Simply Good Monitoring

Digitising process development can reduce the time delay between sampling and availability, and can increase the quality of the data obtained

Paul Kroll and Alexandra Hofer at Securecell AG

Process development aims at fast and efficient designs of new processes or optimising existing ones. Modern development environments use highly automated systems to perform the basic tasks of measurement, monitoring, and control. Additionally, increasing digitisation enables storage, processing, and application of data and model-based methods in real time. Hence, efficiency can be significantly increased in process development, which ultimately leads to a speed-up to market and guarantees process stability over the product lifecycle. Focusing on biopharmaceutical process development, there are still highly manual workflows; for example, the production of a substance mixture (media and buffer), the processing and analysis of samples (draw, prepare, analyse, store), and the generation of process reports. In the present case study, it is shown how the combination of automation and digitisation can accelerate process development, with respect to the processing and analysis of samples. Liquid samples are still the most important source of process-relevant data. For example, in upstream processing, almost all information regarding substrates, biomass, byproducts, and the product itself is based on samples and their analysis.

Typically, the process of sampling and analysis is performed in a separate analytical laboratory, resulting in a time offset. Such at-line or even off-line samples are not suitable for timely feedback and control. To close this gap, firstly, the sampling, the sample processing, and the analysis must be automated. Secondly, the generated data must be digitised to enable feedback and control in time.

With the example of an industrial cell culture process, it will be demonstrated how a robust product monitoring of monoclonal antibodies (mAb) can be implemented and used for control. The basis for this is:

1. A liquid handling system (Numera, Securecell AG), which draws and processes samples from the bioreactor
2. A standard high-performance liquid chromatography HPLC for the analysis (UltiMate 3000™, Thermo Fisher Scientific)
3. A bioprocess software (Lucullus PIMS, Securecell AG) to control all devices and manage the data: this combination results in an integrated PAT solution, as shown in Figure 1

The Digital Sampling Workflow

The typical workflow from a 'sampling and analytical plan' to 'ready to apply data' contains a high number of single operations that influence the time delay between sampling, the availability of the analytical result, and the quality of the result, i.e., data quality (see Figure 2, page 44). The

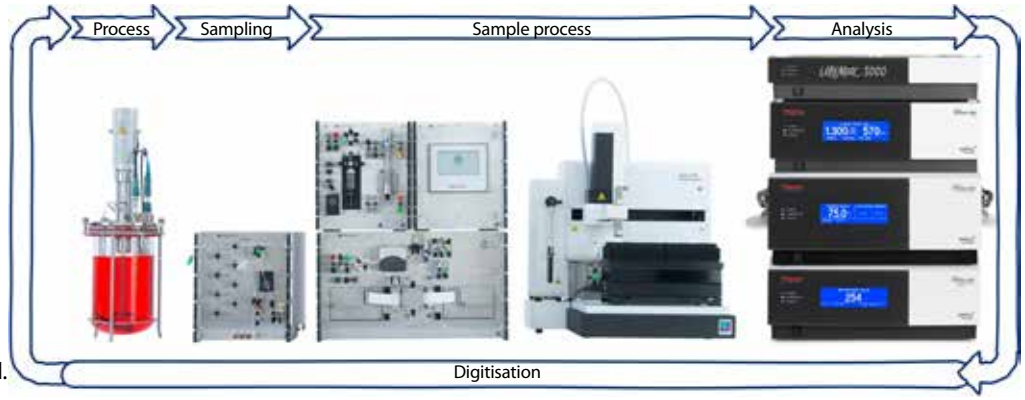

Figure 1: Integrated PAT solution for automated sampling, sample processing, sample analysis, and digitisation, enabling direct feedback to the process (1)

