Technical Note

A Primer on Mass Spectrometry for Non-Chemists

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What is Mass Spectrometry?

Mass spectrometry (MS) refers to the use of a specialized scientific device that measures the masses and relative abundance of individual chemical components within a complex mixture.

What is a mass spectrometer?

It is a tool, an instrument, for analytical chemistry. Mass spectrometers have been to Mars several times. I've not been there once. The Phlebot™ is an intermediary between biology and measurement tools that examine the composition of blood, such as flow cytometers, glucose meters, near-patient clinical analyzers and many others. Mass spectrometers are relatively new to this context but they are an obvious partner for automated blood sampling.

What is analytical chemistry?

Analytical chemistry is the field that deals with the strategy and tactics of chemical measurements. Analytical chemists, environmental chemists, forensic chemists, clinical chemists, organic chemists, medicinal chemists, biochemists, polymer chemists, food chemists and many others rely on such measurements to make decisions. Analytical chemists frequently do research on developing new devices and techniques featuring superior performance - faster, more selective, less expensive, more certain, more reliable, easier to use, smaller and so forth. Other analytical chemists optimize how these tools are used in particular circumstances. Some chemists are in highly regulated environments where the use of the analytical instrument must be rigorously validated for a particular purpose. Clinical, forensic, food and environmental labs are typical of these environments. Data supporting FDA applications for allowance to market new drugs, combinations of drugs and devices and in vitro diagnostic tests are clear examples where getting good information to support decisions is crucial. In such cases there are formalities to define how the data is to be obtained and reported. Quality assurance and method validation are formal elements of the process.

What is measured? What is determined? What is analyzed?

Samples are analyzed and their components are determined in the sample. A sample is a part of a whole. A drop of blood, a piece of tissue, part of an apple, urine from an athlete, a bit of new chemical just made (synthesized) for the first time, a drug tablet from a bottle of many, a piece of metal, residue from an explosion, particles from the atmosphere, water from a river, wine from a bottle are a few of many possible examples. In most cases, the goal is to identify what is there (or what is not there) and if it is there, how much? A recent example would be "Is there melamine in this sample of infant formula and if so, how much?" Typically we look for what we expect to see or what we don't want to see because looking for everything is not practical and the presence of most chemicals is irrelevant to the decision at hand. A toxicologist might be interested in a pesticide on fruit while a nutritionist might be interested in vitamins in the fruit, but not vice versa.

How much?

The term "qualitative analysis" is an old expression for establishing *what* is there, while "quantitative analysis" concludes *how much* is there. “How much” is expressed in two ways: the total amount present (weight of gold in a ring) and the concentration (the % by weight of gold in the ring). In the examples of sample types given above we've taken a portion from something larger such as a drop from the bloodstream, or a cup of water from a river. In such cases, concentration is what we want (the weight of a drug or pollutant in a defined volume

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unit). If the small sample is representative of the whole at a given point in time, we can use concentration to determine the total amount of a substance (the "analyte" of interest) in any volume of the sample.

$$\text{concentration} \times \text{volume} = \text{amount}$$

$$\frac{\text{grams}}{\text{fluid ounce}} \times \text{fluid ounces} = \text{grams}$$

Often a sample is *not* representative of the whole, either right now or at some different time. The object of study (an entire river) may not be uniformly contaminated with a pollutant today. Tomorrow is also a different matter. Thus there is more to making a decision than getting a good number about a particular sample. If it is not a good sample, it's not a good number. Context is very important.

You can learn some things by reading the labels on food packages. Be careful to note the "serving size" and that amounts are expressed in units per serving size. Thus if you consume more or less than the serving size for product A vs. product B, that will be quite relevant to you with respect to salt, sugar, cholesterol etc. Alas, most people don't even look at food packaging. Don't miss this opportunity to learn a little science.

What are the measurement challenges?

