Automation and miniaturization

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Review

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Automation and miniaturization: enabling tools for fast, high-throughput process development in integrated continuous biomanufacturing

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Abstract

Process development in the biotech industry leads to investments around hundred of millions of dollars. It is important to mitigate costs without neglecting the quality of process development. Biopharmaceutical process development is important for companies to develop new processes and be first to market, improve a pre-established process, or start manufacturing a product available by patent expiry (biosimilars). Laboratory automation enables methodical and standardized process development. Miniaturization and parallelization empower laboratories to screen several experimental conditions and define operating windows for purification processes, improving process robustness. Together, they allow for fast and accurate process development in a fraction of the time and cost of nonminiaturized/nonparallel process development approaches. The most widely used High-Throughput Screening technique is a liquid-handling station and microfluidics is taking its first steps in process development. Both are attractive scale-down tools for the characterization of bioprocesses and allow thousands of experiments to be performed per day. High-Throughput Process Development (HTPD) has helped to achieve major breakthroughs in process optimization, both for upstream and downstream processing. Continuous processing is the next step in process development which leads to cost reduction, higher productivity and better quality control; the integration of upstream and downstream processes is seen as a major challenge. In this review, we will focus on the state-of-the-art of miniaturized techniques for process development in the biotechnology industry, and how automation and miniaturization drive process development. A comparison between liquid-handling stations and microfluidics is made and an indication is given of which tools are still lacking for HTPD in the context of Integrated Continuous Biomanufacturing.

Keywords: automation; miniaturization; integrated continuous biomanufacturing; high-throughput process development; microfluidics; liquid-handling stations

ABBREVIATIONS

ADC	Antibody Drug Conjugate
ATPS	Aqueous Two-Phase System
DSP	Downstream Processing
FDM	Fused Deposition Modelling
HTE	High-throughput Experimentation
HTPD	High-Throughput Process Development
HTS	High-Throughput Screening
ICB	Integrated Continuous Biomanufacturing
LHS	Liquid-Handling Stations
mAb	Monoclonal Antibody
PDMS	Polydimethylsiloxane
SL	Stereolithography
UO	Unit Operations
USP	Upstream Processing

INTRODUCTION

In recent years, an evolution in medicine and available treatments has taken place. The available drugs for different therapies keep increasing and competition grows fiercely with patent expiry. Companies that want to remain competitive need fast and inexpensive process development for new products.

With patent expiry, competition rises and consumers benefit, as prices go down. One example of heavy market competition is the monoclonal antibody (mAb) market, where the expiry of patents held by major players in USA and Europe allowed the emergence

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of the so-called biosimilars – molecules similar to the therapeutic mAbs available at a fraction of the price – with the first mAb biosimilar (infliximab) registering a decrease of ≤72% of the original molecule price.[1] Companies have tried to counteract the emergence of similar drugs through the discovery of new applications for already available drugs.[2]

R&D represents a considerable slice of the budget of (bio) pharma companies, but it also is what allows them to differentiate. The challenge in obtaining novel products with profitable processes has led to a decrease of drugs available in the market. In the last 70 years we have seen a ≈80-fold reduction of drugs approved per billion-dollar R&D investment.[3]

High-Throughput Screening (HTS) methods make use of developments in several scientific fields, and combine automation and miniaturization to test and screen products, processes, and conditions inherent to these processes. The use of HTS attracted the attention of (Bio)Pharma companies, that soon shifted to this technology to test and generate data in the order of tens and hundreds of thousands data points per day.[4] Fast experimentation, low sample consumption and reliable data makes HTS attractive for both companies and academic peers.

The true impact of HTS started more than 20 years ago, with a shift being made in early-stage screening. The evolution of this field equipped researchers with powerful tools that allowed for fast screening and generation of genetic libraries of mutants[5] and products.[6] The optimization of microorganisms and the increasing product titres achieved shifted the attention of HTS research from upstream to downstream processing (USP/DSP), that needs to be able to deliver the final product as quickly and robustly as possible (Table 1).[7]

Process development techniques have evolved greatly as they need to adapt to an ever-changing market and capitalize on the availability of cutting-edge technologies. The evolution of the available tools and the introduction of initiatives like Quality by Design and Process Analytical Tools pushed for the need to have better understanding of the process and clear definition of the design space.[15] The increasing computational power enabled researchers to use more complex modelling tools, freeing them from the heuristic modelling chains, that although useful rarely allow for extrapolation and do not promote process understanding. As the industry matured, High-Throughput Process Development (HTPD) combined HTS, a greater mechanistic understanding of processes, and a higher computational capability for smarter process development, which helped to guide experiments in order to achieve better performing processes faster at a lower cost.[8]

Initial evidence of HTS in the biotechnology field started with the appearance of 96-well microtiter plates. These were used to screen chemical compounds and widespread use by the pharmaceutical industry was adopted. Later, with increasing pipetting precision, the 384- and 1536-well microtiter plates were introduced.[4] There also is another option for HTS, namely microfluidics. Microfluidics started more than 20 years ago, gaining traction over the years. These systems are known for the handling of very small amounts of liquids and allow for sample saving taking this one step further. Their small size often allows for the analysis to occur faster than conventional tests, saving time and allowing for multiple data points to be generated with low laboratory space utilization.[16]

