Identification of Glass Delamination Products Using TEM

Introduction
Hello and welcome. My name is Charles Zona (CZ) and I would like to thank everyone for attending today’s McCrone Group webinar. Our presenter today is Elaine Schumacher of McCrone Associates and Elaine is going to talk to us about the identification of glass delamination products using TEM or transmission electron microscopy. Elaine is a senior research scientist with McCrone Associates and has been with the company since 2001. Elaine applies transmission electron microscopy techniques, including high-resolution imaging, electron diffraction, x-ray microanalysis, and electron energy loss spectroscopy to a wide variety of materials. Some of her consulting work for clients in industry and academia has included characterization of polymer nanocomposites, medical devices, pharmaceuticals, minerals, metallurgical specimens, and ceramics. Elaine teaches a hands-on TEM short course for the Hooke College of Applied Sciences.

Elaine Schumacher (ES):
Thank you Chuck and welcome to everyone watching in the audience. The topic of today’s webinar is Identification of Glass Delamination Products Using TEM.

- I will be giving some background on glass delamination and describing the established characterization methodology which is largely based on USP<1660>.
- I’ll be discussing the advantages of TEM for glass delamination characterization, although this is not a method used in, or recommended in USP<1660>, but I’ll show you how well it can serve for identification of thin particulate associated with glass delamination processes.
- I will give some examples including delamination flakes, other particle types that might be encountered, such as glass vial coatings, residues, and other types of particles, and also secondary products that can be formed by reaction of a drug product with a glass container.
- We’ll wrap up to some conclusions.

Some background on glass delamination. This is the formation and separation of glass flakes from the surface of the glass container such as a vial or a glass syringe. It’s the last stage indicator of heavy extraction of glass by the product. So, by the time you see and recognize glass delamination flakes, you already have a problem. The severity of delamination depends on several
factors: the glass composition and manufacturing method; presence of defects; handling and sterilization of glass containers; product interactions—these can be impacted by things such as product pH or presence of buffers; and storage conditions, such as time and temperature. This poses a risk of particulate being present in injectables and parenterals in vials, and it has the potential to affect large amounts of product. As many of you may know, it has been the subject of several product recalls in recent years. As I mentioned at the start, if you are seeing glass delamination, it means this process is already well advanced. I hope to show that TEM can give you the ability to locate smaller particles than you can see by other methods. So, it has the potential to be an early indicator of glass delamination—seeing the effect sooner than you might with other methods.

The methodology that is typically used—and these are all capabilities that we have here at McCrone Associates—are the ones outlined in USP<1660>. The process starts with visual examination of liquid in the containers as received, and also examination of the liquid in the containers using a stereomicroscope. One of the typical features of glass delamination flakes in liquids is a sort of shining or twinkling effect that you will see as you look at the container under light. Sample preparation, then, in order to analyze the materials, is filtration to isolate flakes and residues from liquid sample, and also looking at the glass containers themselves, and this involves breaking of taped vials to maintain the spatial relationship of the vial fragments. This is done because particular areas of the vial, like the curved neck or the base of the vial, see more stresses during manufacturing and maybe more susceptible to delamination, so it’s important to maintain the spatial relationship of the fragments when you break the vial to look at the interior pieces using light microscopy or scanning electron microscopy.

Characterization is then done using multiple techniques which is always a sound approach to materials problem solving. Polarized light microscopy (PLM), scanning electron microscopy (SEM) with energy dispersive x-ray spectrometry—or EDS—which gives you information about elemental composition, Fourier Transform infrared spectroscopy (FTIR), and also XPS, which is a surface technique and it can tell you about migration of elements to the surface of the glass which may be impacting the glass delamination process.

Here we have a polarized light microscopical image—this is taken with coaxial illumination at 50 times magnification. This shows glass delamination flakes on a filter; the colors that you see are thin film interference colors, and this indicates that you have delamination flakes of several different thicknesses present. This is very recognizable by polarized light microscopy.

These are images of the interior of a glass vial after it’s been broken as I have described. You also see the thin film interference colors indicating different thicknesses, and you can see that there’s a lot of porosity and degradation of the surface of the vial. So, this is very severe and advanced delamination; again, readily recognizable by PLM.

