Mass Spec: The Biography of a Scientific Instrument

by

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B.S. Chemistry
University of Wyoming, 2010

SUBMITTED TO THE DEPARTMENT OF WRITING AND HUMANISTIC STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN SCIENCE WRITING
AT THE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

SEPTEMBER 2011

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May 17, 2011

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ABSTRACT

Over the past century, the mass spectrometer has become commonplace in scientific fields ranging from chemistry to geology to environmental science. Its ability to identify compounds and determine concentrations of those compounds leads to a wide variety of applications, from environmental monitoring to disease diagnosis.

This thesis is meant to familiarize the non-scientist with the mass spectrometer. It illustrates the instrument's basic physical principles and the wide range of research that utilize mass spectrometry. The story discusses the development of the mass spectrometer from the early experiments of JJ Thomson to modern uses in proteomics and attempts to miniaturize the instrument.

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Acknowledgement:

All my gratitude goes to Robert Kanigel for his thoughtful advice and constant encouragement.

My friends and classmates at MIT have been invaluable throughout this process. Thank you especially to Emily Ruppel and Saul Glasman for your critiques and encouragement on early drafts of this work.

Thanks also to the mentors, colleagues, and friends who have nourished my love for science and my sense of curiosity. In particular, Mark Mehn and Dylan Houghton.

And finally, thank you to Allen, Glenna, and Ariel Calmes. You are still at the heart of everything I do.

This story has a million data points, and could easily begin in as many different places. It could start in any crime lab in any state in the country, searching white powders for illicit substances, or it could start at Churchill Downs, testing racehorses for stimulants. I could tell you about archaeologists collecting samples of charcoal from old campfires and pigments from cave paintings, using radiocarbon dating to make sense of human pre-history. Or I could tell you about geologists doing the same things with objects so old that nothing was alive when they were created—the rocks making up the Earth's crust. Or about pollution, or disease diagnosis, or author Michael Pollan blending up a McDonald's happy meal to learn how much of it was made from corn. These scenes and stories share a common thread: a character, a living scientific presence, that appears in each of them.

In Rachel Stanley's laboratory at Woods Hole Oceanographic Institute, one entire wall is devoted to shelves of uniform, sealed glass flasks. Each is perfectly round, shiny and secretive, a clear container encompassing a clear liquid. The samples are reminiscent of soap bubbles, whimsical and pretty in their near-emptiness, and each contains nothing more remarkable than a cup of seawater.

Stanley, a marine chemist, collected a portion of those water samples off the coast of Massachusetts. She traveled the world, from Bermuda to Indonesia, to gather the rest. Some of the samples came from surface water; others originated at depths where sunlight cannot penetrate. Each sample by itself is almost useless, a single puzzle piece, but together they will provide clues about the movement of oxygen between the sea and the sky.

Stanley's laboratory is one of seven involved in the Total Oxygen Isotope Project. She wants to count the molecules of oxygen in each of her flasks of seawater and figure out where they came from. On Earth, most molecules of oxygen gas originate in one of two distinct places. They either float down from the stratosphere or they form during photosynthesis. Knowing the amount of oxygen produced from photosynthesis will give Stanley and other researchers a way to quantify the productivity of plants and phytoplankton. "Since photosynthesis is the base of life -- the bottom of the food chain -- it is a really important parameter for understanding ecoystem productivity," Stanley says. "We care about ecosystem productivity for many reasons including fisheries and climate change."

Counting the oxygen molecules is possible because not all oxygen is created equal. Oxygen has three different isotopes, meaning that the atoms can have different masses. The chemical reactions that take place in the stratosphere favor oxygen 18, the heaviest isotope. By determining how much oxygen 18 is in a sample of oxygen gas, scientists can tell how much of the oxygen on Earth comes from the stratosphere and how much comes from photosynthesis.

Each research team working on the project has the same goal, but each has a different, customized instrument for peering into their flasks of seawater and counting the amount of oxygen 18 present. The project is part collaboration. If many of the teams reach the same conclusions, it will add a large measure of confidence to the results. On the other hand, it is part competition. Each laboratory has invested time and energy into its instrument design, and they are vying to create a tool that other groups might use in the future, one that maximizes accuracy and efficiency while minimizing cost. If this

were a science fiction story, or a look into the future, there might be more variety among the types of tools the researchers use. But at the moment, it's a case of theme and variation: The same ubiquitous, molecule-counting instrument plays the starring role in each lab.

The tool that Stanley is using sits in the center of the laboratory, a hulking mass of metal and plastic. Two rows of metallic nozzles stretch across the room at waist height, providing enough spaces to connect 20 of the water samples to the instrument at a time. Metal tubing connects those nozzles to a plastic box almost large enough to be a child's playhouse, a set of two instruments linked together: a gas chromatograph-mass spectrometer. The gas chromatograph is one of many methods of filtering out the oxygen molecules. The mass spectrometer is the common way of counting them. Researchers often refer to the whole thing as a mass spec.

Many people are familiar with words like microscope, telescope, radar, and laser, even if they don't know exactly what they do or how they work. However, unless you work in a lab, you've probably never heard of a mass spectrometer. If you've ever read the science section of your newspaper, though, you've seen the results of mass spectrometry. When an anthropologist reports the age of a bone, when the International Olympic Committee reports that an athlete was doping, or when the Environmental Protection Agency reports the level of benzene in your local water supply, they usually either found or verified their information using mass spectrometry. In 1976, the Viking I spacecraft used a mass spectrometer to analyze the composition of Martian soils, while in 2010, submarines carrying mass specs surveyed the damage of the Deepwater Horizon oil

spill.

The mass spec performs exactly one task: it counts molecules.

