High-Throughput Automation in Chemical Process Development

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Abstract

High-throughput (HT) techniques built upon laboratory automation technology and coupled to statistical experimental design and parallel experimentation have enabled the acceleration of chemical process development across multiple industries. HT technologies are often applied to interrogate wide, often multidimensional experimental spaces to inform the design and optimization of any number of unit operations that chemical engineers use in process development. In this review, we outline the evolution of HT technology and provide a comprehensive overview of how HT automation is used throughout different industries, with a particular focus on chemical and pharmaceutical process development. In addition, we highlight the common strategies of how HT automation is incorporated into routine development activities to maximize its impact in various academic and industrial settings.

INTRODUCTION

The advent of automated, high-throughput (HT) experimentation has enabled step changes both in terms of lab productivity and in the rapid generation of comprehensive data and knowledge across a diverse range of industries and settings, both academic and industrial. Certainly, the growth of this technology has been predicated upon the increasing computing power underlying the software needed for the design, operation, and analysis of the resulting data sets, as well as concomitant advances in automation hardware of increasing sophistication. One of the key drivers for the advancement of such technology is the significant impact that HT automation continues to deliver, specifically in the realm of chemical process development. These impacts are particularly evident in fields where reductions in experimental cycle time are a necessity to meet the ever-condensing development timelines, including but not limited to the chemical and materials industry and, especially, the pharmaceutical and biopharmaceutical industries. It is in these fields where HT automation enables the maximization of data or, perhaps more importantly, knowledge capture per unit of time to inform the design and optimization of fundamental chemical engineering unit operations (e.g., reactions, extractions, fermentations, crystallizations). Although HT automation techniques are applied in a variety of research settings to increase knowledge generation by interrogating comprehensive, and frequently multidimensional, experimental spaces, the primary focus of this review is specifically on HT automation capabilities and workflows that are applied toward the development of scalable, robust chemical (or biochemical) manufacturing processes.

HISTORY OF LABORATORY AUTOMATION

Much of the early history of lab automation derived from demands in the life sciences in the 1980s for more productive means to aliquot and dilute biological samples for testing and analysis. This gave rise to automated liquid handlers that could reliably generate consistent arrays of samples with minimal manual intervention and led to their rapid uptake in both industrial and academic settings, particularly for clinical samples as well as HT screening assays for drug discovery (1). As with the growth of any new technology platform, lab automation's early history has also been associated with cycles of great expectations followed by regression to a steady state. For example, in the 1990s, large investments in infrastructure for the automated creation and testing of combinatorial libraries of drug-like molecules were anticipated to completely revolutionize the pace of drug discovery. But despite this promise and excitement, those large investments in automated, parallel experimentation and screening fell short in meeting those expectations (2). Currently, the application of automated, HT library generation and screening has matured into an important, often critical, tool in aiding drug discovery for both the initial identification of lead structures and focused library generation to optimize against a variety of biomolecular attributes (3, 4). An ancillary benefit from this initial, significant investment in combinatorial chemistry as well as screening was the rapid maturation and broad application across multiple industries of some of these technologies, tools, robotics, and techniques beyond their original intent, as described in the following sections.

An early adopter of these technologies was found in the area of materials science, where the general conceptual underpinnings of combinatorial library synthesis for the serendipitous discovery of bioactive molecules was readily translated and applied to identifying novel materials (5). As early pioneers in this area, Schultz and coworkers (6) provided a compelling example of this approach in the identification of a new blue photoluminescent composite material by generating a combinatorial library of 1,024 unique materials through a quaternary masking strategy. A quaternary masking strategy involves carrying out a series of vapor depositions of N different masks

that successively subdivide the substrate into a series of quadrant patterns. The masks are used four times each and rotated by 90° after a deposition layer. Hence, *N* masks will thereby create 4^N different materials in only 4*N* steps. In this example, the analysis of the resulting materials was a simple fluorescent readout of the array, whereas other applications are oftentimes rate limited by the resulting readout and analysis employed. This general technique and variants thereof have had far-ranging applications for the discovery of new materials in electronics, functional materials, sensors, polymers, heterogeneous catalysts, and biomaterials (7). The unifying element behind all of these applications is the power of automation and HT experimentation to rapidly generate large, diverse arrays of empirical experimental data where first principles and rational design offer an intractable option owing to the innate complexity of the systems and responses under study.

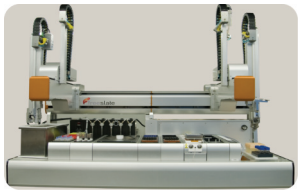
CHEMICAL PROCESS DEVELOPMENT

Evolution of Automated Technology

The underpinnings that enable a diverse set of automated workflows in chemical process development across various industries are built upon a foundation of highly adaptable software and automation hardware that allows for a variety of manipulations of both powders and liquids as well as an IT infrastructure that centralizes the capture of both instrument and resulting analytical data. Some of the leading vendors for these technologies are Unchained Labs (formerly Freeslate, previously Symyx Technologies), Chemspeed, and Mettler-Toledo. The genesis of this technology grew out of Symyx Technologies (8), which developed a set of automated platforms primarily focused on the discovery of novel phosphors as well as new polymerization catalysts (9). This was later adapted to the automated screening of catalysts (10) and crystallizations (11) applied to process chemistry research in the pharmaceutical sector.

In addition, the chemical industry saw the development of parallel microreactor systems, such as Caterpillar microstructured mixer-reactors, routinely used for reaction engineering to simultaneously explore various reaction parameters. However, the adoption of these technologies was slow throughout the chemical industry, as heat and mass transfer rates are rapid in miniaturized vessels, thus making it difficult if not impossible to discern reaction rates for predicting scalability (1). McMullen & Jensen (12) published a review of the various automated microreactor technologies, highlighting how the technology has evolved in the chemical and pharmaceutical industries for applications in reaction discovery and development. These microreactor systems offer potential in obtaining data at extreme conditions (i.e., high temperature and high pressure) in which it is normally difficult, if not impossible, to obtain such data using conventional reactor setups. In addition, this field has seen the development of specific inline analytical monitoring technologies, such as the integration of microsensors with automated feedback control or the incorporation of nuclear magnetic resonance (NMR), Raman, or Fourier transform infrared spectroscopy (FTIR) probes into microreactor systems. As opposed to HT technology, which typically leverages parallel experimentation strategies, most approaches that use continuous-flow microreactor technology involve sequential experimentation toward reaction optimization. Most of these microreactor systems employ continuous-flow operation, which can be useful for a wide variety of reactions but is not necessarily translatable to batch execution.

