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Automated Equipment for High-Throughput Experimentation

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Abstract: Parallel automated equipment in compound handling has emerged from solely liquid transfer systems to integrated tools for sample preparation, chemical synthesis, work-up, purification, analysis, and finally screening. Nowadays, liquids as well as solids can be handled on the very same robotic equipment. Selected examples from the field of organic synthesis, catalysis, and material science show the wide range of applications for high-throughput experimentation. The possibilities for process optimization and process development within the parallel approach are emphasized.

Keywords: Automation · High-throughput experimentation · Parallel chemistry · Process development

Introduction

The field of high-throughput experimentation has recently emerged as a promising technology to accelerate research in biotechnology, the pharmaceutical industry as well as in the chemical industry and materials science. Contrary to its view as a modern topic, the first examples date back more than a hundred years; the first scientist to apply parallel experimentation was Thomas A. Edison [1][2]. In his efforts to find a suitable filament for electric bulbs, he tested more than 1600 different materials before finding carbonized cotton threads as the material of choice. His scientific work is written in more than 3000 notebooks with 280 pages each and provided results for almost 2500 granted patents. In 1912, the Italian chemist Giacomo Ciamician placed hundreds of flasks on the roof of the university of Bologna in search of a photoactive substance for a photochemical process (Fig. 1) [3].

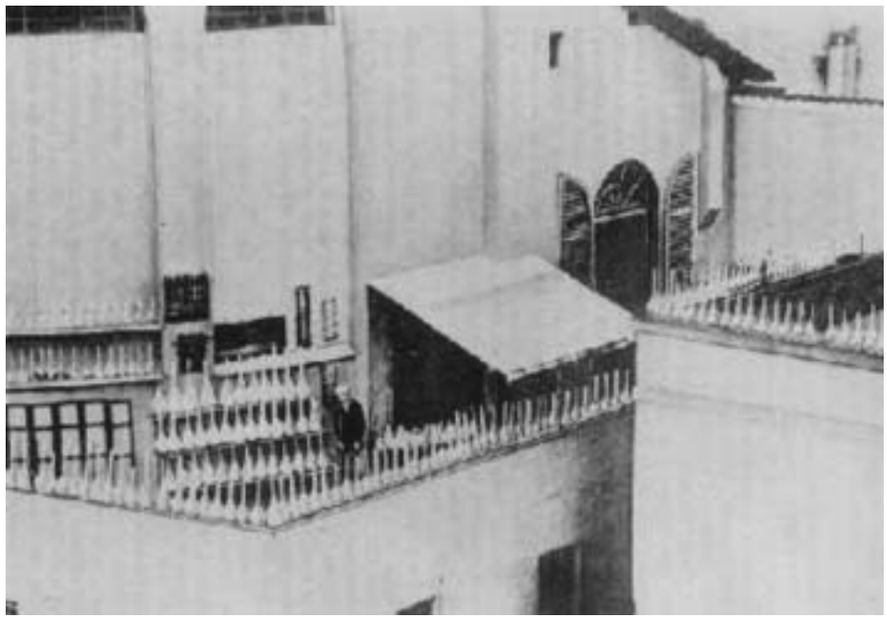

Fig. 1. Parallel set-up for photochemical processes in the early years of the 20th century placed on the roof of the University of Bologna.

A contemporary catalogue supplying chemical laboratory equipment including parallel extractors (Fig. 2a) and stirrers (Fig. 2b) even offered an autoclave suitable for twelve parallel pressurized reactions with up to 10bar (Fig. 2c) [4]. Almost one hundred years later the very same principle is still applied in state-of-the-art equipment for parallel reaction screening under elevated pressure (Fig. 3a, Symyx HiP-reactor and Fig. 3b, Chemspeed parallel pressure reactors) [5]. Although representing an impressive commitment, these first approaches towards parallel and combinatorial experimentation naturally were lacking any automation, so the analysis of the results was an extremely time-consuming task. Consequently, these new ideas were not adopted by other chemists, since they generated new bottlenecks.

