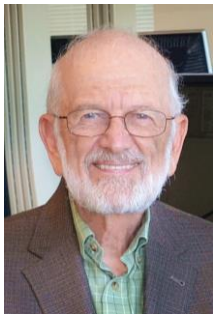
Clinical Chemistry

Ambient Ionization Mass Spectrometry for Point-of-Care Diagnostics and Other Clinical Measurements



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Guest:
Dr. R. Graham Cooks, Distinguished Professor of Chemistry and Co-Director of the Center for Analytical Instrumentation Development at Purdue University in Indiana.

Bob Barrett: This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

A driving force in the development of point-of-care diagnostics is to conveniently provide information without delay so that healthcare decisions can be made while the patient is on-site. Most point-of-care devices are based on immunochemical, electrochemical or colorimetric techniques that can be miniaturized and made portable.

Today's devices were inconceivable just a few decades ago. Can we look forward to a day in the future when mass spectrometry procedures can be brought right to the bedside? That day may not be all that far off.

Ambient Ionization Mass Spectrometry allows direct chemical analysis of unmodified and complex biological samples like whole blood. This technique may provide new opportunities for point-of-care diagnostics and rapid measurements of exogenous and endogenous molecules in small volumes of biological samples, especially when coupled with miniature mass spectrometers.

Ambient Ionization Mass Spectrometry for Point-Of-Care Diagnostics and Other Clinical Measurements is the title of a review article appearing in the January 2016 issue of *Clinical Chemistry*, a special issue devoted to mass spectrometry and the clinical laboratory.

The senior author of that paper is Dr. R. Graham Cooks, the Henry Bohn Haas Distinguished Professor of Chemistry and Co-Director of the Center for Analytical Instrumentation Development at Purdue University in West Lafayette, Indiana. He is our guest in this podcast.

Dr. Cooks, tell us, what contributions has mass spectrometry made to clinical chemistry?

Dr. R. Graham Cooks: So the contributions of mass spec to clinical chemistry are actually summarized quite well in an issue of the journal *Clinical Chemistry*, which will appear in January, so this is a special issue on mass spec, and for anybody interested in the subject that's probably the first place to go to. I will try and summarize not what's in that issue, but rather the way I see the contributions of mass spec to clinical chemistry.

Some of these go back quite a long time; some of them are very recent. So in the area of drug overdose, mass spectrometry has been used for a long time because of its specificity and because of the ability to do complex mixture analysis in combination with gas chromatography or liquid chromatography to identify drug overdose -- identify the drug involved in overdose cases.

Another area which has long since been implemented is breath analysis in the emergency room. That's a simple gas analysis type of mass spectrometry determining oxygen in exhaled breath.

There are several others that are worth talking about; the identification as the causative agent for duodenal ulcers, which was a mass spec success story.

A tremendously important one which impacts every family in the US and in most other countries now is the detection of inborn errors of metabolism. That is done by triple-quadrupole mass spectrometry. That's an experiment in which you identify these metabolic errors using two stages of mass analysis. In other words, the tandem mass spectrometry experiment or as it's often known MS/MS, that is mass spectrometry/mass spectrometry.

So that's a really quick experiment which doesn't require that you do chromatography; in fact, it involves just a pinprick. So it's a very simple test which is applied to some dozens of inborn metabolite errors.

The other items on that list would be the standard test for some of the most difficult compounds to detect in blood, which are the various forms of

vitamin D and also testosterone, both of which appear at very, very low levels.

Similarly, perhaps in terms of transplant rejection drugs, these are best determined by the tandem mass spectrometry LC methodology.

So that's a whole long list; some of them have been around for decades, like the drug overdose and the breath analysis. Some of them have been developed over a period of decades, like the triple-quadrupole or inborn errors of metabolism that have only in the last decade or so been more or less universally used.

And then some are in the process of development, like the transplant rejection drug analysis, which can be done by many other methods, but it looks like mass spec is likely to turn out to be the premier method for that detection.

