THE McCRONE GROUP™
·ANALYSIS · EDUCATION · INSTRUMENTS

Webinar Transcript

Identifying Foreign Particulate in Pharmaceutical Products

Access a recording of this webinar and download the slides.

www.mccrone.com/webinar-archives

PRESENTER:

Scott Stoeffler

Scott is a senior research microscopist at McCrone Associates, and specializes in microanalysis of small particles and contaminants using polarized light microscopy, infrared microspectroscopy and scanning electron microscopy with energy dispersive X-ray spectrometry. He is a member of the Midwestern Association of Forensic Scientists, where he has served as Treasurer and President, as well as belonging to the American Society of Trace Evidence Examiners and the Scientific Working Group for Materials Analysis.

Introduction

Welcome to another McCrone Group webinar. My name is Charles Zona, and today our presenter is Scott Stoeffler of McCrone Associates. Scott is going to talk to us about identifying foreign particulate in pharmaceutical products. Scott is a senior research scientist with McCrone Associates and has over 25 years of experience. Scott specializes in microanalysis of small particles and contaminants using polarized light microscopy, infrared microspectroscopy, and scanning electron microscopy. Scott teaches several courses for the Hooke College of Applied Sciences, including Forensic Fiber Identification, Pharmaceutical Materials and Contaminants, and an Introduction to Forensic Trace Evidence.

Scott will field questions from the audience following today's presentation. This webinar is being recorded and will be available on the McCrone Group website under the webinars tab. Now, I'll hand the program over to Scott.

Scott Stoeffler (SS):

Thanks, Chuck. Welcome to our webinar today. I'm Scott Stoeffler, and, as Chuck said, I work in the light microscopy section here at McCrone. A lot of what I do involves isolating and analyzing contaminant particles in pharmaceutical products. In some cases, the testing is fairly straightforward, and one test gives us the answer that we need. In other cases, after the first pass there are still some unanswered questions about the problems that our client has sent to us, and the testing is a little more complex, so we tend to favor an integrated analytical approach here at McCrone. Starting out with, usually, stereomicroscopy, and all of the different types of small-scale sample prep that lets us do, and then branching out into other techniques depending on the nature of the sample and what the data from each successive step shows us. For example, we can go from stereomicroscopy to polarized light microscopy for particles and fibers; we can go to vibrational spectroscopy, infrared or Raman; we can go to scanning electron microscopy, either for imaging or elemental analysis via energy dispersive x-ray spectroscopy; also liquid and gas chromatography, x-ray diffraction, and some other techniques, as well.

Dr. Gretchen Shearer, senior research chemist at McCrone Associates, uses Raman microspectroscopy to analyze a sample.

I'd like to highlight just a few projects we've worked on here that show how we can coordinate data from various different techniques to provide overall answers to contamination problems.

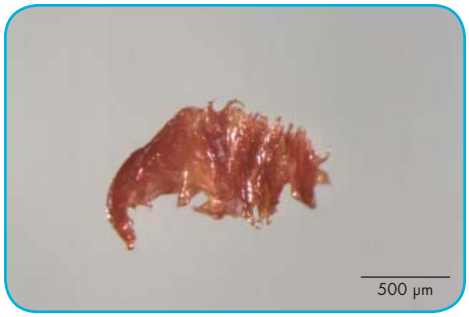
This first one is a fairly common problem that we get submitted to us—a tablet with a foreign particle embedded in it—in this case, a reddish particle. The first thing we would try to do here is dig the particle out for further analysis, but when it's in a dry tablet, there can be a danger of the particle jumping away on you if you don't know what size it is or how firmly it's embedded.

I have a technique that I like to use on samples like this, particularly when I know that it's a single, solid particle—something that is not going to easily dissolve, like a piece of plastic, or a hair or fiber, or a metal particle. I apply a droplet of water to the tablet to soften the matrix; it helps to release the particle and reduces the risk that is going to fly away from a dry, brittle tablet. Unfortunately, I don't have a video of doing it on this particular one, but what I'm going to show you will illustrate how it's done, I think.

Here we have a tablet with the dark flake embedded in the surface. Again, we could go in with a probe and dig this out, but to be safer, I apply a drop of water to the tablet. Now I come in with a sharpened tungsten needle and dig into that softened matrix. I just pluck the particle off, it's a little softer and stickier, so I can transfer it safely now to clean glass slide, clean some of the residue off of it there, and prepare it for further analysis. Here's our particle isolated. We have it in focus, as this is a dark particle, if we want to take a picture of it, we can just slide a white piece of paper under as background and then go on to whatever analysis we want to do.

Tablet with an embedded reddish particle.

So, in this case, here is our particle after being removed from the tablet and cleaned of most of the matrix material.

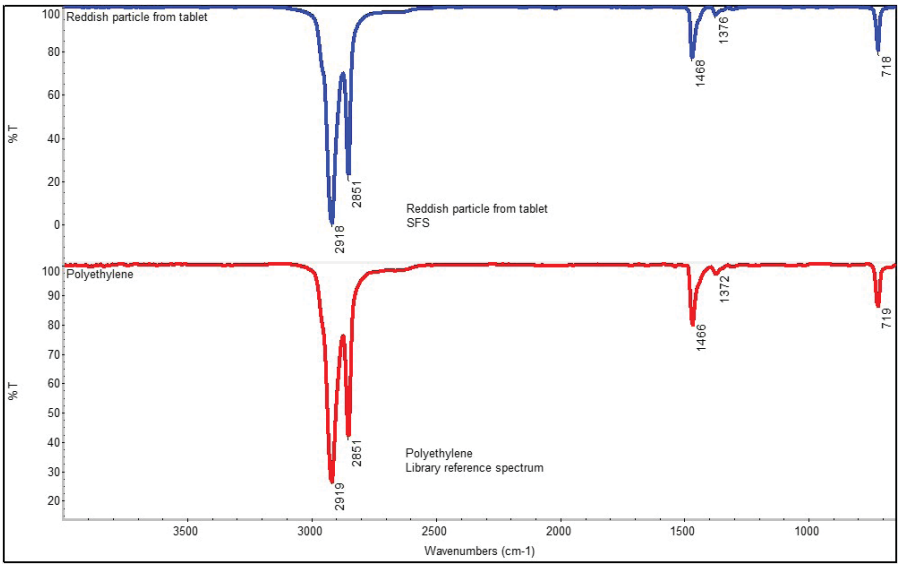


Reddish particle removed from tablet.