Moving then to the scanning electron microscope to look at similar types of samples—on the right we have an image of the interior surface of a vial and again you can see differences in contrast, and evidence of surface texture that indicate delamination is occurring. The SEM also may allow you to see small features, like pits or holes, that may be precursors to delamination.

On the left, we see a delamination flake on a filter. It’s been isolated on a filter, and then the filter and flake have been put onto a substrate for analysis in the scanning electron microscope. So, the darker gray area that you see in the background is the substrate, the filter; the lighter gray angular sort of feature that you see taking up most of the center of the image is a delamination flake, and you can actually see the edges curling up, so it gives you some idea of how thin these flakes are. Now, if these flakes become very small or are extremely thin, they don’t impart a lot of contrast to the image and they may be very difficult to locate in the SEM, which, as I’ll show, is another advantage of going to the transmission electron microscope.

So, some of the advantages of TEM, both in general and specifically for glass delamination: it’s a high resolution, through-sample analysis, so the electron beam is penetrating through the entire thickness of the sample. It provides morphological,
elemental, and crystallographic information, as I’ll show in some examples. We can look at scrapings from the interior of vials; we can look at delamination flakes in residues—these are ideal samples, and I’ll show that we have a couple of methods of easily transferring these thin samples to TEM grids. I’ll also demonstrate that TEM EDS is better suited to very thin samples than is SEM EDS.

This is why. This is a schematic that shows the relative interaction volumes of the microscope electron beam with a sample in the TEM or the SEM. So, in the SEM you typically have a more bulk—or thicker sample—on a substrate, and you have penetration of the electron beam into the sample; and then this large teardrop shaped interaction volume. Now, if you have a very thin sample, like a delamination flake on a filter, the electron beam is penetrating through that sample in the SEM. Most of the interaction volume, most of the volume from which your x-ray signals are coming, is actually the substrate, and not the sample itself.

In comparison, in this red box there is a small hashed area, and this is the TEM interaction volume in the thin sample. So, we are looking at a sample with a thickness of, say, 90 -100 nm, as being ideal for TEM. The electron beam is penetrating through that sample, and all of the x-rays that are being generated are coming from the sample itself and a very thin support, such as a very thin carbon film. So, the TEM is really much better suited to EDS elemental analysis of these thin samples than the SEM.

Another thing to consider is that in the SEM, you can limit the penetration depth of the electron beam by using a lower accelerating voltage or lower energy beam, but this also limits the energy of the x-rays that you’re going to get out in your spectrum and that will be evident in the next example that I am going to show.

So, here we have a typical SEM EDS spectrum taken from delamination flakes on a polycarbonate filter. Liquid product was filtered onto a polycarbonate filter; a section of that filter was then put onto a substrate for SEM analysis. So, you can see that in the spectrum, the dominant peak really is the carbon from the polycarbonate filter. You have an oxygen peak, and you have some evidence that there is silicon present. As I mentioned also, the low accelerating voltage means that you limit generation of higher energy x-rays, so we’re cutting off somewhere below 2keV here. If you had elements in the sample that were of interest but didn’t generate x-rays except at higher energies, you wouldn’t even be seeing those. Now, if we blow up the spectrum, we expand the y-axis, now we can see that we’ve got a minor-to-trace silicon peak here at best, and we’ve got an extremely small aluminum peak. So, if you had already done polarized light microscopy and looked at the morphology of this flake using the stereomicroscope and using the SEM, and you were seeing things that were very typical glass delamination, this EDS analysis in the SEM might be conclusive enough. But if you had a residue, a glass coating, a secondary product; there is really not enough information in the spectrum to make conclusions, simply because most of these, the signal that you’ve gotten going into the spectrum has not come from your sample.

On the other hand, if we go to TEM EDS analysis of a similar flake, and this is supported on a typical TEM grid—it’s a carbon mesh grid with an amorphous carbon film to support the flakes—and we can see that we have a very robust spectrum with a great deal more information in it. We have a very small carbon peak coming from the amorphous carbon support film. The spectrum is dominated by peaks from silicon and oxygen so this is clearly a silicate material. The copper peaks that you see out here are from the copper grid bars from the TEM grid, but we also have several peaks for minor and trace elements, so this can be very helpful in distinguishing different types of glasses, distinguishing a glass from some other kind of residue, or making a distinction between a delamination flake and something that’s formed as a secondary product by reaction of a drug product with the glass container.