After initial work in the 1960s [6][7], Joseph J. Hanak is now recognized as the pioneer of modern combinatorial research. He was the first author to report on the automated preparation and analysis/screening of libraries of inorganic materials in

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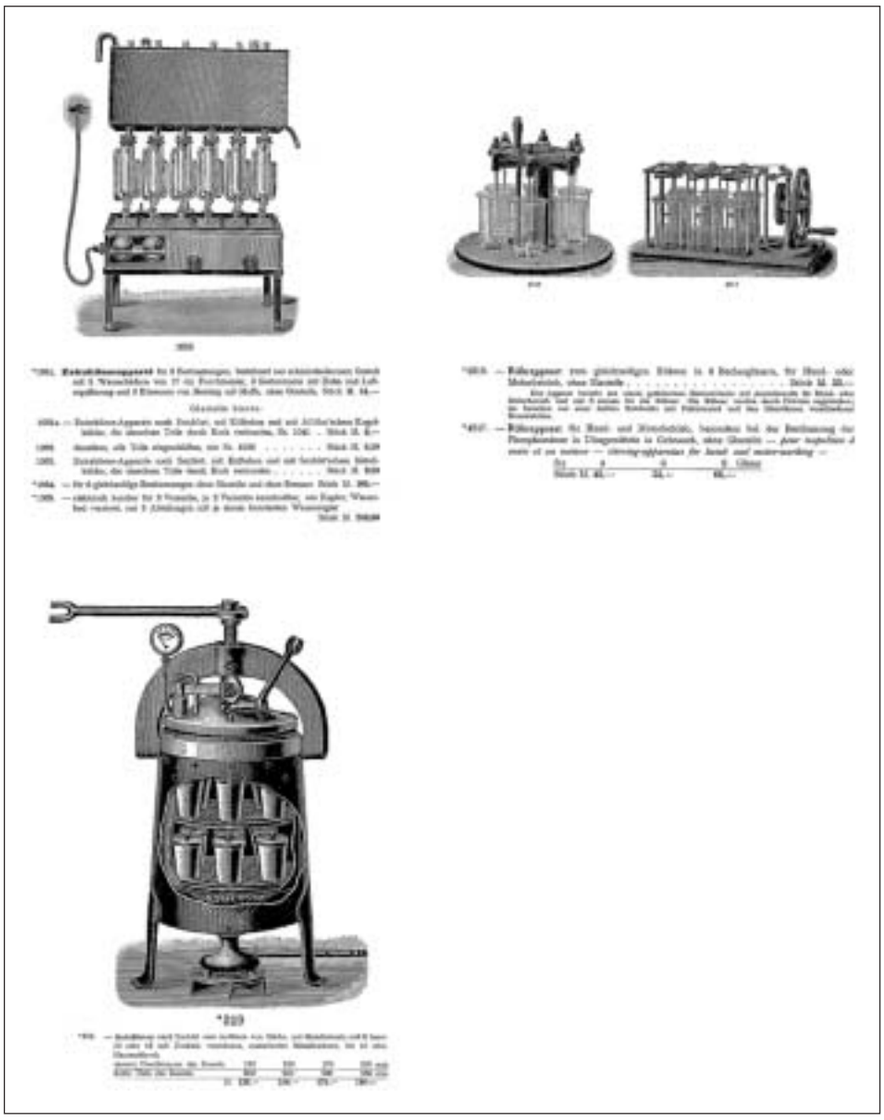


Fig. 2. Contemporary examples of chemical laboratories including parallel extractors (a), stirrers (b) and pressurized reactors (c).

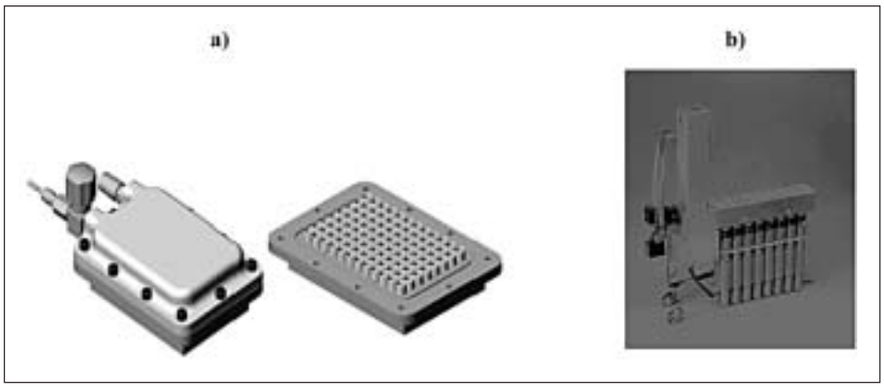


Fig. 3. State-of-the-art equipment for parallel reactions under elevated pressure: a) Symyx's HiP-reactors and b) Chemspeed's array of 16 parallel pressure reactors.

search of new superconductors [8][9]. Nevertheless, this new methodology did not become popular among the scientific community because of the general lack of computers at that time, which were essential for automated testing and data processing [10]. This changed at the end of the 1980s when impressive progress in laboratory automation equipment was achieved through the common availability of computers.