Turning processes that are composed of discrete Unit Operations (UO) into one end-to-end continuous process is a sign of a maturing industry. Operating in steady-state, better equipment utilization, better control and quality, better productivities are some of the advantages of having a continuous process.[17] Biopharmaceutical industries are pushing for this shift which is welcomed by regulatory agencies.[18] All of these advantages culminate in lower cost goods, making this shift ever more necessary and attractive.[19]

The shift of processes from discrete operation to continuous also is achieved through HTPD. This is a key tool for today's process development and by making use of HTPD, researchers aim to achieve continuous processes faster, resulting in Integrated Continuous Biomanufacturing (ICB). To do this, classical HTS methods are used. Although HTS is the cheapest and fastest alternative for process development, the required equipment is expensive. A paradigm shift is needed to achieve lower costs of HTPD tools, together with more adequate analytical tools.[20] Furthermore, some unit operations still lack proper scale-down models and, for the ones already in place, the translation of the results obtained to manufacturing scale need to be investigated.[21]

This article aims to shed a light on the evolution of High-Throughput Experimentation (HTE) and the evolution in the role this approach has gained over the years, providing an overview

Table 1. Examples of HTS applicability in different stages of process development. The three process stages included are pre-process screening, up- and downstream processing.[8,9] HTS also can be used to study formulation which was discussed in other publications[10,11]

Preliminary screening	USP	DSP
• Screening for molecular properties that can help determine processing steps	• Build mutant library - titre and host organisms are important	• Development of complete downstream process[8]
• Determination of critical quality attributes	• Screen for best-producing strains (usually highest titre)	• Definition of UO based on separation efficiency and yield
	• Optimize bioreactor design	• Optimize purification train for minimal number of UO at highest possible yield - expensive steps are usually the ones getting tackled first (e.g. chromatography)
	• Define best operating conditions for fermentation and to test in scale-up setup[12]	• Test new UO for already-established processes (e.g. ATPS for mAb purification)[13]
	• Toxicity testing for producing strains	• Define window of operation for different UO[14]
	• Test processing mode (Batch *versus* Continuous)	
	• Establish KPI for the processes	

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on the automation and miniaturization, and how it has influenced the biopharmaceutical and food industries for the development of continuous processes.

MICROPLATES AND MICROFLUIDICS: AUTOMATION AND MINIATURIZATION

Bioindustries soon captured the advantages of miniaturization of assays for process development, which allows for faster processing using fewer samples. With a smaller footprint needed for the performance of assays, the parallelization of such assays arose innately. This translated to a reduction in cost and time. The technological advances in mechanical engineering in the second half of the 20th Century allowed for an ever-increasing level of automation that benefitted process development, analytics, quality control and quality assurance. Automation enabled the use of automatic equipment for the processing of samples, and the first evidence of automation in the drug discovery industry can be traced back to Japan,[22,23] where the first automated tasks were the transport of samples throughout the laboratory. Shortly after, the technology started making its way to the mainstream and equipment that combines automation and miniaturization arose, allowing for the first HTS, through microtiter plates.[4]

Microfluidics is the area that studies systems that allow for fluid handling in small dimension channels, in the micrometre range, allowing for handling liquids even in the nanoliter range.[16] With the development of technology, microfluidics also has its own subdisciplines such as, among others, droplet microfluidics.[24] Liquid-handling stations (LHS) also allow for the miniaturization of experiments and empowered researchers to have automated systems that could perform trials in the microliter range with great precision.

The importance of automation and miniaturization, for both LHS and microfluidics, will be covered, along with a discussion regarding the different uses these two methodologies have. The rise of 3D-printing also will be covered, as a promising tool for HTPD.

The power and role of automation and miniaturization

Liquid handling is paramount for research in life sciences and is a crucial part of experimentation in this field. As assays moved to a smaller scale, accurate liquid handling became ever more important for the assays to remain reliable. This brought together automation and miniaturization in the form of LHS. Although miniaturization has the power of reducing sample volume consumption to very low volumes, automation has the power of removing humans from the experimental realm, helping to reduce human-prone errors and allowing for more time to be dedicated to designing the experiments rather than performing them.

Liquid-handling robots have proven to be very useful tools for process development and screening, fulfilling the automation and high-throughput needs in such a competitive market as the life sciences. There are several different assays that can be done with robotic workstations and these can be tailored to a laboratory's needs. Accuracy and precision naturally are key performance parameters for LHS, independent of the working volumes. For a more thorough analysis of the advances in the liquid dispensing field, the reader is directed to another review.[25] Here, the authors cover the different components of the robotic workstations (e.g. dispensing parts, robots and sensors), and compare different commercially available systems and their performance regarding the minimum dispensing volume and speed.

The level of miniaturization employed in microfluidics is manyfold higher than for LHS.[16] The advantages of this degree of miniaturization are not exclusive to reagent saving as at such small scales the physicochemical conditions will be different. Besides allowing for the handling of samples in the nanoliter range, microfluidics also allows for a deep understanding of the physical properties of a system. The characteristic dimensions of such systems allow for a precise fluid flow characterization as a result of the well-ordered laminar flow through dimensionless numbers, such as the Reynolds (Re) and Péclet (Pe) numbers.

