MIR Spectroscopy for Continuous Control and Monitoring of Metabolites

Tackling inefficient bioprocesses, one – continuous – step at a time

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A new measurement system, which is based on mid-infrared spectroscopy, allows to monitor multiple bioprocesses online at the same time. Applications of the instrument are foreseen in upstream bioprocessing, in metabolite monitoring and control, as well as in downstream bioprocessing to aid in aggregation studies, contaminant detection or the monitoring of target proteins and excipients.

Bioprocessing is the term used to describe the whole process that occurs from the initial stages of cell harvesting (i.e. extracting cells) to the production of the biopharmaceutical. The most commonly used types of cells are mammalian cells.

Mammalian cells are extensively used in cell biology studies, for model systems of human pathologies or as a source of biopharmaceuticals. The cells themselves, or specific proteins within them, are the raw material for biological drugs. As such, it is vitally important to develop monitoring methods for these cells. Monitoring and control of these cells should give crucial information about the quality, stability, and growth patterns [1].

To go from cells to useful pharmaceutical products is a long, specific, and highly controlled process. Due to the precise nature of this process, batch-to-batch variation, as well as contamination and impurities are major causes of decreased product yield and the need to have more automated processes [2]. Fig. 1 shows the variety of parameters that need to be kept constant and monitored throughout the bioprocess, from cell parameters to product impurities and downstream parameters. As can be seen in Fig. 1, there are many areas of control and validation necessary to optimize processes in the industry.

QUALITY ATTRIBUTES			PROCESS PARAMETERS AND OUTPUTS	
Product-related and process-related impurities	Safety	Others	Cell Culture Control	Downstream Control
Aggregation	Microbial safety (bioburden)	Bulk DS concentration	IgG titer	UV
Fragmentation	Endotoxin	Cell-based potency assay	Cell viability	Conductivity
Charge Profile	Virus safety	Antigen binding	Viable cell density	pH
Deamidation		FcgR, C1q and FcRn binding	Yeast proteins	Pressure
Oxidation		Density	Insulin	Flow
Glycosylation profile		Appearance (color)	Glucose	Turbidity
Glycation		Visible and sub-visible particles	Amino acids	Product concentration/mass
Non-glycosylated heavy chain			Galactose	
Host cell proteins (HCPs)			Glutamate	
Host DNA			Glutamine	
Leached protein A			Lactate	
			Ammonia	
			Other nutrients and metabolites	
			Exit gas composition	
			pH	
			Metals (Cu, Mn, etc)	
			Gas concentrations	

Fig. 1 Process parameters and quality attributes needed to be monitored within the bioprocess. Many critical attributes need to be controlled and monitored in order to adhere to strict standards. (Adapted from [3])

It is relevant to acquire information about the conventional critical variables (such as cell growth, consumption of nutrients, production, and consumption of by-products and the bioproduct production), and the cell metabolism towards a better understanding of the culture process and consequently for more efficient optimization and control procedures. To optimize and consistently achieve high quality products, specific frameworks have been put in place, such as quality by design and process analytical technology (PAT) initiatives.

Quality by design (QbD) is defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" [4]. The need for QbD is evident; in order to have consistently high-quality products, systematic approaches need to be defined and controlled. Part of this type of approach encompasses tools such as PAT, which is defined as "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality" [5]. PAT has been implemented as a pillar of process control for many parts of bioprocessing. The aim is to monitor and analyze processes in real time, enhancing prior understanding and establishing a control strategy to improve both the quality and quantity of produced product in the biopharmaceutical industry. To some extent, large leaps have been made within the last decade, especially with regard to obtaining quality attributes and more consistent monitoring of processes [6]. However, utilizing data, as well as data collection and control of processes, is still lagging. So to fully integrate PAT into bioprocessing, new solutions for data collection and the control of bioprocesses are ultimately necessary for higher product quality and yields. By looking into improved PAT processes, we also must first talk about the two main areas of bioprocessing, upstream and downstream, and how this area of monitoring and continual processing can aid both succinct parts of biopharmaceutical production [7].

Upstream bioprocessing describes all processes from the first steps of cell isolation, to cell cultivation. Cell cultivation, which is crucial, is the process of keeping the cells alive for further steps. This also includes scaling up the process from cell flasks to larger vessels, which are called bioreactors. Bioreactors are vessels in which living cells produce proteins, in a large and highly controlled environment, where factors such as pH levels and oxygen levels are kept consistent [8]. Very large volumes of proteins can then be harvested in these bioreactors. As previously mentioned, the process from cell isolation to biopharmaceutical product is long, tedious, and fraught with many possible failures in both product quality and quantity. Once we have isolated the relevant protein from the cell, the next steps are taken to purify the product. These processes are referred to as downstream processes, involve another array of steps such as filtration (amongst others) so that a stable formulation can be produced. These steps involve different types of chromatography, from size exclusion chromatography to ion exchange and affinity chromatography [9]. Chromatography is slow, expensive, and difficult to automate. Some of the most difficult hurdles of PAT can be found here; scaling up chromatography in downstream processing and more continuous process monitoring and control in upstream processing.