Today, every step of a specific workflow starting from sample preparation, control of reaction conditions, purification, analysis, and finally screening can be automated with suitable robotic equipment. In the following sections applications of such automation equipment in organic synthesis, materials science and catalysis are described.

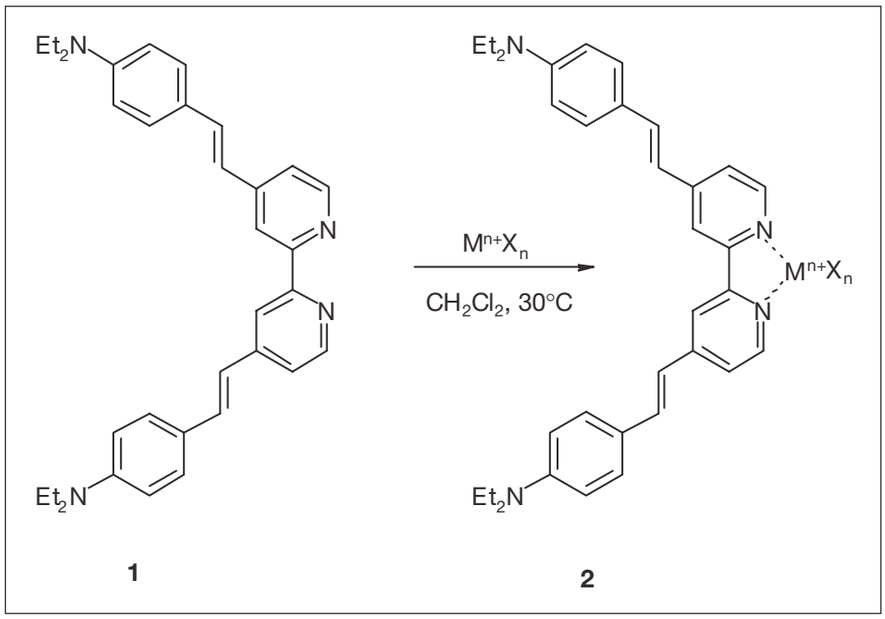
Materials Science

Combinatorial research in materials science is already a well-elaborated field, successful applications cover a very broad range from *e.g.* new phosphors and luminescent materials to high-temperature superconductors [10][11].

Metallo-bipyridine complexes of the general type **2** are a promising class of compounds with non-linear optical properties. They combine advantages like ease of synthesis with a high thermal stability of more than 300 °C and a high N(on) L(inear) O(ptics) efficiency. Synthesis of a library of complexes **2** was performed under inert conditions on a fully automated synthesis workstation (ASW1000, Scheme 1) [12].

Execution of the reaction involved slow addition of a solution of 4,4´-diethylaminostyryl-2,2´-bipyridine (**1**) ('DEASbpy') into the reactors that had been previously charged with different metal salts. After stirring the suspensions at 30 °C, the solutions were filtered from insoluble material and subsequently evaporated in parallel. The remaining material was taken up in CH_2Cl_2 and aliquots were transferred to a sample plate for UV/Vis analysis. The spectra of the derived complexes showed a bathochromic shift that correlates well with Lewis acidity of the metal salt and the NLO activity of the complex (Table 1).

