Webinar Transcription: Sub visible particle assessment for high concentration biologics

Prerecorded Webinar Link: https://curirx.com/video-recorded-webinar-2/

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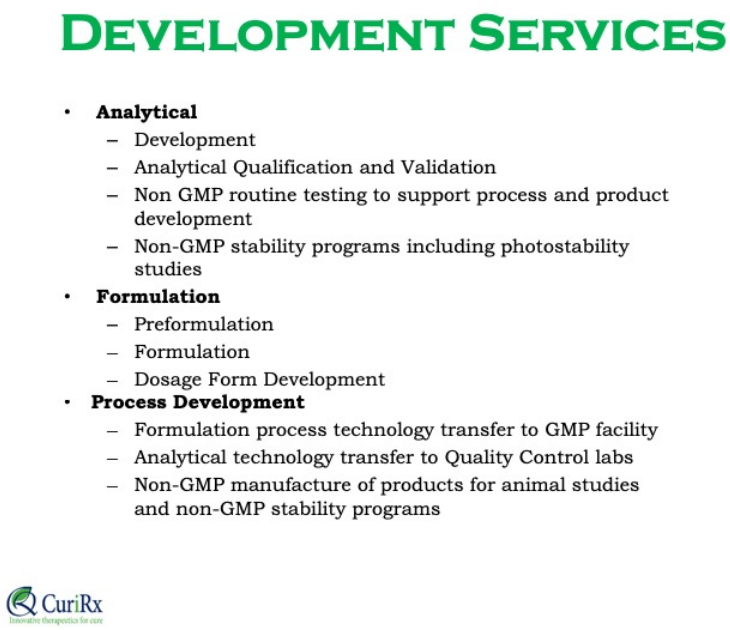
CuriRx, Innovative Therapeutics for Cure.

CuriRx is a Contract Research and Development Organization, established in November 2011 and head-quartered in Wilmington, Massachusetts. Our 13,000 square foot laboratory and office center is highly equipped to provide our customers with excellent product development services.

The services we provide include:

Analytics such as Product Development; Analytical Qualification and Validation and Non GMP stability programs including photo-stability studies. Formulation Services which include Pre-formulation; Formulation Development and Dosage Form Development.

Process Development Services including formulation process technology transfer to GMP facilities; Analytical technology transfer to quality control labs and; non-GMP manufacturing of products for animal studies and non-GMP stability programs.



As you may know, for biologics or any injectable product, the presence of particle impurities in a vial can come from the product itself during bioprocessing, process equipment or from container closures and packaging. The presence of particulates in a drug product can impact safety, efficacy, effectiveness, and the stability of biomolecules.

In this webinar we will be focus on: Key Challenges, Tools and Strategies, Characterization and Risk Assessment during the development of formulations as well as during the processing of the materials.

For particulate measurements, as you may be aware, there are the widely used USP methods 787 and 788 that have been developed for therapeutic proteins and injectables. These methods were essentially created to control levels of extrinsic particles. There is also a new chapter 1787, published in 2014, which includes guidance to characterize sub-visible particles.

Regarding particulate origins .... The list on the slide is not exhaustive, but here are some of the things that one must think about. Extrinsic, intrinsic and inherent particulate origins.

The extrinsic category are things like skin flakes, dirt particles, insect parts, fibers from clothes and hair. Intrinsic particles can come from the process itself, for example, when a product comes into contact with materials like glass/stopper container closures, metals used in the processing equipment, silicon oil, filter-process-particles or other process related particulates. Inherently, the particulates can be generated due to a molecule that is unstable and undergoes a degradation pathway, protein aggregation or aggregation induced by silicon interaction.

Particulate Origins

Extrinsic	Intrinsic	Inherent
Skin Flakes	Glass/stopper	Degradation
Ordinary dirt particles	Metal	Protein Aggregation
Insect parts	Silicon Oil	Silicon interaction
Fibers from clothing and hair	Filter process particles	
	Process related	

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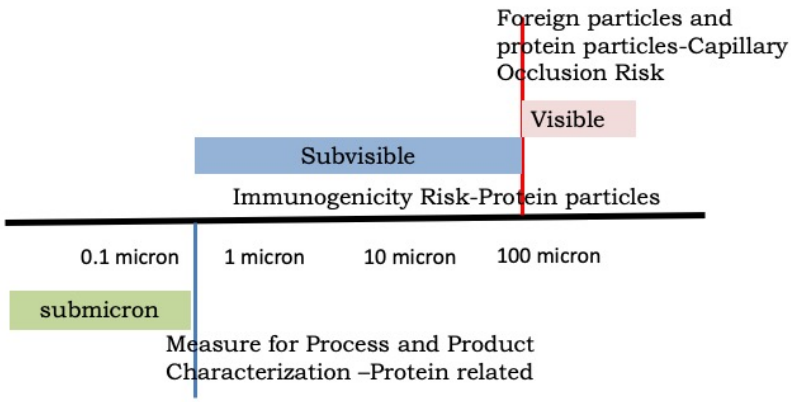
In terms of particle categories and particle risk, one can basically divide them into sub-visible, visible, and submicron.