The Re predicts if the system will be dominated by inertial or viscous forces, whereas Péclet number expresses the relationship between convective and diffusive transport. In microfluidics, laminar flows are dominant with Re values remaining usually <1, meaning that the flow is clearly dominated by viscous forces. This makes it easier for the modelling of the fluid flow and the behaviour of chemical species inside such systems, where mixing, diffusion and reactions can be modelled with great precision.[26,27] The Pe number can help predict the length of a channel and the time needed until a desired degree of mixing is achieved. These characteristics can even improve performance of miniaturized unit operations, a concept described as 'positive downscaling'.[28]

Automation in microfluidic devices

Automation in microfluidics is achieved by integrating different components in the microfluidic device, through implementation of different features in the design, using external equipment or by exploiting the microscale characteristics. Fluid flow in microfluidic devices can take many shapes and forms and several have been applied in different applications.[28] Although pressure-driven flows may seem the most intuitive for microfluidic devices, both this and electroosmotic flow are applied when performing chromatographic separations,[29,30] the latter allowing for flow control without the need for external pumps or valves.

Microvalves and micropumps greatly aid in the operation and automation of microfluidic devices, which come at residual incremental material cost but at a high complexity cost both in design and fabrication.[31] Microfluidic integrated valves and pumps enabled scientists to achieve the concept of Lab-on-a-chip, using a methodology that allows for the discretization of fluid flow in the microchannels, as well as flow control and mixing, which can be important for the micro-integration of several operations in the same microchip.[32] However, it is important to highlight that most of these types of valves and pumps cannot be transversely employed in all microdevices, because for some there is a need to have a flexible material, for example an elastomer such as polydimethylsiloxane (PDMS), and not all devices use this material. The type of materials in which microfluidic devices are built can vary greatly depending on the desired purpose and this has been deeply covered in other publications.[33,34]

Several methodologies with a high degree of automation have been employed in microfluidics experimentation that showed an increase in throughput. Droplet microfluidics makes use of immiscible fluids with different properties and through the manipulation of fluid flow rate, droplets of very precise diameter can be generated,[35] although several advances have been made and different methodologies can be employed for the control of droplet formation,[36] geometry being one of the most important.[37] This discipline of microfluidics has shown good advancements in this field and several studies have showcased its prowess in the screening and selection of microorganisms, from selecting for antibody secretion to the selection through cell viability or to select specific oxygen uptake rates.[38]

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The relevance of automation and miniaturization is evident for the implementation of laboratory HTS. It has empowered researchers to deliver results very fast and reliably with the use of automated systems, while keeping the costs low by miniaturizing assays. Either by using microfluidics or robotic systems, the present and future of HTPD involves automation and miniaturization as there is a push for more automated systems dealing with the least amount of volumes possible.

Brute force (liquid-handling robot) *versus* design freedom (microfluidics)

Robotic workstations have established their role in the biotechnology field through their capability of performing several experiments with minimal human intervention. Besides this, the evolution of such devices has been related mainly to achieving a greater number of tests per unit of time and integrating more systems (both for liquid handling and analytics) in one single equipment. The liquid handling by such devices can be done in several manners, either by pipetting or with acoustic energy[39] and both of these technologies are suitable for the dispensing of very small volumes (as low as the nL range). The dispensing also can be done by having contact or noncontact liquid dispensing, the latter being most suitable to avoid cross-contamination. The LHS often are connected to plate readers, which report results in a very fast manner. Furthermore, LHS software can be tailored to report the readings directly as results, with built-in data analysis. This allows for time saving whilst avoiding human-prone errors in the calculations. LHS have allowed researchers to adopt a 'brute force' method when performing experiments by allowing them to carry out a large amount of experiments in a short amount of time. More advanced process development tools are increasingly available and smart process development is taking over the field.[40] The main advantages of LHS compared to its miniaturized counterpart are the level of automation that can be achieved in such systems and the generalized acceptance from researchers of the field.

Microfluidics is often perceived and portrayed as a cheap screening technique. Although this is true for consumables, the fabrication of mastermolds for subsequent soft lithography is not cheap. Silicon wafers bought in bulk can cost up to US dollar 30 per 4-in. wafer, which translates to US$3700 m^{-2}. Besides the cost of wafers, clean room equipment for the fabrication of the microchips and maintenance of a clean environment inside the fabrication facilities also are expensive. Therefore, research groups usually share facilities and companies outsource the production of devices. The greatest advantage is the level of detail achieved, with structures showing very good accordance to the desired design at very small scales (μm and nm).

Microfluidics has evolved in a different way from the LHS. It also aims to reduce assay time and sample consumption, and although automation is a desired trait it is not mandatory, and most of the times this methodology takes advantage of its very small characteristic dimensions. With technological advancements, more complete systems have been developed, and the design freedom achieved with microfluidics is unprecedented. Unit operations and processes have been scaled down for the separation of products or biocatalysis.[41-43] Complex microbioreactors also have been developed where perfusion bioreactors were developed making using of microbubbles for both aeration and convection of the system.[44] What microfluidics lacks in ease of automation, it makes up for with its design freedom. Effective scale-down models can be achieved with high precision at a sample consumption several orders of magnitude smaller. Furthermore, the entrance cost also is several orders of magnitude lower when compared to LHS.

The rise of 3D printing has enabled researchers to reduce the time from design-to-chip and fabrication costs. Instead of cleanroom facilities, it is now possible to produce microchips using 3D printers,[45] a natural low-cost solution for microfluidics.

3D printing: an enabling technology

3D printing dates back to the 1980s, but major breakthroughs of this technology that allowed it to reach mainstream status happened only in recent years. Commercially available 3D printers have seen a major evolution throughout the past 10 years and printer prices have plummeted.