More precisely, it counts ions, which are molecules with an electric charge. The instrument makes ions with an electron beam, a stream of electrons sent through a vacuum tube. When the electrons hit the gas molecules in the sample, the sample usually fragments into smaller pieces, and some of those pieces pick up an electric charge and can be picked up by the detector. A mass spectrometer cannot detect neutral molecules, because it doesn't measure the mass of the molecule directly. Instead, it measures the ratio between the mass and the charge.

Each oxygen ion has the same charge, but because they have different masses, the mass spec will detect different mass-to-charge ratios and separate them into different categories.

Stanley's model, like every mass spec, has three main components: an ion source that turns neutral molecules of gas into electrically charged ions (that's where the electron beam is located), a mass analyzer that sorts the heavier ions from the lighter ones using an electromagnetic field, and a detector that counts the ions. For each sample, Stanley's instrument spends 2 hours going through that three-step process.

The system in Stanley's lab is connected to a desktop computer, and the monitor shows a diagram of the instrument, taking us on a whirlwind tour through the fate of each sample as drops of seawater move from one of the glass bubbles into the opaque plastic box. Inside that box, the water is turned into a gas, bombarded with electrons to create positively charged ions, and forced through a long, thin glass column that's coiled up and hidden from view. That's the gas chromatograph. All the molecules from the sample

enter the column together, but different gases take different amounts of time to journey to the far end, so the oxygen molecules stay close to each other, and get separated from everything else in the sample. Finally, after 45 minutes, the oxygen molecules enter the mass spectrometer itself. Modern mass spectrometers can measure mass differences smaller than the mass of a single proton.

From sample flask to computer file, the system is fully automated. The data from each experiment is displayed as a mass spectrum, which looks like a bar graph. The horizontal axis represents mass-to-charge ratio, and the vertical axis represents the percent abundance. The ion present in the highest quantity is set to 100% abundance to make the data easier to analyze, and then all the other ions are compared to that peak. Measuring relative abundance allows the scientist to directly compare spectra produced at different times or on different instruments.

Once those 20 flasks have been attached to their valves, the computer will open each in turn and feed it through the experiment.

Though this single instrument, and this single research project, sounds to me like enough complication to keep a team of scientists busy indefinitely, there is a second instrument in the Stanley laboratory, a different model set up to answer different research questions. There are three more mass specs down the hall, in the lab where Stanley worked as a graduate student. Some of them are commercial instruments with only slight modifications; others are made entirely from scratch. Though Stanley does not use any of them for her own research, she wants to share them with me.

"Yeah, show her all the different sports car models," a post-doc quips as Stanley points out the various features on each instrument. The mass spec we're looking at does,

indeed, have a plastic steering wheel attached to it as a way of tuning the magnets inside the detector. The instrument reminds me of the racecar games I used to see at nickel arcades, and I can almost imagine graduate students making up such games late at night when they're sleep-deprived and still in the laboratory, but I'm sure none of them ever do, because tuning the instrument is serious business, and twirling that wheel around at will would cause them a ton of extra work.

Tuning a mass spec is much like tuning a guitar: by physically adjusting the instrument, you change its output. A guitar not used in a while, or that has been jostled about, will probably play flat notes unless its strings are tightened, and they won't sound the same as the notes played on a different out of tune guitar, let alone a guitar that's in tune. To make matters worse, the guitar might get flatter and flatter as the days pass, so if the musician made a recording without tuning, then used it to accompany his live concert a week later, the same out of tune guitar might produce very different notes. Likewise, tuning the magnet and electronic components of the mass spec ensures that the spectra it produces on a given day can be compared to spectra taken on a different day. Analyzing mass spectra is all about ratios and comparisons. Each time the instrument is used, the experimenter has to run a standard, a known sample for which he has previously determined the chemical structure and concentration, to make sure the data is consistent.

The biggest instrument in the room is, once again, something Stanley had a hand in building. This one lacks a steering wheel, and in order to tune it, the heavy magnetic cylinder has to be slid across the top of the lab bench. I have to crane my neck to see to the top of the heavy magnet. The bench has a plastic mat to make sliding the magnet easier, but even at that, Stanley says the job usually takes two people. Setting this

instrument to run a single experiment can be physically exhausting.

"This is my love." Stanley's voice changed in timbre as we circled the massive instrument she had helped build as a graduate student. I traced my finger along the top of the sample holder, which was much smaller than the one on the instrument designed for the Total Oxygen Isotope Project. "Oh, here," she said, suddenly distracted and squirming out of her jacket. She showed me a rough, roundish indentation in her bicep. It looked like someone had tried to shove a drinking straw through her arm. Stanley had gotten her battle scar from leaning into one of the sharp metal tubes of the sample holder when the instrument was still being built. "But it was worth it," she shrugged, and turned again to explaining the various pieces of the instrument. My hand continued its path across the piece of metal that had caused the scar, and I noticed how smooth and safe it had become.

Though that first spectrometer may have seemed a dear friend after it was completed, Stanley had not been attracted to her line of research by a love of gadgets or of tinkering. "I had never done anything technical before I started my thesis," Stanley said. "Well, I had used a screwdriver before, but not very often, and I had certainly never seen a welder."

The mass spec is the most tangible part of Stanley's work. She likes that if it breaks down, she can apply the scientific method to fixing it. And that's good, because there's a good chance that it will break down. The rule of thumb is that a mass spec won't work right about a quarter of the time. Lots of things can go wrong in the tangle of strong magnets and vacuum pumps, long glass columns and specialized computer programs. "There are lots of checks on the system. If something goes wrong, we don't

want to blow through twenty samples before we find out," Stanley explained. Picking up more seawater from Indonesia and getting it back to the United States in a sealed container is a bit trickier than stopping by the grocery store for a quart of milk.