Company

IRUBIS

The Munich start-up was founded in 2017. It is specialized in mid-infrared spectroscopy. IRUBIS has designed an instrument – Monipa – specifically for bioprocess monitoring and control. The hardware consists of a mid-infrared spectrometer, a single-use flow cell and user-friendly software. Applications of the instrument are foreseen in both upstream bioprocessing as well as in downstream bioprocessing.

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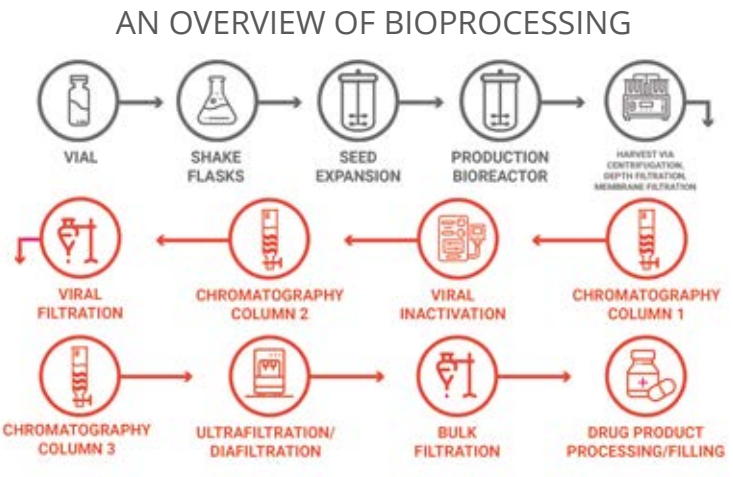

Fig. 2 The overall biopharmaceutical process, both upstream (grey) and downstream (orange); from cell isolation to the production of the biopharmaceutical. (Adapted from [10])

In order to visualize the many steps that are part of bioprocessing, Fig. 2 shows the overall life cycle of biopharmaceutical processes, from cell isolation to eventual biopharmaceutical product.

The eventual goal in PAT for biopharmaceutical production is to have upstream and downstream processes that involve real-time and continuous monitoring and control. Data can then be analyzed on the fly, and problems can be fixed as soon as they occur. Through this, product quality not only increases, but batch-to-batch variation also decreases and hence product yield sees a steady increase. However, in order to do this, the instrument has to be able to take continuous data. This is a difficult task, and there are few instruments that are robust, can provide monitoring for continuous processing and produce useful data to aid in the monitoring and control of such difficult processes.

Our solution

Spectroscopy is a powerful tool to monitor bioprocesses. Molecular spectroscopy describes the interaction between electromagnetic waves and molecules. For bioprocesses, typical wavelengths range from UV to mid-infrared. Spectroscopy enables real-time monitoring of several parameters in parallel. For example, in upstream processing it is possible to monitor glucose, lactate, ammonium, glutamate and glutamine all at the same time. Bioprocesses can be monitored inline, online, atline or offline. These terms are described using the example of the analyzer used for bioreactors. Inline means the analyzer is directly inside the bioreactor, for example a Raman or MIR probe. For online methods, the analyzer is not in the bioreactor but directly connected to it, for example via a loop. Inline and online measurement methods enable continuous real-time monitoring. For atline (nearest to the bioreactor) and offline (may be further apart from the bioreactor) methods the operator needs to take a sample to a separate device.

Today, the established and most used technology to monitor nutrients, and metabolites in upstream processes are offline systems, such as the Cedex Bioanalyzer or Nova Bioprofile. These measurement instruments work offline, which means they are separate and not connected to the bioreactor. Therefore the device cannot provide a real-time and continuous monitoring as with inline or online methods. The samples are taken manually. Manual sample taking is not ideal in the industry, foremost because it increases contamination risk as well as giving poor monitoring and control of metabolites which is only done once a day.

