increase sensitivity. The connection of ionization method selection to changes in chemical noise observed in the mass spectrum is explored in this column. In addition to the implicit goal of a reduction in chemical noise, we also show how the use of some ionization methods contributes to an increase in the chemical noise, and how this deleterious effect is accommodated.

Figure 1 classifies ionization methods using characteristics not usually separately identified. Not all ionization methods used in mass spectrometry (MS) are plotted within this simple two-axis depiction, nor can all methods charted be discussed here in detailed relationship to chemical noise. Selectivity in ionization through chemical or physical processes is ordered on the vertical axis. As an example, electron ionization (as reiterated below) is applied as an all-purpose, non-selective ionization method, and so its vertical position is low. Chemical ionization (CI; described in detail in a previous column, 1) can be applied so as to be more selective, based on, for instance, the energetic balance of the proton transfer reaction. Finally, photoionization using a specific wavelength and energy of laser photons to cause ionization is an example of an ionization method that can be extraordinarily selective, and is therefore higher in vertical axis position. Spatial selectivity is ordered on the horizontal axis. Electron ionization (EI), again as an example, deals with gas-phase sample molecules uniformly distributed in an ionization volume, so the degree of spatial selectivity is low. Field ionization is a general-purpose ionization method (as is EI), but because field ionization ionizes only those gas-phase molecules that come into close proximity to sharp emitter tips, the spatial selectivity is increased. As we discuss below, both fast atom bombardment (FAB) and matrix-assisted laser desorption ionization (MALDI) include several specific elements of spatial selectivity at the microscopic level, justifying their positioning on the chart in Figure 1. Increased chemical/physical selectivity in an ionization method is commonly used as a strategy to reduce chemical noise in MS measurements. In contrast, increased spatial selectivity in an ionization method is offset by most current practice in FAB and MALDI, so that selection of these ionization methods leads to increased chemical noise in the mass spectrum.

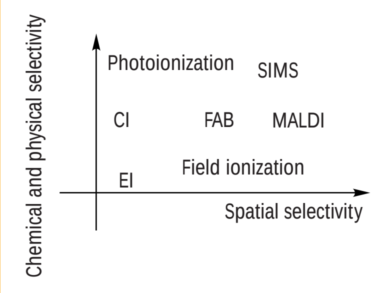

Figure 1. Selectivity factors for ionization methods in MS.

We begin by revisiting EI, a general-purpose method designed to ionize all organic compounds. In EI, an electron of 70-V energy causes the ejection of a single electron from a gas-phase sample molecule M to form the odd-electron molecular ion $M^{+\bullet}$. The mass of the molecular ion $M^{+\bullet}$ is the mass of the sample molecule (with the differing mass of the electron being inconsequential compared with the mass of M). The EI process leaves internal energy in $M^{+\bullet}$ that causes its dissociation to fragment ions, and the pattern of fragment ions (the mass spectrum) can be pieced together to deduce a molecular structure for the compound. The appearance of both $M^{+\bullet}$ and fragment ions derived from the molecular ion — and therefore the deduction of both molecular mass and molecular structure — is the central tenet of EI MS. Remember that 70 V was chosen as the electron energy to provide more than enough energy to ionize all organic molecules, to provide enough additional energy to cause dissociation of the molecular ions, and to provide an overall ionization efficiency not strongly dependent on the exact value of the electron energy. Each of these desired traits is concordant with the use of EI as a general-purpose ionization method.

CI was developed to address the difficulty in identification of molecular ions of low relative abundance (and therefore the difficulty in establishing the molecular mass of the sample) in some EI mass spectra. CI occurs as the result of a collision and chemical reaction between an ion (the reagent ion) and a gas-phase molecule (the sample molecule M). In common applications of CI, the ionization occurs as the result of the transfer of a proton from the reagent ion to the neutral sample molecule to form $(M + H)^+$. The primary reactant ion in methane CI is CH_5^+; this reagent ion acts as a strong gas-phase acid that protonates anything more basic than methane. Almost all sample molecules are more basic than methane, and methane CI is therefore a general-purpose ionization method with wide applicability. The protonated molecule then dissociates to an extent determined by the amount of internal energy left within it as a result of the CI process. The protonated molecule is usually more stable than the corresponding molecular ion formed by EI, and the molecular mass of the compound may be more easily deduced from the higher relative abundance of the protonated molecule in the mass spectrum.