3D-printing techniques breakdown the 3D design into different layers, which are then built additively on top of one another (additive manufacturing), irrespective of the type of technique used. 3D printers have the advantage of easing the fabrication of the devices when 3D structures are desired for microfluidics, as neither extra steps nor skill-dependent assemblies are needed. Within the realm of 3D printing, there are different techniques that are employed: stereolithography (SL), laser sintering (LS), multi jet modelling (MJM) and fused deposition modelling (FDM) (more commonly known as thermoplastic extrusion).[46] SL has been evolving as a natural technique for fast prototyping at a low cost and high resolution. Traditional SL resolution (minimum feature size) is strongly dependent on the laser spot size and the spectrum of absorption of the used resins.[47] Initially, SL was the only technique that was able to consistently fabricate closed channel devices with no extra assembly steps required,[46] despite SL needing a post-processing step for removal of nonpolymerized resin. The fast development of 3D printers and the materials used have allowed for a broader range of techniques and materials to be employed in microfluidics, and FDM also has shown to be a valid option for microfluidics.[48] Moreover, one of the main advantages of cleanroom-fabricated microfluidic devices compared to 3D printed devices was the ability to include valves, to automate the apparatus. The automation of 3D-printed microfluidic devices has been demonstrated by Lee *et al.*[49] The authors printed a device with a 'Quake-style' valve, a technique often employed in cleanroom-fabricated microchips, with a biocompatible resin using SL in a 3D-printer. The proof-of-concept of such valves raised the standard for automation of 3D-printed microfluidic devices for the future to come, as coupling such control mechanisms to 3D designs can yield promising devices.

The fast evolution of 3D printers enabled the technology to fill in the gaps for it to be recognized as a viable alternative to clean room microfluidics. The evolution of the mechanics and the materials, coupled with a considerably lower price and easiness of handling, makes 3D printing a persuasive alternative to cleanroom-fabricated devices, as can be seen in Fig. 1.

HTS revolutionized the biotechnology sector. It allows for time and sample saving while maintaining or achieving greater quality data than possible before. LHS seemed to have moved towards increasing assay performance and microfluidics towards eccentric designs that are able to achieve good results and often mimic laboratory-scale performance. This led to greater acceptance of the LHS from industry and academia, as microfluidics seems unable to captivate industrial attention. That said, microfluidics has the size advantage and its portability also is a differentiation factor, as Lab-on-a-chip is still of great interest for point-of-care diagnosis.[51]

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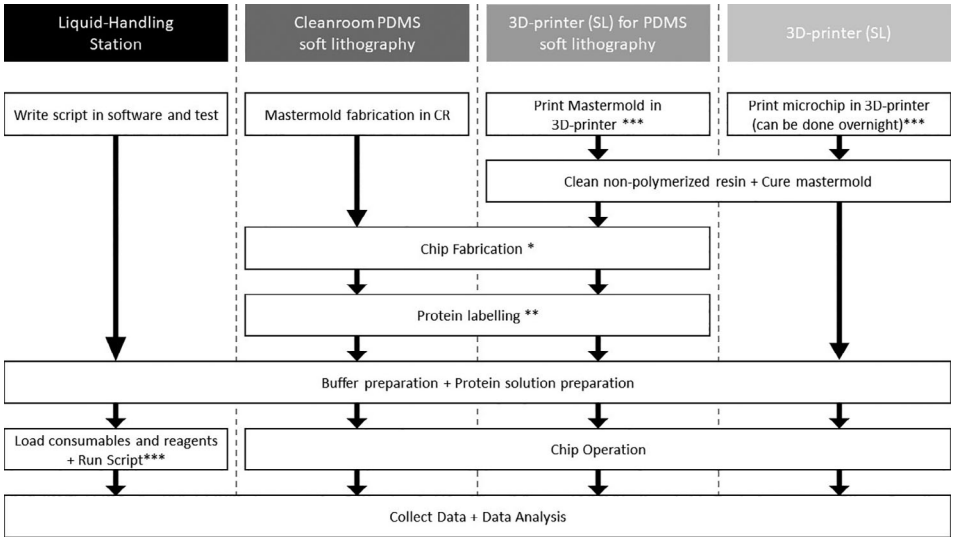

Figure 1. Typical workflows when operating different HT techniques. The operation using the 3D printer for the production of a mastermold for further PDMS soft lithography has the largest number of steps in the workflow; however, this does not necessarily mean that it is the lengthiest process, as a mastermold fabrication in the clean room is very time-consuming. * indicates that there are several steps to consider when doing microchip fabrication by PDMS soft lithography: (i) prepare PDMS; (ii) cure PDMS in mastermold and glass slides; (iii) Aligning (optional: if one or more layers are used in the microchip) and bonding of the layers; and (iv) chip sealing and bonding of the structure to the glass slide (which usually happens overnight).[50] ** indicates that protein labelling may be necessary as fluorescence is still the mainstream detection method for PDMS microfluidics. *** indicates fully automated steps (no human labor is needed – script and printing also run on their own).

ICB AND HTPD

The way that ICB and HTPD are interconnected depends on how the different technologies/tools can come together. Some of these will be further discussed: the need for compatible analytical tools, the lack of scale-down models for different UO, the importance of data management and modelling and the affordability of HTPD tools.