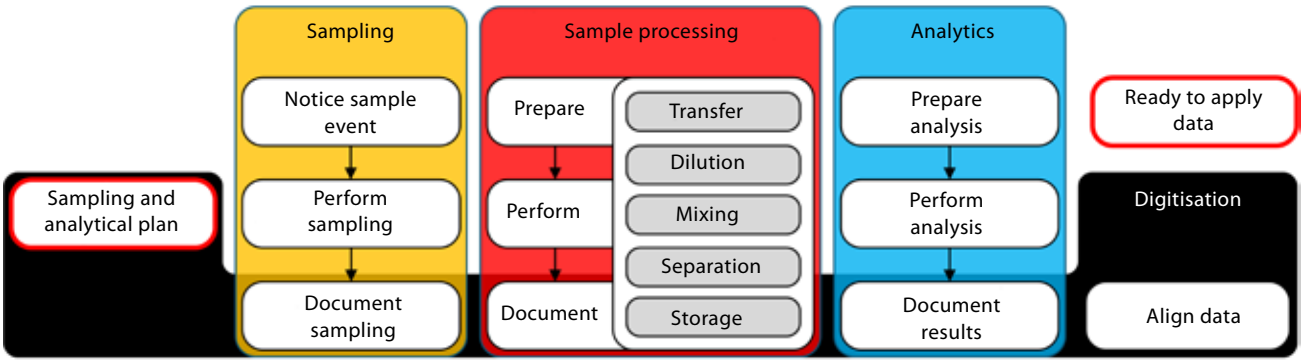

Figure 2: Every process starts with a sampling and analytical plan and aims to end with data that are ready to be applied for process evaluation. An overview of the different single operations in between is given in this figure. Automation and digitisation can transform this workflow in regard to efficiency and data quality

automation and digitisation of this workflow can reduce the mentioned time delay and increase the quality of data at the same time.

Sampling and Analytical Plan

With reference to cell culture processes, samples are often drawn in the industrial process development environment only once or twice a day. Therefore, the time is usually the decisive criterion. The analytical plan normally contains standard at-line analytics that are performed close to the bioprocess and, if necessary, off-line analytics performed in a specialised analytical lab.

The digitisation of the sampling and analytical plan expands the design space of the process developer by enabling higher measurement frequencies and time independent sampling based on process events. This is finally the basis for a higher-information content of a single experiment.

Within the presented integrated process analytical technology (PAT) solution, Lucullus PIMS enables the setup of a process-specific sampling and analytical plan.

Sampling

The sampling procedure itself is typically a critical event because it represents a direct interaction with the running process. This requires special care. The sampling process is performed in five steps:

1. The preparation of vials, labels, syringe, disinfection, etc.
2. The notification of a sample event
3. The performance of the sampling (liquid transfer from reactor in vial)
4. The post-processing (cleaning of sample port)
5. The documentation

In terms of 'time delay' and 'data quality', it is, above all, the reproducibility of the sampling, the required time, and the proper documentation that is important. Automation ensures this reproducibility and complete documentation.
Within the presented integrated PAT solution, the aforementioned steps are covered by Lucullus and Numera. For 'sample preparation', sample-specific labels containing a barcode can be printed. The second step, 'notification of sample event', is performed according to the sample plan, which can be created before the process. The software triggers the sampling based on time or process events. Afterwards, the sampling process is performed by the liquid handling system. It draws a volume of about 3-4mL from the reactor. To ensure an undiluted sample, the first 100µL are discarded before the sample is transferred into vials. Sample processing steps can be performed before sample storage in the vials. After every sample, the 'post-processing' is performed, including an air flush to empty the sample line to the reactor and a cleaning procedure of all other used tubings. Finally, the documentation of the exact sample time is performed in Lucullus.

The example CHO process was sampled every 60 minutes according to the sampling plan. The minimum sample time would be every 15 minutes. The sample volume was 3.5mL.

Sample Processing

The sample processing is dependent on the subsequent analytical method. Most applications can be performed with just a few unit operations (see Figure 2). These are transfer, dilution, mixing, separation, and storage of samples. Each single step must be prepared, performed, and documented. Hence, all steps require various equipment, consumables, and human resources. Like the sampling, the sample processing affects the 'time delay' and 'data quality' by the reproducibility of the sample processing, the required time, and the proper documentation.