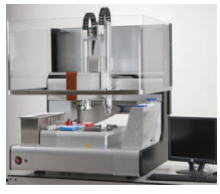
Advances in both the software, which allows for fast, flexible experimental designs and data capture, and the hardware have made these systems much more robust and versatile. Capabilities such as the ability to dose powders and liquids as well as time-dependent sampling for subsequent high-performance liquid chromatography (HPLC) analysis across multiple temperature zones in arrayed vial formats from microliter to milliliter scale enable a wide variety of chemistries and unit



CM3 1
- 9 temps/3 stir zones
- 1 × 16 G and 4 × 22 G needles
- Plate/vial gripper
- Cap/uncapper
- 3 powder dispense options

Chemspeed powder dispense
- 24 96–well plate positions
- 48 source powders
- Walk-up
- Can be inerted overnight

CM3 2 (catalysis) inert
- 6 temps/2 stir zones/1 vortex
- 1 × 16 G and 4 × 22 G needles
- Plate/vial gripper
- Cap/uncapper
- 3 powder dispense options and PDT



Protégé powder
- 96–well plate hotel
- 3 powder dispense options

2 × protégé PharmD
- Solubility and crystallization
- 96–well plate hotel
- Heat/cool/stir
- 4 × 22 G needles

Figure 1

Fleet of automated equipment used for chemical process development at Bristol-Myers Squibb.

operations to be conducted in a parallel, automated fashion. In chemical process development at Bristol-Myers Squibb (BMS), for example, a fleet of automated tools with distinct sets of functionalities (**Figure 1**) are applied to enable various workflows for the development and optimization of robust, scalable processes.

As the visibility of HT technology has grown in the scope of chemical process development, more researchers in both industrial and academic settings have begun to embrace HT technologies with an understanding that the impact of these technologies is primarily in investigating scale-independent factors as well as elucidating trends across wide experimental spaces.

Polymer Chemistry

The rise and evolution of combinatorial chemistry into parallel experimentation platforms helped drive technology development toward automated systems for parallel experimentation. Although the initial adoption of these automated HT technologies was primarily in pharmaceutical discovery, other industries soon followed, including the polymer research and materials industry (13). In one example, automated platforms from Chemspeed Technologies were used to conduct arrays of parallel experiments to investigate the effects of several factors (solvent, temperature, agent) on reversible addition fragmentation termination and anionic polymerization (13). Use of HT automation in the polymer industry has expanded on the analytical front as well, such as the use

of the automatic continuous online monitoring of polymerization reactions, which is executed using automated sampling and subsequent sample preparation for a variety of inline analytical techniques (14). However, although this versatile platform can be used to execute reactions in batch, semibatch, or continuous fashion, the closed-loop self-optimization mechanism does not allow for the incorporation of more strategic experimental design, such as design of experiments (DoE). Other HT analytical techniques have been developed as well to optimize polymer chemistry and processing, although most of these have limited scope to specific polymer chemistries (15, 16). Comprehensive overviews of how HT technology coupled to parallel experimentation strategies and multivariate analysis have advanced polymer synthesis process development have been outlined, showcasing the impacts of HT automation on accelerating the speed to market for new products (17, 18). Applications of HT automation in reaction engineering for organic chemical transformations span multiple industries; we have included details and examples of these approaches in the section titled Pharmaceutical Process Development.

Biological Engineering: Biomass, Biofuels, and Biosynthesis

Analogous to other industries, the subsection of the chemical industry focused on producing biomass-based fuels and chemicals has been shown to benefit from HT automation (19, 20). Microbial biosynthesis of commodity chemicals can be optimized using either rational design or directed evolution strategies, both of which can benefit from HT automation, specifically in the analytical space with colorimetric and fluorometric plate-based screening, as well as biosensor-based approaches (19). In a microbial growth application, Radzun et al. (21) outline a HT optimization approach employed to optimize microalgae production by investigating the primary nutrient effects on cell growth using automated equipment for media preparation via a statistical experimental design. This two-step approach incorporated the use of carefully designed DoE arrays and statistical analysis to elucidate effects of single factors as well as pairwise interactions of various nutrients (e.g., N, P, Ca, Mg, Fe, Mn). Strategic DoE approaches are advantageous for reducing the typically overwhelming, and often impractical, number of experiments resulting from use of fully crossed combinatorial libraries.

In upstream process development for producing biomass-based fuels, HT platforms have enabled the assessment of biomass digestibility using combinatorial arrays of various biomass sources crossed with different enzymes (22). Many other assays and plate-based methodologies to screen pretreatment and enzymatic conditions have been developed on the analytical front, although some of these HT options are expensive and have certain limitations on their applicability (23–25). However, upstream process development for biomass digestion and fermentation can greatly benefit from HT experimental platforms to investigate multidimensional design spaces. In fact, DuPont, in collaboration with MIT, adopted parallel microreactor systems specifically for the development and optimization of fermentations (1). Still, some of the current HT technologies in this space do not adequately translate to large-scale operations (26).

PHARMACEUTICAL PROCESS DEVELOPMENT

In the pharmaceutical industry, automated, parallel experimentation plays a central and ever-increasing role, from the discovery of new molecules and their purification to the development of robust manufacturing routes (27, 28). Laboratory automation has made steady gains in terms of both technologies and the level of implementation and sophistication in chemical laboratory settings over the past few decades. Whereas application of automated, HT screening systems is routine and well established in the areas of biological assays and drug discovery (9, 29), adaptations of such technology for chemical process development have progressed at a slower pace.

This is likely due to the unique demands and rapid adaptability required for the discovery and optimization of diverse arrays of chemistries and unit operations, such as workups and isolations. Despite these challenges, there have been several examples in which automation and HT experimentation have had notable impacts in both academic (30–37) and industrial (38–40) settings for the identification of novel catalytic transformations and new reaction screening methodologies, as well as for the rapid exploration of crystalline forms of pharmaceutical solids (41). Although these reported examples of applying automated screening protocols have been quite impactful for generating hits, the expansion of these tools to fully exploring and defining a chemical process from end to end has not been fully realized.

Investments in automation over the past 20 years by pharmaceutical companies have led to a full suite of workflows and tools to not only discover, optimize, and model complex chemical reactions but also provide deep knowledge sets for workups and isolations of crystalline intermediates (42, 43). The value proposition offered by automation is multifaceted. The most obvious benefits are enhancements in terms of productivity, as these can be readily measured by determining experimental throughput and cycle time when operating in designed, parallel arrays as opposed to iterative modes of experimentation. Another benefit is that experiments of this nature are conducted on smaller scales with little human intervention during the handling of often hazardous materials, and this provides immediate safety benefits by limiting exposure. A less obvious, and more difficult to measure, value is found in the generation of high-quality, internally consistent data sets that are automatically captured in centralized databases. Operating experiments in a parallel, automated fashion ensures much less variation from one reaction to the next or from one array to another compared with generating the same sets of data in a manual mode, owing to the introduction of human error via minor variations in experimental documentation and protocols. Automated, repeatable experimental design execution in conjunction with centralized data capture and traceability provides high-fidelity data sets, enabling a robust foundation for in-depth process understanding and for computational modeling exercises.