Troubleshooting the instrument represents the straightforward, black-and-white approach to science that most students learned in the ninth grade. Stanley can make a hypothesis as to why the instrument is not functioning correctly, test it by manipulating the settings on the instrument or swapping out a part, and then know whether or not her hypothesis is correct based on whether or not the instrument works again. Stanley has samples with known concentrations of oxygen that she uses in tuning the mass spec. When the spectra of the standards look the same way they did before the instrument broke, then Stanley knows it's fixed.

Even though there is a firm answer, a desirable outcome, testing that hypothesis could mean days spent ripping into the instrument's guts, or thousands of dollars in replacement parts. It means that time and money that could have been spent investigating the ocean is fed into the mass spec instead. When the instrument breaks down, it can be an inconvenience. I thought I would hear more sob stories about lost productivity, but the researchers with whom I spoke had learned to expect the delays. And looking at it from a different angle, an instrument down can mean a break, or at least a different kind of work.

For scientists exploring the complex mysteries of the natural world, such enigmatic subjects as sea and space, answers are not always definitive. Scientists know some facts with more certainty than they know other facts. They can design experiments and run tests. However, when they ask a question as broad as 'how does the ocean

work?' they have to settle for incomplete answers. Perhaps the only truths that people can ever grasp fully are the ones we design for ourselves.

A mass spectrometer performs a single, specialized task. Yet, it has managed to find its way into every nook and cranny of the chemistry world, and also into most fields that intersect with chemistry, from geology to mechanical engineering to medicine. Though it is only about a hundred years old, it has played a major role in six different Nobel Prizes awarded in either chemistry or physics. Some of those prizes were given to the inventors of the instrument and the people who improved it, while others were given for major discoveries made using the instrument. Together, they suggest the mass spectrometer's range and importance, its irreplaceable role in the modern laboratory.

The first was awarded to Sir Joseph John (JJ) Thomson in 1906, "in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases." He not only discovered the electron with those investigations, but also determined the mass-to-charge ratios of the gas ions he was studying. Mass-to-charge ratio was the characteristic that Thomson measured with the first mass spectrometer, which he built in 1912, and the instrument still makes the same measurement today.

In1921, Frederick Soddy was awarded a Nobel Prize in chemistry for his investigations of radioactive substances. The very next year, the chemistry prize was awarded to Francis William Aston. Aston, a former student of Thomson's, had helped build the first mass spec. Later, he used the instrument to uncover isotopes in a large number of non-radioactive compounds and to verify the whole number rule, which says

that the masses of the elements are whole number multiples of the mass of the hydrogen atom, meaning that all atoms are made up of the same types of particles.

Advances in mass spectrometry continued at a breakneck pace throughout the 20th century, but no more Nobel Prizes were awarded to scientists using mass spec until 1989. That year, Hans G. Dehmelt and Wolfgang Pauli shared in the physics prize for their development of the ion trap technique in 1953. The ion trap, a combination of electric and magnetic fields, can stop a charged particle and hold it in place, allowing for the study of the structure and properties of that single, individual particle. Their design, sometimes called the Pauli trap, is still common to many of today's mass spectrometers.

The next chemistry award associated with mass spec, given to Robert F. Curl Jr., Sir Harold W. Kroto and Richard E. Smalley in 1996 for their discovery of fullerenes, showed how far the field of mass spectrometry had progressed in the previous hundred years, and also how far-reaching its implications were. A fullerene is a special, systematic arrangement of 60 carbon atoms into a regular geometric shape, a "truncated icosahedron cage." A European football shares its shape with the fullerene, as does the geodesic dome designed by the American architect R. Buckminster Fuller for the 1967 Montreal World Exhibition. When Curl, Kroto, and Smallery identified the structure of fullerene via mass spectrometry, they were unlocking the secret to many advances in materials chemistry and electronics, including new superconductors, polymers, catalysts, and nanotubes.

The most recent Nobel Prize awarded to people working with mass spectrometry was the 2002 chemistry prize, shared by John B. Fenn and Koichi Tanaka, who developed methods to identify biological macromolecules like proteins, carbohydrates

and nucleic acids and analyze their structures. Fenn and Tanaka developed "soft" methods of ionization, alternatives to hitting the sample straight-on with a powerful electron beam. The ionization process is vital to making the mass spectrometer work, but it can also create problems for scientists working with larger molecules. Often, the energy from the electron beam tears through the molecule, causing it to fragment so many times that it is impossible to tell what the original structure was. With soft ionization techniques, it is possible to analyze biological molecules like proteins that weigh tens of thousands of times what a hydrogen atom weighs.

When Klaus Biemann became an assistant professor of analytical chemistry at the Massachusetts Institute of Technology (MIT) in 1957, he had only one problem with his new job: he had to choose a new field of research outside of his area of expertise, organic chemistry. The young professor had spent the previous twelve years polishing a specific skill set as an organic chemist, first at the University of Innsbruck in Austria and later at MIT, but the switch to the analytical division would require new skills, new thinking, and a radical change in direction. It was a bold move that few of his fellow chemists would have made.

In an interview with the Chemical Heritage Foundation, Biemann said that many of his colleagues in the 1960's looked down on analytical chemists. "Mainly the physical chemists considered the analytical chemists as failed physicists or failed physical chemists, which had some truth to it. Analytical methodology was either physics or physical chemistry," he said. Most analytical chemistry at the time was quantitative, where the scientist would determine the concentration of a known element or compound.

(Some of today's examples include determining the concentration of lead in the city drinking supply and testing athletes for steroid use.) Though such work is useful, it's far from revolutionary. "That doesn't lend itself to great research advances that catch the public's eye or even the chemists' or scientists' eye," Biemann explained. He wanted to make analytical chemistry more innovative and qualitative, to use an instrument to discover new molecules rather than just counting the ones he already knew existed.