The first step we would typically take, seeing that it is a polymeric particle, is to shave off a little of the particle, press it out into thin layer on a potassium bromide salt plate that we polish for this type of analysis, and then run transmission infrared microscopy on it. And when we do that, in this case, this is the spectrum that we would come up with a the fairly common recognizable pattern—a fairly simple infrared spectrum. When we go into our infrared spectra libraries and run a comparison search, it comes up as polyethylene. That's a fairly straightforward analysis and identification as it goes.

In this case, though, the client had also sent us a comparison sample, something they suspected of being the source of the particle that they wanted us to compare to what we had taken out of the tablet. And here's the particle, or part of the particle, that they sent as a reference. First thing, again, that we do, just as a preliminary comparison, is to see if it is actually the same type of plastic. And, when we do that, we get pretty much same infrared spectrum also of polyethylene. That's good as far as it goes, but polyethylene tends not to vary that much from one sample to another, so finding two samples of polyethylene that have matching infrared spectra doesn't tell us whole lot about whether they may or may not have come from the same source. There are only a few peaks here to compare. There can be small variations from one polyethylene sample to another, but when we're working with something that has come out of, say, a tablet matrix, or some other drug product it may leave some residue, we may also get some contributions in our spectra from whatever the particle came from. And it's not always easy to interpret whether those extra added peaks in our spectrum are due to something foreign to the polyethylene itself or whether they're indicating an actual difference in composition from one sample to another.

So, we got spectra and they match really closely. They are both polyethylene. But, we want to take that maybe one step further to say yes or no; this is a good possibility for a possible source, or no it can't be a



Infrared spectra of reddish particle and polyethylene.

©2016, The McCrone Group • www.mccrone.com

possible source, so a color comparison is the next step we can go to. We can look at these particles just under the stereomicroscope. And, if we do that—simulated here—they look a little bit different in color. Our unknown on the left there is a little more orangey. The known seems to be more reddish. That may be just due to the thickness of the pieces that we have here—that can affect the color that we see. So, what I would do in this case is go to a little higher magnification; look at the color a little more closely under a polarized light microscope, again, taking thin shavings from both of these. If we do that, the portion from our unknown particle on the left we can see at this magnification definitely has kind of a brick-red or red-orange color, whereas the material from our reference sample is more purplish-red.

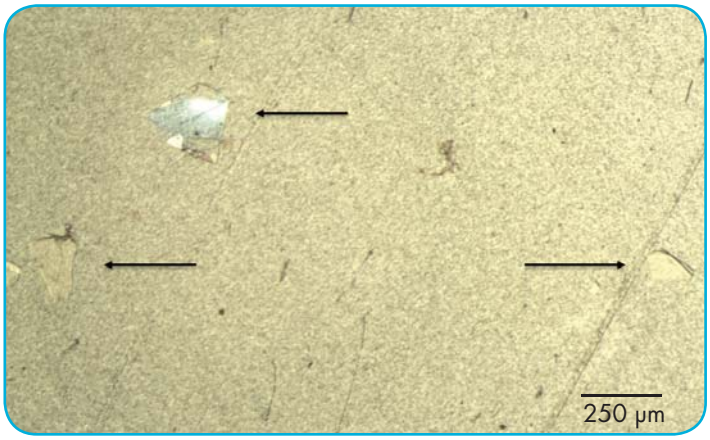
I have cut these so that there are gradations of thickness in the particles; again, so that we're not deceived by the depth of color that we are seeing. We can see here that these two materials definitely have different overall pigmentations, even though they're both either generally the same red color, or close to it. We can see from here that the particle from the tablet is definitely not from the same source as the reference sample. And, that's what we told our client in this case, and they had to look elsewhere for a possible origin of that particle.

This is another type of project that we get pretty frequently. It involves looking for glass delamination particles in drug products that are stored for long-term, usually in pharmaceutical vials. In a case like that, the first thing that we'll do is a visual inspection of the vial(s) in question either just with the eye with side lighting, or under the stereomicroscope. This is a vial with a pretty serious delamination problem. If we just shake and agitate the vial a little bit, you can see the twinkling little flakes moving around in the liquid, and that appearance of particle is already a good indication that we've got delamination flakes—not certain, but that's a strong indication right away.

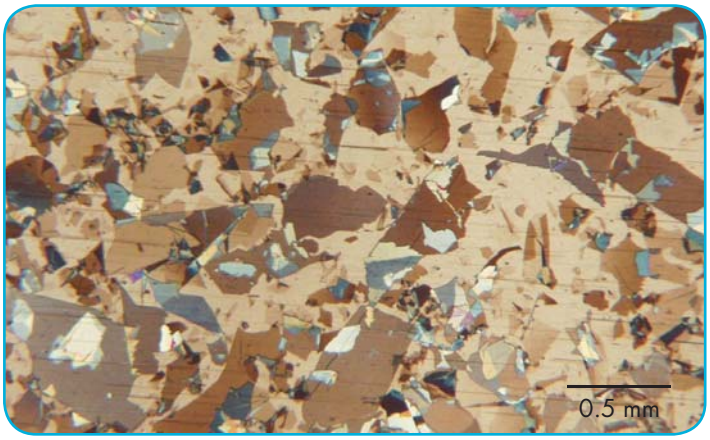
The delamination flakes that we look for in these types of samples are very, very thin; usually thinner than a micron. We would typically try to filter a sample like this onto a membrane filter to isolate those particles, but because they're so thin, they have essentially no relief and they are very difficult, if not impossible, to see on the filter with just ordinary oblique side lighting of the type that we've used for most larger samples. For this type of sample, we use a special attachment on our stereomicroscope to give us what we call coaxial illumination. That sends light down directly at a 90° angle to the sample, straight down through the objective onto whatever we're looking at. And, we attach what we call a quarter wave plate to the front of the objective to highlight any very thin particles, or we can also use it for very thin films on all kinds of surfaces.

