ELSEVIER

International Journal of Mass Spectrometry 212 (2001) 89–95

International Journal of Mass Spectrometry

www.elsevier.com/locate/ijms

Mixture Analysis by Mass Spectrometry: Now's The Time

R.W. Kondrat

University of California, Chemistry Department, Riverside, CA 92521

I entered the last stop on my tour, a lab with bright lights and fancy equipment. I didn't really know what the machine here was doing. Every few minutes whirring and clicking sounds were heard—small lights flashed and then silence. A robot arm moved here and there like they always do, knowing what it needed to do even though I was clueless. My editor had suggested me for this assignment because I had once been involved with science. Seeing this facility and this lab made me feel inadequate for the job. It was not the first time I had felt this way, but I knew I could write a reasonable article for our readers. While I was lost in my thoughts of inadequacy, my host said, "This is an automated tandem mass spectrometer."

I looked it over while he gave a detailed explanation of what was happening. I was told this particular machine might help scientists find a new cure for a disease; something about proteomics, he said. I was also told that there were similar machines scattered throughout the facility monitoring the workers for drugs of abuse, the incoming air to make sure it was clean, testing what went out of the building to minimize pollution, and controlling the manufacturing process real time to ensure product quality. I was informed that many such instruments exist all over the world doing similar things. I heard various technical terms that meant little to me, but MS/MS and tandem mass spectrometry were mentioned often.

At a suitable point on one such mention I asked, "Sort of like tandem bicycles?" The tour continued following a polite smile from my host, and I was told how many samples could be done in a day owing to automation. I saw a picture on the wall nearby, of a short, thin-faced, bearded man standing in front of a machine, wires hanging all about him and, I went over to examine it. My host informed me, "That's what one of these machines used to look like. As you see, the modern equipment is a lot smaller these days. More affordable than they used to be."

"Ah, the wonders of modern technology," I mused. "Who's the man in the picture?"

"Oh, that's Graham Cooks. He did research at Purdue University and played a part in getting us from the old to the new."

I turned and asked my host, "So what did he do?"

"That would take too long, he replied, I'll give you some articles to read about it later. But you asked about tandem mass spectrometry before. That was one thing he played a major role in."

Sensing a story here, I said, "So tell me about that."

"Well, in the early 1970s we had only gas chromatography coupled to a mass spectrometer (GCMS) if we wanted to analyze individual components of complex mixtures. It was no wonder people dreaded certain analyses, and had no chance at all with many others. Chemical derivatization was used but required more time and there still

Email-address: mspec@mail.ucr.edu

Dedicated to R. Graham Cooks on the occasion of his sixtieth birthday.

PII S1387-3806(01)00516-4

were laborious sample cleanup methods that were difficult to do with small amounts of materials. Thanks in part to Dr. Cooks, how we today perceive the analysis of mixtures by mass spectrometry is very different. We are much more optimistic and have fewer limitations since now we have a large arsenal of instruments and techniques available which can be used in an automated manner to perform far more complex analyses like you see here." He swept his arm across the room as if waving a magic wand over it all.

"He did all that!" I said in astonishment.

"No, no, don't misunderstand. All of this didn't happen overnight, and thousands contributed in fields like material science, computing, electronics, and other mass spectroscopists."

Then he motioned for me to follow him. "Come to my office. I can show you something that will give you an idea of how this man was involved in all of this."

While we walked, he explained, "By the early 1990s the technique of tandem mass spectrometry was an accepted method for amino acid sequencing of peptides and other biomolecules, and they didn't even have to be pure! For a long time the journal *Analytical Chemistry* reviewed developments in mass spectrometry every two years. By the mid '90s they no longer had a section called tandem mass spectrometry like before. That's how common it had become."

I followed him through a door in the lab that took us to his office, where he motioned me to a chair. The sound of jazz on the radio was in the background as I sat down, while he turned to look over his shelves. I looked to my right and could see the robot doing its thing through a large window that overlooked the lab.