The foreign particles that you can actually see are the ones determined by The Light Obscuration Method or even by Visual Inspection. The sub-visible particle is what we are most concerned about as these really induce an immunogenicity risk and they are all protein related particles and they range from one or two microns to hundreds of microns. The submicron particles are lower than one micron, measuring and characterizing these for process and product development becomes essential. The USP 1787 is the informational chapter with guidance to characterize these sub-visible particles.

Particle Categories/Particle risk



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The key challenges regarding particulate size is the size range from 150 micrometers to one micrometer or less than one micrometer. The probability of detection decreases as the size decreases.

Here are the domains: Visible, Sub-Visible, Sub-visible and Compendia Threshold and Sub-micrometer. The key challenges come because the probability of detection decreases with size.

Key Challenges: Particulate size

Size	Domains	Probability of detection decreases
>150 µm	Visible	
25 µm	Sub-visible	
10 µm	Sub-visible and compendia Threshold	
1µm	Sub-micrometer	
<1µm	Sub-micrometer	

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The FDA strongly recommends having an in-depth characterization or understanding of the particles in protein therapeutics, especially in the range of two to 10 microns.

The USP 1787 has new information intended to supplement, it's not a substitution, but rather a supplementation of 1787 for method to measure the sub-visible particulate.

The chapter provides guidance on setting strategy for identifying and characterizing the various particle population in such products during the development as well as the lifetime of the product.

The main thing is the characterization of the proteinaceous particles to include size/count, reversibility/dissociable, structure/conformation, chemical modification and composition/ identification.

It includes the Summary Assessment of Methods that can be useful in this effort.

Chapter 1787 provides guidance regarding ranges from two micrometers to one the hundred microns range.

The rationale for using this range is based on one hundred microns as a conservative, lower-limit threshold for visible particles and, two microns as a lower-level limit, or lower size domain, for which the recommended techniques are considered robust and proven.

It is recommended that during the development phase, that you collect the data on the population below 10 microns or in the different two sets of data bins: two to five and five to ten.

Chapter 1787 concerns with all particle species present in the final product. However, it is primarily oriented toward the inherent therapeutical agent’s condition and acceptability.

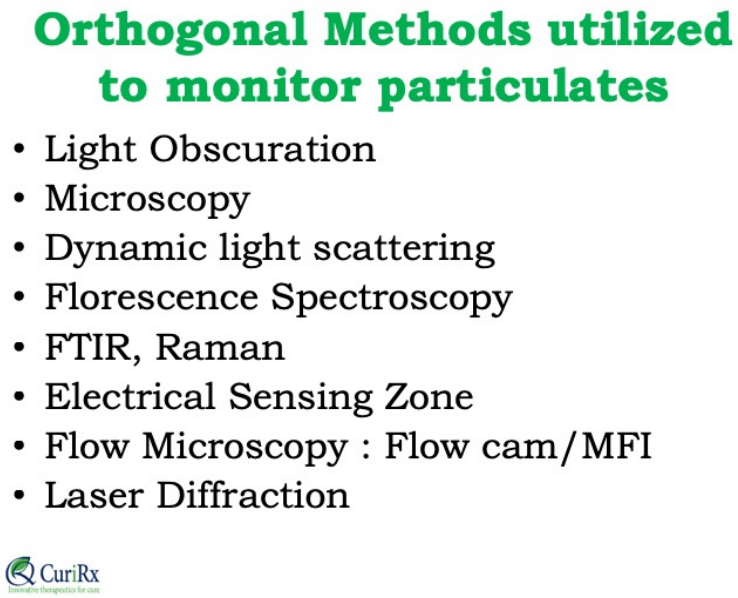
For product release, the particle-size threshold remains the same. So for release, the specifications still calls for 10 micron and 25 micron, with the limit of 6,000 particles and 600 particles respectively.

The recommendation to monitor the population below the 10 micron threshold is done by orthogonal methods to characterize particles beyond size and count ... ID, composition, morphology, reversibility, and conformation.

The methods that are utilized to monitor the particulates are light obscuration, microscopy (which is very much used while releasing the particles.)

With light obscuration method, again, the threshold mentioned earlier is the same.

Other techniques are also used, such as: Dynamic Light Scattering, florescence spectroscopy, FTIR, Raman, Electrical Sensing Zone, Flow Microscopy, Flow-Cam/MFI, or laser diffraction.



USP does not provide the recipe for when to use a method, nor does it provide the methods to use.