Bob Barrett: And what are the characteristics of mass spectrometry that make it important in clinical chemistry?

Dr. R. Graham Cooks: I guess the characteristics; there are two points I would like to make there perhaps. One is the positive characteristics and then the ones that are missing.

So in terms of the positive characteristics, the speed of mass spectrometry in most cases is a big advantage. So I say in most cases, because one has to be careful of how much chromatography is involved and that can vary from many minutes down to virtually none at all, or in fact none at all in the MS/MS experiment.

But flexibility is probably an even more important advantage of mass spec; in other words, you can change the conditions of the instrumentation to allow you to look at compound A or compound B so that you have a fully versatile system for analysis. So general applicability would be the second advantage.

And the third one would be what we have already referred to in terms of the vitamin D and testosterone; that is the extremely high sensitivity of mass spectrometry.

A fourth advantage would be the ability to do quantitation, and quantitation is typically done then by first doing the separation, LC or GC, and then doing an MS/MS experiment, tandem mass spec experiment, in which one goes back and forth

between the precursor ion, which is characteristic of the drug of interest, and its product, so that pair, to find some particular compound of interest.

And then measuring at virtually the same time, within a fraction of a second later, an isotopically labeled version of that compound or some other internal standard and its characteristic fragments.

That experiment is called Multiple Reaction Monitoring or MRM. That's an old experiment. It was described actually out of this lab several decades ago, but it's widely used in quantitation, in proteomics, and in and all the other -omics techniques, and then also in a lot of clinical chemistry.

So those are the principal advantages of mass spectrometry: speed, versatility, sensitivity, and the ability to do quantitative analysis.

In terms of what's *not* there at the moment, is high throughput. And in terms of what is also *not* there is low cost. And so those are the places where mass spec needs to develop, and is in the process of developing.

The high throughput experiment, well, the multiplexed experiment, is almost never done by mass spec. This is an experiment in which you want to go through as many samples as you can using the appropriate performance in the mass spectrometer. And of course the cost, the only way one can afford to use mass spectrometry for things like vitamin D, testosterone, is to essentially take all of the countries' sample for those and put them in a few places where some high performance mass spectrometer is used to run through those many samples, but you are coming just to a couple of labs.

So the developments that one looks for in mass spec are to maintain the kind of performance that one has been used to, but to simplify and in fact to miniaturize the instrumentation so that it can be applied at point-of-care. That's really where the future probably lies.

Bob Barrett: In the area of diagnostics, what modern spectroscopic methods are potentially useful, or for that matter have actually proven themselves to be useful?

Dr. R. Graham Cooks: Yeah. So I guess this question is about spectroscopic methods mostly, and I suppose one starts by saying that it really is the genomics methods which one needs to start from and to ask beyond that, what can one learn by spectroscopy?

The case that everybody knows about is diabetes, and detection of diabetes is now a solved problem of course, detecting blood sugar. Modern methods of spectroscopy have contributed greatly to those commercial methodologies.

Perhaps the more interesting, because it's not anywhere near to being a solved case is cancer diagnostics, and there modern spectroscopic methods, including second order spectroscopy, conventional, laser spectroscopy, and Raman spectroscopy are techniques that are worth watching. This isn't actually useful at this point, but certainly they have potential in those areas.

Bob Barrett: And what are the advantages of mass spectrometry over these methods?

Dr. R. Graham Cooks: So I think what mass spec offers is much more specificity, molecular specificity, than one would get from a Raman or indeed from second order spectroscopy methods, at least in the way in which the kinds of samples, at which one would be asking questions.

So, mass spec has got this phenomenal molecular specificity, which means that it's applicable to complex materials. This of course includes the biofluids, but it also includes much more than that, and I think that this is really the much more than that is tissue, and I see the tissue analysis area as being the area in which there is most room for development, and where mass spectrometry has some really unique advantages.