"Ah, here it is," as he pulled out a book and handed it to me. "You can read what one of the sages of mass spectrometry, Fred McLafferty, said about Graham's contribution to tandem mass spectrometry for yourself."

He opened the book to where a yellow piece of paper protruded and handed it to me. The book was opened to the introduction at the section The MS/MS Explosion. I glanced at the book cover and saw a simple title—*Tandem Mass Spectrometry* [1]. A section was highlighted in yellow, and I read aloud into my tape recorder, "Research and applications have grown tremendously. . . . [S]pearheading this effort, the pioneering work of Professor R.G. Cooks. . . . has attracted broad scientific attention to the promise of MS/MS. . . . [S]pectacular success of such applications has made this area of MS/MS highly visible."

While I was recording this text, my host looked for something else, and when I had finished, he handed me a journal. I looked at the cover, which read *International Journal of Mass Spectrometry*. "Just look at the table of contents and introduction," he said. I saw it was dedicated to Dr. Cooks on the occasion of his sixtieth birthday. I saw topics dealing with ion traps, surface-induced dissociation, membranes, and much more. "Seems this guy was quite busy and well regarded," I offered. "Why do you know so much about him?"

"Oh, I've been intrigued by the man for some time. He even wrote an article comparing art to mass spectrometry, which is very interesting. I like this the best of all his publications." He turned to rummage over the shelves again, now handing me a journal called *Analytical Chemistry*. The cover had a painting of a woman near a table with a caption that read "Creativity through Instrumentation" [2].

My host sat in his chair and turned it so he could see the lab through the window and continued: "Graham often gave presentations at various meetings, where he would make analogies between mass spectrometry and art. A particular group of artists actually, called the pre-Raphelites. I liked this analogy very much and, being a musician myself, figured you could do something similar with mass spectrometry and music too."

"How do you mean?" I asked, quite confused. I looked over the various paintings in the article while he spoke. He turned to face me, and I could see his eyes light up as he began to speak: "Well, in medieval times music was simple, monophonic—sort of like a single-sector mass spectrometer. Eventually, two melodies were played simultaneously; this polyphonic music expanded the musician's ability to express himself. Enter double-focusing mass spectrometers. As time progressed and new musical instruments were

invented, more complex expressions were made possible. Today we have complex symphonic compositions, sophisticated jazz, and a lot more. It's like the many new hybrid mass spectrometers that can analyze inorganic as well as organic species that we have today. You saw one of those hybrids in the lab just now. It's called a TOF-TOF. You could say the modern well-equipped mass spectrometry facility looks like an orchestra, each instrument playing its part in getting the job done, with a director taking the ensemble where he believes it should go. But how an instrument is used, musical or technical, is most important. At the start of the twentieth century in America, people played trumpets, but when Louis Armstrong came along and broke traditions, he inspired many after him. A fellow by the name of Aston really got us started in mass spectrometry, and by the 1940s people were doing lots of work using the technique in the oil industry. . . . By the way, did you know that Graham was inspired by Aston?"

Without waiting for my reply, he continued. "He even named the lab at Purdue after him. I believe the idea was that Graham worked with metastable ions and Aston had first noticed them in his instrument, and called them Aston bands. But don't print that because I'm not 100% sure about the reason."

"Anyway, oil is a messy business, and chromatographic separations or mass spectrometry by themselves gave limited information. It wasn't until GCMS was invented that we could get around the problems of the two individual methods. Sort of like the invention of the vibraphone in the early twentieth century, which then winds up in jazz groups and added new possibilities and sounds to the jazz ensemble. Or take the saxophone, for example: A guy like Charlie Parker comes along and after everyone hears how he plays a saxophone, they all want to play their instrument like him [3]. Parker was someone who clearly changed jazz forever, and sometimes I think that's Graham Cooks in our field of mass spectrometry. After him, lots of scientists wanted to do tandem mass spectrometry. Lots of people may have had the idea of using a mass spectrometer differently, but Graham sort of led the charge thanks to the data/music he got from his instrument."