Within the presented integrated PAT solution, Numera takes over all sample processing steps. It can perform dilutions between 1:2 and 1:30, the addition of and mixing with a defined reagent, as well as separation of supernatant by a filtration module. The filtration module is designed so that for

	Sampling	Sample processing	Analysis (TM=3min)	Digitisation	Time to feedback
Manual	5-10	15	6-10	>20	> 46 best case
PAT solution	3	7	3.5	0.1	<13.6 worst case

Table 1: Comparison between manual sampling procedure and fully automated workflow. The manual sampling procedure is assumed to be performed without interruption and with minimal time delay: time is presented in minutes

every sample, a new piece of filter material is used. Hence, the problems of membrane clogging and cross-contamination can be prevented. The modularity of the system allows individual sample processing strategies for each sample in a fully automated manner.

The later product HPLC analysis (immunoglobulin G [IgG]) of the example process requires a separation of the supernatant from the cell broth. This requires the unit operations, which are:

1. Transfer sample to filtration module
2. Filtrate sample
3. Transfer filtered sample to a sample vial
4. Store sample at 4°C; afterwards, the system cleans itself to ensure the highest sample quality and exclude cross-contamination

Analytics

Analytics generate data by measurements. The quality and reliability of the data depend on the applied analytical method. Reference methods are predestined for the quantification of complex biological samples. Depending on the analyte, these are mostly chromatographic (e.g., HPLC), enzymatic, or microscopic methods. The analysis is typically performed in the following four steps:

1. The preparation of the measurement
2. Trigger and performance of the measurement
3. The data analysis
4. The documentation of results

The manual steps are typically the preparation, the trigger of the measurement, and the documentation of the result.

Within the presented integrated PAT solution, the manual preparation steps for the measurement are performed in an automated manner. These steps include the delivery of the sample to the analyser, as well as sample naming and the choice of the right analytical method that should be applied. The bioprocess software Lucullus triggers the start of the analysis, and the result is sent back to be applicable for monitoring or control actions.

In the exemplary CHO process, the product was analysed by HPLC method using a protein A column and a gradient elution, resulting in a measurement time of three minutes. The liquid handling system is transferring the sample via an injection valve directly in the flow path of the HPLC. The injection and the start of the analytical method are simultaneously triggered by Lucullus. After the run, the HPLC software performs the automated data analysis.

The final measurement result is transferred to Lucullus and automatically aligned with the process data.

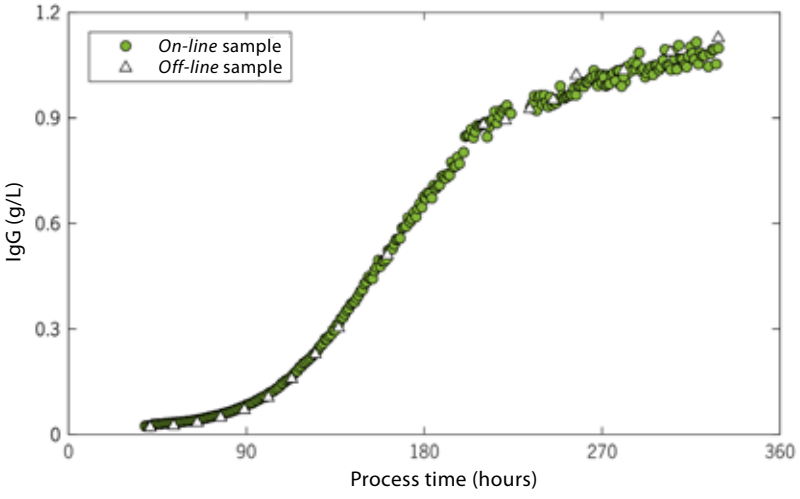

Figure 3: Real-time available product information (IgG) of a cell culture fed batch process (CHO) (1)

“The digitisation based on manual data integration is one of the most critical steps regarding data integrity”

