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SPOTLIGHT

PODCAST INTERVIEW with:

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Advancing real-time monitoring of AAV vector processes

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Q How can we make vector bioprocessing faster and more cost-effective – and improve the identification and measurement of critical quality attributes – by harnessing cutting edge analytical tools?

RL: In order for us to understand how we can make vector bioprocessing faster and more cost effective, it is important to firstly touch upon what are the main technical challenges that currently exist in developing a robust viral vector on time and on budget.

One major challenge is that the process development cycle time is long. When the process development team requires many development cycles for achieving a good process, consistency, good compatibility between the development lots, and optimal product recovery yield, then there is a major delay in proceeding to reliable and low-risk scale-up engineering runs followed up with manufacturing runs.

The current analytical methods for evaluating the AAV titer – for example, an empty-full capsid ratio, during the final steps of purification and during lot variability assessment, a stability shelf-life study, and a lot pooling strategy – are time consuming and do not accurately inform about product critical quality attributes. When you have limited process control, your process is at high risk, and you cannot make the appropriate decisions because you are walking slowly in the dark with minimal visibility to identify what is the true optimal operational design space.

To expedite your development time and improve your purification recovery yield and process consistency, there is an urgent need for robust titer and empty-full ratio data generated in real time, and with faster turnaround time. It should ideally be possible to process a large number of samples with no sample manipulation required.

Currently, more and more gene therapy and vaccine companies want to maintain independence with their supply chain of raw materials for viral vector and mRNA-based products, such as plasmid. Having rapid and reliable titer and purity assays for the starting material is very important for good critical quality attributes of the product.

"The current analytical methods for evaluating the AAV titer ... are time consuming and do not accurately inform about product critical quality attributes."

- Rachel Legmann

Q Where are the key throughput-related issues for vector manufacturing assays/analytical tools currently? And how to address them moving forward?

JF: Currently, much of the work being done with in-process testing requires

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wait times and hold up times while the material is being processed, which unfortunately doesn't expedite the flowthrough of the actual product itself. What we are looking to do is to give scientists a tool to be able to analyze their samples in real time, without any sample manipulation, so they can understand exactly where they are in relation to the process. We are more focused here on giving the scientists the tools to make real-time decisions in the process, without requiring additional time to post measurements, without hold up time, or time to strengthen the analytics technology that exists currently.

RL: The main bottlenecks are in the development of the viral vector during the manufacturing process, and having large amounts of samples, which really presents a big challenge to moving forward with accuracy and making the right decision.

The first point of concern is the last two purification steps – the final run, when you have to remove the non-product-related impurities and the empty capsid from your final product – and during the final tangential flow filtration (TFF) step. In these steps you have so many samples with different formulations and this is where you have a bottleneck, because you need real-time data to make a very rapid decision.

Another key point during development is implementing this into a pooling strategy. When you are working on a pooling strategy and you are not processing the whole production harvest at one time, but instead splitting the harvest into multiple lots, then you have multiple samples that you have to analyze simultaneously. However, you must make a decision rapidly in order to avoid losing the batch and you must check the consistency of each lot prior to the pooling – that is critical.

There are further bottlenecks in development relating to measuring consistency of multiple development lots. For example, a customer running multiple development lots will need to know the optimal time to move forward with the engineering run in manufacturing. At that point you have multiple samples that must be analyzed really quickly and in the manner Joe described. And finally, when you come to define the many conditions affecting product shelf-life for the best possible formulation, storage, and shipment, you once again have multiple samples and another real bottleneck to deal with.

JF: Just to give a little context to Rachel's comments, here's how that would work in the real world.

One of the ways in which we are able to monitor the process is by using a UV-Vis method called Slope Spectroscopy. It is not an absolute absorbance-based technology but is more a technology that looks at the change of absorbance over multiple path lengths of interest, generating a linear slope progression in order to calculate just how accurate that slope is to replace absolute absorbance measurements with a slope regression and R2 value.

The SoloVPE® is capable of undiluted sample analysis in less than one minute. This enables the Slope Spectroscopy method to provide a process feedback control that allows for real-time decision making.