Then he stopped and suddenly leaned back violently. I jumped slightly in my chair. "Do you know that today well over half of all mass spectrometers sold have the ability to do tandem mass spectrometry experiments, but in the early 1970s that number was zero? Graham is responsible for this to a significant extent." He paused to take a breath while I tried to comprehend everything he had just told me, glad that my tape recorder was getting all this for future reference. I asked, "So you're a jazz fan I take it?"

"Well, I like several styles of music and especially the innovations and improvisations. Jazz relies on improvisations, and so does mass spectrometry. Just wait a while and someone else will come out with something new and the rest of us jump on board. In jazz you had swing, bebop, fusion, acid, and others you can't even name. Now we use our mass spectrometers in ways our predecessors never could. For instance, we have many different scanning modes on these new hybrid instruments that didn't exist in the 1960s. And so it goes—one musician/spectroscopist may develop a style which others copy, improve, modify, etc, to fit the needs and desires of the scientific community. We all wonder who will be the next innovator. That's why I have that piece of paper hanging on the wall there. It reminds me that we need to be innovative." He pointed to two lines of sheet music hanging above the door to my right (Fig. 1).

As I looked, he explained. "Each has the same eight notes, those letters you see written there. Ah, but depending on how you emphasize them, I mean timing and rhythm, you get different melodies. So take a mass spectrometer and work with it. Then someone comes along and finds something else to try with it and voila, a new tune!"

"So when did this fellow Cooks do all this research?" I pondered out loud.

"Well, I suppose Graham's contributions to analytical mass spectrometry begin in 1971, when he published two articles together with John Beynon that literally turned the field of mass spectrometry around [4,5]. In these articles, this duo described a reversed geometry mass spectrometer. You could say that this was the first hybrid instrument in mass spectrometry. Prior to the '70s, mass spectrometry was performed

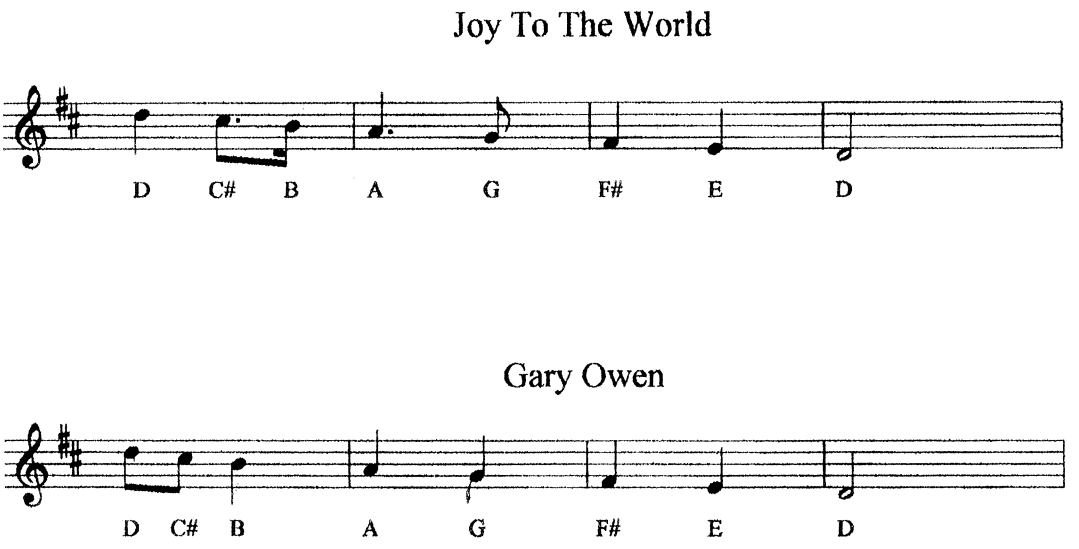

Fig. 1. ●●●

on sector instruments designed with a forward geometry—meaning the electric sector preceding the magnetic sector. At that time, John Beynon and Graham Cooks were interested in studying the fragmentation processes involved in mass spectrometers via the investigation of metastable decompositions; i.e., those that occur outside the ion source of the instrument. Their goal was to better understand the how and why of fragmentations. Reminds me of playing harmonica: You can play it backwards too, if you can get used to it. I know several people who do it. Like with mass spectrometry today, people don't even think about it anymore."

