New methods in combinatorial chemistry—robotics and parallel synthesis
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Technological advances in the automation of parallel synthesis are following the model set by the semiconductor industry: miniaturization, increasing speed, lower costs. Recent work includes preparation of high-density reaction blocks, development of ink-jet dispensing to polypropylene sheets and synthesis inside customized microchips.

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Introduction
During the past five years a variety of technologies have been developed that enable parallel synthesis of libraries of individual peptides, nucleic acids and small molecules. Libraries of up to 11 200 compounds have been published [1]. Ontogen Corporation (San Diego, CA) has synthesized 100 000 individual compounds by parallel synthesis (Adnan Mjalli, personal communication). Most of these libraries were prepared by synthesis on solid phase. Each solid phase technology balances the numbers of compounds that can be synthesized per unit time against the quantity of compound that can be synthesized and the range of reaction conditions that can be accessed. These technologies may be classified according to the type of solid-phase substrate that is utilized for synthesis, the means of introduction and removal of reagents, and the design of reaction chambers. Typically, a polymeric resin with high internal surface area is utilized as a substrate for solid-phase synthesis. In this case the resin may be enclosed in a fine mesh tea-bag or placed in a reaction chamber with a fritted bottom. Alternatively one may utilize a solid polymeric pin or a flat polymeric sheet as a solid-phase substrate. In closed reactor systems a small number of glass reaction chambers are permanently plumbed to a series of liquid and vacuum lines for the introduction of and removal of reagents. In reaction block systems an array of glass or polymeric reaction chambers is held inside a metal or TeflonTM block. Typically the reaction blocks are sealed with a septa material and reagents are introduced into the reaction chambers via pipetting. In another approach one may utilize a microchip to synthesize large libraries of compounds in very small quantities. Lindsey [2] provides an excellent review of automated synthesis prior to 1992; in this review, we discuss progress made since then with the techniques mentioned above.

Closed reactors
Closed reactor systems are distinguished by one or more fixed reactors that are connected to fluid and vacuum lines by a set of fixed connections and valves. These systems typically support solid-phase synthesis and offer a wide range of reaction conditions with compound synthesis of up to 50 millimoles. Automated closed reactor systems are desirable because they use glass reaction chambers, support the widest range of temperatures and maintain a true inert atmosphere. They are deficient in that they can only produce a few (1–48) compounds at a time. The advances described below are significant because they increase the number of compounds that can be produced and allow selection from a large number of reagents.

The first automated systems of this type were developed for automated peptide synthesis. In order to support nonoligomeric and nonpeptide chemistries, these systems have evolved to provide more versatility in temperature control and reagent introduction. For example, the Symphony/Multiplex system (Rainin Instrument, Emeryville, CA) provides 12 reaction vessels that can hold up to 400 mg of resin (5 micromoles–0.4 millimoles). Although peptide synthesizers such as the Symphony are being modified to synthesize small molecule libraries, they may not offer the flexibility of devices designed exclusively for this purpose.

Argonaut Technologies (San Carlos, CA; http://www.argotech.com) sells the 24 chamber NAUTILUS system (discussed in [3*]). This device provides three banks of 8×15 ml glass reaction chambers. The reaction chambers rotate through an arc of 200 degrees to provide agitation for solid-phase synthesis. Up to 176 reagents are available for use and are introduced into the reaction chambers through glass valves. Individual temperature control of the chambers from −40°C to +150°C is provided. Products may be isolated from any of the 24 reaction chambers and delivered to any position on an integrated fraction collector. The NAUTILUS represents the current state of the art in closed reactor systems. However, it is a complicated and expensive device and can only produce 24 compounds in one experiment.

Chiron (Emeryville, CA) has built a semiclosed reactor system with 48 glass reactors (discussed in [4]). Each reactor is plumbed to a vacuum and to a nitrogen line for solvent removal and agitation respectively. In this design, a robotic arm delivers resin and reagents into each reactor.
Nitrogen bubbling is used to provide an inert atmosphere as well as to facilitate agitation of the resin. However, the system is not a fully closed design since the reactors are open to the atmosphere. Although the open reactor design restricts the reaction conditions that can be achieved it also simplifies the design of the device and allows more compounds to be produced in a given experiment than other closed reactor instruments.