In this case, the oblique side lighting that we would aim in from, say, a 45° or 60° angle won't really bring out the types of particles we're looking for. If we're very lucky, or unlucky, depending on your point of view, we see this kind of thing on a filter. Here, this is from a vial again with a very severe delamination problem. We have got hundreds, if not thousands, of very distinctive particles on the filter. Again, this is viewed with coaxial illumination. On the morphology, these particles with kind of sharp, jagged edges, the number of them, and their appearance, is a very strong indication, all by itself, of delamination. The different colors that we're seeing in these particles are due to slightly different thicknesses of the particles, depending on what area you're looking at.

More often, we don't get quite this many delamination flakes in a single vial. What's more common is something like this, where we have a membrane filter that we've filtered the contents of one or more vials onto. We've got just a few thin flakes seen with coaxial illumination, pointed out by the arrows. They have the fairly typical appearance of delamination flakes, but there are not that many of them. And, not everything that is thin and flake-like on a filter is going to be delamination, so we want to go to some additional techniques to give some complementary chemical information about the particles we're seeing here to try to confirm that we do, in fact, have delamination, because we don't have such an abundance of particles that nails it down like the previous filter would have. The first thing we can do is scrape a little of this flaky material off the filter with a fine tungsten needle, and transfer that to a potassium bromide plate, and press it out for transmission IR. When we do that, this is the spectrum we got in this case. And, these particles came from a protein drug product. Some of the bands that we see in the infrared



Filter with limited (possible) glass delamination particles.



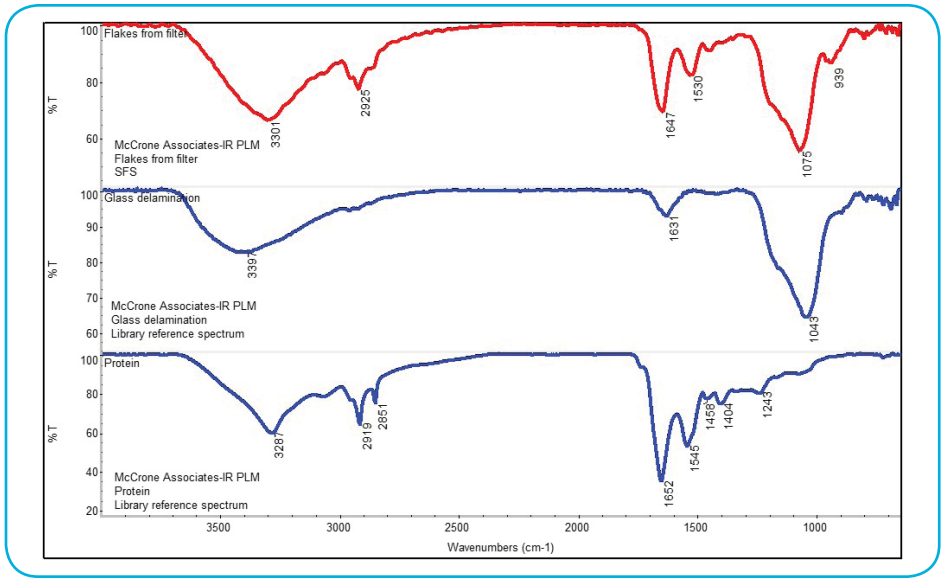
Filter with abundant glass delamination particles.

©2016, The McCrone Group • www.mccrone.com

spectra are residual protein from that product. The bands at 1647 and 1530 are very typical of protein; the strong nitrogen and hydrogen absorption up around 3300 we also see from protein. But, the strong, broad, single band that is centered on 1075 wave numbers, here, is very characteristic of glass-like or silica-based materials, which is what we would expect from glass delamination.

If we superimpose this with a couple of reference spectra, one of glass delamination and one of protein, we can pretty much account for all of the bands in our spectrum. Again, the protein has typical amide bands around 1650 and 1540/1530, nitrogen and hydrogen absorption is up a little higher, and our glass delamination or silica-like material has a strong, broad band between 1100 and 1000 wave numbers. Because the chemistry of glass delamination flakes can vary from one case to another, we don't look for a band at one particular wave number to identify glass delamination. Here, there's a little bit of difference between our reference and our unknown, but that can happen just because glass delamination varies from one source to another. So, this is still a good indication that in addition to the product residue, we've got something silica-based and possibly glass delamination.

The next thing we can do to give us more chemical information is an elemental analysis on these flaky particles. In this case, I removed some more of the flake material onto a beryllium stub and looked at it under the scanning electron microscope and used the elemental analysis capability with energy dispersive x-ray spectroscopy. This is the elemental profile we got. There is some carbon and a little nitrogen, and attributable to the protein residue from the product. Some of the oxygen is probably due to that as well. We have a lot of silicon here, from some kind of silica-based material, and some of the oxygen is also attributable to that. We also have a little bit of aluminum and that's also common to see in delamination flakes. That's a typical component of the Type I borosilicate glass that's commonly used in pharmaceutical vials and that is the



Infrafred spectra of flakes and references.

source of delamination flakes very often. That type of glass also has sodium (Na) in it, and sometimes glass delamination flakes will show some sodium; sometimes not. It's actually erosion and leaching of sodium from the glass that is one of things that precipitates delamination in the first place. We don't necessarily expect that the composition of glass delamination flakes that we've filtered out of a product and taken off the filter will match exactly with a chunk of the vial from the glass itself, if we were to analyze that; again, because in the process of delamination, we get chemical alterations on the inside surface of the glass, so what flakes off has been changed somewhat from the original glass. We can look at this and see that we do have a silica-based material; we've got some of the aluminum that we expect to come from the glass itself. That's all strong support for having glass delamination flakes here.