Compatible analytical tools

With the increased miniaturization of assays, the analytical tools used needed to adapt. A further development of analytical tools was needed and lower volume requirements for analysis helped to propel the shift. The incorporation of multi-well plate readers into LHS and adaptation of the microfluidic device analytics, both on- and off-chip, for accurate assays that have results in real time highlight the importance of having analytical tools that are adequate for the screening scale.[52] Furthermore, using the already established tools for more complex or precise analysis, such as precise determination of resin volume using optical methods in a 384-well plate,[53] or coupling the analytical tools with models to aid in the analysis of complex systems, where recent work highlights the use of such methodology for the study of complex systems as multicomponent isotherms in HTS platforms.[54-56]

Scale-down models for different UO

Another important aspect to consider is the feasibility of scale-down models in translating the results obtained at smaller scales into manufacturing scale processing.[21] Although some UO have favoured from a lot of attention from research peers, such as chromatography, some still lack feasible or practical scale-down models. Only recently proper scale-down models for fermentation processes have arisen, both in LHS (with the Ambr® systems) and microfluidics.[44,57] Moreover, membrane processes are present in every (bio)pharmaceutical process and are crucial to the ICB mode of operation. However only recently studies on the adaptation of this unit operations have been published,[58,59] denoting significant room for improvement and can help to justify why these processes often operate in suboptimal conditions in manufacturing. These scale-down models need to be accurate representations of their production-scale counterparts in order to add value to the process development stage. This is why models that can accurately translate miniaturized-scale data into production-scale are so valuable.[60]

Data management and modelling

HTPD makes use of HTS methods and models for the development of optimal processes. It will in turn lead to the generation of large amounts of data both from experiments and simulation. Now that most of experimentation is automated, data analysis needs to be automated too.[61] This is of paramount importance for the successful implementation of HTPD.

Affordable HTPD

HTPD for continuous manufacturing follows similar trends to HTPD for batch processes: fast and cheap process developments are achieved owing to lower consumption of materials. However, the platforms used for HTS usually come at a high price tag, this is why some companies keep away from such methodology. The advancements in both automation and miniaturization are reducing this gap and helping to democratize such equipment, by lowering the price and reducing the equipment complexity.

ANALYTICAL METHODS IN HT METHODOLOGIES

Analytical methods are pivotal in every experimental field. For HT methodologies to be efficient the detection must allow for 'high-throughputness'. Optical methods are the most widely used in HTS as they are easily adapted to such equipment: for LHS it is absorbance analysis and for microfluidics there is a wide range

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of methods available, although microscopic based assays, such as fluorescence, remain amongst the most popular.

Several assays in the biopharmaceutical industry rely on optical analytical methods (absorbance measurement of samples). The detection of impurities in a bioprocess is of the utmost importance and usually leads to tedious and time expensive laboratory work and LHS allow for more automated analytics. However, microfluidic devices allow for the use of different analytical methods owing to their very small size, as they can be fitted to numerous spectroscopy equipment (contrary to microplates). This has shown a wide variety of applications and analytics implemented in microfluidic assays.[62,63]

LHS can be tailored to a laboratory's needs. This is the great power of automation and the advantage of increased miniaturization of assays. As equipment size decreases the integration of analytical equipment in one single workstation becomes easier. Workstations working in 360° offer the possibility to integrate a greater range of equipment in the same space.[64] However, laboratory space often is limited, and linear workflows are often preferred (like the solutions offered by Tecan®, Männedorf, Switzerland). These systems are frequently commercialized as a package but are limited to a smaller number of plates that can be handled and to limited analytics to be performed (optical analytical methods with plate readers, such as absorbance, fluorescence and luminescence).

Raman spectroscopy has been used for upstream process development for some years, and more recently this analytical tool is being considered for use in DSP. It offers a broad range of applications from screening raw materials and culture media, to the monitorization of the process and assessment of chemical or structural changes in proteins.[65] It has not been until recently that the adaption of Raman spectroscopy to HTS platforms has taken place;[66] further implementation to downstream process development could bring important developments to the ICB landscape.

In Table 2 we can see an overview of commonly used detection methods in HTS with LHS and microfluidics. The discussed analytical methods do not cover all of the available methodologies for both LHS and microfluidics. However, it is possible to conclude

Table 2. Comparison of some of the different analytical methods available in LHS and microfluidic devices

	LHS		Microfluidics	
Analytical method	Ref	Comments	Ref	Comments
Absorbance	There are plenty of commercially available microplate readers that are easily integrated in the liquid-handling stations (e.g. Tecan)	Any type of absorbance assays can be performed (ELISA, UV–visible measurement, etc.)	27,67	-
Fluorescence		-	68	-
Luminescence		-	69	-
Mass spectrometry (MS)	70	This paper has a workflow where the MALDI-TOF MS is integrated in the HTS workflow, with several liquid handlers and the MALDI-TOF MS analyser in the end	62,71	-
Raman	66	Sample volumes of 160–200 μL are analyzed in the Raman module coupled to the Ambr® system	72,73	-
Near infrared (NIR)	-	-	74	The authors correlated the absorbance difference spectra with the solute concentration, and were able to obtain clear images of the acid–base reaction and the salt formation from the neutralization reaction
Dynamic light scattering (DLS)	75	There are commercially available DLS plate readers: DynaPro II Plate Reader DLS instrument[1] and Zetasizer APS[2]	76	-
Surface plasmon resonance (SPR)	-	-	BIAcore X100 (BIAcore, Cytiva)	Commercially available device

[1]Wyatt Technology Corp., Santa Barbera, CA, USA.
[2]Malvern Panalytical, Malvern, UK.