Various automated workflows are described throughout the following sections (**Figure 2**) that can enable the efficient generation of knowledge critical for developing and defining a robust, scalable chemical manufacturing process. These range from identification of the ideal combination of reagents and catalysts, as well as the span of operating conditions, that maximize the productivity of a given chemical reaction to understanding the means by which the desired product can be isolated at acceptable levels of quality in a predictable manner. Taken as a whole, these workflows

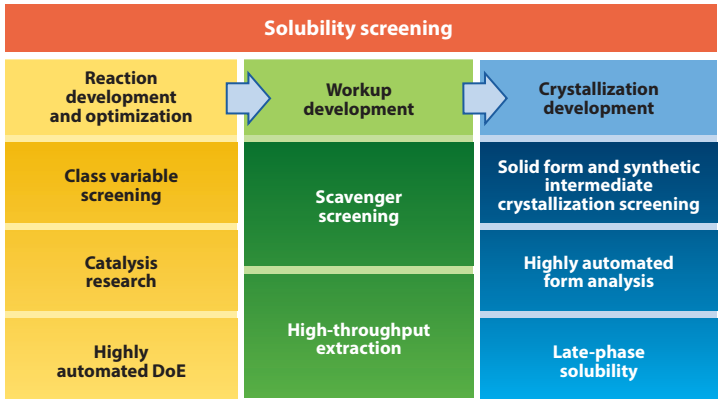


Figure 2

Automated workflows for process invention and development. Abbreviation: DoE, design of experiments.

encompass most unit operations and activities that a typical chemical process may encounter and serve a critical purpose in generating the fundamental knowledge to allow for rapid selection of a chemical process route as well as a clear understanding of process limitations and acceptable operating conditions.

Solubility Determination

Thermodynamic solubility data are critical pieces of information that influence many decisions during organic process development (44). In the context of a synthetic step, every operation, from reaction to extraction to crystallization, can benefit from the knowledge of solubility on relevant compounds (e.g., starting materials, products, reagents, impurities) in pertinent solvent systems.

Numerous HT methods can be used to measure thermodynamic solubility in organic and aqueous solvents. At the highest level, they can be broadly separated into two factions: excess solid methods and excess solvent methods (45). The excess solvent methods operate by adding solvents to a slurry or increasing its temperature (because solubility and temperature generally have a positive correlation) until all solids go into solution. Various detection techniques may be employed to determine the exact point at which the last bit of solid disappears (46). In contrast, the excess solid methods incubate a compound and solvent slurry for a lengthy period of time until equilibrium is established with excess solids still present. The concentration of the solute in the solution phase can then be determined by several different analytical methods. Generally speaking, the excess solid method is more advantageous for process development purposes given its more accurate measurement of thermodynamic solubility, which the excess solution method does not necessarily provide. A key product enabling the excess solid methodology is the commercially available Symyx solubility workflow (47) (**Figure 3**).

Within the excess solid method, the most critical operation is the filtration step, in which a sample of the slurry is filtered to remove excess solids, followed by immediate dilution of the filtrate to prepare HPLC samples. The Symyx Filter Assembly technology was elegantly designed with a seal during filtration to prevent solvent evaporation; in addition, all components (Filter Assembly, slurry plate, and liquid transfer source line) can be temperature controlled to prevent solution temperature fluctuation during filtration (**Figure 4**). The critical role of the Filter Assembly makes it the keystone of the Symyx solubility workflow (**https://www.unchainedlabs.com/freeslate-small-molecule**).

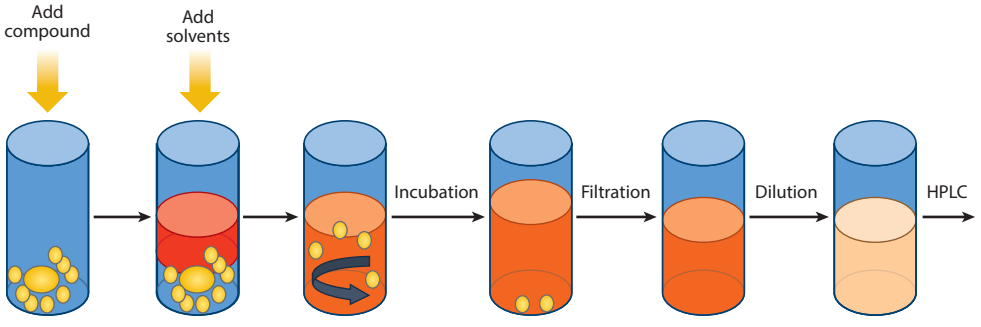


Figure 3

Symyx automated solubility workflow (**https://www.chemspeed.com/**, **https://www.unchainedlabs.com/**). Abbreviation: HPLC, high-performance liquid chromatography.

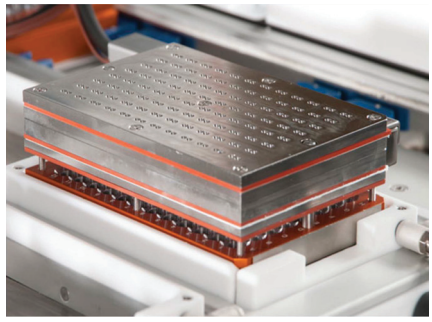


Figure 4

Symyx Filter Assembly.

The parallel experimentation platform allows solubility screening studies to incorporate designs that include common solvents and frequently used binary solvent mixtures, although customized designs can be easily implemented (48, 49). Ultimately, this carefully sequenced workflow has been integrated in pharmaceutical process development and continues to significantly impact the scouting and optimization of numerous reactions and isolations, as well as influence the final decision on route selection.

In the later stages of pharmaceutical process development, the development of a robust, controlled crystallization is critical for the isolation of an active pharmaceutical ingredient (API) or a synthetic intermediate. To design a controlled crystallization process, the solubility of the compound of interest must be mapped within the given solvent system. Cohen et al. describe a late-phase solubility (LPS) workflow to map solubility using a similar automated technique to the solubility screening workflow via the excess solid method described above (50). In LPS, however, an array of slurries are prepared at various conditions (solvent ratio, salt content, ± additive) at a larger scale (~500 mg per condition) than a typical solubility screen (<50 mg per condition). In addition, LPS studies often include an internal standard, allowing for a compound's solubility to be calculated in wt% from the mg/ml concentrations of the compound and the internal standard, as measured by HPLC-UV analysis. Acquiring solubility measurements in wt% allows for accurate solubility modeling to further assist in controlled crystallization development and crystallization modeling. The increase in scale and modified process result in an increase in solubility measurement accuracy, but the calculated tradeoff for this type of study is a slight decrease in overall experimental throughput.

Chemical Reaction Development and Optimization

Advancements in automated technologies have allowed researchers to design innovative platforms geared toward efficiently developing and optimizing chemical reactions in a variety of industries. Descriptions of such platforms are reviewed in this section, with a particular focus on applications in the pharmaceutical industry.