So the advantages of mass spectrometry in terms of tissue analysis are that it represents an imaging technique that one can do mass spectrometry, make mass spec measurements at a point on a two-dimensional surface, for example. One can move the beam that one uses to create the ions over that surface and one can then create a map, and such a map would be a molecular map. So it's a molecular map of the compounds that are present on, let's say, a tissue section.

That molecular map could then be read in terms of the chemical nature and to some extent the amounts of the particular molecules that are distributed in that tissue.

And so now we have a new method, aspect imaging method of looking at tissue. And so the advantage of that would be that you could recognize from the mass spectrum whether that tissue is normal by comparison with the mass spectra of reference tissue which are normal, or whether it's cancerous by comparison with the mass spectra of cancerous tissue samples from some library that you create.

Or indeed, in even more detail in some instances, what the stage of that disease is, what the grade of the cancer is.

These experiments have been tried using proteins. They have actually been more successful with much smaller and simpler molecules; that is with the phospholipids.

And so phospholipids are turning out to be treasure troves of information, which is way down from the genomics level, and way down even from the proteomics level, and it's in the level of the metabolome. So we are looking at compounds which reflect the state of the system as it is at a particular time, and that state is most particularly the disease state of that system.

So information on disease states, in other words molecular diagnosis by mass spectrometry, is I think one of the great emerging areas of mass spectrometry.

Bob Barrett: Dr. Cooks, can you describe how molecular diagnosis is achieved by mass spectrometry and what are the strengths of this approach?

Dr. R. Graham Cooks: Okay. So I think the strengths I have indicated already briefly. The strengths are the fact that this experiment doesn't require any sample preparation, that it can be used for samples of intrinsic interests, like tissue, for example, during surgery.

I can describe this experiment perhaps in a little more detail by referring to just one of the methods that are used to implement.

So the mass spectrometer in these tissue imaging experiments is an ordinary mass spectrometer of

some type; ordinary let's say commercial bench top mass spectrometer, and so there is not anything further to say about the mass analyzer component. What one needs to talk about is the way in which ions are created.

And if we are talking about tissue analysis, if we are aiming to do something about intrasurgical analysis, then we need to be able to take this data as quickly as possible, essentially instantaneously. We need to be able to take the data in the open environment; for example, during surgery and intrasurgical environment.

The methodology that is being used for this is mostly a method known as Desorption Electrospray Ionization, abbreviated as DESI. So this is a method in which charged droplets, in fact, a mist of charged droplets of solvent is directed at the sample, at a spot on the sample. And in the course of the interaction of these tiny micro droplets with the sample, compounds from the sample are extracted, and then as additional solvent droplets impact the sample, secondary droplets are created, and they are pulled through the air to the mass spectrometer, and they give rise to a mass spectrum.

So the mass spectrum is a simple low resolution unit mass resolution mass spectrum, and it contains signatures for a variety of phospholipids.

This particular experiment doesn't require that one identify those compounds. It's a bar code type of an experiment, so you simply have a characteristic bar code, which you compare with your reference library of bar codes for different tissue types; for healthy tissue; for diseased tissue; and for various grades of cancer.

That experiment can be done extremely quickly. It has been done for something like 8 different cancer types. This publication started in 2005, the joint publication that we and this group at Purdue did with Richard Caprioli at Vanderbilt, and it has continued up to present, including a longish collaboration that we had with Nathalie Agar at Harvard Medical School, in which we looked at brain tissue.

So brain cancer can be detected and the diagnosis can be performed on that tissue using this kind of mass spectrometry, where you spray solvent as a sample and make a very rapid comparison, an automated comparison with a library of spectra and

come up with an answer. That answer might be, there is no disease in that tissue, the answer might be that there is a 25% glioma, tumor cell concentration at such and such a point, which then represents a margin for that tumor.

So the exciting news about this is that this methodology is giving tumor margins, and there really isn't a rapid way of doing that at the moment.