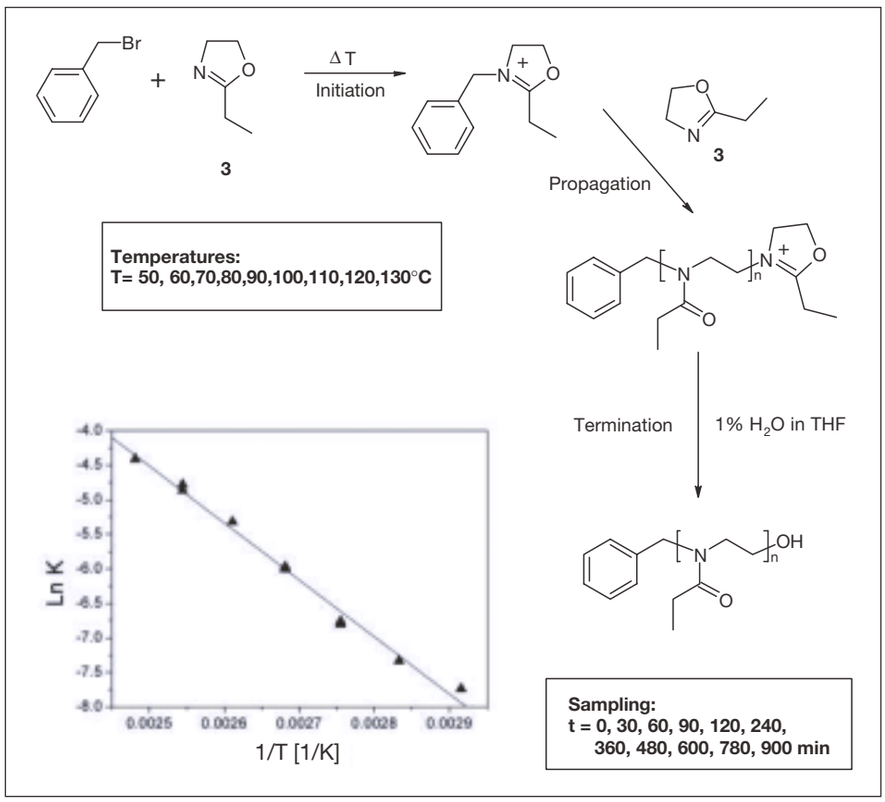
The optimization of reaction conditions is another powerful application for automated parallel synthesizers, since variations of all parameters such as reaction temperature, reactant and reagent ratios, solvents, catalysts *etc.* can be performed in parallel and subsequently analyzed online and/or offline. A recent example is the optimization of the living cationic polymerization of 2-ethyl-2-oxazoline (**3**) and the determination of its activation energy [13]



Scheme 1. Reaction scheme for a library of non-linear optical materials based on metallo-bipyridyl complexes.

Table 1.

Metal salt	λ_{max} (nm)
free ligand	397
$Zn(OAc)_2$	444
$FeCl_2$	466 - 595
$FeCl_3$	466 - 595
$AlCl_3$	488
$NdCl_3 . 6H_2O$	no complexation
VCl_3	no complexation
YCl_3	no complexation



Scheme 2. Optimization of a living cationic polymerization reaction at different temperatures. The Arrhenius plot is shown for the living cationic polymerization of 2-ethyl-2-oxazoline (**3**).

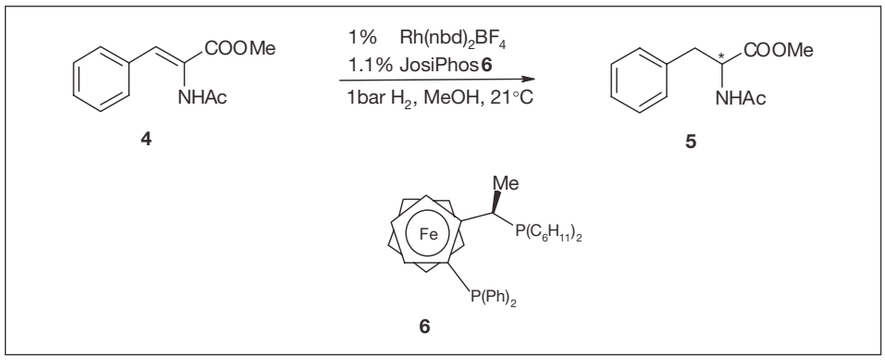
(Scheme 2). After (parallel) evaluation of the solvent of choice, sixteen polymerization reactions were screened in parallel at different temperatures. The temperature in each reactor was set and recorded individually. Aliquots for online GPC as well as offline GC were taken automatically at specific times during the reaction. From the different reaction rates at specific reaction temperatures the activation energy of the polymerization was determined (Scheme 2). The result was in accordance with the previously reported activation energy of the polymerization of the closely related 2-methyl-2-oxazoline (68.7 and 72.9kj/mol, respectively) [14]. The authors mentioned that parallel experimentation reduced the time for solvent and temperature optimizing and evaluation of the activation energy from 6–7 weeks down to 2–3 days.