Therefore, a Quality by Design approach during the formulation dosage-form development is highly recommended. This is primarily achieved by instituting methods during formulation development that will allow for monitoring particulates during the screening of excipients, buffers, stabilizers, lyoprotectants, cryoprotectants and surfactants, to understand the liability and thereby create proper control strategies.

Basically, understanding or knowing your product, knowing your process becomes important. Monitoring the particulates during development to create a product, during manufacturing and stability history recording and understanding the liabilities. This is how we create a proper control strategy.

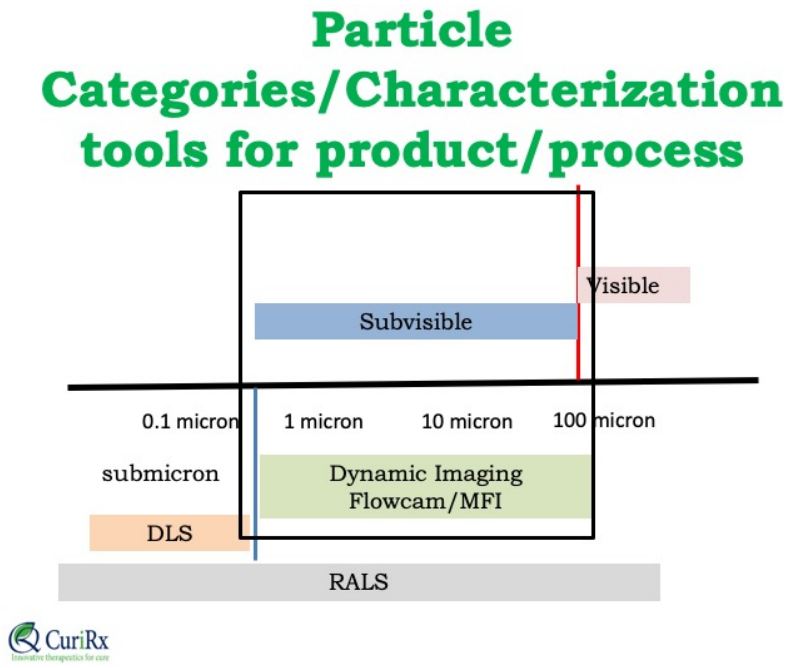
Methods utilized during formulation development is what we do at CuriRx.

When we are developing the formulation, these are some of the methods that we use in order to control the particulates in the final product formulation:
FlowCam/Micro-Flow Imaging system
Right Angle Light Scattering and Dynamic Light Scattering

This is a diagram of particle categories and characterization tools for product and process.

We look at the visible category on the right-hand side of the slide. The visible range exceeds one hundred microns, so one micron to one hundred microns is the sub-visible range, and below one micron is the submicron range. Within visible range, there are many ways to identify the particles, even visually, but the sub-visible range is where we can utilize Dynamic Imaging, FlowCam/MFI.

Below one micron, we can utilize Dynamic Light Scattering, and at CuriRx we rely heavily on gaining understanding even at the submicron range, using Right Angle Light Scattering. Right angle light scattering helps to understand the product going from the subvisible to visible range, or in other words how product degradation induces aggregation.



The FDA strongly recommends in-depth characterization of particles in protein therapeutics. For this purpose, a Dynamic Imaging Particle Analyzer is increasingly being utilized. Dynamic Imaging Particle Analysis can provide incredible insights to your formulation, allowing you to see your particles, especially in the two to 10-micron range.

At CuriRx, we use FlowCam.

FlowCam is a technology that uses Dynamic Imaging for direct particle detection, which enables rapid quantification of particle size and shape for tens of thousands of particles per sample, and sensitivity to detect translucent protein particles.

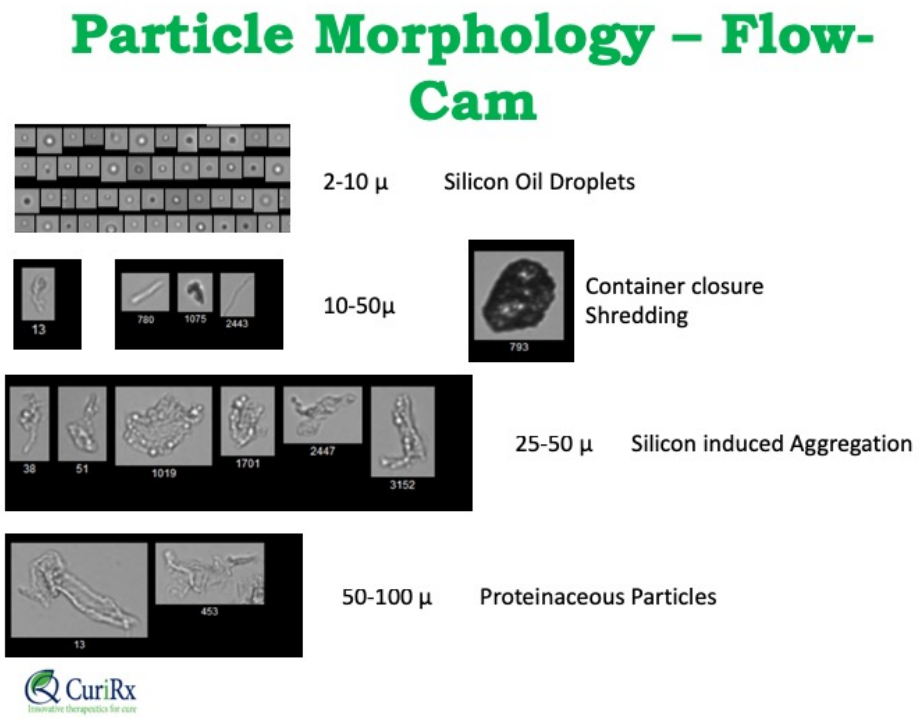
FlowCam is a sensitive, simple, and automated method for the analysis of sub-visible particles and protein aggregates. Its ability to provide particle size, count, and morphology enables our

