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Tutorial

Concentration Measurement with Refractive Index

Utilizing Index of Refraction Monitors in Biopharmaceutical Downstream Processing and Purification

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When the FDA published the 2003 document, "Pharmaceutical Current Good Manufacturing Practices (CGMPs) for the 21st Century—A Risk Based Approach," it was intended to encourage the early adoption of new technological advances including process analytical technology (PAT). It has since led the industry to a much wider use of PAT for reducing risk in manufacturing steps.

As a PAT technique, many processes require liquid concentration monitoring to optimize and ensure performance. Errors in liquid concentration at any step can result in loss of high-value product, reduced product quality/efficacy, and reduction in productivity. There are many methods used to measure concentration including refractive index, conductivity, pH, and osmolality. However, when the FDA document was published, index of refraction (IoR) as a method of concentration measurement was limited. It is worth another look at current and emerging technologies to satisfy the spirit of risk-based approaches to manufacturing.

IoR is a nonspecific and direct fluid concentration measurement using refractive index, which is the measurement of light refraction. IoR has been shown experimentally to be a significant if not superior means of detection and identification of process fluids as compared to inline quality control methods like conductivity, pH, and osmolality. Unlike nonspecific indirect methods, IoR can be used to both positively identify and display concentration measurements. Furthermore, previous experiments have shown the usefulness of IoR as a quality control metric for buffers and media components. *Figure 1* shows the **Entegris** IoR Concentration Monitors.

Positive identification and quantification of biopharmaceuticals and other protein-based products during manufacturing are often performed by taking a sample and using offline tests including: electrophoresis, high performance liquid chromatography, and immunoassays. Inline methods for product identification are limited to indirect methods such as conductivity (a measure of the solution's ability to conduct electricity), which can become susceptible to the effects of low ionic concentration and increased fluid turbidity. The newer IoR technology works well for highly ionic solutions and nonionic chemistries.

This tutorial outlines applications for the IoR method as PAT for monitoring and control of biopharmaceutical purification processes. Included are examples of key process steps where the technology can be used.

Cell Removal and Clarification

The process of removing cells and cellular debris from a harvested culture prior to purification is typically performed using depth filtration, tangential flow filtration, centrifugation, or a combination of methods. Filters are often placed downstream in order to capture any material that escapes the separation method. PAT methods are

Figure 1. Entegris IoR Concentration Monitors

utilized to identify incoming buffers, and to ensure the breakthrough detection of cells and/or process debris during processing.

As a PAT method, IoR monitors can identify incoming buffers used to rinse and equilibrate microfiltration (MF) filters prior to use. IoR can also identify buffers for diafiltration and recovery of product, while detecting even small concentrations of cells or cellular debris breakthrough. Due to this identification and detection, processes can be designed to bypass cellular breakthrough filters until, and only if, they are needed. This can lead to a cost savings.

During MF and depth filtration, IoR monitors optimize the diafiltration process by detecting when a product is below its target concentration. Utilizing real-time IoR monitors enhances these processes by reducing process time and product dilution.

Affinity Chromatography

In many processes, affinity chromatography performs most of the product purification process. During cell removal, IoR monitors can identify each incoming buffer that prepares and equilibrates the cell removal system. Especially during affinity chromatography, an incorrect buffer can inadvertently damage the resin, causing thousands of dollars in lost material, before processing even begins.

Regarding inadequate resin concentration, IoR monitors detect product breakthroughs, channeling, or high asymmetry in the bed, potentially saving product loss.

To optimize product peak detection during the collection process IoR monitors provide augmentation or replacement of ultraviolet-visible spectrophotometry (UV-VIS) technologies. Additionally, IoR can determine the post-use removal completion of bound contaminants to ensure a faster and more optimized cleaning process.

Size Exclusion (SEC) Chromatography

Size exclusion (SEC) chromatography is commonly used to separate product subtype by molecular weight or to remove product fragments and/or aggregates. As in affinity chromatography and other chromatography steps, IoR monitors can be used to positive identify all incoming buffers and product, as shown in *Figure 2*. IoR can also act similarly to UV-VIS in identifying each of the process fractions: high molecular weight, product, and low molecular weight. Consequently, IoR can control when each peak is collected. Therefore, these monitors identify process fluids and control the entire process more notably than in other downstream processing steps.

Table 1. IoR Process Matrix

Process Step	Identification of Incoming Buffers and Product	Detection of Product Outflow	Control of Product Collection	Process Optimization
Harvest	X	X	X	X
Microfiltration	X	X	X	X
Affinity Chromatography	X	X	X	X
Ion Exchange Chromatography	X	X	X	X
Size Exclusion Chromatography	X	X	X	X
Viral Inactivation	X	X	X	X
Ultrafiltration	X			X
Formulation	X			X

Ultrafiltration / Diafiltration

The ultrafiltration (UF) process step offers the highest potential for process optimization using IoR monitors. There are three distinct process points where positive fluid identification and product concentration knowledge will achieve more process control and faster overall process time. During product concentration, the process operates until the product is at a target concentration, often verified by sampling and offline quality verification. These sampling and verification steps could be eliminated if IoR monitors are used as a real-time detection method.

Next, during diafiltration and buffer exchange, IoR can verify the completed exchange step. Finally, quality lab sampling and offline verification during the final concentration point would not be required using IoR measurements. With real time process fluid identification and concentration measurements, process time is reduced while production quality and optimization are increased.

Conclusions

The effects of errors in chemical concentration can range from minimally impactful to entire batch loss. Concentration monitoring technology that can meet the challenges of accuracy, timing, or speed of data availability, and the ability to perform across a broad range of solutions ensures quality at virtually any process step.

While there are highly valid uses

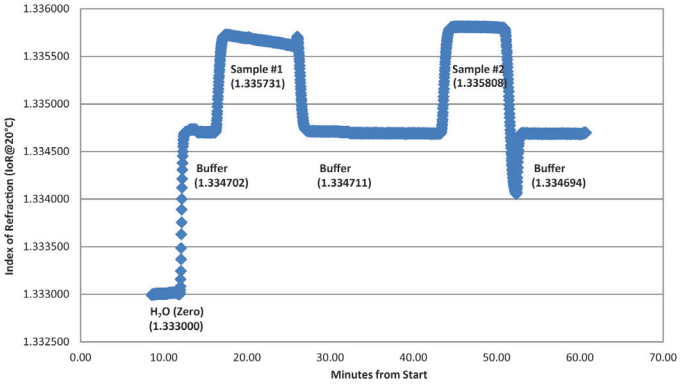

Figure 2. IoR detection of product and buffers in chromatography.

Graph courtesy of Biogen Idec®

for conductivity, pH, and osmolality in biopharmaceutical manufacturing, the IoR technology brings a higher level of accuracy and repeatability to a broader range of process fluids. IoR monitors positively identify and display solution concentration unlike other methods. It is shown to be a significant method for detection and identification of fluids and a companion metric to traditional detection measurement for improved overall PAT. For inline requirements, this technology provides information in real time, allowing for fast response to process variations. As shown above, the IoR method for monitoring of purification processes provides improved process quality control and operations. GEN

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