Class variable screening. The primary application for HT approaches in the area of synthetic organic chemistry has been for the rapid identification of catalysts and new modes of reactivity in both academic and industrial settings (12, 51–53). In most cases, the central aim of an academic HT screening endeavor is typically to unveil new modes of chemical reactivity with broad applicability across a spectrum of substrates. In contrast, a typical HT study in chemical process development in the pharmaceutical industry is to deliver maximum molecular complexity toward a given new

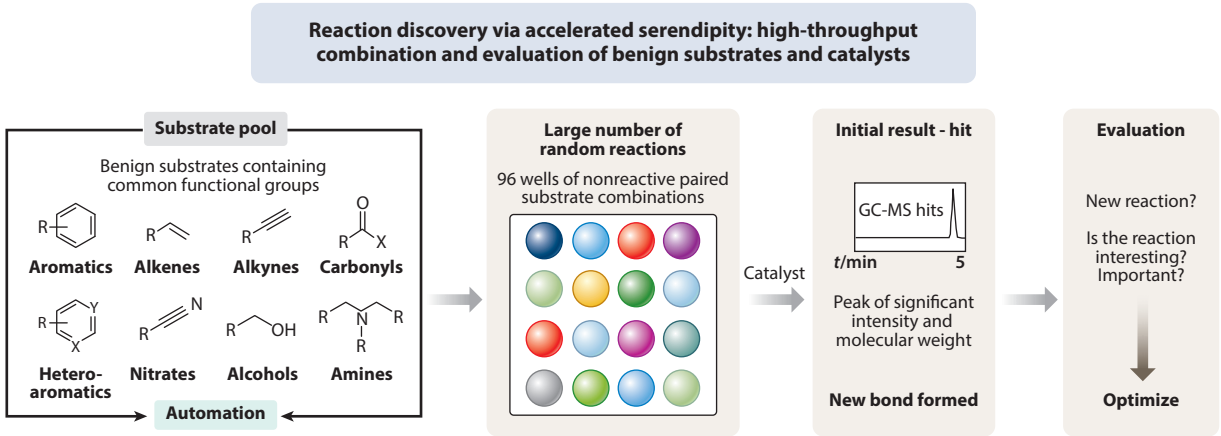


Figure 5

Accelerated serendipity strategy. Reprinted from Reference 31 with permission from AAAS. Abbreviation: GC-MS, gas chromatography–mass spectrometry.

chemical entity as efficiently as possible. At present, an overview of the HT reaction discovery engines that power chemical process research-and-design organizations in the pharmaceutical industry has yet to be delineated for a broader audience but remains of paramount importance for innovation in chemical synthesis.

Arrayed experimentation is harnessed to look at the impact of class (discrete) variables, such as metal salts, catalysts, ligands, solvents, and additives, to empirically derive optimal homogeneous conditions to effect a desired transformation. This can operate in a nondirected fashion, as with the concept of "accelerated serendipity" by McNally et al. at Princeton University, wherein a pool of reactive substrates was crossed with an array of inorganic photoredox catalysts (31). The resulting reactions were then analyzed using gas chromatography–mass spectrometry (GC-MS), with data analysis examining the presence of mass indicative of a novel bond-forming event having occurred (**Figure 5**). In this example, they were able to uncover new photoredox conditions to enable an alpha-arylation of a cyclic secondary amine.

Similarly, Robbins & Hartwig (35) used a multidimensional approach toward HT reactions to discover new catalytic reactions. Arrays of catalysts with 15 metal centers and 23 common phosphine and amine ligands along with 17 diverse substrates were screened and analyzed via mass spectrometry to find high–molecular weight transformed products. This multidimensional approach represents more than 50,000 reactions and can be performed in a few days (**Figure 6**). To this end, two new reactions were discovered: a copper-catalyzed alkyne hydroamination and a nickel-catalyzed hydroarylation.

The more usual approach is directed in the sense of screening class variables to mediate or catalyze a desired chemical transformation or bond-forming event (51). This is often informed by the literature, past data, and intuition, with automation being leveraged to explore chemical reactivity space in a more comprehensive, parallel manner rather than an iterative, one-factor-at-a-time approach. Perhaps the apogee of this methodology is best exemplified by researchers at Merck and Co., Inc. (54). They demonstrated the miniaturization of this catalysis screening methodology to a 1,536–well plate format using 0.02 mg of substrate per well. Operating on this nanomole scale, they clearly demonstrated an ability to optimize class variables (base, catalyst, and nucleophile) as well as run a nanomole-scale DoE against a few continuous variables (equivalents

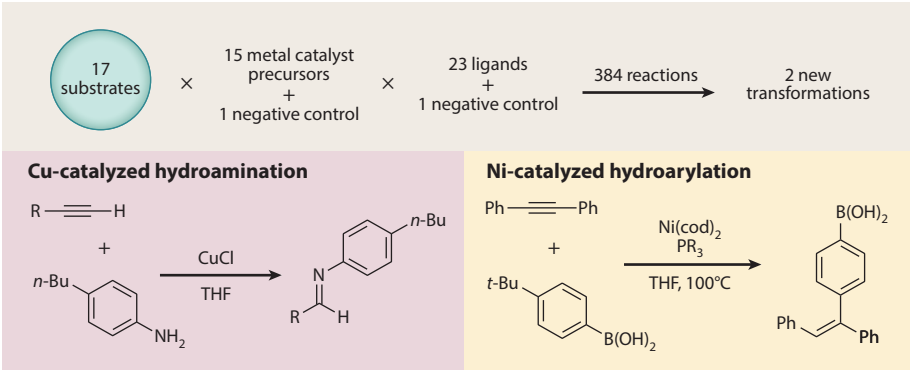


Figure 6

Multidimensional array that led to the discovery of two new reactions. Reprinted from Reference 35 with permission from AAAS.

of nucleophile, base, and catalyst loading). In addition, they were able to complete their analytical assays in 2.5 h for 1,536 samples using flow-injection liquid chromatography–mass spectrometry (LC-MS) for this challenging palladium-catalyzed C-N cross-coupling reaction. On this scale, they were limited to highly soluble reaction components in dimethyl sulfoxide (DMSO); nonetheless, they demonstrated the power of this screening platform to quickly optimize class and continuous variables in a complex, homogeneous catalytic transformation (**Figure 7**).

HT technology is leveraged to rapidly discover novel transformations and conduct initial reaction optimization through screening libraries of catalysts while capitalizing on the commercial availability of large numbers of metals and ligands. The experimental setup for such catalysis screening studies has several nuances and caveats that must be considered in the design (32). For example, the additional parameters of catalyst loading and ligand-to-metal ratio, along with generally more pronounced solvent effects, can have a significant impact on the success rate of a screen. For this reason, two-way interactions are crucial to consider when evaluating a potential system. Furthermore, the catalyst precursor identity, addition order, and aging protocol, each of which is informed by mechanistic understanding of organometallic chemistry, are also critical variables (55).