researchers to discriminate groups of particles from each other, and monitor changes in the particles over time.

The FlowCam examines the fluid under a microscope. It takes images of the magnified particles within that fluid and then characterizes the particles using a variety of measurements.

It is a high-precision computer-controlled syringe pump that pulls the fluid sample through a flow cell perpendicular to the optical path. The optical system is similar to a microscope, and is used to capture real-time images of the particles in the fluid as they pass through the flow cell.

Here are a few examples of what you can see utilizing a FlowCam instrument. The first top panel over is two to 10 microns, silicon oil droplets. As can you see, the silicon oil droplets are very small, and very precise round circles. From 10 to 50 microns, there are have various proteins, fibers or protein fragments, and you can also see container closure shredding. The silicon induced aggregation can also be viewed at the range of 25 to 50 micron. And, of course, the proteinaceous particles are larger, 50-100 micron range.



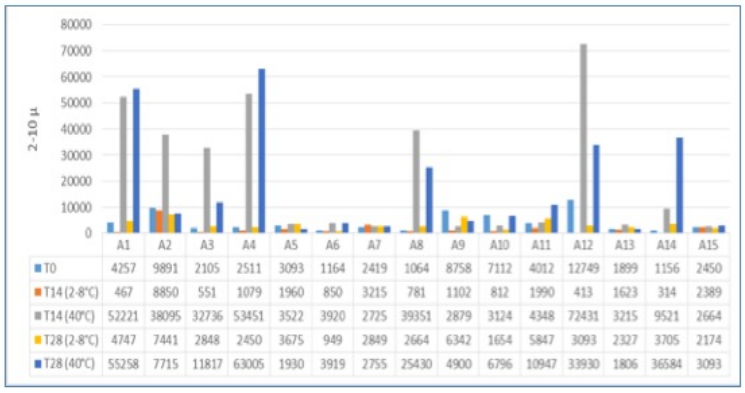
This is a case study utilizing FlowCam as a technique. This involved 15 different formulations, examining formulations from time zero to 28 days.

Here we wanted to identify the formulation, so this data was collected during formulation excipient screening. We wanted to examine and understand the number of particles from the two to 10 micron range, detectable during the excipient screening methodology.

As you see, different formulations over different periods of time will result in the number of particles remaining static or increasing in number.

This analysis enables us a reliable way of distinguishing particle counts between the formulations, and also helps to assure us, that at the end of the day, a particular product formulation will not undergo degradation thereby creating more particulates in the product during storage.

CASE STUDY 1: Short-term Stability Study- Flow-Cam



	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
T0	4257	9891	2105	2511	3093	1164	2419	1064	8758	7112	4012	12749	1899	1156	2450
T14 (2-8°C)	467	8850	551	1079	1960	850	3215	781	1102	812	1990	413	1623	314	2389
T14 (40°C)	52221	38095	32736	53451	3522	3920	2725	39351	2879	3124	4348	72431	3215	9521	2664
T28 (2-8°C)	4747	7441	2848	2450	3675	949	2849	2664	6342	1654	5847	3093	2327	3705	2174
T28 (40°C)	55258	7715	11817	63005	1930	3919	2755	25430	4900	6796	10947	33930	1806	36584	3093

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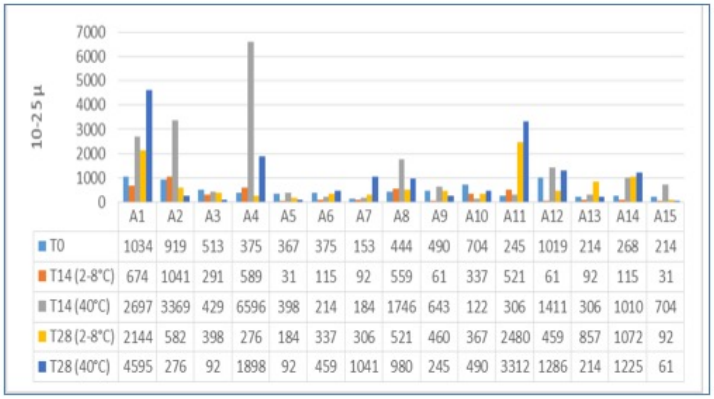
In this Case Study, “Case Study 2”, in a short-term stability study, we are looking at particles in the 10 to 25-micron range. The previous study was two to 10 microns, this is 10 to 25 microns.