The ability to test samples without the need for any manipulation or sample dilution allows for a highly accurate reading of that sample made at the time of measurement. These slope values can then be used to assess where particular batches are within a process, and allow the scientist to make real-time decisions based on which samples continue through the process, which are

pooled with other processes, and which are potentially held at time, allowing further processes occurring in the future to be added to the particular lots going out for continued analysis.

Q What tools are available for measuring the impact of various AAV vector engineering methods on the capsid and its transfection profile – and what do these data mean for vector manufacturing at large?

RL: As we all know, the adeno-associated virus (AAV) is the most popular viral vector used in gene therapy today. Currently, there are four main workhorse techniques to measure AAV titer and empty-full capsid ratio: digital droplet PCR (ddPCR), ELISA, transmission electron microscopy, and analytical ultracentrifugation. ddPCR is precise but has a smaller dynamic range, therefore requiring exact sample dilution. Other methods that are currently being evaluated in the gene therapy market include high-performance liquid chromatography (HPLC), capillary electrophoresis, dynamic light scattering, and traditional UV-Vis spectroscopy.

Traditional UV-Vis spectroscopy utilizes a standard UV-visible spectrophotometer that uses a 1 cm fixed path length. When analyzing samples that are outside of the concentration range of the spectrophotometer, it is necessary to dilute or perform other manipulations on the sample in order to obtain a reliable reading. Careful manipulation of a sample can take between 30 minutes and 3 hours, depending on the operator and their expertise. And more importantly, it increases the risk of error in the final measurement data.

Essentially, data that are processed immediately allow more rapid process understanding and a design of experiments (DoE) approach that can potentially reduce process development time for titer impurity measurement for multiple drug modalities, including proteins, plasmid, and AAV. However, none of the aforementioned methods deliver results quickly or easily, so that they can be implemented in real process time. This impacts process control ability and creates process risk, which can cause complete shutdown, and most unfortunately of all, impact the patient who is looking to receive a safe drug.

The problem in the gene therapy market remains the fact that analytical tools are insufficient due to high variability (of between 20–40%), low throughput, and a lack of capability to execute in real process time. Therefore, there is a great need for a better in-line process control, offering quick and direct total viral vector analysis with high dynamic range during development to enhance throughput and improve decision making. Real-time monitoring will reduce the risk of batch loss by eliminating the dependency on offline testing and indeed, there is a tremendous ongoing effort in the field to decrease labor costs through in-process tools that allow real-time measurements. The SoloVPE system is one such solution that addresses the disadvantages of current analytical methods.

JF: When we started exploring our methodology for Slope Spectroscopy to see if it would be applicable for AAV, one of the things that really startled us was the average 20–40% acceptance range that is currently tolerated by the industry. This was a world that was not well known to us: when we talk about either high concentration

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monoclonal antibody, plasmid purity, or oligonucleotide concentration, we are typically used to working with groups that are looking at under 5% variability.

We wanted to understand how and where a UV-Vis technique could potentially offer a solution to the industry – to leverage our experience gained and the progress we've made in reinventing how UV should be done to see if we could potentially influence lowering that range, increasing the throughput due to no sample manipulations or dilution, and getting rid of the variability within the measurement itself.

"...the technology has now advanced to the point where Slope Spectroscopy represents a better analytical tool for providing real-time feedback, and eliminating the wait times..."

- Joe Ferraiolo

It is important to emphasize that we are not looking to remove or replace those current analytical methods we described earlier – that is not a viable approach. However, through collaborations with our customers, the technology has now advanced to the point where Slope Spectroscopy represents a better analytical tool for providing real-time feedback, and eliminating the wait times associated with the current process to influence how quickly or slowly the process goes through step-to-step changes.

RL: Indeed, the great correlation between the SoloVPE and ddPCR/ELISA (the two separate methods that between them form the gold standard in the market right now) demonstrates that this system enables real-time process decision making, thereby mitigating risk for pooling strategy and improving the overall decision. So again, it's something that can be used not instead of the standard analytical tools approved by the regulators, but specifically to deal with multiple samples.

Q Can you provide an overview of current Repligen analytical solutions for therapeutic product quality attributes – what are the current limitations, what constitutes acceptable variability, and how we can get better in this department?