We have a couple of ways of easily getting material onto TEM grids here at McCrone, and up at the top on the left you see a picture of cleanroom microscopists that work in our ISO class 5 clean room; they have combinations of polarized light microscopes and stereomicroscopes for examining and isolating a variety samples. In the center at the top you see, in this case a glass slide, but it could as well be a filter or some other type of substrate, a blue area has been circled and there is particulate in the center of that circle that is of interest. At higher magnification you see the end of a very fine tungsten needle, and the microscopist is using that needle to pick up that particulate, after which it’s going to be transferred either to a scribed SEM substrate as you see on the right, or at the bottom we see a TEM locator grid. So, this is what I’ve described—there’s a copper mesh, it’s got an amorphous carbon film over it, in this case it’s what’s called a locator grid with letters and numbers in the grid. The clean room microscopist can put the particulate of interest into a specific grid square and then give me a map so I know exactly where to locate it. So, this is one easy way of directly transferring particulate from the filter to a grid for TEM.

Another methodology that we worked out here recently is direct filtration onto a holey carbon grid.

So, now that amorphous carbon film, instead of being continuous, has holes in it. And you can put it onto a standard filtration apparatus as you see here, with a polycarbonate filter, and the grid has been placed off to one side of the center of the filter.

Material can be alternately dropped directly on to the grid, and also onto the center of the filter, so you can concurrently prepare a TEM grid and an SEM sample on a filter from the same liquid sample.

We’ve done some comparisons and some studies using this filtration technique. You have to be careful that there is not background particulate that might interfere with your analysis.
So, we looked at as-received grids; we also looked at blanks prepared in the clean room using our particle free water, and in both of those cases there was little to no background particulate on the grids, and certainly not anything that would be confused with the kind of particulate that you would see from glass delamination. So, we felt fairly confident that we could move forward with this filtration method. The other concern, of course, especially with TEM because you’re sampling very small amounts of material, is whether your sampling is representative. Are you actually going to capture enough particulate to give you a conclusive finding? And we have found consistently that we do capture delamination flakes even in the small 3 mm diameter area of a TEM grid.

We did a comparison study with a set of samples looking at results from the SEM and from the TEM. This study involved multiple vials of product, and for each lot of product, one vial was selected for preparation of an SEM filter and a TEM grid on the filter at the same time. And you can see that in two cases, for samples A and B, delamination flakes were captured on the TEM grids, but were not seen in the SEM. These may have been cases where the delamination flakes were so small and so thin that they weren’t evident in the SEM, but were readily found in TEM examination. In the case of sample C, which seemed to have much more prevalent delamination, thirteen glass flakes were observed in vial one, and one large delamination flake was found by TEM. And then in the case of sample D, delamination was not was not found by either method.

This is an example of what I’ve seen analyzing these types of grids in the TEM. From sample A, in the upper right, we see a TEM image; this is quite a low magnification image, the very dark area is actually the copper grid bar, and you see that a large delamination flake has landed on the grid bar and is overhanging the carbon film area that you would use for analysis. So, this presents a very large area several micrometers in size—very thin material suitable for TEM analysis, and you can see that we have got spectrum there that I think you probably already recognize as being very characteristic of a glass material. This is confirmation of what was seen by PLM, and also, actually in this case, it was not seen by SEM, that we do have delamination flakes present.

In the case of sample B, again, delamination was not seen in the SEM, but in the TEM I found evidence of smaller flakes, or aggregates of small fragments, as you see in the image. This was out in the middle of the grid square—the grid squares are about 100 x 100 µm square—so, you can see that with the one-micron scale bar we have a small cluster of glass delamination fragments a few micrometers in size, and again, plenty of areas for analysis and an EDS spectrum that is absolutely characteristic of glass delamination.