Methane is not the only reagent gas used to create reagent ions that react via the proton-transfer reaction in CI. Isobutane introduced into the CI source generates primarily $(CH_3)_3C^+$ as an acidic reagent ion, again resulting in formation of $(M + H)^+$. Ammonia as a reagent gas creates NH_4^+ as a reagent ion, which forms (*inter alia*) $(M + H)^+$. Protonated molecules can therefore be formed by methane, isobutane, or ammonia CI, but these ions differ in their internal energies. The CH_5^+ reagent ion is a stronger acid, and, in transferring the proton to the sample molecule, produces a protonated sample molecule with a higher amount of internal energy. Since the amount of internal energy controls the extent of dissociation, the protonated molecule formed by methane CI will tend to fragment more extensively than that produced by isobutane CI. We discussed this aspect of CI in a previous column (1).

We did not previously discuss using CI as a selective ionization procedure that can reduce chemical noise. The use of methane creates a strongly acidic reagent ion that will protonate most any organic molecule, creating a general-purpose ionization method. The reagent ion from isobutane is less acidic, and therefore will not protonate water or normal alkanes. The use of ammonia in CI is particularly useful in a selective ionization experiment. The ammonium ion will protonate only those sample molecules with a higher proton affinity than ammonia itself. Hydrocarbons, for example, will not usually be protonated in a direct proton transfer reaction by the ammonium ion. A component of a mixture that is not ionized cannot directly contribute to chemical noise that may appear in a mass spectrum. The distinction between an ionized molecule and a neutral molecule is easily accomplished, and the early discrimination against the ionization of certain classes of molecules of great benefit in producing high quality mass spectra with reduced chemical noise. For example, ammonia CI is often used for selective ionization of heterocyclic compounds in a hydrocarbon mixture (2).

The MS world, however, does not come into a selective balance so easily. In the case of ammonia CI, the protonated molecule $(M + H)^+$ can also be formed by loss of ammonia from another adduct ion formed, namely $(M + NH_4)^+$. The rules for formation of the stable ammoniated species are different from those that govern formation of the protonated molecule (3). Our predicted thermodynamic selectivity may not be displayed in practice. Furthermore, for the purposes of exploring selectivity, and the reduction of chemical noise, we have assumed that sensitivity is equal as CI gases are switched, and there is no requirement that this is true (but neither is it often rigorously explored). However, with care, selective ionization reactions can be achieved in CI, and in the quest for specificity and sensitivity, the analytical consequences may be worthwhile to explore how to do so. Changes in proton affinities, or the tendencies to undergo addition reactions, have even been shown to reflect the stereochemistry of the sample molecules, and therefore the reagent ion can be chosen to selectively or (more usually) preferentially ionize one form over the other.

Summarizing, the simplest strategy in CI for the reduction of chemical noise (but one seldom perfectly attained) is to choose a reagent ion system that selectively ionizes the molecules of interest and not other molecules in the mixture, and to exploit the easy discrimination between an ionized molecule and a neutral molecule. This scalpel-like desire is compromised by the reality of gas-phase energetics and reactivity, which often seem painted with a wider brush. Less explicitly discussed in strategies for reducing chemical noise is the additional fact that CI is a mass-altering ionization process. The mass at which an ion appears can be selected to some extent using CI. The mass difference between the molecular ion $M^{+\cdot}$ and the protonated molecule $(M + H)^+$ is obvious, but conditions can also be chosen to favor the creation of ions at other masses further afield such as $(M + CH_3)^+$, $(M + C_2H_5)^+$, or $(M + NH_4)^+$. Other reagent gases create still other reagent ions (forming ions under a different set of ionization rules), and the array of adduct or other ions appears at appropriately different masses. For example, dimethyl ether CI has been described as appropriate for analysis of a wide variety of organic compounds (4). Dimethyl ether CI can produce $(M - H)^+$, $(M + H)^+$, $(M + 13)^+$, $(M + 15)^+$, or $(M + 45)^+$ ions, depending on source conditions and the type of sample molecule. If the mass of an ion formed in CI (whether a molecular mass ion or a fragment ion) is also the mass of an interferent, then isotopically labeled reagent gases can be used to shift ion masses. The use of an isotopically labeled reagent gas in ammonia CI has been discussed by Ligon and Grade (5). The expense of isotopically labelled reagent gases must be considered in view of whether the quality of the analytical result — that is, the potential reduction in chemical noise — justifies such an expense. But the use is also squarely within the armamentarium of experimental procedures that can reduce chemical noise, and increase the ability to recognize signal. We will discuss characteristic isotopic patterns as a specialized technique to reduce chemical noise in the final installment of this four-column series in "Mass Spectrometry Forum."