Again, the utilization of this work allows us to not only understand the degradation profile but also aids in the selection of the formulation, looking at to see which one we can forward as a lead. Which formulation will not create degradation pathways or create aggregation that would result in sub-visible particles.

This analysis helps enables us to form a reliable way of distinguishing particle counts, between the formulations, and also helps to assure us, that at the end of the day, a particular product formulation, will not undergo degradation, thereby creating more particulates in the product during storage.

CASE STUDY 2: Short-term Stability Study- Flow-Cam



	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
T0	1034	919	513	375	367	375	153	444	490	704	245	1019	214	268	214
T14 (2-8°C)	674	1041	291	589	31	115	92	559	61	337	521	61	92	115	31
T14 (40°C)	2697	3369	429	6596	398	214	184	1746	643	122	306	1411	306	1010	704
T28 (2-8°C)	2144	582	398	276	184	337	306	521	460	367	2480	459	857	1072	92
T28 (40°C)	4595	276	92	1898	92	459	1041	980	245	490	3312	1286	214	1225	61

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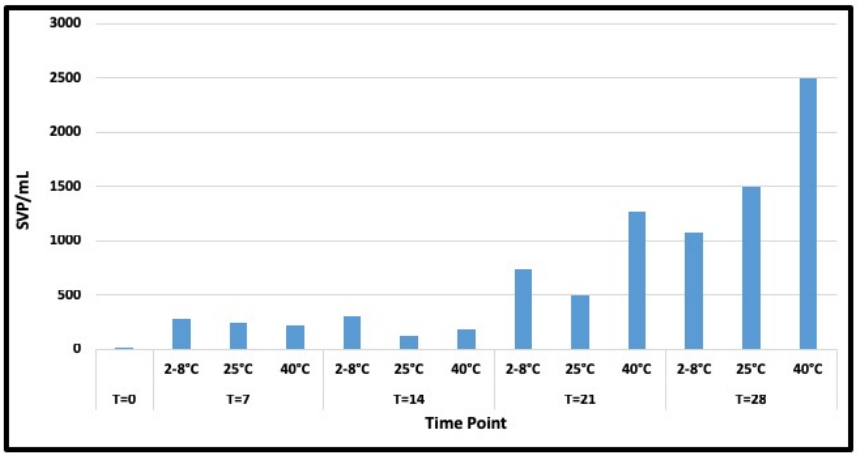
This is Case Study Three, we're examining the utility of FlowCam. Here, we are analyzing a sub-visible particle per mL.

We have looked at the total number of sub-visible particles over a period of over 28 days, and for this particular formulation you can see as the time increases, the number of particles also increase.

As you can see, understanding Sub-visible particles by FlowCam or by the Micro-Fluidic Imaging System are sound tools during, process development; formulation process; or screening of excipients during formation development to help ensure the molecule is more stable, which is to say, helping to ensure that particles are not being created during the formulation process or manufacturing process.

CASE STUDY 3: Utility Of Flow cam: Particulate in formulation



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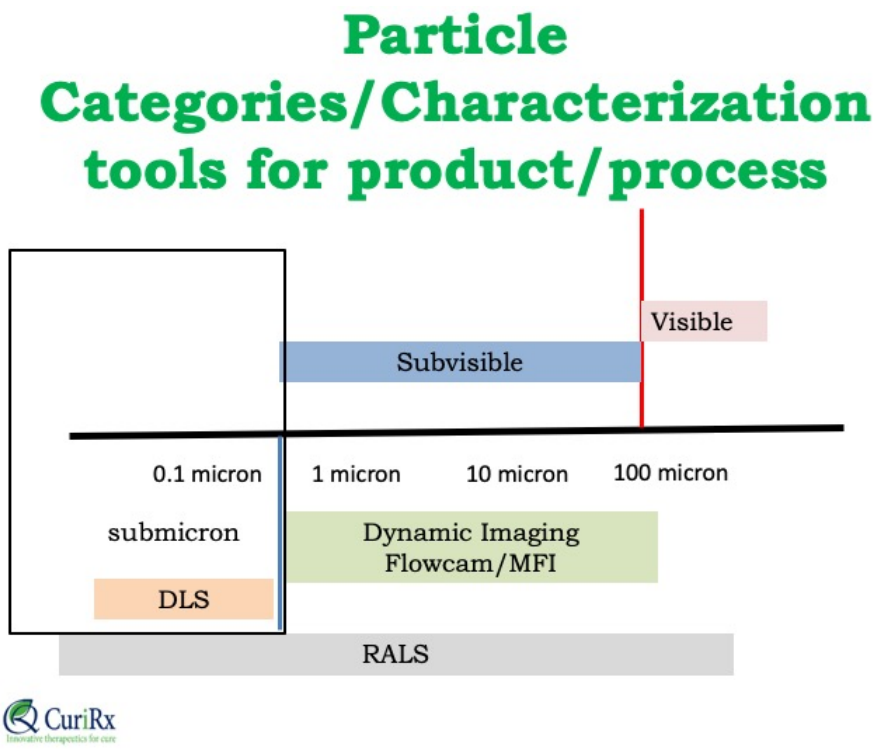
The FDA requires an understanding of the two to 10 micron range. However, we believe it is best to really understand a step beyond that requirement.