Another thing that we can do when we're trying to identify a delamination problem is to actually look at the inside surface of the vial itself. We rinse it out, dry it, and then crack the vial open and look at areas of the interior wall for signs of delamination, or a precursor to delamination. We can look at that under the stereomicroscope, again, with coaxial illumination. In this case, we actually did it on the scanning electron microscope at a little higher magnification. This is one area that we looked at. This is from a vial that did not have a particularly severe delamination problem. We saw a few flakes from this vial, but this area has fairly heavy pitting, and this pitting is really a precursor to delamination rather than delamination itself. You can see from the scale bar these features are fairly small. Pretty much all of the pits are less than 5 µm, a lot of them are smaller than a micron. So, they're difficult to see, but it is a very strong indication that you have started the process of delamination on this vial.

Now, if we were to look at the interior of a vial, like the one we saw under the microscope in the video that has a lot of delamination particles—our filter with very abundant delamination, we might see something more like this, where you have whole bands or strips of surface material on that inside glass wall flaking or peeling away into the liquid. We also have some pitting here, too, and this is a little different in appearance than the last one. Here, we have full-blown delamination—a pretty serious case of it. So, with all these things combined with the microscopical appearance of the vial and the particles on the filter and chemical compositions, we've got very strong support for having a delamination problem in this type of vial. And, it can depend on not only the type of vial, but how long it's been stored, what's been stored in it, what conditions the vial was manufactured, sterilized, and processed under; all those can contribute to a delamination problem.

©2016, The McCrone Group • www.mccrone.com

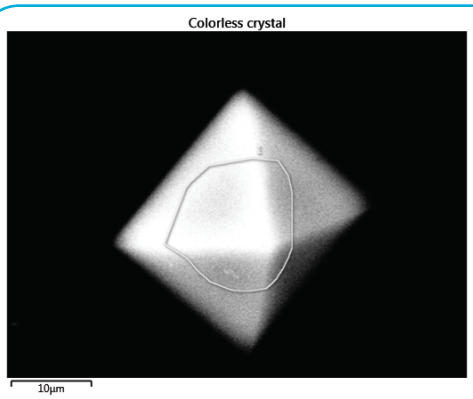
Here's another case where a client had a lot of small colorless particles, they said, in their product, and wanted to identify them. So, we did a standard filtration in our cleanroom of some of the particle-contaminated product and got this. We have lots and lots of small colorless equant, almost cube-like, crystal-looking particles on our filter. We have a fair amount of material to work with here. One of the first things we can do is to try to obtain an infrared spectrum of this material, and see where that leads us. When you've got chunky, equant particles that are not flat, not thin, flakes, not particularly soft, pressing these out directly onto a potassium bromide plate or some other substrate doesn't necessarily work very well, because your IR prep is going to be too thick. At that point, you're not going to get good interpretable spectra; you are going to get too much absorption. So, there's a technique I like to use on particles like this to get them into a more usable condition for IR, and it works really well for pharmaceutical materials that are kind of chunky particles, and some other crystalline materials that are fairly frangible. It doesn't work so well for things like glass, which is too fragile, or things like minerals that are a little too hard, but for quite a lot of things it's worth trying.

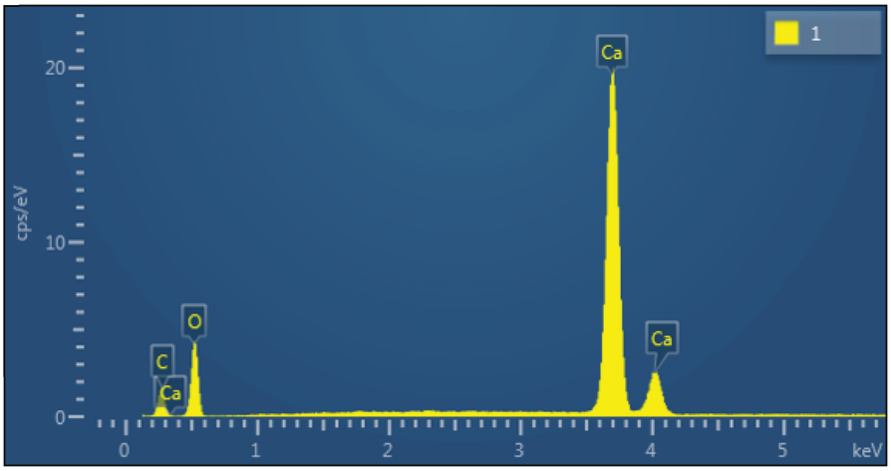
You can do ATR on this type of material, as well. That can work. It solves the thickness problem in some cases, but if you only have one or two crystals to go with, that may not be enough material. This technique that I am going to show you can work just on one fairly small crystal and give you more than enough material to get an identifiable infrared spectrum.

So, what I will do is, I have kind of chunky, colorless crystal on a slide with a light-colored background I can see against. I bring another clean glass slide, just a corner of it, over the top of that and press down, and crush it gently to break it up, push down slowly, and then start to rock and twist that slide back and forth to crush and grind the material into a fine powder between those two plates. Now, I have a nice film of fairly well-ground-up powder. I can go in with a fine tungsten needle, pick off some of that powder, and transfer it to a polished potassium bromide plate. I've laid out a few clumps of different sizes in a small area to be pressed out. And, if I put down some different size clumps to press out, I'll get areas in my prep of different thicknesses, and I can aperture down on various of those to get just the amount of absorption I need for a good-quality infrared spectrum. That works much better than just trying to press a single large particle out on the potassium bromide plate and not really have it break up, just get one big chunk. That doesn't work all that well.

That's what we did in this case, and this is the infrared spectrum that we got. It came up very nicely, and looking at the spectrum, I had some indications of what it was. I'd seen this before as a calcium oxalate, and when it went into the infrared spectra libraries, that came up as the closest match, just regular calcium oxalate. The two main bands around 1646 and 1327 corresponded pretty close to the library spectrum, but there are some other areas in here, up above 3000, that don't really match that closely; a few things down below 1000 are not a real great match. At this point, one possibility is that we got a different form of calcium oxalate than our reference sample in the library, or it may even be a different material that happens to have a couple of major bands that correspond. So, we want to go on to some other techniques to see if we can resolve that unknown.