Almost nothing of interest is a single pure substance. Aluminum foil and distilled water are pretty close to being pure. They are better than 99.9% of one chemical entity. Most samples include many thousands of different substances over very wide ranges of concentration - often a range of a billion or more. In such cases we need a measurement tool that is *selective* for each chemical of interest out of the many of no interest. Thus the needle in a haystack idea is applicable and implies the need for a *chemical separation.* We also then need a tool to look at very small amounts, very low concentrations. Informally we often hear the term *high sensitivity* to describe this. Other (better) language used in the jargon is a *low limit of quantitation* or a *low detection limit*. Samples are often chemically diverse, meaning the personalities of what is there varies widely. Blood, for example, is cells suspended in salty water, but also includes fat, proteins, nutrients, amino acids and all sorts of waste products of the metabolism of food. Quite a mess! It's all held together by a solvent known as water. Most of the chemical entities in blood are in concentrations far below 0.000001% of the total, yet many can be easily determined today, both qualitatively and quantitatively.

Mass spectrometry?

This tool is just a bit over 100 years old, but has only been a practical instrument for life sciences since about 1970. In the last 10 years, MS has come into routine use by non-experts as the technology became more reliable and more economic, while also becoming more capable. It began as a tool of physicists interested in electricity, particularly the basic electrical nature of matter. We have become very good at handling electrical things such as charged particles; electrons being one example. This is well-reflected in television, radar, microwave ovens, computers, cell phones and the like. We are especially good at working with charged particles in a vacuum where they are unlikely to encounter other particles (atoms or molecules) with which they may inconveniently collide. On a larger scale, it's similarly nice that Earth also moves in a vacuum with a rather low probability of hitting an object of similar weight even on a time scale of 1 billion years. Another way to think of this is reduced traffic. When we are in the only car on the highway we're closer to an ion in a mass spectrometer than when driving at rush hour.

A few very simple electrical laws discovered in the nineteenth century enable very precise control of the motion of the charged particles we call “ions”. We know how to accelerate them in a straight line and put them into orbits of various shapes by the influence of electric and magnetic fields that modern electronics allow us to control with great accuracy. We can therefore make ions go where we want them to go and we can even count them over time. Ions are differentiated by their positive or negative charge: cations (+) and anions (-). They are also differentiated by their mass (weight). The ratio of the mass of an ion to its charge determines where it will go when it encounters an electric or magnetic field. A mass spectrometer takes advantage of all this. We can tune the instrument to select for ions of a given mass-to-charge ratio and do this with great precision. Thus a mass spectrometer is first of all an elegant separations tool. We can separate charged chemical entities that are very close in mass, allowing those we want to pass through and not those we don't want. The term *mass filter* comes to mind. This greatly simplifies a sample since most chemical entities are filtered out of a mixed population. It is a tunable filter though, like a radio tuning in different stations. The tuning can be programmed automatically so we can see which ions are available in the sample over the range we are tuning. Basic mass spectrometers can easily separate ions differing in mass by 1 unit, as in 372 vs. 373. More costly instruments can distinguish 372.4873 from 372.4869. If these were the weights of two football players, we wouldn’t care. In chemistry, we do!

Once we've focused on an ion in a beam coming through the instrument, we can do things with it downstream, such as collide it with another entity and cause it to break into pieces. Those pieces which are charged can then be filtered again with another stage of mass spectrometry and we can learn the properties of those pieces. Knowing something about the pieces helps with selectivity and also with identifying more details about the first ion. If the first ion were a house, the pieces might then tell us that it was a three bedroom house with a two car garage and two bathrooms. We could repeat the process a third time and learn that one bedroom has a crib and another stacked bunk beds. Thus mass spectrometers can be used in stages. When there are two stages we use the term tandem mass spectrometry or MS/MS. At the end, we count the ions with given ratios of mass-to-charge and convert that count into an electrical current, an *ion current.* This is quite easy to do. The ion current tells us what is coming through this elaborate system of filters at a given mass-to-charge ratio. The more current, the more ions per unit time and the more concentration in the sample we started with. Thus MS is a very elegant qualitative and quantitative tool with superb performance for the money spent. While the analogy isn't perfect, think of ions like a marble, golf ball, baseball, softball, soccer ball and basketball. Can we easily separate two golf balls differing in weight by only 0.001%? A mass spectrometer can easily do this with ions that close together in weight. Amazing!