Therefore, to help in understanding the propensity of a particle to form via any degradation mechanism, it is advantageous to utilize Dynamic Light Scattering and Right Angle Light Scattering for monitoring of the submicron range.



We routinely use Dynamic Light Scattering at CuriRx. Dynamic Light Scattering is generally utilized to determine particle size and particle size distribution of protein therapeutic formulations. DLS is used for the screening and development of bioformulations and to predict and monitor the colloidal stability of such formulations.

Dynamic Light Scattering measures Brownian motions and relates this to the size of the particle. The particles in the solutions are illuminated using laser and the intensity fluctuation in the scattered light is analyzed. Basically, when a particle becomes two or more, or it comes together, it scatters more light, and that's the basic principle of DLS. Even Right Angle Light Scattering has the same principle.

Hydrodynamic diameters are reported as the intensity-weighted average and as the volume-weighted average over a particular range of size populations corresponding to the most prominent peak.

The Intensity-Peak value is used as the hydrodynamic diameter of a particular species.
The percent volume values are used to approximate relative amount of various species in the formulation. And the Z-average are generally used to assess batch-to-batch variability of a sample.

When CuriRx employs DLS into our screening program, we look at everything. We look at Z-average. We look at polydispersity index, we look at intensity and the volume because it gives you a better, whole picture.

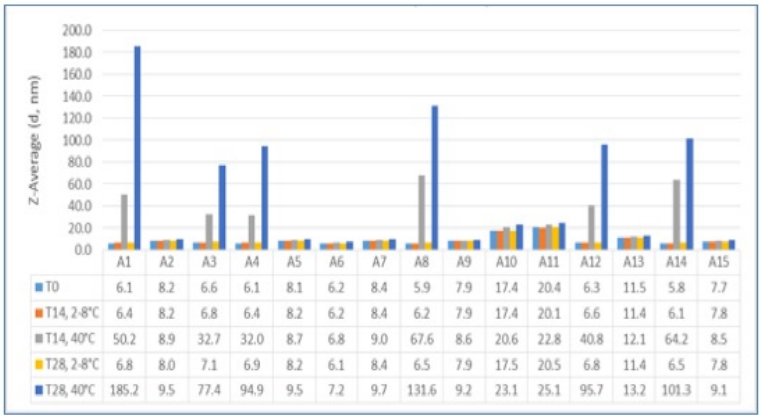
Here, we are presenting some data that only looks at Z-values.

There are many different formulations, from A to A15. Over a period of 28 days, we can see if there is an increase in Z-average based on Dynamic Light Scattering.

It becomes an added tool, along with the FlowCam, along with size exclusion, along with all other analytical tools we use, as this becomes additional information to help us gain an understanding about the control of degradation by the excipients being used.

These are the degradation pathways that can lead to aggregation or a “coming together” of the molecules. Here, as you see, in this example, we can distinguish between different formulations and we can characterize which formulation behaves better, and under what conditions.

CASE STUDY 1: Short-term Stability Study- DLS



	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
T0	6.1	8.2	6.6	6.1	8.1	6.2	8.4	5.9	7.9	17.4	20.4	6.3	11.5	5.8	7.7
T14, 2-8°C	6.4	8.2	6.8	6.4	8.2	6.2	8.4	6.2	7.9	17.4	20.1	6.6	11.4	6.1	7.8
T14, 40°C	50.2	8.9	32.7	32.0	8.7	6.8	9.0	67.6	8.6	20.6	22.8	40.8	12.1	64.2	8.5
T28, 2-8°C	6.8	8.0	7.1	6.9	8.2	6.1	8.4	6.5	7.9	17.5	20.5	6.8	11.4	6.5	7.8
T28, 40°C	185.2	9.5	77.4	94.9	9.5	7.2	9.7	131.6	9.2	23.1	25.1	95.7	13.2	101.3	9.1