In the case of sample C, in this case delamination was seen in both the SEM and the TEM, an extremely large flake was overhanging the edge of the grid bar, and you see the carbon film has been kind of disrupted underneath it. As we go up in magnification, even though that delamination flake looked very large and quite thick, there are still thin edges of the areas that are very amenable to TEM analysis. And you can see that along this area where there’s some alternation of contrast—and this is probably just differences in thickness and fracturing of the glass, but all of the numbered areas indicate places where I was able to get EDS spectra, just to see if the differences in contrast, or very small dark contrast features might reflect a difference in elemental composition; but in this case, everything looked very uniform and again looked very typical of a glass material.

Other types of particles might be captured on these filters as well, and in this sample, a mixture was actually found. Again, on the left we have a very large delamination flake spanning most of the grid square, but as we go up in magnification we can see that there is quite a bit of

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vial 1 SEM</th>
<th>Vial 1 TEM</th>
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<tbody>
<tr>
<td>A</td>
<td>No flakes observed</td>
<td>Two delamination flakes</td>
</tr>
<tr>
<td>B</td>
<td>No flakes observed</td>
<td>Two delamination flakes</td>
</tr>
<tr>
<td>C</td>
<td>13 glass flakes observed</td>
<td>One large delamination flake</td>
</tr>
<tr>
<td>D</td>
<td>No flakes observed</td>
<td>A few C-rich particles, two possibly graphite</td>
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Comparison study with a set of samples, looking at results from the SEM and the TEM.
other particulate that’s been captured in this filtration; certainly far beyond any background particulate that you might expect. And what was found in this sample was actually a mixture of things. The three images at the top show particles that were found throughout the sample that contained both silica and stainless steel. So, you have a very large silicon peak in the spectrum, but you also have the iron, chrome, manganese, and nickel, characteristic of the steel material. And this was quite common in a lot of these particles. Down at the bottom we have a higher magnification image of the edge of that big glass delamination flake, and you can see dark contrast features in that flake. In those spots, the elemental analysis showed them to be rich in phosphorus and rare earth elements. So, a somewhat different glass composition, but easily recognizable in the TEM.

And this is just to illustrate some of the differences in texture that you might see—this sample was actually looked at in another microscope, another TEM, at the University of Illinois at Chicago—and this was a case where extreme delamination had occurred. You see that the glass is very badly degraded, very porous. This is advanced delamination, and on the right, the scale bar there is 20 nm; you can see that a lot of these holes are actually extremely small, and again, seeing this can be an earlier indication possibly you have glass delamination going on. You have the opportunity and the capability in the TEM to see much smaller features, such as pitting and porosity, that you might not recognize in the SEM.

We tried something else with this sample as well, operating in scanning transmission mode. So, here you take your parallel beam of illumination that you would use in the TEM and you turn it into a fine scanned probe like what you use in the SEM. So, you have the scanning capability of the SEM, combined with the high spatial resolution, through-sample analysis of the TEM, appropriate for these thin samples. Because you can scan the beam, you can collect two-dimensional EDS maps to show you elemental distributions and give you an indication of how the elements are associated. So, in this case we see the red and blue maps for silicon and oxygen, which are associated as you would expect, and then the yellow map is for calcium, which is a minor-to-trace element distributed throughout the glass, but there is also a small concentration of yellow there, and by taking a spectrum right in that area we can see that there is a concentration of calcium present there. So this is another approach in the TEM for getting even more elemental information about these extremely thin samples.

I mentioned that formation of secondary products is a concern, and this is an example of something that you can easily see in the TEM, possibly not in the SEM. This was a filtered sample from a product known to contain delamination flakes, but looking at the material on the filter using light microscopy, there were brown particles evident that looked different from the delamination flakes, and the clean room microscopist was able to selectively pick out those types of particles for transfer to a TEM grid. And you can see that it has got a texture and almost a layering or needlelike appearance to the microstructure readily evident in the TEM that tells you this is probably something other than glass delamination.