The next thing we can try is elemental analysis to see if the composition matches up with what we think it might be. I took one of these crystals and transferred it to an SEM plate for EDS analysis. This is a pretty well-shaped crystal here, you can see. It's very well-formed; it has nice flat sides and well-defined edges, and the elemental composition of this came up with calcium, carbon, and oxygen, and that was it. From that, calcium oxalate is still in the ballpark as a possibility. This could also correspond, for example, to calcium carbonate. If you're familiar with crystal forms, you may recognize that this particular type crystal is a little bit different in shape than you'd expect from calcium carbonate. You might not want to hang your hat on that, but it doesn't really look like a calcite crystal. Seeing that it's a very well formed, very well shaped particle, in this case, it's probably highly crystalline, so another possibility that we could do to get more specific phase information is to go to x-ray diffraction.





Crystal from filter: elemental analysis by SEM/EDS.

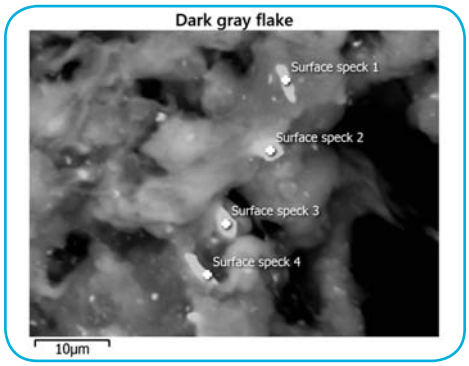
©2016, The McCrone Group • www.mccrone.com

We have a micro x-ray diffraction capability here at McCrone, so we could, in theory, look at even one particle like the one I have here that is just a few tens of microns in size, or we could take one or several of these particles, crush it up the way we did for the IR prep, and make a micro-powder preparation, as well. That's actually what we did in this case. When we did that we got this peak pattern, and searching that against our diffraction libraries, what we came up with was actually a hydrated form of calcium oxalate; the mineral name is weddelite. If we superimpose the red stick pattern of the reference sample on our peak pattern of our unknown it is pretty much a perfect match, so we can be very confident that that is the crystalline phase that we are looking at here. I have, just for interest's sake, superimposed on those two the pattern of regular anhydrous calcium oxalate, in this case, the blue stick pattern. We can see that there are a lot of areas—a lot of peaks—that don't correspond to our unknown at all. We can be quite sure that we don't have an anhydrous calcium oxalate, but the hydrated calcium oxalate is a very confident identification. The fact that in this case we had a very large number of small, very well-shaped crystals is an indication that these particles did not just get in there as accidental contamination. It is more indicative of their having formed right there in the solution, probably from the interaction of soluble calcium salt with soluble oxalate salt, or even oxalic acid. Since the calcium oxalate compound is actually very insoluble, anytime you get those two ions together in solution, it's almost certain that you're going to start to get some precipitation and forming of very nicely-shaped crystals that are not crushed up or broken like samples out of, say, a chemical bottle up in the laboratory. That is where we pointed the clients in this case: to look for possible sources of either soluble calcium or soluble oxalate ions getting into their sample. Water that's too hard is one possibility. Sometimes people have one of these ions in their product and the other one gets in it inadvertently and causes precipitation where there should not be any.

This is one more example of a project where we had a multi-analysis approach to the solution. This is a case where a client had some flakes of a drug product that was a protein-based product. We did filtration in our clean room, isolated some of these flakes. Some of them are whitish, some of them are lighter gray, some of them shade to a fairly dark gray.

The first step that we take in handling them—we felt they were fairly soft, they were likely to be organic—so infrared spectroscopy was a good first step. We isolate a little bit of one of the whitish particles and one of the dark gray particles, and press those out for infrared spectroscopy. If we look at the spectrum of one of the whitish flakes and we see bands above about 1400 that are all attributable to proteins, we can put that down as residue for product. We also have this fairly strong closely-spaced doublet here at 1212 and 1155 wave numbers; that's very indicative of Teflon™. If we look at the spectrum of the grayish flake, it's basically the same protein bands and bands of the Teflon. We don't really see any significant difference between the spectrum of the grayish flake and the spectrum of the whitish flake, which tells us that whatever is causing that dark discoloration either is not present in large enough amounts to be picked up on IR, or it's something inorganic, like metallic, or possibly carbonaceous, which wouldn't give us much of an infrared signal even if there is quite a bit of it there. So, we can pretty confidently nail down Teflon as the material that is forming the substrate of these flakes. Again a reference spectra of Teflon overlays very nicely with the doublet we see down low in the fingerprint region, and then the remainder of the bands up above1400 all correspond to protein, likely residue of the drug. But, now we would like to get some idea of what's causing the discoloration on these particles and what that might indicate as far as a source for them.

We can go on to elemental analysis using the SEM, and we can take an overall elemental scan from one of the darker grayish flakes. We figure whatever were looking for is going to be most abundant there. If we do that, the general elemental scan shows us largely carbon and fluorine that's all coming from the Teflon substrate, a little bit of nitrogen from the protein, a little oxygen—some of that may also be coming from the protein; but we see low levels of elements indicating the presence of a steel, in this case, iron, chromium and nickel. We can go a little further—zoom in to higher magnification— and try to look at individual particles of whatever that discoloring contaminant is, and if, in fact, it is steel. In this case, we had the electron microscope set up in what is call the backscattered electron imaging mode (BEI). When the instrument is configured like this, particles that have higher average atomic number, like metals, will appear brighter on the screen. Whereas materials or particles that are carbon rich and have lower average atomic numbers will appear gray or black. We can highlight the particles of interest in this case and look at them individually. When we do that, we can see that we have lots and lots of very bright, small specks all over the surface of this particle, and we can be pretty sure that that is what is causing our problem.



BEI image of the dark gray flake.

We can zero in on individual particles here and get specific elemental composition on pretty small areas, down to even smaller than a micron. If we do that, I posted up here elemental profiles on four of these selected surface specks. We're still getting some contribution from carbon and fluorine in the organic portion of the substrate.