Selectide (Tucson, AZ) has built a library synthesizer [5] which uses resin distribution by sedimentation in a reactor assembly with 20 compartments. Sedimentation is one of the simplest and most accurate means to achieve uniform distribution of resin. The biggest limitation is that the number of reaction chambers cannot be modified without redesigning the instrument.

Reaction block systems
Reaction block systems are the most popular mechanism for parallel synthesis. Reasons for this popularity include: a lower cost than closed reactor systems, a greater number of compounds produced per unit time, an 8×12 array format, a block can be conveniently moved among a set of reaction chambers which matches the microtiter plate format and assembly line processing allows production of up to 1000 compounds per synthesizer per day. The instrument is not commercially available.

Bohdan Automation (Mundelein, IL) sells a reaction block which houses an array of up to 48 glass reaction chambers with a total volume of approximately 4 ml [8]. As in the Ontogen system the reaction block is constructed of aluminum. The advantages of aluminum are that it is inexpensive, easy to machine to high tolerances and has good heat conductivity. In the Bohdan reaction block the glass reaction chambers rest on spring-loaded supports in order to provide a consistent seal against an (optional) top septum. A novel mechanical valve assembly is used to seal the bottom of the reaction chambers. An (optional) system of reflux condensers may be fixed to the top of the block. Compounds may be cleaved from the reaction block into an array of test tubes. A variety of workstations are available for agitation, heating/cooling, cleavage of product from solid phase, and reagent preparation. Each reaction block contains a fairly complex set of valves and valve-actuators.

CSPS (San Diego, CA; http://www.5z.com/csp) has constructed a reaction block from Teflon™ using an array of 42×3 ml polypropylene syringes as reactors. The advantage of polypropylene syringes is that they are commercially available, inexpensive and resistant to many (but not all) common reagents. The system was integrated in several laboratories with various types of pipetting stations, creating a semiautomatic parallel synthesis system.

Diversomer Technologies (Ann Arbor, MI; http://www.diversomer.com) sells a reaction block which provides an array of 40 5 ml gas-dispersion tubes enclosed in a plastic housing [9]. An (optional) top septum is available. An integrated system of reflux condensers is built into the reaction block. The block must be manually disassembled in order to remove solvents from the reaction chambers. A variety of pipetting and agitation workstations are available. The instrument is best for manual use by a single chemist or for semiautomated use in the production of smaller libraries.

Advanced Chemtech (Louisville, KY; http://www.peptide.com) manufactures a 96×2 ml well TeflonTM reaction block [10]. Heating and cooling are available although there is no provision for internal pressurization or reflux condensing. A set of four septa may be used to seal the top of the reaction block. A pipetting workstation with integrated agitation is required to utilize the reaction blocks. Products are collected after cleavage from solid phase resin into an array of small beakers. The reaction block is best left on the workstation for the entire duration of the reaction. Because the reaction block dimensions are larger than a standard microtiter plate the reaction products must be reformatted from beakers into a
syringes placed on the platform of a pipetting station. The reaction block of 40 flitted 10ml (or 60x5ml) glass synthesizer (Synthesis Robot) which is built around a MultiSynTech (Bochum, Germany) is selling the 'SYRO' and product storage.

Microtiter plate or array of microtubes for solvent removal

MultiSynTech (Bochum, Germany) is selling the ‘SYRO’ synthesizer (Synthesis Robot) which is built around a reaction block of 40 fritted 10ml (or 60x5ml) glass syringes placed on the platform of a pipetting station. The unique feature of the block is a magnetically suspended stirring ‘flea’ in each syringe. Experience from the use of this synthesizer was recently reported [11].

Charybdis Technologies (San Diego, CA, http://www.charybtech.com) provides a semiautomated workstation this synthesizer was recently reported [11].