The most important energy source for the cell is glucose that is fed daily to the bioreactor. The glucose concentration has been shown to have an influence on antibody production, cell growth and

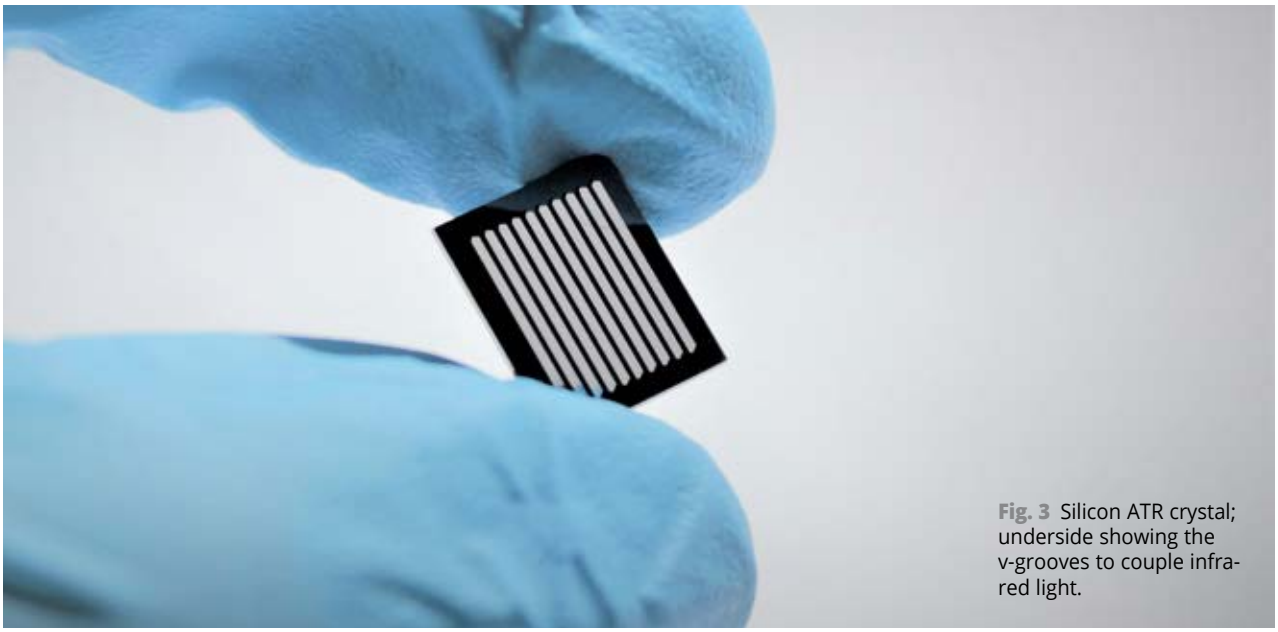

Fig. 3 Silicon ATR crystal; underside showing the v-grooves to couple infrared light.

glycosylation [11]. Improved glucose control is therefore crucially important for an increase in production of the factors mentioned above, and to avoid large fluctuations.

It has been shown by Sandor et al. [12] that infrared spectroscopy provides high sensitivity in monitoring metabolites such as lactate and nutrients, e.g glucose. For the continuous monitoring and control of bioprocesses in the industry, a single-use sensor and plug-and-play device would be the ideal solution.

So we have developed an instrument specified to the customer's need called 'Monipa'. The core element is a cost-effective ATR crystal as the sample interface. ATR is the abbreviation of attenuated total reflection. This established infrared spectroscopy method does not need any sample preparation and is independent of the sample thickness. Transmission methods are limited in relation to the maximum sample thickness, otherwise the absorption of the light is too high. So our device relies on the measurement principle of ATR spectroscopy: the light passes through the ATR crystal under a certain angle, and is totally reflected at the interface between the sample and the ATR crystal. A small part of the beam interacts with the sample, which is called the evanescent wave. One important factor is the material of the ATR crystal, as the angle of total reflection also depends on the refractive index. Therefore only materials with a high refractive index can be used, such as diamond, zinc selenide, etc. However, these materials are expensive and therefore not suitable for single-use application in bioprocess monitoring. Irubis optimized the ATR crystal (**Fig. 3**) published by Schumacher et al. [13] to enhance the light throughput. By using silicon material and well-known semiconductor manufacturing processes, large quantities of ATR crystals can be fabricated, reducing the price. This makes them suitable for single-use application. This ATR crystal is integrated into a single-use flow cell that can be attached to our MIR system Monipa. The flow-cell is easily connected via a loop to the bioreactor. As a demountable component, it is autoclavable and suitable as a disposable element. Alternatively, it can also be gamma sterilized if necessary. Compared to inline probes, the external flow-cell provides a constant measurement environment, without influences of gas bubbles or the stirrer in the bioreactor. Our MIR system Monipa evades problems with absorption from water vapor by background measurements. These are reference measurements which are used to correct the spectra for changes of the instrument or the environment.