I’ve seen examples of this from certain types of products where you actually get a mixture of textures. You can see that there is a large fairly amorphous looking area or particle captured on this grid, and then this other sort of flocculent-looking or fluffy-looking particulate—two distinctly different textures present in many areas on the grid. Looking at them at higher magnification, we can see that the one on the left has this sort of needlelike microstructure to it, or possibly some layering. The one on the right has a much more amorphous, kind of fluffy, appearance. An analysis of several areas showed that these actually had consistently different compositions. This was an iron-containing API and the products that I found were high in silicon and also high in iron, but consistently showed two different silicon to iron ratios, depending on whether the morphology was the needlelike structure, or the more fluffy or amorphous-looking structure. So, if you look at these spectra which have been normalized on the silicon peaks, you can see that you have a lower iron to silicon ratio for the yellow, the spectrum 2. So, two distinctly different products forming, but possibly also an indication that you’ve got a process going of continuous dissolution of glass, reaction of glass with the iron-containing API, and then forming a product that may eventually stabilize (finalize) at a given iron to silicon ratio. But, certainly something is going on beyond just glass delamination.

And this is a similar sort of product. Let me go back to the low magnification image. You see that this image has a lot of texture in it, it looks like something other than glass delamination. Looking at it at higher magnification, you can see that there are areas where there are bands of light and dark contrast; these are showing you the edges of a layered, or somewhat ordered structure that formed. That’s reflected in the electron diffraction pattern, which shows two faint rings which are evidence of this sort of large-scale ordering or layering of the structure; and this analyzed as a silicate with a distinct and uniform composition resulting from reaction of the product with the vial glass, and it did have an ordered layered structure. So again, something beyond glass delamination and now actually reaction of your product with the glass container to form secondary materials.

So, I just want to reiterate at this point—I hope that the examples have shown you some of the advantages that TEM EDS and other TEM capabilities offer for analysis of these very thin samples. And to show you again the typical spectrum that you get off of SEM EDS of these very thin samples, as is recommended in USP <1660>, you really don’t get a tremendous lot of information compared to what you can get from TEM EDS of delamination flakes and other types of particulate isolated from these samples.
So, in conclusion, glass delamination is a complex process. Understanding the mechanisms is crucial to ensuring pharmaceutical product quality.

TEM, I hope I’ve shown, is an ideal technique for analysis of thin residues and particulate isolated from liquid pharmaceutical formulations, providing high-resolution morphological, elemental and crystallographic information.

Unambiguous TEM EDS spectra can be obtained from flakes and residues that are too thin for SEM EDS analysis.

TEM grids can be prepared here at McCrone by direct transfer of glass delamination materials, or by filtration. And we’ve shown that filtration of vial contents onto holey carbon coated TEM grids provides representative samples of particles, residues, and delamination flakes.

Detection of smaller and thinner flakes by TEM may aid in earlier detection of glass delamination.

Because it is a high-resolution, high-magnification technique, TEM samples small volumes of material. It shows you things that you can’t see in other ways, but you have to keep in mind that you need to be careful about representatively sampling, and you should use it routinely with other complementary techniques. Use it in conjunction with other techniques that give you the bulk information, as well. All of them are of value.

And with that, I’d like to thank you for your attention. I’d be happy to answer any questions.

CZ: Thanks Elaine. Thanks to everyone for attending today’s webinar and Elaine will be taking some questions here. If you have questions, go ahead and type them into the question field.

We have a question from Jan: “TEM’s are big, or are there benchtop TEM’s available?”

ES: There actually is a benchtop model that exists. There actually is a benchtop model that exists. There is a benchtop model that exists. That’s a really excellent question, and I didn’t point out boron on any of the spectra that I showed, and that’s because being such a light element it is difficult to detect by EDS. The x-rays are so low energy that they have trouble escaping from the sample and making it to the detector, even in very thin samples. The detectors are getting better and more sensitive all the time, so you stand a better chance of detecting boron, but it would be going out on a limb to say that you could really get good stoichiometry to look at boron in glass. You would have to go to something that is a better light element technique. Actually, in the TEM, electron energy loss spectroscopy is better. It complements EDS and it’s better for light elements. The thinner the sample the better, for EELS. So, things like delamination flakes are better suited for it than a lot of materials are.

