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Monitoring Monoclonal Antibody Breakthrough Curves On Chromatographic Columns

Raman spectroscopy is an excellent non-destructive analysis technique which measures the frequencies of chemical bond vibrations. As these occur at very specific energies dependent on the exact chemical environment around the bond, a survey of different frequencies can be used to 'fingerprint' specific chemical compounds, even for complex chemical species such as monoclonal antibodies.

Raman spectroscopy can be used to verify that the desired products have been produced and to quantify the levels of impurities. One of the biggest challenges in the preparation of monoclonal antibodies is often the purification process using chromatography. Complexities and inefficiencies in this step often lead to higher prices. This is partly why therapies with monoclonal antibodies are expensive.[2]

Purification Problems

Chromatographic separation processes need to be well-controlled to obtain maximum product yields with minimal contamination. This means that the chromatographic peaks associated with the product elution are sharp and well-separated from all other compounds.

For industrial scale synthesis, minimizing the overall time taken for column separation is optimal, as is finding procedures and approaches where the synthesis and purification can be carried out in a continuous system.[3] One of the properties that is carefully monitored as part of process optimization is the breakthrough curve of the column.

Breakthrough curves indicate when a column is completely saturated, or when the amount of target input compound (for example, the unpurified monoclonal antibody) is equal to the amount of antibody leaving the column. This is the point at which adding additional material to the column would simply result in product losses but also where the column is being most efficiently utilized.

Monitoring breakthrough curves in situ is therefore crucial for the optimization of chromatographic antibody purification. Development of systems capable of this is the subject of ongoing research: for example, researchers at the Institute for Chemical and Bioengineering in Zurich developed sample delivery systems that use Raman spectroscopy in conjunction with physical modelling to monitor monoclonal antibody breakthrough curves on a chromatographic column.[4]

[Multispec Raman System]

[tecRaman Probe 785 Cuvette]

[Hellma Flow-through Cuvette]

High Performance Raman Spectroscopy

One of the challenges with using Raman spectroscopy for sample analysis is that the Raman signals can be quite weak. The team overcame this by combining the Raman analyzer with an extended Kalman filter approach – an algorithm that combines multiple measurements made over time to estimate statistical quantities.

Such analyses are being enabled by a new generation of high performance Raman systems, such as the MultiSpec Raman system (versatile R&D system) or CompactSpec II Process Raman spectrometer developed for hazardous locations by leading spectroscopy company tec5USA.[5, 6] The MultiSpec utilizes 600µm fibers for high optical throughput. Use of accurate and high-quality optics is crucial for accurate Raman spectroscopy – the MultiSpec system uses a temperature-stabilized 785nm laser in conjunction with VPH transmission gratings that reduce straylight, enable excellent spatial uniformity and have very low, transmitted wavefront errors. Powered by 1MP/s 16bit AD conversion the tec5USA hardware allows fast data acquisition. Real-time data processing can be utilized to customize control procedures or to perform extensive averaging and smoothing to increase measurement accuracy.

The result is a system capable of accurately monitoring antibody breakthrough curves in-situ, allowing the optimization of chromatographic antibody purification processes. tec5USA's spectroscopy systems haves several unique features which facilitate this type of analysis. Their inline spectrometers are flexible and scalable, offering a range of enclosures, electronics and probes to suit any application. Their spectrometers are also outstandingly robust and are suitable for use in harsh industrial environments and extreme temperatures and pressures. tec5USA systems also include the latest generation of data acquisition and analysis software MultiSpec Pro II.

tec5USA Spectroscopy Solutions

As well as antibody measurements and optimization solutions, tec5USA offers a range of different products and expertise in UV-VIS, NIR, Raman and LIBS spectroscopies. These include mobile and high speed spectroscopy devices and a number of different solutions for industrial process control. Process evaluation and custom solutions are also available, drawing on tec5USA's extensive experience as both OEM and high-grade industrial system manufacturers and designers.

Many biotech firms involved in vaccine and antibody development need fast and reliable sample identification that is also non-destructive, so no precious samples are wasted. Raman spectroscopy is already a common process analytical technology for such applications but for many labs, spectrometers need to be integrated with existing workflows to avoid costly redesign and upgrades. This is exactly the type of in-situ or in-line measurement tec5USA's systems are intended to handle, as well as offering faster routes to receive important data on the state of reactions.

Process automation requires accurate, ideally non-invasive, measurements in an online format and tec5USA's systems have been successfully used in a range of applications, including bioprocess monitoring of cell cultivations and reaction monitoring in microchannels. One of the benefits of tec5USA's systems are the high stability of the Raman set ups that make it possible to detect even small changes in concentration accurately, whether immersion probes are required or just flow cells for passing the laser beam through the sample.

With the possibility of custom configurations of spectrophotometer devices and an extensive portfolio covering many aspects of spectroscopy, tec5USA are your partner for spectroscopy for any application.[7]

Works Cited

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