JF: When Slope Spectroscopy was invented, it was born out of frustration with the current methodology available at the time. Any UV-based technology developed in the last 30 or 40 years has been based on a 1cm path length. If the sample happens to fit within the linear range of that spectrophotometer, it is possible to make the measurement; if not, it is a case of having to introduce sample prep and dilution error to the potential assay. So, the goal was to provide a platform solution regardless of concentration of sample. When compared against other fixed path length technologies, even though the path length might be smaller, it still relies on that sample fitting within the linear range of the given path length to make the measurement. So unfortunately, it still has the same problematic issues as traditional fixed path length UV at 1cm.

> "...the SoloVPE system can transfer more easily into GMP environments where a trained scientist may not be operating the system."
>
> - Joe Ferraiolo

Fundamentally, the SoloVPE or FlowVPX® technology establishes the best linear regression fit within Beer Lambert's law to provide a slope value which is paired with an R2 value of three 9s or higher, assuring accuracy in the measurement. What we have essentially done is taken spectroscopy and made it into a pass-fail method based on the R2 criteria of three 9s or higher.

With proteins and plasmids, and any DNA/RNA, we are looking at what is essentially a two-wavelength measurement, at 260 and 280 nanometers, and the system calculates concentration by either taking these measurements by themselves or taking a ratio of the two. Most plasmids and oligonucleotides are highly concentrated and very viscous. The only action the scientist has to take is pipetting their sample (undiluted and with no sample prep) into one of the SoloVPE vessel and pressing the 'Start collect' button. The software is completely automated and will generate that linear curve based on multiple path length reads within the measurement. This is obviously very quick – each measurement takes about a minute to run – and you get a real-time picture of exactly where that process is.

More importantly, the SoloVPE system can transfer more easily into GMP environments where a trained scientist may not be operating the system. It may be a lab technician instead, who has no fundamental idea of what Beer Lambert's law is, but who is more than capable of pipetting sample into a cuvette and running the measurement on the SoloVPE, because there is no sample preparation or dilution required.

We typically leverage plasmid purity, and anything related to high concentration nucleotides, because of the ease of transfer. Once the method is validated, it's easily transferred to sister sites or contract manufacturers using the same slope method.

We are not only interested in helping one part of the process. We are keen to find out where Slope Spectroscopy becomes the platform from company to company and from site to site, and achieves a repeatability of <+/-2%. This to our minds is what the platform approach means for the industry: to not only expedite the way that data goes from one lab to another, but to reduce the amount of time and error associated with each method of transfer.

Regarding AAV process monitoring and screening, it is about getting away from some of the theoretical values that have been published (or not published, in some cases) and addressing the fact that there is no real, fundamental, defined method for this analysis. All we know for sure is the two wavelengths of interest when we are talking about UV, where the measurement should be made. In our case, we take a dual wavelength slope ratio, which essentially becomes our R value, our ratio. That OD value can be used as a tool for process monitoring, pooling applications, or final concentration.

There are some published extinction coefficients available for the AAV material, so we are now talking about leveraging a one-minute method that can be locked down in GMP environments to calculate concentration, as well as monitoring any steps post-chromatography.

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RL: From the process development perspective, when I am producing a plasmid using *E. coli* fermenter, I like to use the system on the upstream side, when you are actually using the OD600 in order to rapidly measure the density of the microorganism with no dilution. It is a major benefit not only for the evaluation of the quality of the produced plasmid, but also for the option of very rapidly monitoring the growth during the fermentation process.

JF: That is one of my favorite applications to work on, not because of the type of sample but more for the benefits that using this technology gives to the process.

One of the challenges that currently exists with OD600 is the fact that you always have continued cell growth throughout the entire process. One can imagine that if a scientist or analyst is trying to leverage traditional UV-Vis method, they would have to pull samples at the time of measurement, and they would then have to potentially store those samples, dilute them, and then report back the measurement. And while all of this is happening, the cells are growing, so it's a bit like chasing a moving target. With the at-line version of the SoloVPE System, it's as simple as pipetting a sample from the process into the system, undiluted and with no sample prep, and then you have your measurement in less than a minute.

With the FlowVPX technology, which is our in-line solution for Slope Spectroscopy, this process becomes even more robust and is also simplified. It also allows us to leverage the kinetic software to assign the time points when we would like the analysis to be made. Everything is completely automated, so there is zero sample manipulation. Essentially, you are watching concentration over time within your process.