That opportunity presented itself in the form of a mass spectrometer. A few months before Biemann accepted his MIT professorship, scientists at the Swiss perfume company Firmenich had asked Biemann to attend a flavor and fragrance conference in Chicago on their behalf. "I gladly agreed," Biemann remembered, "not so much because I was interested in the talks but more because I could take my first ride on an airplane." But after the plane landed, Biemann took a greater interest in the conference. Another Massachusetts scientist, W.H. Stahl of the Quartermaster Corps Laboratory in Natick, presented research on how the small molecules that give fruits and flavors their characteristic smells could be identified with mass spectrometry, an instrument that Biemann and many of his colleagues in organic chemistry had never heard of, let alone used.

When the instrument was first developed, mass spectrometry and organic chemistry seemed incompatible. Organic chemists sometimes joke that, of the 118 elements of the periodic table, they only care about 6 of them. Organic chemistry is the study of carbon, and most organic substances are made of long chains of carbon and hydrogen, with an occasional atom of nitrogen, oxygen, phosphorous, or sulfur sprinkled in for dramatic effect. It's as if nature has written an entire book of profound and

insightful poetry with only six words. Order matters. A lot. But the mass spec jumbled everything up. Exposing those delicate assemblies of carbon and hydrogen to the electron beam of a mass spec is tantamount to opening fire with an assault rifle. They get splattered into tiny pieces, which can make it difficult to tell heart from nose from celery.

At the conference in Chicago, Stahl showed that he could identify very small organic molecules from their mass spectra. The molecules break in statistically predictable ways, so each chemical's mass spectrum is like a fingerprint. It's a bit as if a bartender decided to chuck all of his glasses out a window and then asked you to count them. Most of what you had would be indistinguishable piles of glass. But the wine glasses would all break at their weakest point, and you would be able to tell how many there were by counting the number of stems. The size and positions of the peaks that show on the spectrum depend on the abundance of the different ion fragments.

Mass spectrometry was not a new technique in 1957. It was widely used in the petroleum industry for quantitative analysis of crude oil and gasoline, but its uses were limited to substances with known chemical structures. Now, though, Biemann left the conference convinced mass spectrometry could be a very powerful tool for organic chemists studying larger molecules, maybe even those as large as proteins, and that by obtaining the mass spectrum of an unknown compound, he should be able to determine its chemical structure.

Arthur Cope, Biemann's boss and a fellow organic chemist, was hesitant to buy a mass spectrometer for the department. At $50,000, it cost much more than any instrument organic chemists were using at the time. Cope had also heard that the instruments were quite labor-intensive, and worried that he would have to hire an

engineer to look after it. Biemann convinced Cope to purchase the instrument by promising to do the maintenance himself. He remembers Cope saying, "If you promise that it will not collect dust, I will come up with the money."

Both men, Biemann said, kept their promises. He assembled his laboratory's first mass spectrometer in May of 1958. In the meantime, he had applied for grants from the National Institute of Health and the National Science Foundation, promising to study the chemical sequences of the small peptides that make up proteins. With the help of two post-doctoral researchers, he started working on the peptide project, which he described as "high risk," and at the same time set about proving that mass spectrometry could be used to determine the structure of complex organic chemicals, such as plant products that are used as pharmaceuticals.

Biemann's work started catching the interest of other scientists, and during his tenure at MIT, he collaborated with researchers from all over the United States and Europe. His skill attracted scientists from institutions as disparate as NASA's Jet Propulsion Lab at the California Institute of Technology, the University of Wisconsin Department of Zoology, and the FBI. Since few organic chemists had their own mass spectrometer, scientists would send samples to the Biemann laboratory for analysis. Sometimes, they would send students to be trained in the basics of mass spectrometry in his laboratory, or visit themselves, or write to ask advice on how to purchase and set up an instrument.

By 1962, he had become one of the world's experts in mass spectrometry and had written a textbook to introduce organic chemists to it. In the preface to *Mass Spectrometry: Organic Chemical Applications*, he shared the secret to his success. "As is

the case with similar techniques, the experience gained by thoughtful consideration of many spectra of widely differing compounds is the best basis for the correct interpretation of the spectrum of a substance of unknown structure." Organic chemists were on the verge of discovering a tool that could make their projects infinitely easier, one that would open up new lines of inquiry to them, but only if they could conquer the learning curve and really get to know how to run the instrument.

"The actual determination of the spectrum will have to be varied from case to case, depending on the nature of the compound and of the information desired," he said. A versatile tool is capable of producing many results, and many of them will be bad unless the instrument is properly steered by its master. "It is for this reason," Biemann concluded, "that the most useful data are obtained if the originator of the problem and the person determining the spectrum are in close contact." Better yet, they should be one and the same. The mass spectrometer, like the computer, does exactly as it is told. The skill of the human operator, and his overall understanding of a specific project, determines the quality of the results.

By 1965, it was apparent to Biemann and his contemporaries that mass spectrometry would play a vital role in the future of organic and biochemistry, and the National Institute of Health granted his laboratory a new type of grant to fund the development of a training program.

Some of his greatest innovations involved linking the spectrometer to other instruments and making the instrument easier to use. Between the visiting scientists training on the mass spec and the samples arriving through the mail, the laboratory was dealing with an unprecedented number of spectra, so they were among the first lab

groups to attach their mass spectrometer to a computer. The IBM model that he obtained, which was dedicated entirely to mass spectrometry, occupied an entire room and foreshadowed the merging of the two technologies: given the number of samples run in a busy laboratory like Biemann's, the field could not have grown into what it is today without computers.