Catalysis

Optimization of a catalyzed reaction is often a difficult task, since minor modifications can have major impact on conversion and/or selectivity [15]. Therefore, all parameters of catalytic reactions have to be optimized carefully; often the (repeated) robotic/automated dispensing of the reagents proves to be more accurate than manual handling of reagents.

The enantioselective hydrogenation of methyl 2-acetamido cinnamate (**4**) to N-acetyl phenylalanine methyl ester (**5**) was chosen as a test reaction to evaluate the transferability of this reaction from classical to an automated equipment (ASW2000P, Scheme 3) [16].

As catalyst, 1 mol-% of an *in-situ*-formed catalyst from $Rh(nbd)_2BF_4$ and the chiral biphosphine *JosiPhos* (**6**) was employed. Scaling effects had to be taken

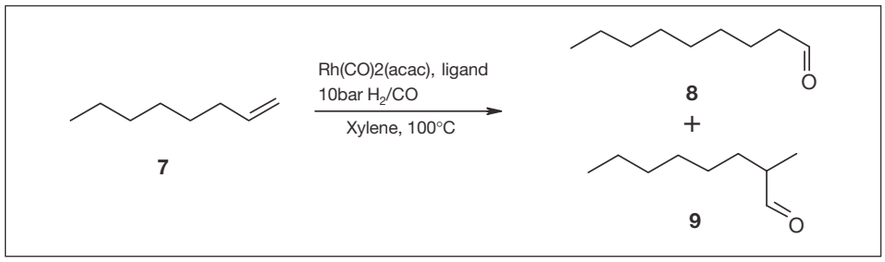


Scheme 3. The Rh-catalyzed hydrogenation of methyl 2-acetamido cinnamate (**4**). Josiphos **6** was used as the chiral biphosphine ligand.

Table 2.

Entry	Set-up	Reaction volume [ml]	Time [h]	Enantiomeric excess [%]	Standard deviation [%]
1	Classical	2.5	2	79.6	–
2[a]	ASW2000P[b]	2.5	2	78.1	1.3
3	Classical	5	2	87.2	–
4[a]	ASW2000P[b]	5	2	87.0	1.2
5	Classical	10	2	90.7	–
6[a]	ASW2000P[b]	10	2	91.1	1.4

[a]The results are averaged over eight reactions. [b]Chemspeed's automated synthesis workstation.



Scheme 4. Rhodium-catalyzed hydroformylation of 1-octene to the linear (desired) nonanal (**8**) and the branched iso-nonanal (**9**).

Table 3.

Entry	Phosphine ligand	Reaction time [min]	Experimental set-up	Agitation mode/ reactor type	Ratio [8:9]
1	1	60	ASW2000P	Vortex/ steel reactor + glass inserts	90:10
2	1	60	Classical	Aeration stirrer/ glass reactor	85:15
3	2	60	ASW2000P	Vortex/ steel reactor + glass inserts	75:25
4	2	60	Classical	Aeration stirrer/ glass reactor	72:28

into account both for the classical as well as the automated experiment. The results are shown in Table 2.

All hydrogenations were carried out under 1 bar of hydrogen and repeated seven times. The results between classical and automated experimentation are in complete alignment and show the accuracy of control of reaction conditions, which may influence, in particular, the enantiomeric excess.

In contrast to classical batch experiments, where shaking of the reaction solution is accomplished by magnetic or overhead stirring, agitation of the reactors on these automated workstations is accomplished by a built-in vortex shaker.

To assure the comparability of the results of automated parallel experimentation with classical experiments, the rhodium-catalyzed hydroformylation of 1-octene (**7**) to the linear *n*-nonanal (**8**) and the branched *iso*-nonanal (**9**) was chosen (Scheme 4). This reaction involves a gas–liquid phase-transfer and consequently is prone to mass transport effects influenced by differences in shaking efficiency. The results gained in collaboration with BASF, Ludwigshafen, together with results from classical set-up, are shown in Table 3 [17].

All reactions showed quantitative conversion of the starting material after the stated reaction time.

Comparison of the parallel *vs.* classical results (entry 1 *vs.* 2 and 3 *vs.* 4) show good correspondence between the classical and parallel set-up with a slightly enhanced *n*:*iso* ratio in the case of automated experimentation.