Calibration model building is an important step for spectroscopy. The spectra depend on the molecules in the measured samples. Different cell lines, cell culture media, metabolite or nutrient concentration lead to changes in the spectra. Therefore, calibration models are built by online monitoring of the process, as well as reference samples by offline methods (e.g. a Cedex HT Bio). The spectroscopic measurements are correlated to the reference method building a calibration model. The additional bioreactor runs and offline measurements to build the calibration model are costly and time intensive. A new model is necessary for every change in the cell culture or media. This can, in theory, be solved by building a generic model. However, this creates even more work, with many more additional bioreactor runs and can lead to reduced accuracy. As well as the large cost and time, the compliance regulations of biopharma industries are not compatible with creating generic models based on the data of a large array of pharmaceutical companies.

To tackle this setback, Irubis created two options to reduce the effort of building a calibration model for our spectroscopy method. With our system, it is possible to measure the samples offline. Samples are taken and archived for almost every bioprocess run. These archived samples can be used to create a calibration model. So instead of measuring three to five bioreactor runs, which takes up to two to four months, our calibration model can be built in just one week. The second option is to keep the glucose concentration at a constant level. Irubis created a relative measurement to address both the control of the glucose concentration and to avoid extensive calibration model building. A known start value, such as 3 g/L is given for the relative measurement as the input to the Monipa system. The algorithm of the system then compares the actual spectrum to the spectrum with the known start value. If there is a change, the coupled pump adds glucose to the bioreactor system; see also **Fig. 4**. This

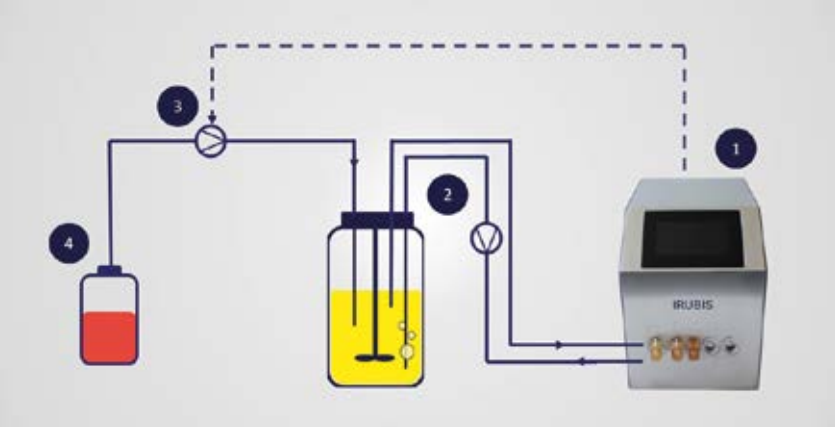

Fig. 4 Schematic of the setup to monitor and control glucose. The numbers indicate different parts of the setup, with number 1 being the MIR instrument Monipa. The instrument contains orange flow cells, which contain the ATR crystal. Number 2 refers to the bioreactor, which as can be seen by the schematic, is connected to the instrument through tubes. Number 3 references the control loop, which runs from the instrument to the feed to increase the amount of glucose in the bioreactor (dependent on measurements). Number 4 is the feed, which goes directly into the bioreactor.

created the first of its kind of spectroscopic mid-infrared system as a plug-and-play device for bioprocesses.

In the previous paragraphs, the methodology of the instrument was discussed for its application in glucose and metabolite control and monitoring in upstream bioprocessing. This is just one of the many applications of the instrument in the field. For downstream use, MIR has been shown to be used in aggregation and conjugation studies, as well as for determination of concentrations of antibodies and proteins [14, 15]. So the applicability of the system for bioprocessing is not limited to its use upstream.

The Monipa system tackles some of the many challenges faced in bioprocessing. Circling back to the introduction, obstacles faced by the industry to obtain high yields and quality products are vast. However, being able to continuously monitor and adapt concentrations of nutrients and metabolites in real time in the upstream processing steps is a huge advantage over older, conventional approaches.

Additionally, using MIR for downstream applications to monitor aggregation or the conjugation of antibodies or proteins would also be highly advantageous for the field of bioprocessing [16]. The opportunity for a fast acquisition time and little sample preparation is even more vital here than in upstream processing, because processes are faster as they do not require cell cultivation and growth.

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Anja Müller holds a master's degree in physics from the Technical University of Berlin. Over the course of her studies, she acquired relevant knowledge and skills in the field of MIR spectroscopy. During her master's studies, she was awarded a scholarship for the Career Building Program at Femtec, where she acquired important management and leadership competencies. At Irubis she is responsible for funding and project management as co-founder and COO.

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