The first papers that described the development of FAB MS in the early 1980s included an admission that the mass spectra were "noisy." In fact, it could be seen that the native mass spectra contained a peak at every mass, and those non-sample–related peaks were derived from the FAB "matrix." In some of the very early experiments, this characteristic could be construed as a convenience, since a manual counting of mass was used to determine the masses of protonated molecules significantly higher than the usual operating range. Clearly, however, the presence of such noise, which is by clear definition chemical noise, would become a barrier to the identification of low intensity signals, and a limit to the S/N ratios that could be measured. In FAB, the sample is dissolved in a (usually) liquid matrix. That sample/matrix solution is then bombarded by a fast-moving particle beam. The impact of the primary beam causes evaporation and desorption of the sample/matrix solution, a variety of sputtering processes, and (ultimately) the creation of charge-carrying species related to the solvent that will react to ionize the sample molecule. Sample ions created as small droplets sputtered from the sample solution become smaller through neutral matrix molecule evaporation, ultimately leaving the charge carriers to ionize the sample molecules. In most situations, the ultimate charge carrier is the proton, and the sample molecule is ionized to form $(M + H)^+$. Concomitantly, simple desolvation of the droplets can lead to ions of a pre-charged sample (onium salts, for example).

The placement of FAB in Figure 1 reflects the spatial selectivity inherent in the ionization process. The sample molecules must enter into a solution with the liquid matrix, the matrix must exhibit the proper physical characteristics to form the droplets, the matrix must exhibit the proper chemical character to support the creation of a reactive charge carrier, and all of this must happen within the time and physical constraints of the ionization source. The search for useful FAB matrices indirectly reflects these selectivity factors (6), even if the searches were almost always completed through empirical observation rather than by predictive design. The chemical noise observed in the FAB mass spectrum is a direct consequence of the large surfeit of matrix solvent in comparison to sample, in combination with the physical and chemical processes involved in ionization. The progenitor of FAB, secondary ion mass spectrometry (SIMS), also exhibits a high degree of spatial selectivity, but usually without the concomitant increase in chemical noise. In fact, the ionization process in SIMS can be both spatially as well as chemically and physically selective. The discrimination against chemical noise can be high, and both the surface specificity and high sensitivity of SIMS are characteristics of that technique in consequence.

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Exploitation of the spatial selectivity of FAB to reduce chemical noise in a mass spectrum has been sporadically pursued. A more common approach by users was to follow a (not usually so successful) strategy of background subtraction, artificial thresholding to remove the apparent contribution of the noise, or abandonment of the lower mass regions of the mass spectrum in which the higher intensity of the chemical noise was especially problematic. Fundamentally, however, the preponderance in FAB mass spectra of ions that correspond to surface-active species in the solution was well known, along with the predominance of ions that corresponded to pre-charged organic sample ions. A robust analytical strategy to create such sample molecules through derivatization was germinating when the use of FAB by the MS community was supplanted by electrospray ionization and MALDI. The derivatization concept survives, however, in the guise of experiments called "polar derivatization" or "charge derivatization" (7), and it is used for peptide and oligosaccharide sequencing.

MALDI can be seen to be following many of the same developmental routes as its FAB predecessor. The use of different matrices is explored experientially, with broad groupings of "hotter" or "cooler" matrices. In MALDI, the sample molecules are again mixed in solution with an excess of a matrix, although in MALDI, the end result desired is a thin film of solid crystals supported on a metal surface. These crystals are then irradiated with a pulsed laser; the high instantaneous energy input leads to a mixture of desorption and ionization processes, some of which ultimately lead to sample-derived ions. The excess of matrix leads, not unexpectedly, to a great deal of chemical noise in the MALDI mass spectrum, the noise here again being ions that are derived from the matrix itself. Studies have documented spatial elements of MALDI requisite to successful sample ion production; most studies concentrate on crystal morphology. Because the dependences are known, the spatial selectivities of MALDI are a potentially useful element that can be used to reduce chemical noise in a mass spectrum. We see the beginnings of such analytical acumen in experiments that study desorption from tailored surfaces, including those containing specific adsorption sites. In common practice, however, the potential is unexplored. The near exclusive use of MALDI with time-of-flight mass analyzers engenders an automatic averaging of individual mass spectra that can mask the lower or higher quality of individual mass spectra. The use of MALDI as a molecular mass instrument, with the desired information limited to the usually high molecular mass of the sample, along with the fact that the intensity of chemical noise falls off with increasing mass, means that the centroid of sample molecular mass is easily discerned above the threshold of the chemical noise, even for low sample amounts. When the analytical problems to which MALDI is applied change to require more attention to the chemical noise inherent in the ionization process, new approaches to reduce that chemical noise will be developed, based on the common underlying principles that this series strives to identify.

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