©2016, The McCrone Group • www.mccrone.com

Spectrum Label	Surface speck 1	Surface speck 2	Surface speck 3	Surface speck 4
C	47.27	35.16	35.75	49.06
N	8.94	3.69	4.02	8.46
O	10.05	4.29	4.67	9.96
F	11.85	14.16	15.85	13.72
Si	0.72	1.54	1.48	0.66
P	0.18	0.32	0.46	0.25
S	0.40	0.50	0.66	0.42
Cl	0.06	0.08	0.10	0.06
Ca	0.15	0.29	0.54	0.18
Cr	3.62	7.06	7.19	3.20
Fe	14.16	27.67	24.65	11.93
Ni	2.20	4.74	4.26	1.92
Mo	0.41	0.50	0.37	0.18
Total	100.00	100.00	100.00	100.00

Grayish flake: elemental analysis by SEM/EDS (BEI).

We're getting pretty high levels of iron, chromium, and nickel; also a little molybdenum. Those elements in the proportions we have here are strongly indicative of 300 series stainless steel. The molybdenum indicates possibly a 316 series alloy.

Next, something we actually see pretty commonly on Teflon flakes, and we've pinpointed sources of this before. What happens is that Teflon gaskets of different types that are used in pharmaceutical processing streams will collect or accumulate metal wear particles from stainless steel components in the system, eventually degrade, and those flakes of Teflon that are contaminated will wear off and shed into whatever material is being processed. This is just an example of a braided Teflon gasket that's white to begin with, but that over time will accumulate these metal particles at some chokepoint in the processing stream. They degrade or break down, shed some of the flakes from its surface with the metal particles on there, and end up in the product. That is where we were able to point the client in this case. Once you know that your problem is a contaminated Teflon gasket, it's usually pretty easy to go into your system and pinpoint where those particular components are and find which one of them is the problem.

That's the end of our presentation. I would like to thank everyone for joining the webinar and I hope that was informative. I will be happy to take any questions now, or if you also like to get in touch with me afterwards, if something a little more involved to ask about, my contact information is up here on the screen, and you're welcome to get in touch with me at any time.

CZ: Great. Thanks, Scott. As Scott mentioned, he'll take questions now, so go ahead and type your questions on into the questions field, and we will begin answering them. It looks like James has a question, "How do you know that the particle being removed using water isn't soluble?"

SS: That is a good question. I would only try that on particular types of particles. If I can see, for example, that I have hair, or fiber, or metal flake, those I can be confident are not going to dissolve if I apply water to them. But, there are quite a few types of particles or things we see embedded in tablets that I wouldn't try that on. It's not uncommon to see a chunk of one of the tablet components that has been cooked, or degraded a little bit, and taken on a yellowish or orange color. When I see something like that in a tablet, then I would always try to pick it out manually, dry and not use water on it. You have to be careful about what kind of particle you apply the water to. You are very correct.

CZ: "What is the smallest particle you can isolate from a tablet?"

SS: If it's a single, individual particle, I can get down to isolating particles in maybe in the 25 to 50 µm range. In some cases, what we see are deposits of multiple very small metal particles, for example. We may get a drop of lubricating oil with metal wear particles in it that spots onto a tablet—you get a dark spot that actually has many hundreds and hundreds of metal particles in there. You can dig out that entire chunk and isolate many, even sub-micron particles all together in the matrix. You can't really isolate individual particles down that small. If it was just a polymer flake, for example, again, a few tens of microns is probably the practical lower limit.

CZ: "Do you have a favored spectral library that you use or is it in-house McCrone library?"

SS: We actually have quite a few different spectral libraries on our instruments, and we do have a library of materials that we've built up ourselves. Some of the older spectral libraries, the quality of spectra is not as high. We substitute ones that we generate on our own for the same materials. There is the HR Polymers and Additives—a pretty common commercial library, the Georgia Crime Laboratory library of drugs, there are some libraries of pharmaceutical materials and excipients. Those are probably the three most common that we use; that tends to be the particles that we see a lot of, but there are quite a number of other ones out there. Those are the three most used ones here, I'd say.

CZ: "When a particle does not stick to your tungsten needle, what can you do?"

SS: A couple of things, again you have to be a little careful about what it is so that you don't damage it. The first thing I usually do is to take just a little bit of skin oil, I'll rub my finger on the side of my nose and my needle through—between my fingers—just to get just the tiniest bit of oil on the surface of the needle, and then try that

©2016, The McCrone Group • www.mccrone.com

to pick it up. If that doesn't work, there are some other adhesives that you can use to just put a tiny blob on the end of your tungsten needle. I've used black carbon tape, or just ordinary double stick tape that I put down on a slide, and then under the microscope, I'll pick up a tiny bit of adhesive on the tip of my needle and use that to touch to the particle and then transfer it somewhere else. If you want to release that, you can just put the particle into a drop of, say, amyl acetate and that will disperse the adhesive. You don't want use a 20 µm blob of adhesive to pick up a 2 µm particle. You want your blob of adhesive to be a lot smaller than what you're picking up, so you have to keep that in mind, the size of your the particle and how small a bit of adhesive you're able to handle. Those are couple of ways to do that. Another thing that I've used is Elmer's makes some water-soluble glue that you can get. Put a tiny little bit of on the tip of your needle and then just release the particle in water later on.

CZ: "What is the way to analyze silicone oil in a syringe sample?"

SS: What we'll typically do there is go in with a needle, or some other kind of probe, and remove some of the oil from the surface that we're interested in or whatever the oily material is. It tends to be fairly viscous in a syringe, so we can get some to stick to the needle and we can transfer that directly to a potassium bromide plate, and run that in transmission mode. We can spread it out a little bit to as thin a film as we want, and then get a transmission spectrum of the oil. That can also be done, if you work this way, on a reflectance slide, a polished aluminum slide, or a gold mirror, and get an infrared spectrum of that oily film in the reflectance mode. Either one of those works.

CZ: "Can you compare/contrast benefits to micro FTIR versus micro Raman?"