But urine is not a vacuum is it?

This suggests a real challenge. How do we get solids and liquids into an exquisite instrument operating internally at near the vacuum of space? Mass spectrometers were first used with gases because it is easy to leak a little bit into the vacuum. Solids and liquids were then vaporized in a very hot chamber under vacuum, ionized with an electron beam (as in a television tube) and a few of those ions could be accelerated and swept into the mass analyzer stages. In the last 40 years or so, all sorts of means have been employed to create ions under a variety of conditions. These methods are too complex to explain here, but all of them have the purpose of getting chemical entities from the real world into the mass spectrometer world. Our partner company, Prosolia, is noted for desorption electrospray ionization (DESI) described at www.prosolia.com. DESI operates in the laboratory, or even in locations remote from the laboratory, enabling chemical entities on a surface to be picked up and transferred into the world of mass spectrometry. The surface could be your finger tip, an explosive residue

on a gun, an apple peel, a spot of blood, a slice of tissue from a tumor and so forth. Prosolia applications notes provide some examples. The present section is not for chemists and I hope they will not be too hard on me. This description is for their relatives who are cooks, accountants or went to law school and want to invest in Phlebotics or Prosolia. Here are some take home messages:

1) MS is the most powerful analytical chemistry tool available today
It comes in a wide variety of formats and prices. It is used for an extremely wide range of applications on Earth as well as Mars. To learn more, visit the web site for The American Society for Mass Spectrometry (ASMS) at www.asms.org. The Journal of the ASMS regularly runs features on the history of this fascinating tool and the remarkable personalities who helped it to evolve, step-by-step.

2) MS relies on very simple electrical principles
These are well established for charged particles in a vacuum. These principles are generally covered in a freshman physics book.

3) MS is a very selective chemical separations tool
It is improved even more by placing other separations tools before it to "clean up" or "prepare a sample" to enter this new environment. As a rule, in analytical chemistry the sample seen by any instrument should be no more complicated than it needs to be and no less complicated either.

4) MS also provides very valuable confirmatory information about the identity of chemicals
While being capable of determining very small amounts of chemical entities that are selected, it is also capable of discovering unanticipated chemicals in complex mixtures. However, the user must look for them.

5) Components of any sample under study must be ionized and placed in a vacuum
Samples can also be placed in the vacuum first and then ionized. Then the fun begins. Uncharged, or 'electrically neutral' chemical entities cannot be examined until they acquire an electric charge by adding or removing electrons (-) or protons (+).

6) While mass spectrometry is a powerful tool, it is very rarely used alone.
It is frequently combined with a variety of sample isolation, sample storage, sample preparation and other separation tools (chromatography being most noteworthy) used prior to MS. It is also combined with optical tools (lasers) on a regular basis. The art of mass spectrometry is very rich indeed.

What's next?

I predict the bedside or nursing station mass spectrometer for intensive care and personalized medicine will be with us in less then a decade. Look for one in a hospital near you. We have the beginnings of portable, even battery powered instruments capable of use in the environment and for monitoring the atmosphere in public facilities and on battlefields. A few years ago we assembled an Indiana Mass Spectrometry Alliance (IMSA) in conjunction with the national meeting of the ASMS in Indianapolis (2007). It is very impressive how many of these advances derived from academic and commercial developments in Indiana, particularly at Purdue and Indiana University. If the reader puts these institutions into a Google search with the additional words "mass spectrometry," you'll no doubt be very impressed. If you have questions, the MS teams at Prosolia, Purdue University, Indiana University, Phlebotics, Inc., AIT Bioscience and ASMS will be pleased to respond. If I can help you find the right expert, don't be a stranger (petekissinger@phlebot.com).