

Challenges in the Interpretation of Data from Hyphenated Techniques

Part I: General Principles

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"I have yet to see any problem, however complicated, which, when you looked at it in the right way, did not become still more complicated."

— Poul Anderson (1)

"What counts in making a happy marriage is not so much how compatible you are, but how you deal with incompatibility." — George Levinger (2)

This first installment in a series about challenges and pitfalls in interpreting data from hyphenated techniques explores general concepts in initial approaches.

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In the expanding world of hyphenated instrumentation, some people act as spectroscopy marriage counselors, interpreting the data offspring of unions of contraptions—unions that can be polygamous and even intrafamily. But this type of person understands that no union was ever achieved without conditions.

In the 1960s, even before attempts at uniting gas chromatography (GC) and mass spectrometry (MS), the principal focus was on getting these hyphenated unions off the ground and flying in formation. Much less thought was given to the influence of the union on the data produced. Part of this may have been due to the sole focus of one technique's experts considering the other technique no more than a useful accessory. Technique moat-digging persists as a significant impediment to interpretational success.

This article is the first installment in a series about the special challenges and pitfalls incurred in the interpretation of data coming from hyphenated tech-



niques. We will consider techniques that involve chromatography and other means of species separation in combination with molecular and even atomic spectroscopies. We will look at multiple spectroscopies and their unique sets of challenges. We will also consider the synergistic use of data from each of the instruments used in the combination and pitfalls that wait for us in this attempt.

We will call on experts in all of these areas to aid us in our search for pitfalls, things to be aware of, tips, techniques, incantations, and prayers for enlightenment. Consider this an open invitation to actively participate in the production of this series of articles. This invitation extends to users, vendors, students, teachers, and all practitioners who have experience or ideas worth considering. One of the great benefits of our corner

of the science community is our ability to share knowledge and information.

Let's first consider some general concepts that may extend over a broad range of hyphenated techniques. Consider these first principles of which to be aware when approaching interpretation from any hyphenated technique.

In the vast majority of spectroscopy techniques, certain spectra can be sensitive to the matrix in which they exist or are produced. Change the matrix, and the spectrum changes. In hyphenated techniques where separations are involved, this possibility must be considered. The separation from its native matrix could change the form of the species and therefore its interpreted identity. Looking beyond simple identification, one must also be aware that the use of the spectral information to extrapolate chemical dynamics may well be influenced in the transfer from one end of the hyphenated technique to the other. At the very least, this valuable information could be lost by isolating the species of interest using a separation technique. Once the species is identified using the hyphenated technique, an attempt to locate the species in the spectrum of its native matrix may produce some interesting information.

Another area to consider is the instrumental systems of the hyphenated technique and their interfaces. In the transfer from one environment to another, changes in temperature, pressure, chemical, and even catalytic interaction can affect the condition of the species of interest.

Consider the early GC-MS systems that used platinum-iridium transfer lines. Until the technique was used by those who had experience in catalytic chemistry, many users and vendors were unaware of the chemistry that might occur in their transfer lines at elevated temperatures. Indeed, catalysis can happen on many surfaces under the right conditions.

A few more examples from the days before the word "hyphenated" applied to instrumental techniques may help to better illustrate this principle. Before the days of GC-Fourier transform-infrared (FT-IR), our laboratory used a

preparative-scale (3/4-in.-i.d. column) GC system to enable trapping of the species of interest. The trapped diffraction would then be run on a dispersive infrared (IR) spectrometer. Usually, it would take several runs to produce enough of the fraction to run on the IR instrument. One day long ago, in this primitive environment, while separating a substance using this ancient technique, I found myself periodically getting extremely nauseated. It wasn't until we interpreted two of the component's spectra as phosgene and thiophosgene that it dawned on me what was happening. The sickening compounds had actually been formed in the chromatograph itself and were not part of the substance under investigation. Breath-

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ing a sigh of relief, I continued to lunch in the company cafeteria and immediately ordered exhaust trunks that would go from the exit port of the gas chromatography to the fume hood system in the laboratory.

This story brings up an interesting side note. Although the practice of trapping the fraction coming out of a gas chromatograph may seem like an idea whose time has long since passed, it may well be that if the component of interest is well separated in the chromatogram, this practice may indeed be preferable to the more expensive hyphenated technique, along with the time spent waiting in the queue for the more popular and fashionable answer.

Another technique that appeared on the scene before the invention of the light pipe was something called *stop flow GC-IR*, which involved the use of a gas cell equipped with IR windows directly attached to the exit port of a gas

chromatograph equipped with a thermal conductivity detector. The flow through the gas cell would be halted when the component of interest was detected by shutting off the carrier gas to the chromatograph. The fraction of interest, trapped in the gas cell, could then be scanned by the IR instrument. In early designs, the principal problem with this technique was that during the time that flow was stopped to trap the component of interest, diffusion within the chromatographic column would destroy the rest of the separation, making it impossible to trap and analyze more than one component per run. Although subsequent designs eliminated this problem, the lesson was that these marriages of instrumentation almost always involve concessions that may affect how we interpret the spectra emanating from our hyphenated techniques.