Biemann was also the first researcher to connect a gas chromatograph directly to the mass spectrometer. "I wanted to do it because we had to collect [samples] tediously, peak by peak, in one [test tube] after the other, put it aside, label it, and make sure we didn't confuse it. Then we put them into the mass spectrometer, one after another," Bimann explained. "This takes a long time. But it also gives you a lot of time to think about how to convert that tedious manual process into an automatic one."

Rachel Stanley and her contemporaries can attach twenty samples to their mass specs, push a few buttons on her computer, and then walk away. The free time she earns because she does not have to babysit her instrument and change part of the setup every few minutes is a direct legacy of the time that Biemann spent watching liquids collect, drop by drop, in the bottom of a small glass capillary. The time and the tedium gave Biemann and his colleagues a sense of connection to the instrument, and perhaps a deep sense of understanding. By the time Biemann closed his laboratory in 1999, researchers no longer had to ship their samples across the country for analysis, and the mass spec had become a common fixture everywhere from universities to crime labs to government offices.

By 2008, the year I was a junior in college, I knew how to analyze data from a

mass spectrometer. I practiced that skill in my sophomore-level organic chemistry classes and laboratories, then again at my summer jobs. However, I never got to use the instrument myself. Throughout that two-year period, I saw only the end of the process, the printed-out pages of ion peaks.

In my last year of school, I took a course called "Instrumental Methods of Chemical Analysis." Our professor split us into teams and assigned each to an instrument in the laboratory. We had an atomic absorption spectrometer, a UV/Vis spectrometer, a fluorometer, and a mass spectrometer. Each team had a simple problem: find the concentration of iron, or lead, or mercury in the sample. We had six class periods to figure out how to use the instrument and write a procedure that our classmates would be able to follow. Then, we would rotate to a new instrument and test our colleague's procedures. Our professor was clever enough to assign us instruments that we had never used before, and we flailed heroically through most of the course. Over the semester, we wrote or edited procedures for three of the instruments and then designed and executed an experiment using one of them.

My lab partner, John, worked for the professor who ran the course and had been running a mass spectrometer daily for nearly four years. He was incensed that we were never allowed to use the mass spec during class. When it came time to pick a final project, he insisted we use his favorite instrument. I protested, loudly and often, that I had never used the damn thing before, whereas I had written or edited documents pertaining to every other instrument in the lab. But John had his mind made up, and so we decided to analyze the caffeine content of various types of coffee and energy drinks using mass spectrometry.

Most chemistry students learn the law of conservation of mass. It says that there is a given amount of matter in chemical reactants, and all of it will still be present after the reaction is finished. If I burn a piece of wood, what's not left as charcoal has been converted into gas, but if I could gather all the products back together, they would weigh the same as the piece of wood. Well, it seems that John and his lab mates also had a law of conservation of mass spectrometers. Based on the patterns they had seen from the instruments in their laboratory, they hypothesized that there is only a given number of working mass spectrometers in the world at a particular time, and that if one instrument starts working again, it means another one has broken down. It's karma, they said. When you're spending your day cussing at your instrument, at least you can console yourself with the idea that, somewhere in another laboratory, a frustrated fellow scientist is finally getting good data again. I had been nervous about this whole endeavor from the start. But I should have been extra worried when the grad student across the room from us magically started getting good data again the day before we started our caffeine project.

It took nearly a week's worth of class time to acquire and prepare our samples. When they were finally ready, we set the vials in the autosampler and watched as a robotic arm stuck its proboscis-like needle into the first standard. Since it would take fourteen hours for the experiment to finish, we went home. That night, I did my best to keep my mind off the experiment. When I got back to the lab the next day, John was staring at the computer monitor and frowning. We had a caffeine peak, but we had lots of larger peaks that we couldn't identify. John thought there was something wrong with the way we had prepared our samples, or maybe something wrong with the mass spec.

When we told our professor we were having trouble with our project, he looked at us and grinned. Then he said something to the effect of, you guys better figure it out, and disappeared into his office.

There are entire books showing the mass spectra of possible impurities, many of them plastics and other common materials, that can taint a sample being prepared for an experiment. We looked through the book. We walked back through the steps of our sample preparation process, trying to find the place it had failed. We sat in silence for what felt like hours.

"Column bleed," John finally muttered. He tracked down some of our classmates' work. The strange peaks hadn't come from our samples, but from inside the column itself. As best as we could guess, one of the students working in the laboratory had turned the temperature on the gas chromatograph too high, and in addition to vaporizing their sample, they had cooked the inside of the glass column, and bits of it were sloughing into our samples during the experiment.

As soon as one fiasco ended, a second one started. The instrument's $10,000 software program crashed. The project, the instrument, expanded to fill all the time we could possibly give it. We spent a week sitting in front of the mass spec, John tinkering, me looking up terms and procedures in instruction manuals and silently cursing him for choosing to work with this rotten, infuriating, impossible mess of magnets and computers. Throughout the project, I could feel myself growing more and more wary of the instrument, and increasingly terrified that I had to rely on John to troubleshoot it.

As nights stretched into mornings, John told me stories about why he loved mass spectrometry, and my anger started to soften. I had seen this troubleshooting as a waste

of time without ever stopping to consider that fixing the instrument could be a puzzle, could be fun. Perhaps just as important, I had never realized that the thrill of working with the instrument could provide as large a sense of achievement as writing a paper or giving a presentation could. I had my ideas about what made being a scientist fun and about what type of work was fulfilling, and John shared none of them.