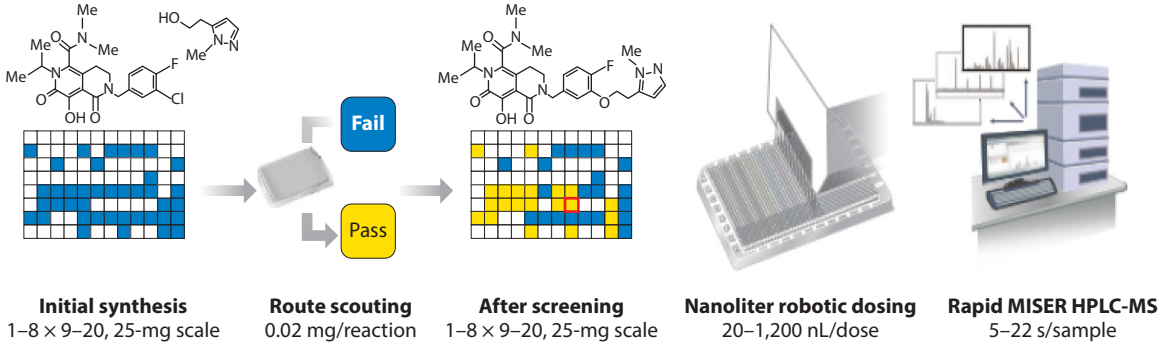


Figure 7

Nanomole-scale homogeneous catalysis screening. Reprinted from Reference 54 with permission from AAAS. Abbreviation: HPLC-MS, high-performance liquid chromatography–mass spectrometry.

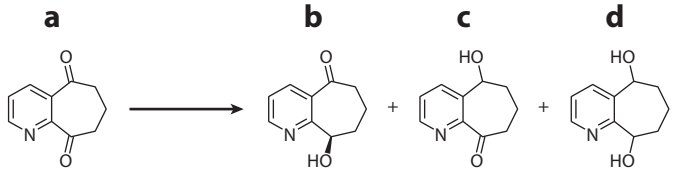


Figure 8

Chemo- and enantioselective reduction of (*a*) dione to (*b*) ketoalcohol with side products (*c*) and (*d*). Adapted with permission from Reference 56, ©2012 American Chemical Society.

A prime example of catalysis screening involves a chemo- and enantioselective reduction of dione *a* to ketoalcohol *b* (**Figure 8**) (56). The reduction was complicated by the presence of an additional ketone that generated regioisomeric alcohol *c* as well as the over-reduction product, diol *d*, as impurities. Prior work involved an asymmetric enzymatic resolution that provided the requisite material in 92.5% ee, albeit as an 84:3:13 mixture (**Table 1**). Although the enzymatic system provided material in good enantioselectivity, enzyme ES-KRED-119 was costly, required high loading, and was difficult to source. Thus, a chemo- and enantioselective transition metal–catalyzed approach was desired.

In considering the molecule, the pyridine handle may serve to differentiate reactivity by altering the electrophilicity of the ketone as well as directing the catalyst to the target moiety. However, the different electrophilicity alone may not be sufficient to suppress reduction in the regioisomeric position. Thus, to exploit the divergent chemotypes, a screen of 96 different conditions with 48 distinct metal ligand combinations was designed specifically to target systems that are bidentate in nature. Within the first screen, Rh(R-Binapene)(COD)BF_4 provided ketoalcohol *b* in remarkably high enantioselectivity without any trace of the undesired regioisomer or diol by-products (**Table 1**). To confirm that pyridine behaving as a directing group is the origin of the observed selectivity, isomeric substrate *a* was generated and subjected to reaction conditions. The side-by-side comparison revealed that even with 20% loading of catalyst, none of the desired mono ketone reduction product was observed, and that chelation of pyridine and the ketone is the primary mode of reactivity and selectivity (**Figure 9**).

The newly found conditions required only 0.02 mol% loading and were further demonstrated on a multikilogram scale (**Table 2**). Subsequent studies of >2,000 reactions showed there are an additional 5 catalyst systems that provide the desired product in >95% ee. Careful design experimentation and the use of automated technology provided multiple hits, which may provide more processing flexibility as well as streamline reaction discovery and optimization.

The driving force behind laboratory automation capabilities should focus on specialty designed HT experimentation. For class variable screening, customized arrays of class variables, including solvents, reagents, and catalysts, provide opportunities to interrogate a desired chemical transformation from numerous angles (57). An extensive library of reagents and catalysts coupled with automated solid dispensing allows for these highly customized reaction plates to be prepared with

Table 1 Comparison of enzymatic approach and Rh-catalyzed reaction selectivity[a]

Entry	Conditions	Conversion (%)	ee (%)	Ratio (A:B:C)
1	ES-KRED-119	99	92.5	84:3:12
2	Rh(R-binapene)(COD)BF_4	100	>99.9	100:0:0

[a]Adapted with permission from Reference 56.

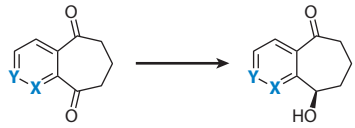


Figure 9

Screening to identify chemotype effect where X and Y (*blue*) were varied (see **Table 2**).

blinding speed. The use of specialty designed plates (versus prepackaged plates) allows teams to pull inspiration directly from the most recent literature in combination with existing in-house knowledge for a given synthetic challenge. Furthermore, these experimental arrays are tailored to fit the needs of a given program at its respective stage of development, focusing on new reactivity at early clinical stages versus improved safety and greenness at later stages (39).

Another example of the impact of HT class variable screening on the manufacturing process during the development phase involved a drug candidate that had achieved proof of concept for a streamlined route to produce the complex molecule. A key obstacle for implementation of this route on a commercial scale was the ineffective oxidation of an azaindole to the corresponding *N*-oxide (**Figure 10**) (58, 59). The present approach used a rhenium-based oxidation protocol that afforded the azaindole product in high yield; however, an estimated 0.4 metric tons of rhenium would be required per year with a cost of $100,000/kg. To overcome the need for a prohibitively expensive metal catalyst, a HT screening campaign was initiated to find alternative conditions.

Several reports of azaindole oxidation were known in the literature, but these options were found largely ineffective in single-lab runs for the desired substrate. The in-house knowledge was collated with the existing literature, and a plan to evaluate a custom array of anhydrides, oxidants, and solvents was executed. It was discovered that phthalic anhydride in the presence of hydrogen peroxide was able to carry out the desired transformation after four rounds of screening (**Figure 10**). The resulting conditions saved an estimated $330/kg drug substance by using a commodity chemical versus rhenium, which requires mining, and decreased reaction time sixfold.

Other impactful examples of HT applications and novel experimental platforms exist in the realm of reaction discovery and exploration of novel chemical transformations, but they are not necessarily driven toward process development and are thus not outlined in detail in this review (37, 51, 60–62).

Highly automated DoE. As demonstrated throughout multiple industries, statistical software and lab automation in chemical process development are two complementary technologies. Lab automation enables the implementation of parallel experimentation, whereas DoE provides an efficient, well-designed array of experiments for interrogating a multidimensional experimental space (39, 63). Statistical designs and a Freeslate-based automated platform have been integrated into a unique, highly automated DoE (HAD) workflow, which has evolved into a routine process understanding technique (64, 65).