The progress in automation equipment strongly improves the throughput resulting in reduced time expenditure on discovery of new targets or materials. In order to avoid any bottleneck that would diminish this time gain, the other steps of the overall research process, optimization and process development, have to be accelerated in a comparable manner to finally achieve a reduced time-to-market.

Process Development

Process optimization is traditionally accomplished by first determining the parameters that influence the outcome of a certain reaction or process followed by optimizing the reaction towards the desired response (yield, turn-over, time *etc.*). It is a fact that for complex reactions with several reagents, side products and interdependent parameters, process optimization is a tedious and demanding task.

Contrary to the classical approach, optimization with the aid of statistical programs

is done by performing given experiments while changing several parameters within predefined boundaries *simultaneously*. The results are then analyzed by statistical means and represented in plots showing areas with optimum regions regarding the target for different reaction variables.

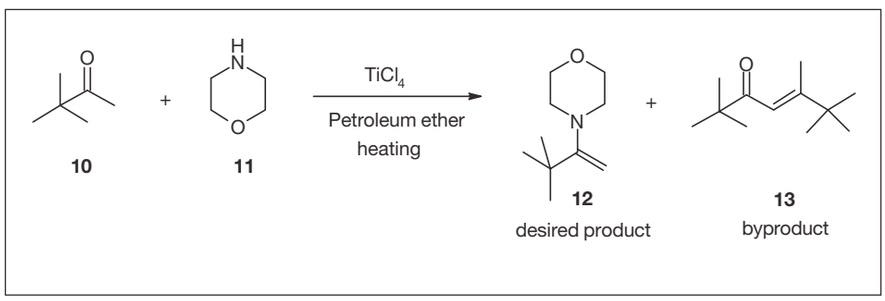
The combination of high-throughput experimentation with DoE (design of experiments) therefore offers a very efficient way to accelerate the entire research process with reduced total costs.

An application of DoE on our automated synthesis workstations is the optimization of the $TiCl_4$-mediated condensation of pinacolone (**10**) and morpholine (**11**) to the enamine **12** [18]. The major side product in this reaction is ketone **13** arising from homo-condensation of pinacolone (**10**) (Scheme 5).

The influence of temperature, the ratio pinacolone (**10**)/morpholine (**11**) and pinacolone (**10**)/titanium tetrachloride (the 'factors') towards the yield of product and byproduct ('responses') were investigated. For this purpose, *MODDE 6.0* DoE software from *Umetrics* was employed. The results from parallel experimentation on Chemspeed's ASW2000 were then compared to the results of the classical experiments reported in the literature [19]. They proved to be in complete alignment. Execution of the experiments was analogous to the classical approach; preparation of a $TiCl_4$–morpholine complex at 0 °C followed by addition of pinacolone (**10**) and heating the reaction mixture to the specific temperature. The results from parallel experimentation on the ASW2000 were then compared to the classical experiments and are shown in Fig. 4a and 4b.

Contour plots show areas of experimental parameters with optimized yields of product **12** or minimized yield of byproduct **13** (Fig. 5). As can be seen, increasing ratio $TiCl_4$:**10** increases both the yield of product and byproduct; the optimum ratio between product and byproduct however can be calculated from the data.

A recent publication describes the use of the very same equipment (ASW2000) for crystallization studies at *DSM* [20]. The aim of this work was to find optimized conditions for the diastereomeric crystalliza-



Scheme 5. $TiCl_4$-mediated condensation of pinacolone (**10**) and morpholine (**11**) to the enamine **12**.

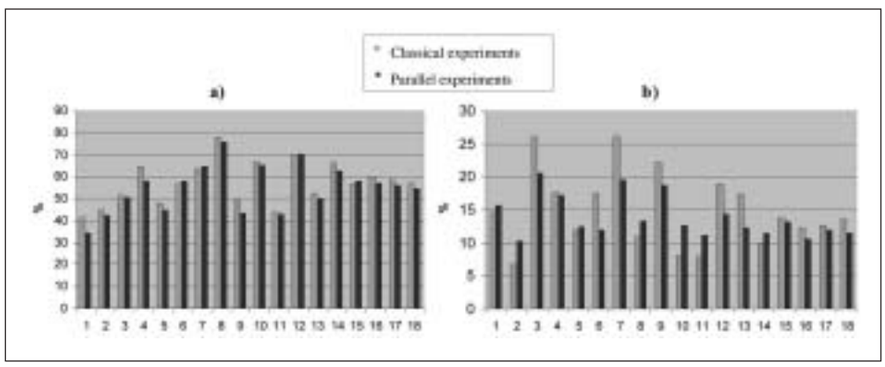


Fig. 4. Comparison of yield of products (a) and byproducts (b) from classical and parallel experiments.