For AAV empty-full correlations, we have done a lot of work with companies that have certified standards for AAV. We have leveraged these standards samples to test and compare comparability studies conducted using SoloVPE System and qPCR, which is the current technique being used. There is excellent agreement between both techniques, meaning the technology can not only be used to leverage process monitoring – if it appears within the given range of about 1 e+11 and higher, we can assess concentration as well.

Q How to drive further improvements in AAV vector process analytics?

RL: Coming from the monoclonal antibody world and moving into a far more complex product such as AAV, I would love to improve the process in several places. It is really exciting to see if I can use this platform (or any other platform) in order to get better production and performance of the process, which means having a better yield and a better purity of my product.

One of these points is in the purification steps with chromatography. Obviously, people are using the regular UV spectroscopy, but it's very important to have a more reliable and rapid tool with lower standard deviation – a quick result from which to make the right decision. I'm talking about the final chromatography run, which is the critical point where you are separating the empty and full capsids. And we all know that it's not only important to have the population with a certain amount of full capsids. It's also important that it is consistent – that

every time we are getting the same thing. Otherwise, we cannot move forward. This tool will help us to be able to develop and rely on the best final chromatography process.

As mentioned earlier, I would also use the system for the final process step before fill-finish (TFF), when it's also very important to know the titer and the impurities of the empty-full capsid ratio. And product stability is a further point of application, in order to measure and monitor the product under different extreme conditions to identify the best shelf-life. I would definitely use it for lot-to-lot variability, too – both in development and in the manufacturing process.

As Joe mentioned, the system is not going to replace ddPCR and ELISA, or maybe future accurate tools. But it will certainly help us to understand the consistency and the reproducibility of the process, in addition to the other assays that we will be doing at the end on the final product. It will also establish robustness through conducting a real-time risk assessment for many engineering runs, and that will significantly shorten the validation time. That means better performance for the process itself as well as the monitoring of it. And our pooling strategy, as we mentioned, is mitigating the risk of batch failure.

Lastly, I would like to see the improvement delivered through using a rapid analysis tool in the quick testing of samples before the drug substance formulation. Off-line measurements are of course required, but you do need to have a degree of consistency between all the drug substances you are analyzing.

JF: The current methodologies that are used all come with their own sets of issues. But the common thread that runs through all of this – and especially in relation to what our customers need – is that it's not just about being quick, it's also about being accurate.

Unfortunately, a lot of the current technologies out there don't cover both. That is really the need we are trying to address. We know the limitations of UV-Vis and what it can and cannot do, but in the places where it can be leveraged, the technology now exists to provide the marketplace with a better tool for the job than those that are to hand. Whether it's monitoring the process steps or implementing the system in a GMP environment where the final release test has been done with the technology, we can provide the industry with a better tool that does go after both speed and accuracy.

What we try to achieve with this is not just to claim the technology is good for literally anything you can think of that has a UV chromophore, but it's understanding where that tool is best deployed within a process, and giving our customers a better way to leverage the information from the analytics to make better decisions in a much faster timeframe.

Q How is Repligen driving innovation of in-line process analytics?

JF: Slope Spectroscopy has been a game changer within the industry. I mentioned earlier that there hadn't been significant progress in the evolution of UV-Vis in decades, frankly.

What we have tried to do over the years is not only to understand where the best fit for the technology might be, but to provide our customers with a true solution. As I mentioned earlier, we are not just interested in one area – we like to see where the technology can be implemented, and then give our customers the best support moving forward in how to implement

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the technology at that particular process point. That's the case whether it is in development, where we are just monitoring the process (particularly in the non-regulated environment), all the way through on-the-floor manufacturing and QC, to where the technology is being used for final release – and ultimately, transferred to other companies, sister-sites or contract manufacturers.

Looking forward, we are looking to drive the innovation of the technology in-line – that's really where the technology is headed. Both Rachel and I feel the best use of the technology within the process flow is being able to give scientists real-time information by literally just watching a screen and understanding where they are within a process, related to either concentration or slope optical density readings, in order to make real decisions. And not just making those decisions quickly, but having the backing of each linear regression providing the proof and evidence that the scientist needs in order to have confidence in the values that are being reported by the technology.