Charybdis Technologies (San Diego, CA, http://www.charybtech.com) provides a semiautomated workstation which utilizes commercial 2 ml microtiter filterplates, a commercial robot pipettor and a proprietary housing for the filterplate. The use of filterplates for solid-phase synthesis was developed by Sphinx [12] utilizing a manual apparatus. The device is reasonably inexpensive for a room temperature synthesizer and includes software for compound structure management and instrument control. The chemist must manually disassemble the reaction block in order to remove solvents from the reaction chambers.

Synthesis in syringes

Selectide (Tucson, AZ) has constructed a synthesizer based upon the use of plastic syringes equipped with frits, commercially available from CSPS (San Diego, CA) as reaction vessels for both peptide and nonpeptide solid-phase synthesis. In this technique, the syringes ‘pull’ reagents up from reservoirs into the closed reaction chamber provided by the syringe barrel. One compound is produced in each syringe. The movement of the syringes is accomplished using a robotic hand. An automatic synthesizer utilizing syringes for continuous (non-batch) operation was built recently [discussed in (13)]. Although the synthesis throughput of the instrument is slower than many of the reaction block designs reviewed here, the use of a robotic hand allows the system to operate unattended for longer periods of time.

Synthesis in tea-bags

An alternative to parallel synthesis is the ‘split-pool’ synthetic method. In this method a pool of resin beads is split into a set of reaction chambers, a reaction step is carried out in each reaction chamber, and the resin is then mixed back into a common pool. The iterative application of these steps can be used to generate large mixtures, in which each bead of resin contains a single compound. For a recent review of this technique see Lam et al. [14].

Houghten Pharmaceuticals (now Trega Biosciences) practices ‘tea-bag’ synthesis in which solid phase resin is placed in a set of mesh enclosures which can then be utilized in parallel synthesis [15]. In this approach each tea-bag is written with a unique code that allows identification of the tea-bag by visual inspection. Because the identity of each tea-bag is known, it is possible to utilize the split-pool technique to produce individual compounds in tea-bags rather than to produce mixtures of resin beads.

The split-pool method has advantages over other methods of parallel synthesis in that the number of distinct reaction chambers required is dramatically reduced. For example, in order to synthesize all peptide trimers one would require 8000 separate reaction chambers. Using the split-pool approach one would require only 20 reaction chambers. During synthesis each reaction chamber would hold 400 synthesis packets.

An automated synthesizer based on the tea-bag technique was constructed [16], and is commercially available (Spyder Instruments, San Diego, CA). This synthesizer utilizes the principle of ‘inclusion volume chemistry’—reaction vessels in the classical sense can be eliminated, since only the internal volume (0.4 ml per tea-bag) of the polymeric carrier is used for the reaction [17]. In this technique reagents are sprayed directly onto tea-bags using a pneumatically activated pump. The tea-bags are mounted on a centrifuge so that they can be spun dry when desired.

Synthesis in tea-bags with radio tags

A drawback of the Houhten tea-bag method is that the label of each tea-bag must be visually inspected and a human being must distribute each tea-bag to the correct reaction chamber at each synthetic step.

This problem has been overcome by a new technique independently developed by Ontogen and Irori (San Diego, CA) [18*,19]. In this technique a small microchip is placed on each tea-bag which carries a unique identification code. The code can be read at a distance by pulsing the microchip with radiofrequency. Sorting of the tea-bags can be automated. The capacity of the sorting machine built at Irori is approximately 10000 packets per night. At the end of the synthesis the structure of the synthesized compound in a particular tea-bag can be decoded from the radio tag. By using radio tags, the split-pool technique may be used as an efficient means of automated parallel synthesis.

Synthesis on polymeric pins

Chiron Technologies (Clayton, Australia) has commercialized a system for solid-phase synthesis on polymeric pins following work by Geysen et al. [20]. In this system an array of 96 pins is ‘dipped’ into a sequence of reagents distributed in microtiter plates. The variety of chemically functionalized surfaces available on polymeric pins has recently expanded greatly and is available commercially from Chiron Technologies. However, the cost of these polymeric pins is considerably higher than polystyrene-based resins. Surprisingly, little work has been reported in the automation of this technique. The advantage of using pins is that they are considerably easier
to work with than aliquots of resin. On the other hand, the surface area of pins is much smaller than the same physical quantity of resin, and thus, much smaller quantities of compound can be synthesized per gram of solid-phase synthesis support.