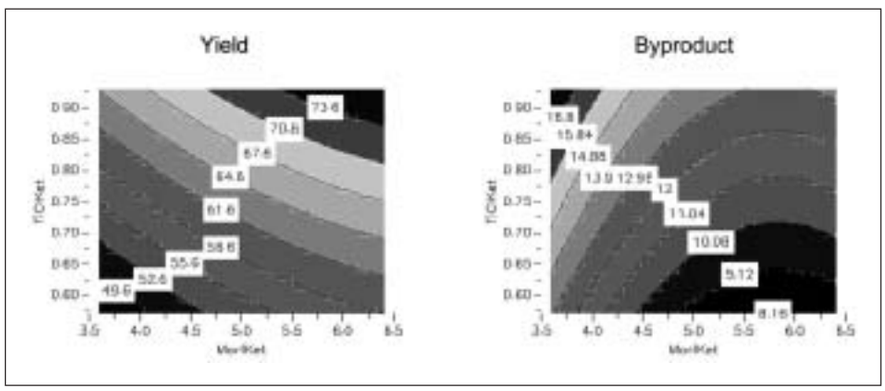


Fig. 5. Contour plots showing areas of optimized yields of products (left) and minimized yields of byproducts (right).

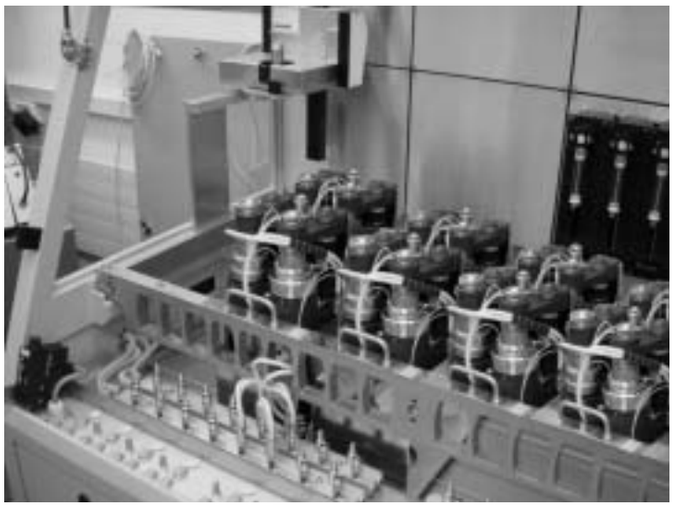


Fig. 6. Chemspeed Accelerator Process Development Station with individually controlled and operated tank-reactors. Up to 20 modules, each equipped with two independent tank-reactors, can be placed on an automated station including liquid and solid dispensing tools resulting in a powerful environment for process development tasks.

tion of racemic amino acids with enantiopure resolving agents ('Dutch resolution'). Online HPLC enabled completely automated determination of solubility phase diagrams of the resolutions.

Summary and Outlook

As shown in the previous sections, parallel synthesis has not only been successfully applied to medicinal and pharmaceutical chemistry. Materials science is one of a growing field of uses for automation and parallel equipment. In areas such as analytics, fragrances and flavors, crop science, and healthcare, chemists are becoming more and more aware of the possibility of accelerating the discovery process and general workflow by the use of non-traditional methods. Applying parallel methods in the discovery process increases the output of molecules and materials tremendously. It is therefore essential to handle the subsequent steps such as optimization, process development and scale-up by parallel methods as well. In many cases, these steps are still performed in a traditional way using the one-experiment-at-a-time approach. Companies can no longer afford the costs and time delays associated with traditional process development. A new product from Chemspeed aims at exactly this target: the Accelerator PXT100 Process Development Workstation focuses on the parallel implementation of optimization and scale-up applications (Fig. 6). This may enable laboratories to cover each single step of the value chain (from product discovery to process development) with modern high-throughput experimentation equipment.

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