Article

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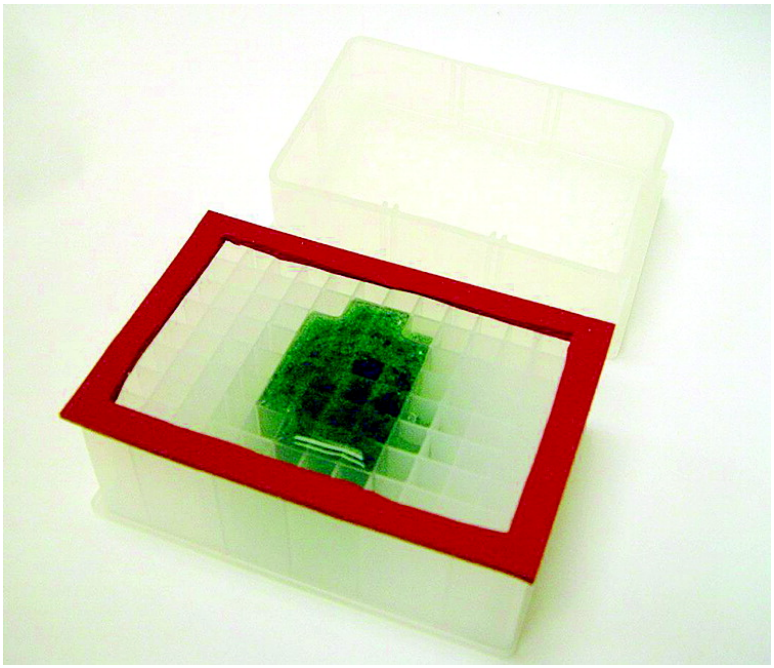

Simple Tools for Resin Distribution

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Two simple instruments allowing uniform distribution of resin beads for solid-phase synthesis are described. The first tool simplifies distribution of resin into microtiterplates. The second tool was designed for distribution of a variable amount of resin beads into any number of reaction vessels, and the technique is applicable to resin beads with a wide range of physical properties (density).

Introduction

Application of solid phase synthesis increases the productivity of organic chemists by simplification of the synthetic procedures. Orders of magnitude increase of productivity results from the parallelization of the synthetic process. Combinatorial chemistry simultaneously processes a multiplicity of reaction vessels, one example of which is synthesis in microtiterplates.[1] Our contribution to this area was introduction of a "surface suction" concept, simplifying processing of multiple microtiterplates and eliminating the need for filtration,[2,3] and "tilted centrifugation" achieving the same goal and allowing efficient automation.[2−5] Parallel solid-phase synthesis starts with resin distribution into reaction vessels. Successful synthesis in multiple microtiterplates depends on a uniform distribution of solid support into individual wells. Solutions for uniform distributions include pipetting isopycnic suspensions,[6] pipetting continuously stirred suspensions,[7] distribution of premanufactured pellets,[8] automatically weighing the dried support (see, e.g., http://www.bohdan.com/ba.html), volumetric distribution of solid support into an array of filled compartments (manual parallel, e.g., http://www.radleys.com, or automated individual, e.g., http://www.zinsser-analytic.com/48.asp), or sedimentation into a uniform arrangement of connected vessels.[9] These techniques have their advantages and drawbacks, the most significant drawback being the relative inflexibility in the distribution format or used solid support, or such desired flexibility achieved only at the cost of expensive automation.

We describe two simple techniques applicable with minimal investment in hardware. The first technique is useful for distribution of resin into the wells of a microtiterplate; the second was designed for distribution of variable (small) volumes of resin into any number of reaction vessels.