Synthesis on polymeric sheets
Recently scientists at Glaxo [21] reported the use of flat sheets of polymer for solid-phase synthesis. The 'ChemSheet' system uses polypropylene sheets with an arrangement of 2304 wells of 8μl. On the bottom of each well is the grafted polymer used for the synthesis. Since traditional means of solution delivery by pipetting fail for precise distribution of sub-microliter amounts of liquid, some form of ink jet handling is required. The first automated system of this kind was built at Glaxo around a solenoid ink jet valve from the Lee Valve Co. (Westbrook, CT). Arrays of ink jet valves can deliver the particular building blocks and reagents into all wells of a chemsheet in 10 seconds. At the end of the synthesis, the products can be used directly for binding assays, or they can be cleaved from the solid support and a biological assay can be performed directly in the well. Synthesis on the planar carriergs was pioneered by Frank [22] and was automated by Abimed (Langenfeld, Germany) [23]. The main advantages of this technique are the large number of compounds that can be produced and the lower costs of reagents. One challenge facing the practitioners of this technique is that all biological assays must be made on the planar carrier. In addition, one chemsheet must be synthesized for each biological assay. Another challenge is to demonstrate synthesis at reduced or elevated temperatures and in inert atmospheres.

Synthesis on a microchip
A massive parallel synthesis system is being developed by Orchid Biocomputers (Princeton, NJ). This system is based on microfabricated components, allowing pumping of reagents and building blocks inside a microchip without any moving parts. Liquid transport is achieved by application of an electric field and utilization of capillary forces. Prototypes of the synthetic chip which will allow parallel synthesis of 10.000 compounds from up to 200 building blocks are expected to be available by 1998 [24]. This technique promises to fulfill the dream of 'millions of compounds' per synthesis that was so exciting during the development of the split bead techniques, and at the same time avoid all the limitations of pooled libraries made using parallel synthesis. This technique also promises to greatly reduce costs. On the other hand, it will be very difficult to access the majority of chemical techniques utilized in the lab today on a chip, as well as replace the current bioassay techniques on a chip format so that the results of the chip synthesis can be screened.

Conclusions
The application of robotics to the automation of parallel synthesis is still in its infancy, but various practical applications have been described [25, 26]. At this moment, many techniques exist, and new techniques are added to the portfolio every day, however, exponential growth cannot be predicted. Darwinian selection will help narrow the field, and only those which will fit the need of multiple laboratories will survive. Closed reactors provide access to the broadest range of chemistry and are useful for the development of complicated chemistries—but they are expensive and produce relatively small numbers of compounds. Reaction blocks have proven successful and will probably become the basis for further development. They are less expensive and can produce larger numbers of compounds. On the other hand, they may not provide access to the same range of chemistry as closed reactor systems. Oddly, synthesis on pins remains manual, although this area seems amenable to automation. Synthesis in 'tea-bags' has been automated—but widespread adoption of this technique seems unlikely. Synthesis on polymeric sheets provides many challenges but with rapid evolution in technology for biological screening on high-density planar surfaces we may see a lot of activity in this field. The techniques utilizing radio tags is a relatively unproven technology (it is being practiced by two companies) and could be overtaken by improvements in parallel synthesis. Synthesis on 'chips', even though very attractive, will probably remain unrealistic in the average laboratory for several years. Hardin and Smitian [27] provide a good source of practical advice for labs wishing to adopt automation. A list of references dealing with automated synthesis is available on the Internet (http://www.5z.com).

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

The authors describe a comprehensive system for parallel synthesis, including detailed drawings of a reaction block and several processing workstations. New software for compound registration, bioassay, and mass spectrometry is discussed.


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The authors discuss the design and use of a pioneering reaction block design for parallel synthesis.


The authors describe the use of a radiofrequency tag system which may be used in place of chemical tags in 'mix and recombine' synthetic methods. See also [19].


An independent development of the radiofrequency tag technique (see [18]).


This is the first published description of high density parallel synthesis using inkjet technology.


Useful advice for those contemplating the use of robotic systems for parallel synthesis.