He leaned forward again and continued: "Anyway, the idea of switching the positions of the two sectors allowed a more specific type of investigation to be performed. It allowed one to isolate a particular ion for study, thus being able to examine all metastable decompositions from a chosen parent in a single experiment. They labeled this approach MIKES (mass-analyzed ion kinetic energy spectrometry). In 1973, the quartet of Beynon, Cooks, Caprioli, and Lester published a book detailing what they had discovered about these processes [6]. Of course, today we modern musicians of mass spectrometry see the utility of this concept to mixture analysis in the technique referred to as MS/MS or tandem mass spectrometry. Of course, many more steps were needed to achieve what is today a practical real-time analytical tool."

"One of those steps was the incorporation of collisional activation (CA) that another spectroscopist/musician, Keith Jennings, had developed. CA experiments provided complimentary information to metastable ions and were also similar to conventional mass spectra. So by the time of their 1973 publication, Jenning's observations had paved the way for researchers to use CA to enhance otherwise weak metastable signals."

I was consciously aware of the fact that I was starting to doze off at this point and was desperately trying to avoid my host noticing this fact. However, I did not know that he was so engrossed in relating this story to me that he was not aware of my state.

My host continued, "At the time, the Purdue group was mostly a physical/organic research effort. How they got involved with a more analytical effort can be traced to two factors. First, the work of other performers in mass spectrometry, which had shown the ability of metastables ions to assist in obtaining information that could be used for analysis of very simple mixtures. Djerassi [7] did this with a steroid mixture in

1974, and that same year McClafferty [8] was able to distinguish small peptide isomers using the CA process. The following year, Graham was appointed to the faculty of Purdue University in the analytical division, so it set the stage to explore these findings more completely."

Suddenly, a crashing sound awakened me, and I saw my host was standing by the shelves again and had dropped a journal on the floor. I glanced at my tape recorder and noticed it was time for a new tape and asked politely for his patience while I made the change. He handed me another *Analytical Chemistry* journal. I nodded that I was ready again, and he proceeded. I looked down at the journal in my hands and saw "Direct Mixture Analysis" on the cover [9]. "Coffee?" my host asked. I nodded, "black please."

He continued speaking as he went to the corner of his office and poured two cups of coffee. "Less than 3 years after Graham started to look into the analytical aspects of the Purdue instrument, he published the paper you'll find in there. It's a summary of the Purdue group's efforts to that point. Whenever an innovator comes along, be it in music or mass spectrometry, there are the skeptics. One of Graham's first students, Kondrat, was a skeptic when confronted with the idea of using MIKES for mixture analysis. He thought it had limited possibilities and might not really be such a great thing. Boy, was he wrong!"

He returned to his chair, faced me, and handed me my coffee as he continued.

"Jim Litton and Terry Kruger, who you never hear about anymore, got the first results and slowly converted the others into believers [10]. So it was natural for someone to eventually decide to push the idea of doing mixture analysis without sample cleanup. One day Kondrat got some freshly cut weeds, poison hemlock actually, ground them in liquid nitrogen and put the pieces in the instrument hoping to identify the alkaloid coniine. It worked surprisingly well. A few weeks later he gets a phone call at home on a Sunday afternoon from Graham, who tells him this technique really does work! Apparently Graham decided to see for himself if those results were reproducible and found they were. The two were enjoying a beer in the lab a bit later."

"Must have been an exciting time in the lab then," I said.

"I suppose it's always exciting when you have something new. If you can find other people excited by what you have, it makes it better. Graham found such a man in the Department of Medicinal Chemistry and Pharmacognosy at Purdue, Dr. Jerry McLaughlin. He was a big fan of the new technique and he really helped a lot, not just by providing real-world samples of plant extracts but, may be most importantly, his enthusiasm about it all. Terry Kruger was also such a man. He was a visiting professor from Ball State University with all sorts of ideas about samples that could be amenable to the new technique. Graham had to turn down lots of possibilities—those he was not comfortable with. There were certainly no shortages of ideas in those days though. You wouldn't believe all of them if I told you."