You typically may want things that are in the 40 - 50 nm range in thickness. EELS is not as standard and pushbutton a technique as EDS. In order to do quantitative work, you do need reference materials and you need to be very careful about that. So, that would be one approach in the TEM, other than that I think you would have to be looking at other types of techniques that are better suited to light elements. Possibly, mass spec techniques, I don’t have anything off the tip of my tongue that would give you really reliable stoichiometry for glass delamination flakes; but it’s a great question and if that’s of interest, it’s something we should be thinking about.

CZ: Another question from Jan: “How small are the delamination flakes that you can detect in pharmaceutical products using polarized light microscopy?”

ES: Chuck, you might be better suited to answer that than I am. Thickness is going to have something to do with it.

CZ: That’s a great question. We may have to follow-up with you on that. We have your contact information so we’ll contact you off-line and give you some more specifics. I think it varies depending on what instrumentation you’re using and such. We’ll follow up on that one with you.

ES: Another thing to consider, too, if you’re looking at what has been filtered onto a polycarbonate filter and you’re examining that filter by PLM, if there’s any significant amount of delamination, you’re probably going to get something like that image that I showed, where you actually have multiple flakes present and you have fragments of various sizes. So, there’s probably a minimum size in that field of view that you showed—it might be quite small compared to some of the other things that are there, but because it’s in the context of this field of view with a lot of particles in it, it’s still recognizable. If you just had a very few delamination flakes of very small size on a filter, I think we’d need to check with one of our cleanroom microscopists to see what’s the lower limit of what they can see with that particular type of material. That’s a good question.

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CZ: The coaxial illumination is key for that analysis, as well, but we'll follow up with that. There is a question here: “What are the conditions that can be used to evaluate the delamination propensity on long-term stability while a product is being developed?

ES: This gets into the realm of delamination studies, and we do get involved in helping our clients to carry those out, and it is certainly very important when you’re developing a new drug product and trying to select your suppliers for your packaging, to go through a careful study. I would say that here at McCrone, typically, the methodologies are the basic ones that I’ve described, which will be visual and stereoscopic examination of liquid in products, and then filtration and examination of filters, and then analysis with SEM EDS. Those typically will take you most of the way in determining whether you’ve got extensive delamination going on or not. Whether you’re going to catch very early delamination, or something that may not delaminate as severely as quickly, it’s difficult to say; but certainly in many cases, the PLM and the SEM EDS are enough to verify what you have going on. But, if there’s another question about secondary product formation, residues, coatings from interiors of vials, then something like the TEM may be more useful. But certainly the standard methodology of the combination of PLM and SEM EDS, using carefully controlled conditions and a matrix of conditions to test out the different possibilities will take you a long way.

CZ: Another question from Scott, I think it is along the same lines as the previous question. “Some glass processing utilizes sulfur or related surface treatments to toughen or condition the surface of the glass—does this simply render a pre-delamination, or is there another chemical or physical process going on?”

ES: That’s a little bit beyond my understanding of the processes.

CZ: It’s getting a little bit into the manufacturing of the glass.

ES: I think it is a fascinating question. One of the things that we face here, because we do assist our clients with these studies, is not necessarily having examples or participation in studies of the glass itself or knowing what goes into the glass or how it’s being treated. You depend on hopefully going to meetings and picking things up out of literature to give you some background. But, I think there are some of these basic questions for which studies need to be done, and the right methodologies brought to bear. We as analysts don’t always see that kind of information or see those types of studies. More typically, what we’re getting are either studies related to a particular drug product and maybe not getting samples of the starting glass material to look at as a baseline, or dealing with something that is already a problem out in the field and it’s an identification and QA kind of issue. So, I think participation in these basic kinds of studies and getting information from those studies is of value to everyone who’s involved in various aspects of dealing with glass delamination, whether it’s the manufacturers of the glass products or the drugs, or those of us who are doing the analytical work.

CZ: Great questions. I think that’s it on the questions. Again I’d like to thank everybody for attending and we just want to mention our next webinar that’s coming up on November 19 with Dr. Kent Rhodes and Dr. Craig Schwandt. They’ll discuss McCrone Associates Analytical Capabilities: The Particle Approach. So we hope to see you out there again for that one or other webinars. And again, this webinar and all of our other webinars are being recorded, and they’re available under the webinars tab. Thanks again for attending.