SS: Micro FTIR—the sample prep is a little more difficult; you have to press the sample out into a fairly thin film, whereas with micro Raman, you can basically just put it on a Raman inactive substrate and you're good to go. FTIR tends to give you a little more recognizable spectral information—a little more interpretable spectral information. Micro FTIR does not have the types of problems that Raman does with fluorescence. With Raman your exciting your sample with a laser, and a lot of different materials will fluoresce under that laser and degrade the quality of your spectrum, or keep you from getting a good spectrum at all, where FTIR does not have that problem. Another advantage of FTIR is that the spectral libraries are much more extensive and much more transferable from one instrument, or one set of instrument conditions, to another. FTIR can maybe get down to, from a practical standpoint, particles about 20 microns. Raman you can go somewhat smaller than that, and get more information on, say, particles that are just a few microns and inorganic particles.

CZ: Kathleen wants to know, "Where did the boron go from the borosilicate glass from the delamination?"

SS: Good question. Normally, when we look at borosilicate glasses or materials with boron in them, it's difficult to see that under normal operating conditions on the scanning electron microscope, and I don't typically look for it. Under the conditions for the sample that I showed you, I think I that was using an accelerating voltage for the beam of about 15 or 20 kV. That tends to miss boron. If we really wanted to look for the boron, and we're more interested in the silica in that case, anyway, but if we wanted look for the boron we would have to go down to an accelerating voltage of maybe 5 to 7 kV, even that is difficult because those flakes are so thin, even a lower energy beam tends to go right through them and not interact very much with the boron. We can pick up boron on our instruments, but it requires a little more fussing around, so we don't always look for it; sometimes we just focus on the silica.

CZ: "How you get rid of water before running an IR analysis?"

SS: If our sample has been in water, we just let it dry on a clean slide. Sometimes, depending on what material it is, we can do an extra drying step just by rinsing with a little ethanol, but usually just ordinary air drying after we've rinsed the particle with water will get rid of it so there's no interference in our IR spectra.

CZ: Courtney is asking, "Contaminants in the IR sampling can shift the spectra where the peak absorption should take place. Do you have a normalization or standard contaminates that you reference?"

SS: Not particularly, although we do have reference spectra of, for example, polymers that have different contaminants or additives in them that we've seen in the past and then logged into our libraries. That's a big problem with running IR spectra since we don't very often get nice clean particles. We have to take into account contamination that may have come from the matrix or from wherever else in the environment these particles were picked up in. So, getting to be able to pick out the peaks that are of your material of interest, and attribute other bands to contaminants, it takes a lot of experience. It's not easy, and one way to do it—or to help do it—is to do selected area searches on your infrared spectral libraries. Some softwares will just let you pick out an area with just a few bands and search just those, and very often that will point you—if those are coming from the contaminant—okay, you have some residual talc on the surface of this particle even though it's actually polypropylene, for example. But that's a difficult problem.

CZ: Lee is asking, "How do you tell if something is stainless steel corrosion from EDS data?"

SS: What we typically look for in that case, if we have very small particles, we look for the proportion of oxygen to the steel elements, and if we're on an organic matrix, sometimes that can be a little difficult because we may get some oxygen coming from the substrate itself. Usually, if we have an ordinary organic material in the elemental profile, our carbon peak is going to

©2016, The McCrone Group • www.mccrone.com

be quite a bit higher than our oxygen peak. If we have clean, uncorroded steel particles, our steel—the peaks from our steel elements—will also be more prominent than the oxygen peak. If we have a corroded particle, then we'll see a pretty high peak of oxygen; more predominant than the carbon peak. In some cases we just can't tell whether the particles may be slightly corroded or largely corroded, and that in case I have to write in my report that these are particles of stainless steel or steel corrosion; it may be both.

CZ: Linda wants to know, "How do you fracture the vials to look at glass delamination?"

SS: What I do in that case is take some clear Scotch® tape, the normal size you get out of a tape dispenser, tape that in a couple of bands around the outside of the vial so it's completely wrapped in two strips. Then, tap the vial with a hammer inside a plastic bag, or wrapped up in paper towels, until it cracks. When you have done that, peel that tape back and you can kind of open up the vial with all of the pieces still stuck to tape, and then look at the inside surfaces of those curved fragments. That keeps the fragments from going every which way and shattering down to dust. Taping the outside and then cracking it and opening it up is the best way I've found.

CZ: James has a question, "Do you have any physical test to check if the glass vial received from the supplier has an intrinsic defect?"

SS: Not really. Unless it is some type of visible defect that is embedded in the glass or something physical, like a crack. If it's something to do with the chemistry of the vial and its strength or resilience—that we don't really have the capability to analyze here.

CZ: Kind of a general question, "How you analyze dark black particles?"

SS: Okay, it depends a lot on what we see under that stereomicroscope. If we can tell that they are polymeric, we will try to press those out for IR. Plastics generally press out pretty nicely to give a good infrared spectrum. Black rubbers, if we find the particles are elastomeric, those are a little tougher, they tend to be more heavily filled and harder to get a good infrared spectrum on, but we'd still use that method of pressing them out. If we have something that looks more inorganic, corrosion of some type, for example, or just degraded material, we'll try SEM/EDS. In some cases, very often for degraded material, we'll even try both IR or EDS. If it looks like carbonaceous material, something like graphite, or just soot or char, Raman spectroscopy is actually very useful for those. Very often, we have to try different methods on black particles until we get one that gives us the answer.

CZ: "What method are you using to eliminate static electricity?"

SS: Nothing in particular. Sometimes, if I have a really staticky filter, for example, sometimes I'll apply a little bit of water to it, just to infuse the filter and make it easier to pick particles off without static. If I have a really staticy environment, if it's the depths of winter and it is really dry, I'll use a little more adhesive on my needle to help things to stay on there without jumping off. But, that's an ongoing problem.

CZ: Courtney is asking, "Do you always use water for removing a particle or are there other solvents that are used that have to be accounted for in the analysis?"