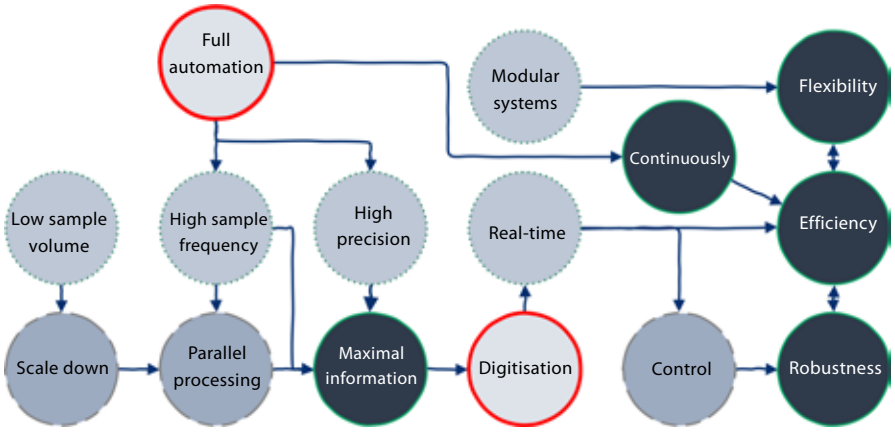

Figure 4: Key features and benefits of an integrated PAT solution enabling full automated sampling, sample processing, analysis, and digitisation. Key features are framed with dots, emerging technology is framed with dashes, and resulting benefits are framed with green lines

Digitisation

The often neglected step of digitisation is necessary to use the generated data for various applications. Therefore, all previously documented information must be collected, digitised, and aligned. This requires various subtasks including:

1. Searching generated documents (paper, data files)
2. Digitisation of the data in a certain software
3. Alignment of the information (e.g., sample time, sample name, analytical result, dilution factor)
4. Secure storage of the aligned data for further applications

The digitisation based on manual data integration is one of the most critical steps regarding data integrity. Additionally, it is known that employees with knowledge-based jobs invest over two and a half hours per day to search the data they need (2). This virtually eliminates feedback in time.

Within the presented integrated PAT solution, Lucullus takes over the whole digitisation. All devices are integrated to be controlled and to feedback data. With a minimal update time of one second, all data are transferred in time. The needed time for the single operations is summarised for the applied method of the case study (i.e., sampling, filtration, three-minute HPLC method). In the best case, the measurement result of a manual performed sample workflow is available for feedback control after 40 minutes. Fully-automated, this can be done, in the worst case, after 14 minutes. It can be observed that even in the best case, the presented PAT solution performs three times faster. Additionally, the automated solution works day and night, and on weekends.

Figure 3 (page 46) illustrates the continuous monitoring of IgG in a cell culture fed batch process over 350 hours. The concentration of IgG in the first 40 hours was under the detection limit of the analytical method and is, therefore, not presented.

Potential of Integrated PAT Solution

The presented integrated PAT solution provides an answer to basic bioprocess engineering challenges. Based on the full automation, a high sample frequency with a high measurement precision can be reached. This is the foundation for the performance of continuous processes. Additionally, a reduced sample volume is necessary, which enables the application of scale-down systems and improved parallel processing (scale out). Finally, the generated information content is maximised. The digitisation enables the real-time availability of this information and data. The real-time availability leads to an increased efficiency and under-usage of control capabilities to an increased process robustness. Finally, the modular approach of the presented PAT solution enables a high degree of flexibility (see Figure 4).

References

1. Visit: www.securecell.ch/domains/securecell_ch/data/free_docs/AN_002_OnlineHPLC_CHO(1).pdf
2. Visit: blog.xenit.eu/blog/do-workers-still-waste-time-searching-for-information

About the authors

Paul Kroll studied process engineering at the TU Dresden, Germany. Early on, he worked on model-based methods for monitoring and controlling bioprocesses. Paul received his doctorate in this field at the Vienna University of Technology, Austria, and later became a postdoc for model-based methods and digital twins. Since 2018, he has worked as a Business Development Manager for the Process and Information Management System, Lucullus PIMS, at Securecell AG.
Email: paul.kroll@securecell.ch

Alexandra Hofer studied pharmacy at the University of Vienna, Austria, and subsequently did her doctorate in the Faculty of Technical Chemistry at the Vienna University of Technology. In particular, she dealt with analytical methods used for process monitoring in biopharmacy. Since 2018, she has worked as a Business Development Manager for the automated sampling system, Numera, at Securecell AG.
Email: alexandra.hofer@securecell.ch