Results and discussion

Simple distribution of resin beads into microtiterplates does not require any sophisticated instrumentation. This technique replaces the distribution based on uniform sedimentation by the "exact volume distribution". The volume of slurry to be distributed into the wells of the microtiterplate is equal to the volume of all the wells to be filled. The simplest way to find out this volume is to fill all wells to the edge. The resin distribution is practically done the following way (see Figure 1): The resin suspended in a small amount of solvent is added into several wells of the microtiterplate (it can be just poured into the middle of the plate without paying any attention which wells the resin fills). The empty wells are then filled to the rim by the additional solvent, for example using a squeeze bottle. A gasket cut out of silicone rubber (1-mm thickness) mapping the outer edge of the microtiterplate is then placed on top of this plate, and the box with volume greater than the combined volume of all wells ("upper box", for example, trough made by Tomtech Inc., Hamden, CT, http://www.tomtec.com/Pages/CustomReservoir.html) is placed on top of the gasket. The assembly is

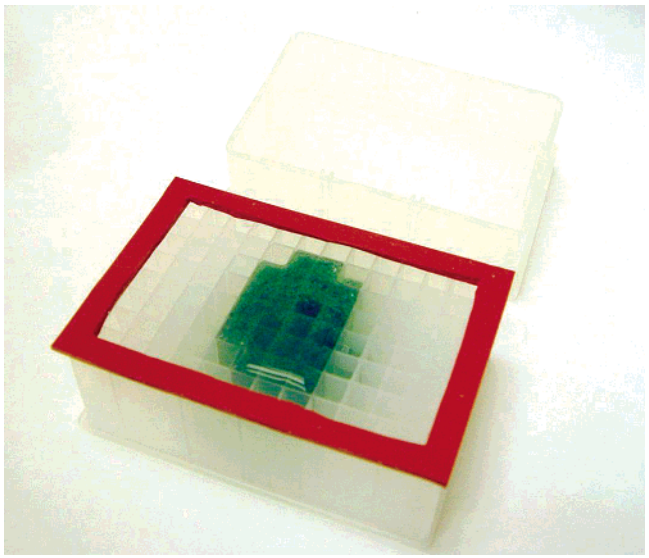

Figure 1. Resin suspension has been poured into the middle of the 96-well microtiterplate, and the gasket has been placed on top of the plate. The "upper box" (in the back) will now be placed on top of this plate.

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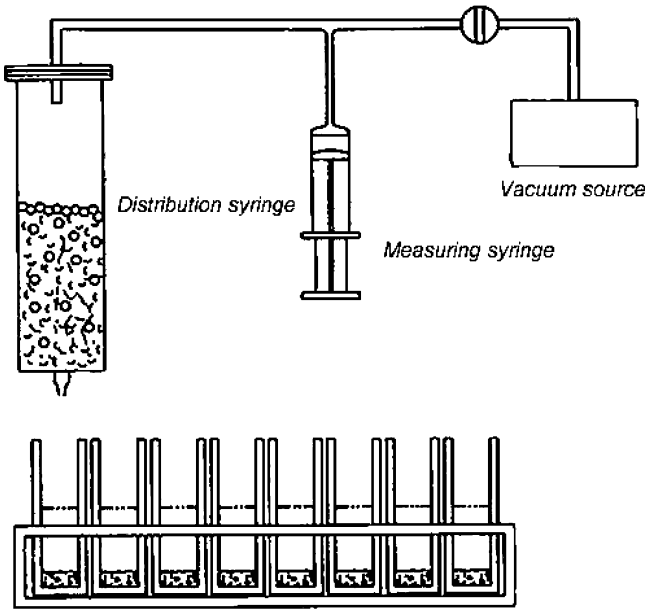

Figure 2. Scheme of the "universal" resin distributor.

then manually firmly pressed together, inverted, intensely shaken, and returned into the original position. After removal of the "upper box" and sedimentation of the resin, the excess of the solvent is removed by decantation or by a 96-well aspirator (assembly of 96 needles applying surface suction).[3] Distribution of resin beads into the filter plate is done the same way; however, the openings of the filter have to be blocked by pressing them into a sheet of silicone rubber (or foam polyethylene) supported by a solid rectangle of the same footprint as the rubber sheet. Obviously, this technique does not apply to the plates with "interwell" openings. In this case, the interface between the plate and upper distribution box has to be constructed, or the plate must be first heat-sealed with foil and the foil coverings of all wells has to be removed by sharp knife or punch, thus creating the plate with only well openings. This technique is applicable also for distribution into only fractions of the microtiterplate (half, third, quarter), but in this case, specialized "upper boxes" must be made.