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that more analytical methods are more easily adapted to microfluidics. It is important to understand the limitations of each device hence why such methodologies are still not widespread and some challenges need to be targeted to allow for a generalized use of the technology.[71]

UPSTREAM AND DOWNSTREAM PROCESS DEVELOPMENT WITH HTE

HTPD – the case of chromatography

HTPD allows for a fast-forward in process development, allowing for a clear reduction in the time needed for optimization operations to be carried and optimum process design. As bioprocesses evolve to the final form of optimized continuous USP and DSP, there comes the task of integrating the bioprocess in one single continuous process.

The transition from up- to downstream in a bioprocess is always challenging. Several factors influence DSP, especially if the process relies on chromatographic steps in the early stages of the process, as small changes in the environment or the handling of the process can greatly affect the product's ability to undergo purification (for instance, the ability of a product to adsorb to a resin), as optimal operating conditions are not always met in a manufacturing environment. HTS is useful to find optimal operating conditions but also a great tool to determine operating windows. This is of great use, in an attempt to minimize the impact that batch-to-batch variations and human error have in downstream processing.[77]

Chromatography is still the workhorse of several biopharmaceutical products, which is reflected both in its high product purification factors as well as percentage of overall process costs, which can be >50% of the total batch costs.[78] In chromatographic separations there are several interactions to consider, and consequently several aspects to optimize: finding the optimal resin (defining the protein–ligand interactions, such as binding capacities) and buffers to use (loading, washing and elution buffers can have different pH and salt concentration), and estimating adequate linear velocity for the desired separation. Consequently, there is the need to comprehend what is happening in the process. Modelling is the state-of-the-art of chromatography process development,[79] especially hybrid approaches that make use of mechanistic modelling and HTS.[80] Although modelling is gaining more acceptance and implementation in process development, it still goes hand-in-hand with experimentation, whether for parameter estimation, 'model training' or validation of modelling results. Hanke and Ottens have reduced the chromatographic process development to three main realms: trial and error, process development based on molecular properties and process development based on molecular interactions.[81]

Examples of LHS and microfluidics for HT experimentation in biotechnology

LHS

Cell culture in microtiter plates offers the advantage of automated pipetting, useful for screening several media components, but can be challenging to achieve proper oxygen transfer to the growth media. Several parameters can influence cell cultivation, and this also holds true for microtiter cell cultivation. Work from Neha *et al*. showed that well format and shaking frequency, among others, were important parameters in achieving cultures of *Pichia pastoris* with a higher cell density in 96-well plates.[82] The advantage of having cell cultures in LHS is that they can be introduced in a workflow for the full automation of expression, extraction, purification and evaluation of the protein of interest, just as Shah *et al*. demonstrated for a HIV-specific mAb produced by *P. pastoris*.[83] Although the aforementioned parameters are important and impactful, LHS remain the state-of-the-art for upstream HTPD in the biopharmaceutical industry.[84,85]

Bensch *et al*. extensively cover in a review the developments and challenges faced when using HTS of chromatographic phases for process development.[86] The authors show the 'thought process' behind the development of this purification step, covering subjects such as resin and column screening. This is very useful in early stage process development. The next step is to verify whether behaviour remains the same in column experimentation and optimization and validation of the proposed experimental protocol is needed. Konstantinidis *et al*. developed a new methodology for the operation of miniature columns in a LHS.[87] These have the advantage of providing more insight on the separation process by mimicking large-scale operation. Miniaturized columns do not allow for linear gradients for elution, as liquids are loaded to the columns discretely. This work shows an automated way of experimenting in eight miniature columns in parallel and the output file already have automated calculation of the blanks and normalized spectroscopy measurements. The power of automation is clear in this study, as it was shown that with the same setup it was possible to study the purification of ovalbumin from a mixture with conalbumin and BSA and capture of mAbs. Recently, implementation of a HTS setup coupled to mechanistic modelling showed how data retrieved from MiniColumns can be translated to laboratory-scale chromatography.[60,88,89] By analyzing the Pe number at different scales, the authors concluded that an increased axial dispersion is observed at smaller scales, compared to larger scales, leading to larger elution pool volumes.[60] The results then were used to correct the model, allowing for accurate prediction of elution pool volumes at larger scales using the MiniColumns for experimentation.

Aqueous Two-Phase Systems (ATPS) recently arose as an important process and can represent an alternative to chromatographic processes for the purification of mAbs.[90] ATPS process development involves the preparation of systems with different phase compositions of polymer–polymer or polymer–salt solutions for the discovery of binodal curves and tie line length, which play an important role in the purification process. Azevedo *et al*. unveiled the potential ATPS and achieved recovery yields for IgG of 99% and purity of 76%. These studies were performed in 15-mL graduated tubes, which represent a great expense in consumables and reagents when considering the number of optimizable parameters, such as phase and salt compositions, and involved tedious and possibly erroneous work.[91] Implementing the same methodology in a LHS would allow the time needed for process development to be reduced.[92] Oelmeier *et al*. also evaluated ATPS for the separation of mAbs from host cell proteins.[20] This was performed in a LHS for a total system volume of 650 µL. The methodology described by the authors highlights the powerful features of LHS, such as liquid-level detection and liquid class definition, for aspiration of liquids with varying viscosities. The authors were able to screen a total of 552 systems and estimated that on microtiter plate could screen 33 systems in 2.5–3 h. Studies with ATPS of 300 µL also have been reported.[93]