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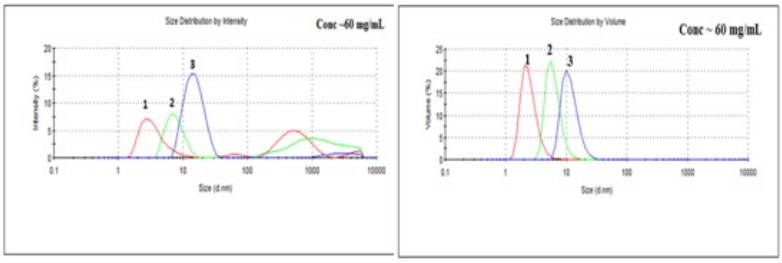
Looking at this case study, of three lead formulation for biologics at 60 milligrams per mL, you can see the size distribution by intensity as well as size distribution by volume. Basically, here, the message is that… all three formulation gives likely different size, and those different size are basically by volume.

This helps us in determining decide which formulation becomes the lead formulation. Obviously, the less size, the better. In this case, number one was the lead formulation.

CASE STUDY 2: Particle Size and High Concentration Formulation - DLS



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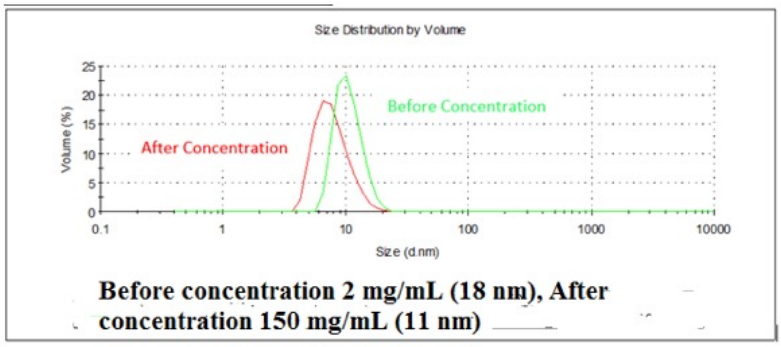
Here in Case Study Three, we looked at a UF/DF process, adding a Dynamic Light Scattering monitor during the process of buffer exchange and concentration.

This is a size distribution by volume and, as we know by Dynamic Light Scattering, if the formulation is a good formulation, it will not show an increase in Dynamic Light Scattering, but, instead, it will actually help in lowering the size.

In the figure, green is before concentration, and the red is after concentration of the protein, and as you see, we are within the range what we were expecting at 11 nanometers.

CASE STUDY 3: Particle Size and High Concentration Formulation - DLS



Before concentration 2 mg/mL (18 nm), After concentration 150 mg/mL (11 nm)

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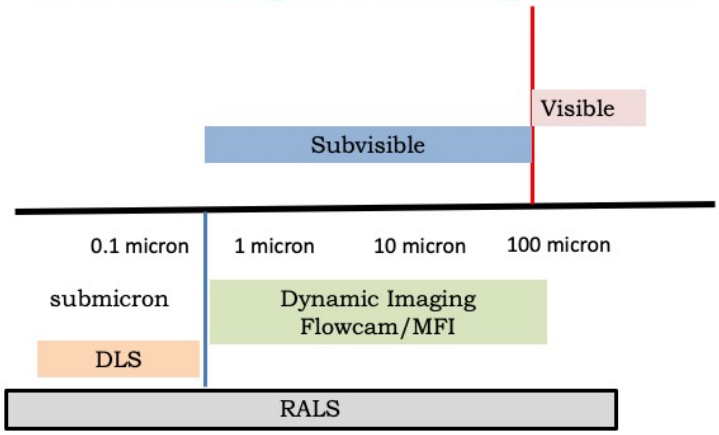
RALS is also a very good tool that we employ at CuriRx to develop the formulation or even develop a purification process and understand how we can mitigate the formation of aggregate during the process.

Particle Categories/Characterization tools for product/process



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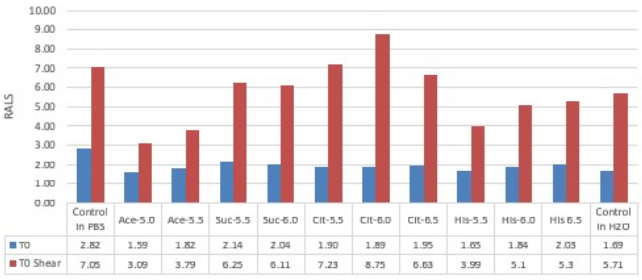
Right Angle Light Scattering (RALS) represents macroscopic changes in an otherwise soluble molecule transferring to aggregation. When a molecule unfolds and aggregates, it scatters light.