Table 2 Catalyst loading conditions for various chemotypes

Entry	Y	X	Catalyst loading (mol%)	Conversion (%)
1	C	N	0.02	100
2	C	C	20	0
3	N	C	20	0

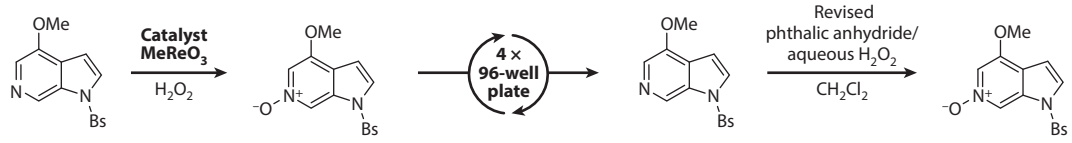


Figure 10

Azaindole oxidation conditions before and after class variable screening.

There are several stages for the implementation of HAD. First, the appropriate experiment must be designed for the chemical transformation that captures the important factors or reaction parameters (e.g., temperature, equivalents of reagents, dilution, solvent composition), as well as a careful setting of ranges on each factor such that the ranges capture perceivable effects on the chemistry. Typically, HAD studies are not conducted until the optimal class variables are defined, as outlined in the previous section. This allows for optimization with respect to the continuous variables, or reaction parameters. The design of these HAD studies does not consider scale-dependent variables, as in most miniaturized HT approaches. Rather, these factors must be studied by other means and at larger scale. Thus, the HAD approach, along with other HT systems, may not be applicable to reactions or other processes where scale-dependent effects, such as mass or heat transfer, dominate the kinetic profile.

Second, the array of parallel experiments is executed on a Freeslate-based automated platform using capabilities of liquid and powder handling, nitrogen inertion, and heating and stirring controls. The automated equipment is programmed to sample each reaction over a time course, capturing time stamps and other metadata for each sample. Third, a structured data table consisting of reaction parameters, time points, and HPLC data providing reaction conversion and impurity level information over time is generated. Data visualization of parallel reactions often incorporates kinetic profile comparisons for each reaction with respect to conversion and impurity levels, as well as a determination of when reaction conversion is achieved (**Figure 11*a***). These types of plots

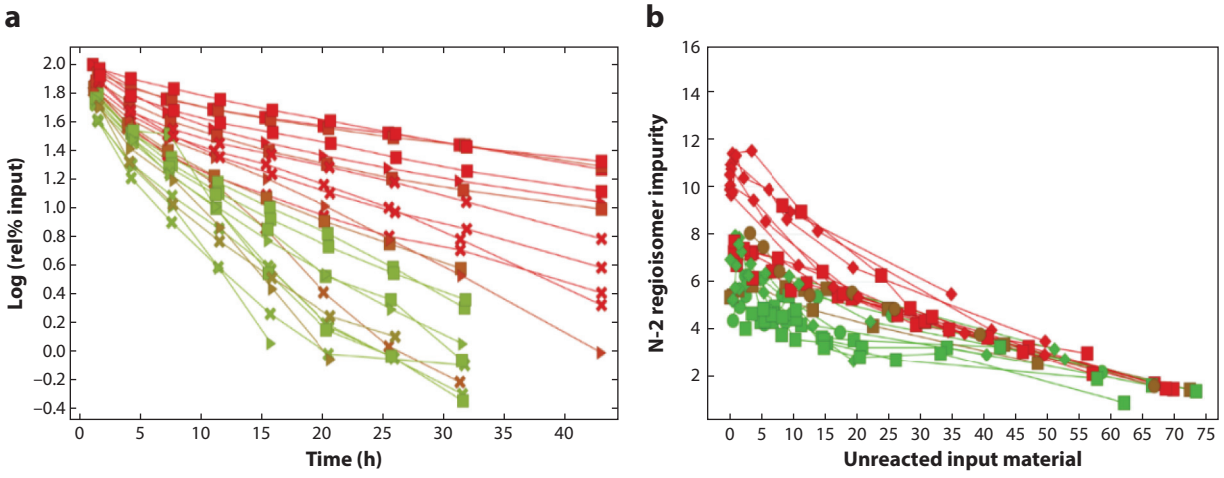


Figure 11

(*a*) Log of relative input material versus time exhibiting kinetics of 24 reactions. (*b*) Impurity formation versus reaction conversion for multiple parallel reactions.

are useful for comparing impurity level versus reaction conversion, as they provide a visualization of how reactions must be controlled to achieve optimal quality and yield (**Figure 11*b***).

Following structured data table generation and data visualization, the fourth step of HAD involves additional statistical analyses, which can influence subsequent DoE studies. The HAD workflow is designed to work iteratively, often using augmented designs. Following multiple DoE studies, models start to become capable of predicting the outcomes in the next DoE or potentially in subsequent lab-scale and pilot-scale batches. Once multiple rounds of DoE studies are completed and assembled into large, internally consistent data tables, the depth and breadth of knowledge of chemical processes are unprecedented, which opens up broad possibilities in the modeling space (66, 67). These data tables become a cornerstone upon which the remaining process development and parameter variation studies are built.

Workups and Separations

The use of solid-state scavengers, such as activated carbons and other agents that coordinate with metals, to selectively remove undesired contaminants that are often present in APIs and synthetic intermediates is a well-established practice (68). Process streams can be forced to pass through premanufactured cartridges that are loaded with scavengers, or alternatively, post-treatment process streams can be filtered if loose scavengers are used. With either approach, removing solid scavengers after use is rather straightforward, whereas solution-type scavengers often require more cumbersome operations like extractions and phase splits.

Several different workflows are available if one wishes to identify the best-performing adsorbents, and the screening protocol is considered the most direct and efficient (69, 70). In some cases, a HT method of screening chelating agents for removing palladium from a process stream was performed using a combinatorial experimental and theoretical approach to maximize the number of candidate agents (71).

Lewen et al. (72) describe a semiautomated HT screening workflow applied to color, synthetic impurity, and residual metal removal from organic process streams. A typical array of process streams are introduced to various scavengers, agitated overnight, and filtered. The filtrate is analyzed with HPLC to determine recovery, and any combination of the following analytics are employed to further analyze the filtrate: (*a*) color determination with UV-Vis or visual observation methods, (*b*) quantitation of levels of remaining organic impurities using HPLC, and (*c*) elemental analysis to quantitate remaining trace metals with inductively coupled plasma-optical emission spectroscopy or portable X-ray fluorescence (72).

After the best-performing scavengers are identified through the screening workflow, further process development is still necessary to define critical process parameters, including but not limited to solvent selection, process stream concentration, scavenger loading, and treatment temperature. Such optimization efforts of scavenger-mediated removal can be performed using more focused automated or semiautomated approaches.