John fell in love with basic research in general, and the mass spectrometer in particular, very early in his academic career, and spent three years working as an undergraduate assistant in the same laboratory at his medium-sized state university. He was part researcher, part mechanic. John has a mindset that is quite common among the chemistry graduate students I've known. He loves science for the tinkering, not for the grand implications it might have concerning life's big questions.

His mentors, the graduate students he worked with, had similar mindsets. They wanted to push the instrument to do new things, more or less for the hell of it. After they had published their research, collected their PhD's, and left, their advisor discovered that some of the experiments they had conducted could not be reproduced, even though they looked perfectly legitimate on paper. Younger students in the lab had been trying to follow their predecessor's work, and though they followed the procedure to the letter, they just couldn't get the samples to run through the instrument. It turned out that the original students had never gotten their experiment to work as it was written, either. They had used a squeeze bottle to add a stream of extra solvent to the sample at exactly the right time, from exactly the right angle, to get the sample through the injection port. John had seen it, but never thought to mention it to the professor. It seemed like such a little thing. But when John finally showed him what the other students had been doing to

make their experiment work, the professor just swore and stormed out of the room. However small such a thing might seem, it's the kind of thing that would make any other scientist question the legitimacy of the research. The results of the project cannot be reproduced, and reproducibility is fundamental to scientific research. It is, essentially, the source of fact.

The laboratory that John worked in specializes in sample preparation. "A lot of times you know what the sample should be, but you don't know if the instrument will process it," John explained. His and his colleagues' work involved figuring out the best way to prepare samples and the best way to set up the instrument to obtain fast, accurate results. Changing the way a sample is prepared is one way to make the instrument perform a new task. If the students had invented a new piece of equipment that basically did the same thing they were doing by hand, it would have been unremarkable but solid research. However, by failing to make their actions repeatable, they called the integrity of their entire project into question. I could never decide if their actions were clever, dishonest, or destructive, but I spent months thinking about it.

On an afternoon in early February of 2011, a group of roughly 30 people gathered in an MIT lecture hall to witness one of the most important rights of passage for an academic: the thesis defense. Most of them wore jeans. They sipped from paper coffee cups as they settled into their chairs, the looks on their faces ranging from mild interest to downright boredom. The young man wearing the suit, Brian Hemond, was about to be grilled by his professors from the mechanical engineering department. He has an intense face, heavy eyebrows set low over green eyes, but they looked relaxed as he made small

talk with members of the audience as he waited for his thesis defense to start. Fifteen minutes into the appointed hour, the last of the professors in charge of examining him finally appeared. As soon as he was given the signal to start, Hemond launched into an eloquent explanation of his doctoral project: the design and production of a miniature, low-cost mass spectrometer.

Hemond hoped that his mass specs would be used as teaching tools in high schools and universities. "Mass spectrometry is such a powerful investigative tool, and it's pretty much out of the hands of everybody except the most privileged university students," he explained. He's right. I was a chemistry major in college, and I never got to use a mass spec by myself until my final year. Hemond's goal is to provide more students with firsthand experience in running experiments. That implies an instrument of cheap and simple design. So cheap, Hemond says, "that you could basically afford to break a few every year and not have to worry about the replacement costs." His goal is a couple hundred bucks—an ambitious number, given that top of the line mass specs can cost upwards of a million dollars.

Hemond expected his instrument would be able to measure chemicals with a mass range of 16 atomic mass units (AMUs), which is roughly the mass of one oxygen atom, to 44 AMUs, the mass of carbon dioxide. With that mass range, it would have been a great teaching tool, but otherwise useless. The instrument showed promise beyond what its inventor was aiming for, measuring compounds as heavy as 80 AMUs, including environmental pollutants like benzene and the smaller compounds created during gasoline production. "The success of the design has taken the project places that we didn't intend in the beginning," Hemond said. His vision changed to include a swarm of

cheap mass spectrometers contributing to fields outside of education.

"Those of you who paid attention to the well failure over the summer probably noticed that they were using a mass spectrometer aboard an autonomous submarine," Hemond said, referring to the April 2010 explosion of the offshore oil rig *Deepwater Horizon* and the three-month oil gusher that followed. The environmental disaster was still fresh in people's minds, and several members of the audience nodded. "Now, interesting as that is, it only gives you one data point in one location at a time." The use of the single mass spec was much like a man who had discovered a major leak in his dark basement trying to assess the damage with a single flashlight. It offered clues, at best. With more mass specs, researchers could have collected multiple measurements simultaneously over a large area. Problem is, a small enough, cheap enough instrument did not yet exist, even though people from various professions had been calling for one for years.

As early as 1974, medical professionals started talking about the potential uses of mass spectrometry in a clinical setting, but 37 years later, those wishes have yet to be fulfilled. True, mass spectrometry has long been a tool for medical diagnostics. By the early 1980's, researchers could use the technique to identify over 200 different chemicals in human breath. They could measure acetone to monitor diabetes, or thio-compounds to diagnose cirrhosis. They could screen for halogenated compounds and other signs of environmental pollutants that a patient might have inhaled or absorbed. But while the mass spec has proved incredibly useful, no one has gotten around to designing one that's portable. Currently, a doctor wishing to analyze a patient's breath must collect that breath in a plastic bag and walk it, perhaps across the hospital campus, to a mass spectrometer

for analysis. Hemond is envisioning an instrument that a patient could breathe into directly. Given its weight, a nurse could carry Hemond's mass spec around, and given its expected price, the world wouldn't end if she dropped it.

A week after he defended his work in front of his professors and peers, Hemond allowed me to visit his laboratory and take a closer look at the tiny instrument. One of the most brilliant parts of the design is that the entire instrument is put inside a low-pressure capsule, eliminating the need for a pair of heavy, breakable vacuum pumps. The capsule amounts to about ¼ of the instrument's total weight and adds several inches to its diameter, so it is an important part of the overall design. On the other hand, it obscures all of the electronics, so the prototype Hemond is showing people is out of its shell.