"Anyway, working with enthusiastic people certainly made it easier to go out and sell the idea to others. One of the first presentations was at a Pittsburgh Conference in Cleveland one year—'77, I believe it was." I looked up, confused, but he quickly said, "Don't ask."

"It was the last paper of an afternoon session, not a big crowd. It went well even though there were some skeptics. Things were different at the same conference a year later, because the people from VG Micromass saw potential in the technique and were handing out copies of the paper you're looking at because they had a commercially available reversed geometry instrument."

I interrupted at this point by saying, "So that's when things took off I suppose."

"Not really," came back the response. "Too many limitations with sector instruments and computers in those days. But the seed was certainly planted, that's for sure."

My host paused to take a sip of his lukewarm coffee and continued. "At first, Graham chose to concentrate his efforts with the sector instrument he had at Purdue. The Purdue group improved their detection limits by doing what they called single-reaction monitoring, just like the selected-ion monitoring experiment used in GCMS. Then they showed

the flexibility of the MSMS technique by doing analyses using negative ions and demonstrating the ability to distinguish isomers via charge stripping reactions. Neutral loss scans came right out of that, sort of fell into their lap."

"But it didn't take long for the next musicians to come along. Trying to get the same data on forward geometry instruments by a new scanning method, for example [11]. Reminds me of those notes up there," as he pointed again to the sheet music.

"Soon Enke and Yost published on how to get MSMS done with quadrupole technology [12]. This really got a lot of people interested because GCMS was quite common in those days and done mostly on quadrupoles since they were smaller and cheaper. So it's not surprising that before long, a commercial triple quadrupole instrument was available from the Finnigan and Sciex companies. Graham got one of the first ones from Finnigan in the early '80s. In the following few years you start to see all sorts of papers and posters at the annual mass spectrometry conferences about MSMS, both applications and new instrument options. From this point on, Graham's group worked on various aspects of MSMS including instrumentation, trying to improve the collision process, and, of course, practical applications to real-world samples. Just to show you how much MSMS affected things in the Aston Lab, in 1975, when Graham launched his efforts, he had about six or seven people working for him. By the end of the decade that number had tripled! He started to keep track of how many people went through the Aston lab, and in 25 years or so he had about 200! That's an average of seven people per year working there on something."

"That's one busy man," I said. "But why all the fuss about different instruments? I thought everyone wanted quadrupoles?"

"Well, turns out every option had its limitations and people were looking to minimize these and get the most bang for their buck. So eventually MSMS was done on all sorts of instruments—virtually every combination you can think of: multiple sectors, a double quadrupole, pentaquad, combinations of sectors with quadrupoles, Fourier transform instruments, time of flight, and new ion trap technology. Graham was also involved with a lot of these. Hell, in the late '90s he even made a movie about ions in a trap and really impressed people with it at one of the mass spec conferences! Later, other musicians/spectroscopists decided that if MSMS was good, why not MS/MS/MS, or MS^n as it became to be called. Anyway, the ability to do MSMS on quadrupole instruments was one of the sparks that drove the technique to where it is today."

"And where's that?" I wondered. I sensed we were getting close to the end of my interview. "Well, it may be ironic, but one thing was people started to do MSMS on GCMS instruments." I looked up, puzzled, and said, "I thought it was all about an alternative to GCMS?"

"Yes and no. People always want more from what they have. Same goes for GCMS. Just like a harmonica player trying to get more notes from his instrument by using bending and overblow techniques. GCMS gave you some structure information, but not enough to identify new compounds always. Getting exact mass measurements only gave composition, but not how that composition was put together. It's like having all the notes of a piece of music but not knowing how they are to be arranged. So MSMS offered everyone, including the GCMS user, the opportunity to get that extra bit of structure information—those few extra notes."