Bob Barrett: And how successful has mass spectrometry been in practice?

Dr. R. Graham Cooks: In this particular -- I guess that's a general question, but I will relate it to this particular example, this particular application area.

As I mentioned, there has been publications on this topic of tissue rather than biofluid tissue analysis by mass spec for the last 10 years, so it's a 10 year old subject.

In the last five years, as indicated in particular by a review example by Livia Eberlin and Demian Ifa in that same January issue of *Clinical Chemistry* that's about to appear, there have been a number of examples of either the DESI method or a related method, which also works in the ambient environment, being used in an experimental fashion to determine disease states of tissue.

Our own work at the moment involves collaboration with a group in Indianapolis. The lead surgeon there is Aaron Cohen-Gadol. He does lots and lots of brain surgery operations a year. And we are in the process of working with him and Hattab Eyas, who is the neuropathologist there, looking at samples which were taken during surgery.

So the normal course of events during a neurosurgical operation, only one single sample is typically taken, it's sent to pathology. The answer comes back some 20 minutes or so later, at least in the two hospitals with which we have collaborated.

In the experiment that we are doing down in Indianapolis, we have got a small mass spectrometer in the operation room. In the last surgery that was done something like 9 or 10 tiny pieces of tissue were taken and smeared. These samples are normally taken for the sort of single pathology analysis that I have mentioned. In this particular instance they were taken from the tumor itself,

directly in the main tumor mass, but also at the margins, and that experiment just worked extremely well.

We get very clear answers with high sensitivity and specificity as to the nature of the tumor and even as much information as the percentage of tumor cell concentration.

So this is all the basic -- basis of that work is all written up, submitted for publication, being considered at the moment. The extension of that work to a considerable number of patients is underway at the moment, and I don't know when that will be finished, but it's definitely in active development at the moment.

There are probably four or five groups in the world who are doing this kind of diagnosis, particularly for solid tumors. We ourselves have done liver and kidney and prostate and are continuing with bladder and also with brain cancer.

Bob Barrett: Well, finally doctor, let's look ahead, how do you see this approach developing over the next five years or so?

Dr. R. Graham Cooks: I think there is actually two ways in which it's going to develop, and I would distinguish them quite sharply between the kind of intrasurgical mass spectrometry diagnosis that we have been talking about, which I think is obviously on track to be successful, but the track is actually quite slow. I think in five years' time I think there is good hope that this will be much more widely used and will be on the point to becoming standard of care.

With regard to the other applications of mass spectrometry in clinical chemistry, and by the other applications I mean biofluids mostly, the much more extensive use than is now the case for mass spectrometry in biofluid clinical analysis will depend critically on the development of and commercialization actually of small mass spectrometers.

Mass spectrometry has the opportunity to be the go-to technique for point-of-care clinical analysis, but the instrumentation has to be in order of magnitude: smaller, and retain the versatility and the other characteristics of the technique. Those applications will then likely be done without any chromatography; that is, they will be MS/MS applications.

And we have an article also in the current issue of *Clinical Chemistry* January 2016 that I have been mentioning which lays out some of the features of that experiment. So that's an article by Christina Ferreira and several other people from this group.

So I see that development is depending in fact on a commercialization success. I am not involved in commercialization, but I know that it's coming, and if that comes quickly and successfully, I think that will be transformative to clinical chemistry.

Bob Barrett: That was Dr. R. Graham Cooks, Distinguished Professor of Chemistry and Co-Director of the Center for Analytical Instrumentation Development at Purdue University in West Lafayette, Indiana.

He has been our guest in this podcast on Ambient Ionization Mass Spectrometry for Point-of-Care Diagnostics and Other Clinical Measurements. His paper appeared in the January 2016 issue of *Clinical Chemistry*, a special issue devoted to mass spectrometry and the clinical laboratory.

I am Bob Barrett. Thanks for listening!