Selekman et al. (73) reported on a systematic yet flexible approach using DoE, laboratory automation, and parallel experimentation to quickly optimize liquid-liquid extractions. In addition to maximizing removal of undesired reaction stream components, this novel HT extraction (HTEx) platform has the potential to broadly impact development by improving process greenness, process mass intensity, cycle time, and ease of operation. HTEx allows for large arrays of extractions to be performed in parallel to identify optimal conditions while considering operational limitations.

This versatile approach involves two steps. First, similar to reaction development and optimization, the optimal discrete or class variables (e.g., solvent, scavenger, base, acid) are identified based on extraction efficiency and operational considerations (e.g., settling time, emulsion risk, phase

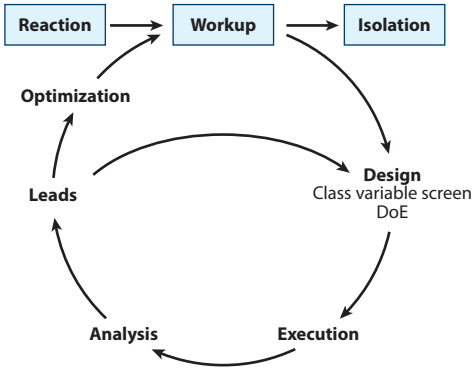


Figure 12

The high-throughput extraction (HTEx) approach. Abbreviation: DoE, design of experiments.

split quality, rag layer presence). Second, the extraction process is optimized with respect to continuous variables (e.g., organic/aqueous ratio, pH, volume, temperature, time). These two steps of the HTEx approach can be performed iteratively to ultimately achieve process optimization (**Figure 12**).

One example highlights how HTEx was leveraged to identify optimal conditions for simultaneously removing residual *N,N*-diisopropylethylamine (DIPEA) from a reaction stream while hydrolyzing a genotoxic impurity (GTI). Although an initial workup procedure had been shown to be effective for removing the GTI and the DIPEA to specification, the phase split quality following the initial wash was inconsistent and operationally intensive, with a moderate risk of emulsion and significant rag layer formation. An iterative HTEx study found that a potassium benzoate solution successfully removed the GTI and excess DIPEA from the reaction stream and afforded superior phase split quality, minimal rag layer, and quick settling time to improve the process. This new workup procedure was successfully performed on a pilot scale (~50 kg), resulting in a 25% reduction in waste generation and cycle time. In addition, this parallel experimentation strategy, in contrast to conventional, large-scale experimentation performed in series, consumed 2,500% less material and required 600% less experimental time, ultimately showcasing the impact of the HTEx approach (73).

Crystallization Development

The evolution of HT and automated technologies continues to benefit scientists and engineers in designing and developing robust crystallization processes for isolating chemical or synthetic intermediates, as well as APIs.

Solid form screening. Solid form screening on APIs is a routine and, to a large extent, mandatory activity in pharmaceutical development (74). Polymorphs, salt forms, and cocrystal forms are thoroughly searched by experimenting with different solvents, counterions, and coformers, often in combinations (75). These screens can be performed manually and one at a time; however, the large number of trials makes it common practice to incorporate automation and parallel experimentation techniques wherever possible.

One well-known pharmaceutical compound that experienced significant form challenges was ritonavir, which eventually led to the widespread acceptance of solid form screening on APIs

as a common practice in the pharmaceutical industry (76). The compound itself was screened by TransForm Pharmaceuticals, Inc. During one set of HT screening involving ~2,000 experiments, a total of 5 forms were found, more than double the number of forms known prior to the screening (77). This HT solid form screening example clearly demonstrated the power and effectiveness of such an approach. Other novel HT platforms have been developed to investigate polymorphism through other kinetic processes, such as polymer-induced heteronucleation (78).

Aaltonen et al. (79) have described some of the standard and prevalent HT API solid form screening workflows in the pharmaceutical industry. Solvents, counterions, and coformer incorporated in the screening process are limited to those considered pharmaceutically acceptable, and the primary analytics, such as powder X-ray diffraction (PXRD) and Raman spectroscopy, are used to characterize API physical properties (in particular, crystal form identity).

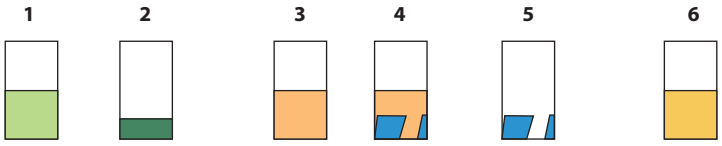
Highly automated (crystal) form analysis. As stated above, it is often imperative to crystallize a specific crystal form of an organic compound, which may possess preferred physical and biopharmaceutical properties (41, 80). Although a variety of approaches are used for crystal form screening, these screening-scale studies produce a few milligrams of material and require careful handling and manipulation of minimal solid material for PXRD analysis. In contrast, once a preferred crystal form is chosen for an API, or occasionally a synthetic intermediate, few reported HT methods exist for systematically interrogating process design space with respect to crystal form. To this end, Selekman et al. (81) developed a highly automated form analysis approach, which uses DoE and lab automation to generate arrays of parallel slurries under varying conditions followed by the sampling and acquisition of PXRD data, used to map crystal form space in an effort to mitigate the risk of forming undesired crystal forms on scale. This workflow has impacted process development across a variety of assets, advancing and expediting crystallization development, guiding control strategies for product isolation, identifying new crystal forms within the process design space, and derisking crystallization and coprocessing procedures.

Synthetic intermediate crystallization screening. For API solid form screening, the primary concern is always crystal form identity (82). In comparison, this is rarely the case for synthetic intermediates, which are pharmaceutical compounds that are isolated during the synthesis of an API. The reason for this is that crystal form identity is often irrelevant to a chemical process, owing to its eventual dissolution in the next reaction. However, significant efforts in chemical process development are often focused on identifying conditions to crystallize synthetic intermediates and/or purge challenging impurities from synthetic intermediates or APIs. Given the successful track record of HT solid form screening and an opportunity for similar HT technologies to address a significant gap in synthetic crystallization development, HT synthetic crystallization screening workflow can be designed specifically for synthetic intermediates (**Figure 13**).

Unchanged from the API solid form screens, the synthetic intermediate crystallization screens operated by removing mother liquor prior to analysis. The primary analytics, however, was altered to chromatography, as opposed to Raman or PXRD, as the best tool for determining yield and chemical purity of the solids afforded by the screens. The use of HPLC-compatible vials allowed for microscopy imaging with custom plates specifically machined to allow for light transmission through the bottom of the vials (**Figure 14**).

As with any miniaturized HT study, follow-up studies at the lab scale should always be carried out to confirm and optimize screening hits, and orthogonal crystallization methods, such as evaporation, antisolvent addition, and seeding, need to be incorporated as appropriate.

This synthetic intermediate crystallization screening workflow is a powerful tool by itself, and when strategically synergized with the solubility screening workflow, it has the capability to solve



1. Add compound (and coformers if any) as stock solutions.
2. Remove carrier solvents.
3. Add crystallization solvents.
4. Temperature cycle.
5. Remove solvents by wicking. Take pictures of solids.
6. Redissolve solids for HPLC analysis.