I think 'quicker' and 'more accurate' – the buzzwords that are typically thrown around AAV analytics – hold true for this technology. What we're really trying to do is place an emphasis on showing everybody examples of how this technology has been implemented with real evidence, and getting our customers to share that experience with us – whether it's related to turnaround time, cost savings within the lab related to not having to serial dilute samples, or ultimately moving each example into a fully-fledged, in-line process monitoring tool.

RL: What is critical for me is that after we have done a long, extensive, and tedious process development, we want to know that this will help us in moving from lab bench scale (where we're doing all the developments that are critical for reducing and mitigating risk) to drug substance, and continuing to progress to a pilot with a FlowVPX and then maybe at GMP

Another key point is the importance of having in-line measurements, especially for product titer impurities. In the world of gene therapy, with AAV vector as a product, it is important for the future when we are going to move into continuous processing, following the footsteps of the monoclonal antibodies. The trend is the same: we want to reduce the cost, so we need to move to continuous processing. And in-line analytical tools will enable this transition.

Even if someone is currently working with a semi-continuous or batch process, inline Slope Spectrscopy is an excellent way to have better feedback control. And it paves the way for the future use of continuous processing for gene therapy utilizing single-use, closed systems, where you can actually do all that analysis inline. This is the dream for everyone who is developing AAV vector-based products currently.

"Slope Spectrscopy ... paves the way for the future use of continuous processing for gene therapy utilizing single-use, closed systems, where you can actually do all that analysis inline."

- Rachel Legmann

JF: One final note. We have talked about the fact that the system is quicker, there are no sample dilutions, and it's very accurate

– but the amount of time savings cannot be overstated. Especially, as Rachel mentioned, in relation to high concentrations of product.

We have discussed multiple applications for the technology and in several cases, once the method has been properly developed and transferred into GMP, there are multiple scenarios where you will not potentially need staff on hand 24 hours a day, 7 days a week. A lot of that time is currently spent sending samples out for analysis and waiting for the results, during which time the technicians are literally doing nothing. It is just holding up the process until QC has analyzed those samples and sent them back, determining whether the process can continue or if it needs any type of modification.

We have multiple publications, posters, presentations, and examples from companies that have eliminated some of their shifts from the production area, and that are simply providing a better quality of life for the individuals who are working there.

So again, the faster turnaround time is great, making the product more robust is great. But I think one of the things that tends to be overlooked is the fact that we are not looking to save you a couple of minutes off your day; this technology is designed to take weeks or months off your year, by saving you having to wait around for those processes that are no longer applicable because you are using the SoloVPE or FlowVPX technology to make those decisions in real time.

RL: Furthermore, every engineering run in the 200–500-liter scale range can cost almost a million dollars just on the plasmid and other materials. Reducing the cycle time and doing fewer development processes means really significant cost savings.

BIOGRAPHIES

Rachel Legmann

Director of Technology, Gene Therapy, Repligen

Rachel has more than 25 years of experience in the field of scalable biologics and gene therapy manufacturing of therapeutic products, viral vectors and proteins for gene therapy and biologics. She completed her Ph.D. in Food Engineering and Biotechnology at the Technion-Israel Institute of Technology, Israel. Rachel joined Repligen in 2021 as a subject matter expert leading the global gene therapy organization helping customers achieve their technical and operational objectives in their manufacturing of vector-based therapeutics and vaccines with a focus on gene therapy processes including upstream, downstream, analytics and scalability. In addition to supporting global customers and building high level networks, Rachel is supporting various internal cross-functional activities and external collaborations. Prior to joining Repligen, Rachel held several scientific and leadership roles at Microbiology and Molecular Genetics department at Harvard Medical School, CRO SBH Sciences, Seahorse Biosciences part of Agilent, CDMO Goodwin Biotechnology and Pall Corp part of Danaher.

Joe Ferraiolo

Associate Director Bioanalytics, Repligen

Joe is the Associate Director Bioanalytics for Repligen. He is in charge of the Bioanalytics Applications department related to the SoloVPE Variable Pathlength UV solution. He has been with the company for more than 20 years, with over 10 years of development and validation

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experience in analytical applications. He specializes in UV analysis and leads the development and commercialization of high-value products and flexible solutions that address critical steps in the production of biologic drugs, gene therapy solutions, and monoclonal antibodies.

AUTHORSHIP & CONFLICT OF INTEREST

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