Another example of this point can be seen in the design of the GC-FT-IR-MS systems. Although the first component of this polygamous marriage is obvious, the configuration of the next two components is not, until one considers the concept of interface volume. One could either have the flow going from the gas chromatograph through the FT-IR and then into the MS system, or split the flow from the gas chromatograph into the FT-IR and MS systems simultaneously. Interface volume, a concept perhaps more familiar to chromatographers than spectroscopists, involves the comixing of separated components, thereby destroying the chromatographic resolution attained. The larger the volume, the more the comixing and the more the resolution of separation is destroyed. This process has a profound effect on the interpretation of what we hope is a separated species. The choice then for a GC-FT-IR-MS system designer is based on the effect of chromatographic resolution on the simultaneous interpretation of data from both spectroscopy techniques. If one designs a system in which the flow from the gas chromatograph is simultaneously split to the MS and FT-IR systems, the chromatographic resolution to the MS system is maximized but does not match the chromatographic resolution in the

FT-IR system, due to the volume of the light pipe. If one designs a system in which the flow is linear from the gas chromatograph through the FT-IR light pipe to the MS detector, chromatographic resolution is the same for both techniques, but is not maximized.

Tough decision for a spectroscopy marriage counselor? Probably not.

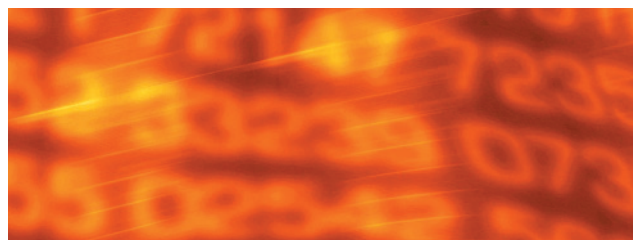
In dealing with biological materials, one should always be aware of the possibility of a substance being labile to thermal or pressure/vacuum extremes. In thermal hyphenated techniques, in which the species of interest is liberated to the analyzing component instrument — by means of thermal ramping, pyrolysis, chemical, or ionic interaction, and so forth — one should be aware that overzealous application could render a species unrecognizable. Kind of like hitting a gnat with a cannonball: Not much left to tell you what it once was. Those who understand the principle of MS electron bombardment recognize this phenomenon.

Spectral libraries of known compounds — reference spectra, used to compare and identify unknown species — are an important part of the interpretation process. The difficulty with many standard libraries is that reference spectra are made in a single, standard, instrumental condition. The hyphenated techniques can generate spectra that are different from the standard references due to many of the conditions alluded to earlier. The best course of action is to generate your own spectral libraries from the hyphenated systems that you'll be using. This is equivalent to removing the instrument function from the spectrum. Let's call it "removing the hyphenated system function." Although various spectral search software packages attempt to do this through data manipulation, the best matches will usually be generated by the do-it-yourself technique. The time required to do this, however, may indeed be a prohibiting factor. Some vendors are starting to present specialized libraries for hyphenated techniques. We hope this trend continues as more hyphenated techniques come into the mainstream of analytical chemistry. In the future, possi-

bly we will see pools of donated spectra, coordinated by our various spectroscopy societies, that could contribute to vendor collections and financially benefit our various societies.

Let's look at the data production phenomena: In the process of merging instruments, some unique advantages seem to have disappeared. In some cases, we find one of the instruments losing its data production capabilities to be used as a more or less sophisticated inlet system. At times, in the interest of affordability, detectors and data analysis systems have been eliminated from the first component instruments in a hyphenated technique line. Again, consider early GC-MS systems in which the only detector available was the chromatogram reconstructed from the total ion data, or systems with which the thermal conductivity detector was the only option available on the chromatograph attached to the mass spectrometer.

This brings us to our second message concerning data production. Although the detectors available to each of the individual instruments in a hyphenated technique may seem to overlap in information content, a closer look usually reveals distinct differences that could prove to be valuable in the interpretational process. Chromatographers have learned this lesson well. In the graduation from generalized detectors — such as the thermal conductivity detector or the flame ionization detector — to the species-sensitive detectors — such as the electron capture detector and chemical ionization detector — chromatographers have discovered the power of using multiple detectors in tandem to produce a more synergistic interpretational effort. The addition of atomic and molecular spectroscopic techniques, including nuclear magnetic resonance (NMR) spectroscopy, is really a continuation of this dawning of capability. The message on the spectroscopy end is to recognize the value of the detection tools the other portions of our hyphenated systems bring to the table.



In covering the general principles to be aware of in the interpretation of hyphenated technique data, we should also briefly touch on some general tips in the interpretation of spectroscopy data as a whole. The first and foremost of these is to learn all you can about the sample and its history. Remember, all is fair in love, war, and interpretation. Being an adept interrogator is as much an interpretational tool as a good library search program.

Remember also that the three great secrets to world-class spectroscopy are

- sample preparation
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Of course, this means different things to different spectroscopy techniques. A former mentor, Daniel Marks, summarized one of these principles for interpretation very nicely whenever someone presented him with a sample. He would simply say, "All I can tell you is what was in the sample container at the time I analyzed it." This simple statement took into account a multitude of possibilities from an interpretational point of view: contamination introduced by the sample container or sampling process; the effect of time on the sample matrix; the effect of removing the sample from its environment; and the assumption that the examination of the leaf will tell us everything about the forest.

References

1. P. Anderson, *New Scientist*, September 25, 1969.
2. G. Levinger, quoted by Daniel Goleman in "Marriage: Research Reveals Ingredients of Happiness," *New York Times*, April 16, 1985. ■