SS: When I'm taking a particle out of a tablet, I normally will start with water. I'll also use ethanol, sometimes, depending on what's in the tablet; that can help to remove some of the matrix material, also, as well as water. Sometimes, again, I kind of have to know what the particle I am dealing with is before I just apply solvents willy-nilly. But, if I have metal particle and I was trying to clean tablet matrix material off, I'd try water, I'd try ethanol, I might try amyl acetate or nonane. All of those can remove different contaminant materials. You don't always know which one is going to work from the start. You have to be careful and have some idea of the particle you have before you just start applying solvents that might conceivably dissolve it.

CZ: Back to identifying black particles, Scott, "What specifically makes them more difficult to ID, if that is the case? Are they more difficult?"

SS: They can be. With polymers, some black particles are difficult to identify because they are very heavily filled with carbon black, and it's hard to press them out thin enough to get an identifiable infrared spectrum. Things like char or graphite, you really can't get a transparent sample out of. We have to go to a method like EDS or Raman, and in that case, it works very well. If you have black particles that are just degraded; degraded product, degraded API, you sometimes can get them pressed thin enough to get a good infrared spectrum on. Sometimes elemental analysis will give you some information if that compound your suspecting has, say, sulfur, phosphorus, chlorine, and some other tag element other than carbon or oxygen. Usually, it's that the opacity that gives you difficulty, or if a material is degraded, it may not have all of its original chemical nature anymore, and you can't get a spectrum that's identifiable with the original source because it has been so badly charred.

CZ: Okay. Nancy wants to know if you know of a source where they can purchase beryllium stubs.

SS: I'm not sure where we get ours. I think we had the stubs cut and polished specially. Unfortunately, it's very hard to find places that will work with beryllium, because when you're grinding and polishing it, the dust is very, very hazardous, and poisonous. I think they're just not sold that much in general, because beryllium is known as being toxic—I think it's more the dust than the bulk metal. I'm not sure if there is a general supplier out there, unfortunately.

CZ: We can follow up with Nancy on that one and see if we can track something down for her, in that regard.

Melissa says, "We've noticed the protein signals on your IR spectra, can you suggest an optimal way to wash off the protein contribution?"

©2016, The McCrone Group • www.mccrone.com

SS: The ones I showed you, in this case, were actually a little more unusual. Normally, we're able, if we're taking a drug out of a protein product, for example, when we do the filtration, we run the product through the filter and then we rinse the filter through with some deionized water. That usually takes off most of the residual product. Or, if I just have a particle that I think may have come out of a protein product, I'll rinse it with a little water on a microscope slide to get rid of as much of the protein as possible. In some cases, it happens that the protein has been denatured and isn't very soluble. In that case, if you can't remove it physically from the surface of the particle, then you kind of just have to live with it.

CZ: "How do you tell if a particle is thermally degraded?"

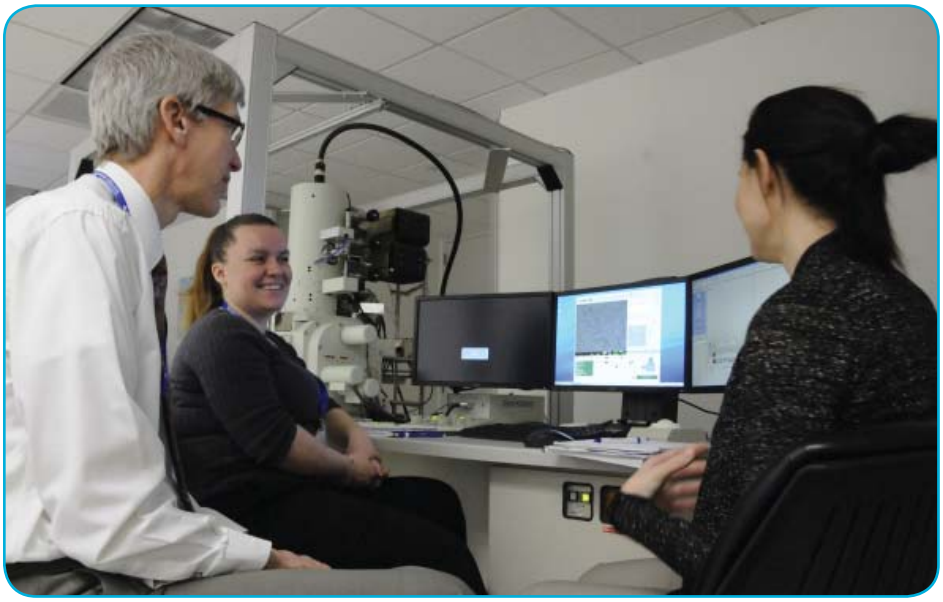
SS: One of the first indications is just based on the color. If you start off with a white organic component, an API or excipient, for example, thermal degradation will typically produce a spectrum of colors from a pale yellow to yellow-orange to orange-brown to dark brown to black. We kind of get to recognize that kind of color gradation. Those colors trigger a flag that it may be thermally degraded. The other thing we can do is, when we run an IR spectra, there are certain bands that we see that are typical of thermal degradation or oxidation of normal organic material. If we see a band around 1700 or 1710, that's a common oxidation peak. Or if we just see a few strong broad bands in the infrared spectrum of an orange-brown material, then that's a good indication of thermal degradation to the point where the original chemical nature of the bands don't show up anymore.

CZ: From Joe, "Do you ever use ATR or micro-ATR in place of transmission IR?"

SS: I don't. Sometimes people do. I've always found that if I work at it, I can get just about any sample thin enough to run transmission on, and I just prefer that. The micro-ATR, at least the ones that I've worked with, have been a little more trouble to work with. Some people do use it, and it does work, but I always work with transmission whenever I can.

CZ: Okay. Great, Scott. I think that does it for the questions.

Be sure to join us for our next webinar on April 21, when our presenter will be Wayne Niemeyer of McCrone Associates. Wayne's presentation is titled, "Defects in Food Packaging." We hope to see you then. Thank you.



Hooke College of Applied Sciences students learn to conduct SEM/EDS analysis and interpret results during an SEM course.

Samples are prepared in McCrone Associates' cleanrooms, which are certified to ISO 14644 Class 5 standards.

©2016, The McCrone Group • www.mccrone.com