The instrument, is roughly six inches long and four inches wide at the base. In Rachel Stanley's troupe of mass specs, an instrument the size of a microwave looked miniature. This one was smaller than a toaster. The bright, electronic guts looked like a maze, or like an abstract gameboard where half of the illustrations had been rubbed off. The c-shaped magnet fit snug into the palm of Hemond's hand, and when his fingers extended outward, they covered nearly half of the green and yellow circuit board. I did not even notice the device's tiny battery until Hemond pointed it out to me, and I also had to ask him to point out the electron source, which is actually a flashlight bulb. The instrument was delicate in both its petite size and in its intricacy, and yet sturdy enough that Hemond feels confident putting it into the hands of high schoolers or dropping it into the sea.

I was surprised when Hemond explained that he had started designing the device a mere seven months earlier, in July of 2010, and my respect for his ingenuity only grew

as he explained the process. "I lost the better part of a month trying to figure out where the primary ion beam in the mass spectrometer was going," Hemond explained, turning the instrument over in his hands. "I was pretty sure that I was making ions, but I couldn't detect anything," and it turned out that there was a stray magnetic field that was just enough to steer the ions off their trajectory so that they would never make it through the system. I ended up having to redesign it to take that into consideration."

The most rewarding part of the project for Hemond was seeing the instrument produce its first spectrum. "You spend a lot of time poking and prodding and testing and fixing. To finally actually see that it's working the way it's supposed to, that you can put a gas into it and actually see the result of that come out, and more importantly that you can put in a gas that isn't naturally present in the air and see the result of that come through, it was really pretty rewarding." That was the moment he knew the project was going somewhere, and it happened in December of 2010, roughly two and a half months before Hemond defended his work. "I did end up scrapping the design afterwards," he said, and built a new version between December and February.

At Hemond's thesis defense, his advisor called him "a beautiful example of the renaissance type we like to have in the lab." In addition to the mechanical engineering and machining required to make the mass spectrometer, Hemond also did the necessary optics, electrical design, and chemistry.

The 2-kilogram prototype that Hemond engineered cost around 1000 dollars to produce, but he already has a plan to simplify the electronics in the next iteration of the design, which could reduce costs by as much as 300 dollars. He is currently in negotiations with investors, and plans to start a company to carry out the final pieces of

the research and turn the tiny mass spec into a commercial product as quickly as possible. "I'm quite certain that it's an interesting enough piece of equipment to find a market," Hemond said. As is often the case, though, there is more than one research team chasing the same great idea. "There are other groups that are miniaturizing mass spectrometers. This one may be—*may be*, I don't want to say that it *is*—ahead of the curve at the moment. I'd like to keep it there."

Adam Catherman is a third year PhD student.

"Ah, you're almost done, then?" I asked him.

He snorted with laughter. "I wish. We recently moved to a new university, and changing labs is eating up a lot of time." Moving to a new laboratory is much like moving to a new house, except nearly everything in the lab is heavy, fragile, and toxic, so taking inventory and packing are even bigger nightmares. Catherman and his colleagues in the field of proteomics use some of the biggest mass specs available.

Proteomics, the large-scale study of the structure and function of proteins, is a relatively new field. Its name is derived from the way scientists describe it, as "the *prote*in complement to the gen*ome*," the simultaneous analysis of the entire protein content of a cell, tissue, or organism.

Now that the entire human genome has been sequenced, many researchers feel that the next big challenge is to specify the function of each gene and determine the roles of the individual proteins encoded by those genes. The long-term goal of proteomics, to better understand the interaction between genes and proteins, might help scientists find markers for various diseases. That, in turn, would help them design more effective

therapies and pharmaceuticals.

Given the intricacy of a single protein, the long chain of amino acids (usually several hundred) folded into a unique 3-D shape, proteomics is a messy, complicated business. Current estimates suggest that the human body contains between 60,000 and 70,000 different proteins. That seems like a huge number, but the challenges of proteomics start to pile up long before researchers start talking about analyzing a whole organism. A single human cell contains around 10,000 proteins. Mass spectrometry continues to be one of the most important methods for analyzing proteins.

I asked Catherman what other techniques he and his labmates might use in their experiments. "Mass spectrometry seems to be the only show in town," he said, but then he back-pedaled a little. Though many scientists think "mass spectrometry" immediately upon hearing the word "proteomics," because so much proteomics work has been done via mass spec, the instrument is not an ideal tool for the field. Even the softest ionization methods cause proteins to fragment, further complicating an already messy situation.

Some researchers study proteomics using a separation method called the western blot technique, but western blots require specific reagents and antibodies, so the scientist has to know the exact identity of the protein he's working with before he can start the experiment. Despite the limitations of mass spectrometry, at the moment there is no other option for a researcher trying to discover unknown proteins.

Though Catherman spent part of his undergraduate career working on research projects, he only came to mass spectrometry after he started his graduate studies. "It was the power of mass spectrometry that appealed to me," he said. "It has the ability to detect so many species, so many protein types at once."

Like Brian Hemond, Catherman says that the best part of his job is transforming a gas into a tangible record, a spectrum that can be analyzed and parsed on a printed page. "When you see good data come off the instrument, that's the defining moment. There's lots of tedium in this field, but when you get quality spectra, suddenly it's all worth it."

Some scientists don't see the mass spectrometer as a means to a better understanding of the sea and the sky, but as an interesting game. Carl Johnson and Melissa Soule work a couple of buildings over from Rachel Stanley, at the WHOI Department of Marine Chemistry and Geochemistry's Organic Mass Spectrometry Facility. Johnson describes himself as a "hired gun." Rather than designing research experiments of their own, he and Soule spend their days running samples for other scientists who do not have their own mass spectrometers. Johnson has intense eyes that light up a bit when he asks me, "Do you want to see the toys?"