Recently, antibody drug conjugates (ADCs) have captured the attention of industry for its potential in cancer treatment. Andris *et al*. developed a HT process for the development of new ADC molecules and showcased how LHS can aid, through

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parallelization and automation, achieve faster process development.[94] An HT-compatible monitoring tool also was developed for the monitoring of these conjugation reactions.[95]

Microfluidic devices

Downscaling operations like fermentations offer great advantages and can provide valuable insight. PDMS is the go-to material for microfluidic devices, and its gas permeability and elasticity features can be used to the researchers' advantage for the production of microbioreactors. Microreactors' ability to be assembled in a microscope set-up allows for real time *in situ* visualization of the experiments. The very small scale means that the analytical methods need to be accurate and have a low limit of detection (LOD), hence explaining why the use of very sensitive techniques such as fluorescence are popular. A picoliter-volume bioreactor has been described for single-cell cultivation of *Escherichia coli* and *Corynebacterium glutamicum*.[96] That study demonstrated that the behaviour of the culture under specific environmental conditions could be tested in a smaller amount of time. It was used to screen the influence of different media in cell growth, and an increase of 1.5-fold growth rate was registered for *C. glutamicum*. Different studies also have showed the use of microfluidics for the cultivation of *Saccharomyces cerevisiae* with integrated sensors in microbioreactors[97] and the cultivation and transfection of CHO cells.[57]

Microfluidic particle liquid chromatography has been reviewed recently.[98] With the design freedom available and advanced manufacturing techniques, there are many possibilities to study chromatography. Several applications, modes of operation and analytical techniques are discussed in the publication. Pinto *et al*. demonstrated an efficient screening of different operating conditions using multimodal chromatographic resin for the purification of a mAb.[99] The authors achieved recovery yields of 95% at the microscale, compared to 98% of laboratory scale, with 100 nL resin per reactor. Furthermore, an automated device that makes use of Quake valves has demonstrated the usefulness of microfluidics, allowing for the determination of one full chromatographic isotherm (with nine different protein concentrations tested in parallel).[100] Although this device only allows for small-sized beads, it portraits the powerful combination of miniaturization and automation.

ATPS also have been explored in microfluidic devices for the determination of binodal curves[101] and purification of mAbs.[102] By using the same systems studied at macroscale, the authors of those studies took advantage of the miniaturization feature of an increased surface area:phase volume ratio, which allows for comparable extraction at a fraction of the time.

Microfluidics still lacks generalized acceptance and widespread implementation of the many versatile devices that have been produced. Small steps have been taken in this direction and there are already commercialized microfluidic devices for different applications. Examples of these devices are the 2100 BioAnalyzer from Agilent Technologies (Santa Clara, Ca, USA) that provides an automated electrophoresis with very high resolution, Biacore™ X100 from Cytiva (Marlborough, MA, USA) that provides a microchip for the analysis of samples using SPR or the LabChip GXII from Perkin Elmer (Waltham, MA, USA) used for automated SDS-PAGE analysis.

The described examples for LHS and microfluidic devices are a few representations of what is being done in process development with both technologies. Microfluidic applications are not exclusive to bioprocesses, as there are many examples of diagnosis applications; however, one aim of this review was to shed the light on process development in the biotechnology industry. Microfluidics still is aiming for general acceptance and validation of the technology for a broader audience, and 3D-printed microfluidics can help to achieve this.