Figure 13

High-performance liquid chromatography (HPLC)-compatible synthetic intermediate crystallization screening workflow.

even the most difficult challenges. A recent example at BMS involved purging an impurity B from a synthetic intermediate A. Both compounds differed by only one methyl group, and solubility screens showed that A was more soluble than B in all solvents: These unfavorable solubility ratios explained why our colleagues experienced difficulties in the purging of B. Because both compounds could form salts with either acids or bases, purging B in its salt form was an option worth exploring. A comprehensive multi-plate crystallization screen (96 counterions versus 8 solvents) was carried out, and some purging was observed under certain conditions. From these hit conditions, (1S)-(+)-10-camphorsulfonic acid (CSA) was selected as the counterion for further study. The CSA salts of A and B were synthesized and their solubility data collected in many solvents, respectively. Surprisingly, the solubility ratios flipped to favorable (i.e., B-CSA salt is more soluble than A-CSA salt) in most solvents. After conducting lab-scale follow-up experiments in different solvents, BMS process scientists successfully developed an effective process that afforded excellent purging of B as its CSA salt.

BIOPHARMACEUTICAL AND BIOCHEMICAL PROCESS DEVELOPMENT

Some of the most recent adopters of HT automation are in the relatively new industries of biochemical and biopharmaceutical process development (83, 84). In upstream biopharmaceutical

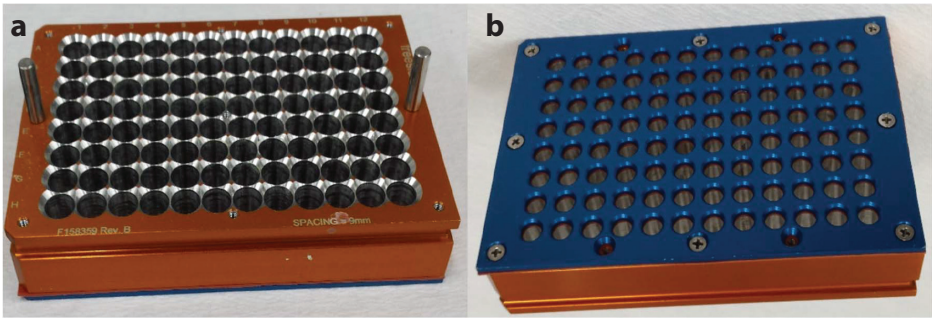


Figure 14

(*a*) Top and (*b*) bottom view of crystallization plate with holes drilled at bottom.

processing, cell culture conditions must be optimized to produce clinical material. To enable rapid development and optimization of these cell culture processes, miniaturization and parallel experimentation, in combination with various technologies, are used as platforms in HT process development (85). These automated platforms range from using microscale bioreactors (<1 ml in volume) (**http://www.iclickmedia.com/bioprocessors/system.htm**) up to mini- or intermediate-scale bioreactors (up to 4 L in volume) (**http://www.tapbiosystems.com/tap/cell_culture/ambr.htm**, **http://www.m2p-labs.com/**). One example of the microscale technology, the SimCell Micro Bio Reactor system, has the ability to run up to 1,260 experiments in parallel, with features such as automated filling of bioreactors, managing of temperature and mixing, monitoring of key parameters (pH, O_2, CO_2), and bioreactor sampling (**http://www.iclickmedia.com/bioprocessors/system.htm**). This HT technology was leveraged to carry out a DoE to explore five factors to optimize production of recombinant antibodies in Chinese hamster ovarian cells, the results of which agreed with data collected from conventional, bench-scale bioreactors (86). An example of an intermediate-scale automated bioreactor system is the Sartorius ambr^{TM} system, which allows for the parallel execution of 12 or 24 bioreactor experiments at 100–250-ml scale with automated liquid handling, sampling, and environmental control (**http://www.tapbiosystems.com/tap/cell_culture/ambr.htm**). The ambr^{TM} system has been shown to be effective in several applications for optimizing various biopharmaceutical production processes, including recombinant protein and monoclonal antibody (mAb) production, with results that successfully and reliably translate to large-scale bioreactors (87, 88). Bareither et al. (83) provide a comprehensive review on various HT platforms using scaled-down bioreactors for upstream biopharmaceutical process development.

In both the biopharmaceutical and biochemical industries, the separations space has seen the development of HT platforms leveraging automation and parallel experimentation, in conjunction with statistical DoE, to produce high-fidelity data sets (85). These data sets are subsequently used to optimize binding and elution conditions in process chromatography for maximizing purity and yield of the desired biochemical product (89–94). For example, one study used an automated liquid-handling system to simultaneously evaluate several factors, including various resins, sodium chloride concentrations, and pH, for defining operating boundaries and progressing toward the optimization ion-exchange chromatography conditions for purifying mAbs (95). A similar approach was used for isolating mAbs via hydrophobic interaction chromatography, where hundreds of elution conditions were screened to identify and rank the most promising operating conditions (96). Because solubility data of a protein of interest are critical to process design, automated HT technologies have also been leveraged to collect large experimental solubility data sets in parallel, helping define the feasible operating ranges for chromatographic separations (96, 97).

In addition, HT techniques combined with thermodynamic modeling have been shown to be useful for predicting protein adsorption, providing a foundation for chromatographic process design (98). The emergence of membrane chromatography for isolation of biopharmaceuticals and therapeutic proteins as an alternative to conventional column chromatography has spawned the development of another area of HT process development (99). Scalability of miniaturized HT platforms has been demonstrated in various systems for isolating therapeutic proteins, offering a means to efficiently develop and optimize membrane chromatography operations (96, 100–102).

CONCLUSION

Through the preceding examples and descriptions of HT automation applied to the generation of process knowledge, we hope to have illustrated the value proposition these platform technologies provide as well as the innate versatility and broad applicability they offer across the entire range of

process development activities. By its nature, this modality of miniature, parallel experimentation does have limitations with regard to scale-dependent variables such as mass transfer, stirring, heat flux, and other factors, which often play a pronounced role in determining the outcome of a chemical or biochemical process. Despite this, the high-quality, internally consistent data generated from automated, parallel experimentation provides invaluable knowledge of feasible operating space that forms the basis for developing a robust process. Moreover, HT automation enables a range of activities from discovery of new areas of chemical reactivity to construction of models and simulations. To this end, research-and-design scientists and engineers are using HT technology and predictive modeling capabilities synergistically, along with cutting-edge analytical technologies, to rapidly develop robust manufacturing processes.

Applications for the pharmaceutical industry specifically, such as those for understanding macromolecular processes (antibody drug conjugate production, antisense oligonucleotides, others), solid phase synthesis, biocatalysis, flow chemistry, and other modalities and technologies, will certainly be adapted and incorporated into these platforms, thereby driving innovation as these challenges arise. Like the pharmaceutical industry, other industries will likely continue on a similar trajectory of increasing the applications of HT automation toward accelerating chemical process development and speed of products to market.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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