Two of the mass spectrometers in the laboratory are sitting on the floor, forming the base of the lab bench that the rest of the equipment sits on. The third, a million-dollar, high-resolution instrument that draws collaborators from across the country, gets to sit on top of the counter, instead of under it.

Johnson has used these instruments to analyze "just about any fluid that can go through a gas chromatograph, and just about any solid that can be dissolved in a fluid that can go through a gas chromatograph." That includes oil components ranging from single-carbon methane molecules to 30-carbon hopane molecules, modern plants, and archaeological remains. "Basically, everything from dead stuff to living stuff," he says.

Since Johnson works with a wide variety of researchers, from archaeologists to

petroleum engineers, every project is a new challenge. He says the only thing that stays constant in the work is that, "it always takes longer than you expect."

One of his fastest projects involved analyzing a single sample from the recent oil spill in the Gulf of Mexico. The scientist showed up with all the necessary paperwork in hand and a properly prepared sample, and he got data by the end of the day. It took six hours to design the experiment, then 20 minutes to produce each measurement. (Samples are always run in triplicate to verify the precision of the measurement.)

At the other end of the spectrum, Johnson remembers a scientist who wanted to look at a rare isotope of nitrogen in 15 different plant pigments. She had such tiny quantities of each pigment that they could hardly be seen with the naked eye, but Johnson says she told him, "That's all I can possibly collect. Run it." It took six weeks to develop an experiment, and the procedure they used required retooling the instrument with specialty parts.

"Scientists want to hand you a vial of goop and say, 'Here, Dr. Spock, tell me what's in this,' and I have to disappoint them. It doesn't always work that way."

"My favorite part of my job is when someone says, 'I've got this project, and everyone tells me it can't be done, but I think it can be done and I think you're the guy to do it.' And then they give me two weeks' salary support and I just get to sit there and tinker with the instrument. I like pushing the instrument to the edge of its capabilities. I like calling up the company that makes the instruments and saying, 'Hey, did you know your instrument could do this?' and hearing them say, 'No. We didn't.' But really, it's not about bragging rights. I just like tinkering with the instruments." Johnson enjoys contributing useful data to scientific research, but what he really loves is the sheer joy of

playing with the mass spec, just for the sake of doing it.

Johnson once spent three days tuning the high-resolution instrument so he could run a single experiment. Some scientists might call such fervor a huge gamble, or a monumental waste of time. "You've gotta be bold," Johnson said. "You've gotta just do it." Collectively, we have the technology and imagination to do just about anything. The limiting factors are time and money.

When I visited her laboratory, Soule was helping a student troubleshoot an experiment. The two of them wore labcoats, safety goggles, and rubber gloves, but the problem appeared to be somewhere in the communication between the spectrometer and the computer, because they were both crowded in front of a monitor, pointing at things on the screen.

"For a million dollars, you would think it would work perfectly, but that's not the case."

How long it takes to collect data depends on the type of sample and the goal of the experiment. Often, with a high-resolution instrument, running the spectrum takes only 20 minutes, but figuring out the best way to run the experiment can take a full day.

Soule's least favorite part of her job is dealing with the broken instruments, which are usually casualties of wear and tear rather than human error. I asked her if the rule of thumb I had heard, that the instrument would be down 25% of the time, seemed to hold in her laboratory. She pursed her lips and thought for a moment before she said, "Yeah, that's about right. It's definitely less than 50."

Fascinating. Versatile. Powerful. As I speak with chemists, geologists, biologists, and oceanographers about mass spectrometry, I hear the same words come up,

time and time again. The scientific community has a lot invested in mass spectrometers, and apparently the instrument is versatile and powerful enough to be worth the time and trouble.

In addition to the behemoth instrument that she uses in the Total Oxygen Isotope project, Rachel Stanley has a smaller mass spectrometer that she can take to sea with her. She uses it to study the concentrations of the non-reactive noble gases, helium and neon and such, in the ocean. Stanley recounted the story of her mass spec's last boat trip. The day they loaded their vessel was a stormy one, and the narrow gangplank quickly grew slippery. "I held my breath the whole time they were putting it on the boat," Stanley said, "but it made it safely."

Once aboard the research vessel, Stanley's mass spectrometer collected data from the ocean every two seconds for three weeks, amassing tens of thousands of samples. "Nobody would be willing to stop the boat that often," she joked. I asked how long it would take to analyze all that raw data. She explained that this system, like the first one we had looked at, was completely automated, and that she had needed to purchase a faster computer in order to process all the data files.

One day, maybe Stanley will have a whole swarm of miniature mass spectrometers of the type Brian Hemond hopes to build, seeds of inquiry to scatter across the ocean floor. I find myself wondering if a computer exists that could keep up with the amount of data such a fleet would produce. The mass spec may have been built on physics, but it has been shaped by chemistry, biology, computer science and medicine. In turn, it has helped to shape those fields.

When I arrived at Stanley's laboratory, she was filling out an order form to acquire a new mass spec. When I went to visit Bryan Hemond, he had turned in all his paperwork to graduate, but was still at work, crafting the next iteration of his tiny instrument. Meanwhile, in each of the thousands of laboratories I didn't visit, in crime labs and pharmaceutical companies and universities all over the country, a researcher leans over her instrument, feeding it samples, fine-tuning its magnets, puzzling over its results. Sooner or later, she will get tired and go home. But once she's got her instrument set up correctly and has designed her experiment well, the mass spec will hum on.

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