Then my host paused for a few seconds and seemed to reflect on something while he looked at me. "I honestly think that MSMS was ahead of its time, really."

"How do you mean?" I scratched my head in puzzlement.

"Well, Graham and others knew the limits of tandem mass spectrometry. The CA process on sector instruments was inefficient, limiting sensitivity. Softer methods of ionization were available but not routine enough to simplify things and to allow nonvolatile species to be analyzed. Computer interfacing to speed it all up and to perform new experiments like neutral loss-linked scans was important. Although MSMS allowed you to get more information about ions and structure than before, people couldn't generate many of the ions that needed to be studied or couldn't get

samples into the instruments. But as with every new improvisation, the limits are explored and removed. Graham started the ball rolling. Like I said earlier, his group explored the possibilities while others contributed by inventing new ionization processes or new liquid chromatography interfaces to mass spectrometers. Combined with faster computers, you now have so much more power to analyze samples that you see these instruments everywhere." He turned in his chair toward the lab once again and waved his hand over it. (The reader is here invited to the web site *http://www.charlieparker.com/pinkpan.html.* Once there, select "Play Now's the Time" before reading further in this article. This will require a sound card and speakers.) Then my host stood and slowly walked over to the window and looked at the instrument quietly working there.

"Now people who never thought about mass spectrometry 20–30 years ago use it routinely for things that were only imagined then. You can see that by the attendance at the annual mass spectrometry conference. Used to be about 1200 people in the early '80s—by the turn of the century it had nearly tripled! Lots of people in the biotech business showed how they used MSMS in their work on combinatorial chemistry, proteomics, genomics, and lots more. A whole new bag of terms became standard jargon, like triple quads, linked scans, MRM "(here he quickly glanced my way and said" means multiple reaction monitoring"), "*de novo* sequencing, hybrid instruments, chemical noise, and others. The last one came from Dr. Jon Amy of the Purdue instrumentation facility that helped explain one of the advantages of MSMS. Dr. Amy pointed out how it was 'chemical noise' from column bleed that affected detection limits in GCMS and how MSMS nearly removed this problem."

Suddenly, he turned back and pointed to the radio while he listened. "Hear that tune? That's Charlie Parker playing 'Now's The Time,' one of my favorites." He turned to look back at the lab and continued.

"You know, Graham was once overheard saying at one of the conferences that perhaps they had oversold the technique."

There was a pause as he continued looking at the lab through the window. For several seconds, he tapped his fingers on it in rhythm with the song. Then he finally said aloud, as if he were speaking to the picture I had noticed in the lab, "Don't worry Graham. You didn't oversell it at all. Now's the time."

References

[1] Tandem Mass Spectrometry, F.W. McLafferty (Ed.), Wiley-Interscience, New York 1983.
[2] R.G. Cooks, Anal. Chem. 57(7) (1985) 823A.
[3] All Music Guide to Jazz, M. Erlewine, V. Bogdanov, C. Woodstra, and S. Yanow (Eds.), 3rd ed., Miller Freeman Books, San Francisco 1998, p. 872.
[4] J.H. Beynon, R.M. Caprioli, T. Ast, Org. Mass Spectrom. 5 (1971) 229.
[5] J.H. Beynon, R.G. Cooks, Res. Develop., 22(11) (1971) 26.
[6] R.G. Cooks, J.H. Beynon, R.M. Caprioli, G.R. Lester, Metastable Ions, Elsevier, Amsterdam 1973.
[7] D.H. Smith, C. Djerassi, K.H. Maurer, U. Rapp, J. Amer. Chem. Soc. 96 (1974) 3482.
[8] H.K. Wipf, P. Irving, M. McCamish, R. Venkataraghavan, F.W. McLafferty, J. Amer. Chem. Soc. 9 (1973) 3369.
[9] R.A. Yost, C.G. Enke, Anal. Chem. 51 (1979) 1251A.
[10] T.L. Kruger, J.F. Litton, R.G. Cooks, Anal. Lett. 9 (1976) 533.