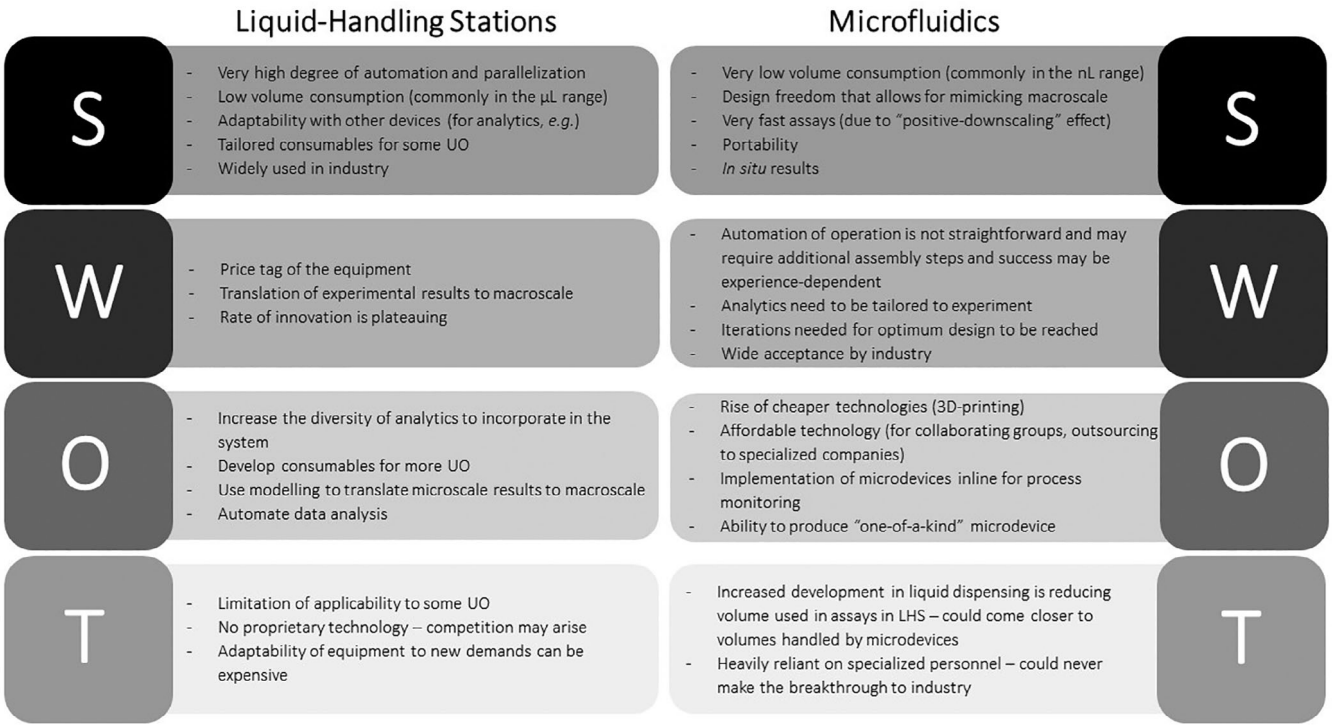

Figure 2. SWOT (strengths, weaknesses, opportunities, threats) analysis of LHS and Microfluidics.[25,50,107]

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CONCLUDING REMARKS

Drastically reduced process development costs and major time savings have been achieved through the use of LHS and microfluidic devices. Automation and miniaturization have increased the throughput of data and reduced time-to-market. Although LHS have a higher price tag, the widespread use of the technology and regulatory acceptance make it an in-demand technology. Microfluidics offers a bigger versatility in analytical methods that can be used, and its major banner is the technology's portability. 3D-printing technologies will enable laboratories to have a very cheap prototyping and manufacturing equipment for microfluidic devices.[103]

The greater need for deep understanding of processes and a more widespread use of modelling does not leave room for 'blind' testing in the hope of a technological breakthrough. When such screening technology is so easily available, it is tempting to perform a multitude of experiments leading to needless over-screening of the systems, while adding little to our understanding on the underlying process mechanisms. As regulatory agencies are pushing for a greater process understanding, rational and more standardized approaches are needed.

This is achieved mainly by hybrid process development which is the combination of mechanistic modelling with HTE.[40] These two methods can be coupled and will form a symbiotic relationship in process development, where the strengths of one can easily make up for the flaws of the other.[81] Opting for a mechanistic model for process-development allows for a great process understanding with low experimental effort. This process understanding is pivotal in current manufacturing strategies and facilitates significantly decreased experimental effort compared to having no available models, while improving process robustness.[56,80,104] Although mechanistic models still need calibration/parameterization partially via a selected set of experiments, this can be achieved quickly and at minimum experimental effort in the current landscape of HTS.[105] The available methods for HTE have shown that with minimum effort a wide variety of experiments can be carried out in the same equipment or set of equipment which will alleviate the financial and learning endeavour of researchers. The need for rapid screening and fast process development is far more evident when occurrences like the current pandemic caused by the virus SARS-CoV-2 arise. The development of the vaccines for this virus often made use of already established processes that needed tailoring to the specificity of the current virus.

The last 10 years in manufacturing saw an evolution in bioreactors where smaller reactors are preferred, outputting lower volumes and higher titers.[106] However, the industry has not stagnated and is moving towards continuous manufacturing, where higher productivities and facility flexibility are needed, and ICB caters to this.[15] The productivity driver is still in place and the key to dealing with this is to find the necessary process innovations, such as the ones offered by continuous processing.

It is only a matter of time before it is possible to achieve a full integration of biopharmaceutical processes into one single end-to-end process. HTS permitted the push for highly optimized upstream and downstream processes, which now need to be integrated into one single process. This is desirable not only for manufacturing companies, as it allows for cost savings, but also for regulatory agencies. Automation and miniaturization have enabled faster process development, and are pivotal for the continuous integration and improvement of these processes.

Moreover, although LHS seem to have reached a plateau in terms of new applications, microfluidics is constantly mutating and evolving and is perceived more and more as a valuable asset for HTPD; the emergence of 3D-printing microfluidics is a perfect example and is starting to get traction. The authors expect that LHS will continue to see the integration of more systems and will see a diversification in the investigated processes within a single piece of equipment. Microfluidics has reached mainstream use in a few instances. It is expected that the near future will show the emergence of novel applications of single-use disposable systems through 3D-printing technologies making this technology more readily available at low cost to the biopharmaceutical process development and analytical community. A further increase in automation together with simpler production and operation will probably push microfluidics one step further into research laboratories worldwide (Fig. 2). The opportunity now lies in being capable to provide HTS solutions at affordable prices for the different processes and develop analytics that can keep up with the increased reduction of volume for assays, while implementing models that are capable of correlating miniaturized-scale experimentation with manufacturing-scale operations. These developments, which are expected in the near future, will broaden applications of LHS and will pave the way in integrating microfluidics as an additional tool in biopharmaceutical process-development.

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CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

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