RALS measures the scattering light intensity at 90° to the incident beam. From the measured intensity and relative comparison allows for qualitative analysis from soluble aggregates. RALS becomes a good tool to monitor soluble aggregates to insoluble precipitant during the formulation process development. RALS represents macroscopic changes in an otherwise soluble molecule transferring to aggregation (soluble and/or insoluble). When a molecule unfolds and aggregates, it scatters light. Larger the particle size, higher the light scattering.

Here, in Case Study One, where we are looking at different buffers we apply Right Angle Light Scattering in regard to agitation or simulated shear stress. As you can see, we can screen many different buffers for this particular protein, and within this buffer range, you can identify which buffer performs better and which buffer will not create more aggregate. Hence we can control or select the buffer that is leaning toward better formulation development.

CASE STUDY 1: Buffer Screening - RALS



	Control in PBS	Ace-5.0	Ace-5.5	Suc-5.5	Suc-6.0	Cit-5.5	Cit-6.0	Cit-6.5	His-5.5	His-6.0	His 6.5	Control in H2O
T0	2.82	1.59	1.82	2.14	2.04	1.90	1.89	1.95	1.65	1.84	2.03	1.69
T0 Shear	7.05	3.09	3.79	6.25	6.11	7.23	8.75	6.63	3.99	5.1	5.3	5.71

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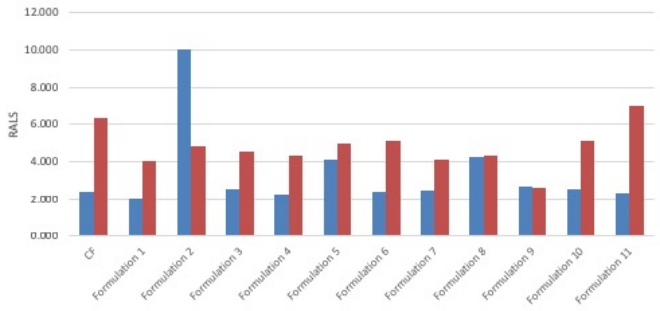
Here, in this case, we are looking at a stabilizer screening, where we are examining different formulations, again, at different time points. This is an experiment that involved 11 formulations at T-0 and T-14, measuring to see which one does not increase in Right Angle Light Scattering.

Basically, the information derived suggests that there are a few formulations that will not change over a period of time, and those become good formulations to take forward.

CASE STUDY 2: Stabilizer Screening - RALS



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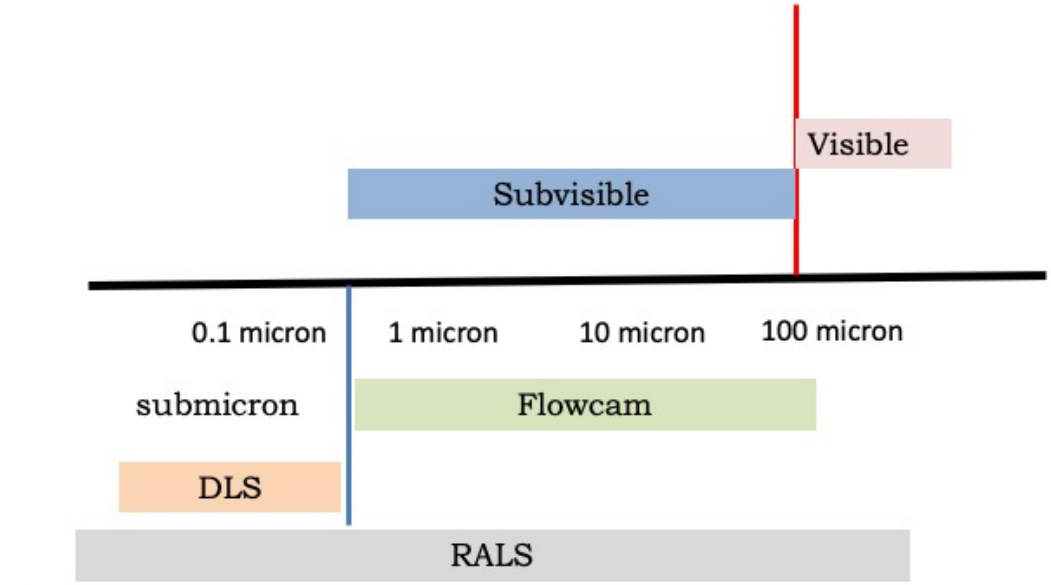
In summary, there is much to focus on regarding sub-visible particles. And, of course that is what the FDA wants you to do. To be able to understand your product, understand your process, and to make your product in such a way that you can control sub-visible particle formation. However, we hope to also show you that it is not only the sub-visible particle that requires focus, but also that the submicron particles are important to control.

In Summary



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In conclusion, presence of particulates, especially derived from biologics or inherent product attributes is controlled by:

- Controlling the quality attributes by knowing your product
- Controlling the quality attributes by knowing your process
- Introducing quality by design into your formulation/dosage formulation development.