Quantitative evaluation of this technique has shown CV of less than ±4% in distributing polystyrene-based and grafted synthetic supports with a wide variety of functionalizations, with size distributions from 35 to 200 μm. Distributions were performed in solvents selected with relationship to the resin: the only requirement is the prevention of formation of resin aggregates. Alcohols, dimethylformamide, or even water (for hydrophilic resins) were used successfully in hundreds of resin distributions.

The goal of distribution of various amounts of different resins into a range of vessels is more challenging; however, even this task can be solved with a simple apparatus. The principle of this technique is illustrated in Figure 2. Resin suspension is continuously stirred with a gentle stream of bubbles created by suction through a vertically positioned syringe ("distribution syringe"). The tubing connecting the source of the vacuum is branched before the valve, which can disconnect the vacuum source. The calibrated syringe ("measuring syringe") is attached to the branch. While the valve is opened and suspension is stirred, the measuring syringe is filled with a volume of air equal to the volume of the suspension to be distributed into the vessel. Then the source of vacuum is disconnected (valve is closed, solenoid is actuated, or tubing is pinched) and the measuring syringe volume is discharged to the system, expelling the volume of resin suspension equivalent to the volume of air in the measuring syringe into the container. A vacuum is then restored, and the measuring syringe is recharged with the volume of air for the next distribution.

This simple arrangement allows distribution of variable volumes (100 μL to several milliliters) into any type of reaction vessel. Very accurate distribution of even submilligram amounts of resin is possible by using high-suspension dilution. Typical CV of polystyrene-based solid support (distributed amount, 5 mg; particle size, 130 um) distribution is below ±5%. Higher accuracy is achieved by a two-step process, when ~85% of the resin is distributed in the first pass, the remaining 15% of the suspension is diluted to original volume, and the rest is distributed in the second pass. This arrangement allows for CV better than ±3%.

The process is very flexible: either the same amounts can be distributed, or every distribution can be individually defined. It is important to use a solvent with a high boiling point (dimethylformamide) to minimize evaporation during bubbling. Accurate distribution is difficult when the sedimentation of the resin is very fast and bubbling is not capable of keeping the resin uniformly suspended. Solvents with high surface tension, e.g. water, should be excluded. The last bubble created at the distribution syringe inlet/outlet at the moment of the discontinuation of evacuation may not detach from the opening, and the volume of this bubble may diminish the volume of suspension delivered by discharging the measuring syringe.

Figure 3a shows the simple tool with two disposable plastic syringes used for manual operation. One operator's hand disconnects the vacuum by pinching the flexible tubing and the other hand discharges the volume from the measuring syringe into the system. Figure 3b shows a semiautomatic device of the distributor. A motorized syringe (Cavro XL 3000, Tecan Systems, Inc., Maennedorf, Switzerland, http://www.cavro.com/) and solenoid valve (Bio-Chem Valve, Inc., Boonton, NJ, http://www.bio-chemvalve.com/) are driven by the Pocket PC (iPAQ 3670, http://www.hp.com/country/us/en/prodserv/handheld.html) program written in Visual Basic. This device is commercially available (http://www.torviq.com).

Experimental Section

Combinatorial Library of 172 800 Unnatural Peptides. Synthesis was performed in plastic syringes equipped with a frit. One gram of TentaGel resin (Advanced ChemTech, Louisville, KY, 130 um, 0.4 mmol/g) was deprotected by piperidine (2 + 10 min, 25% in DMF); washed by DMF (5×), DMF with 0.1 M HOBT, and 0.01% bromophenol blue; and suspended in 10 mL of dimethylformamide (DMF). The suspension was aspirated into a 20-mL syringe resin distributor described above. In 10 cycles of distribution, 0.8 mL of the suspension was distributed into 10 2-mL fritted syringes, the volume in the distribution syringe was adjusted

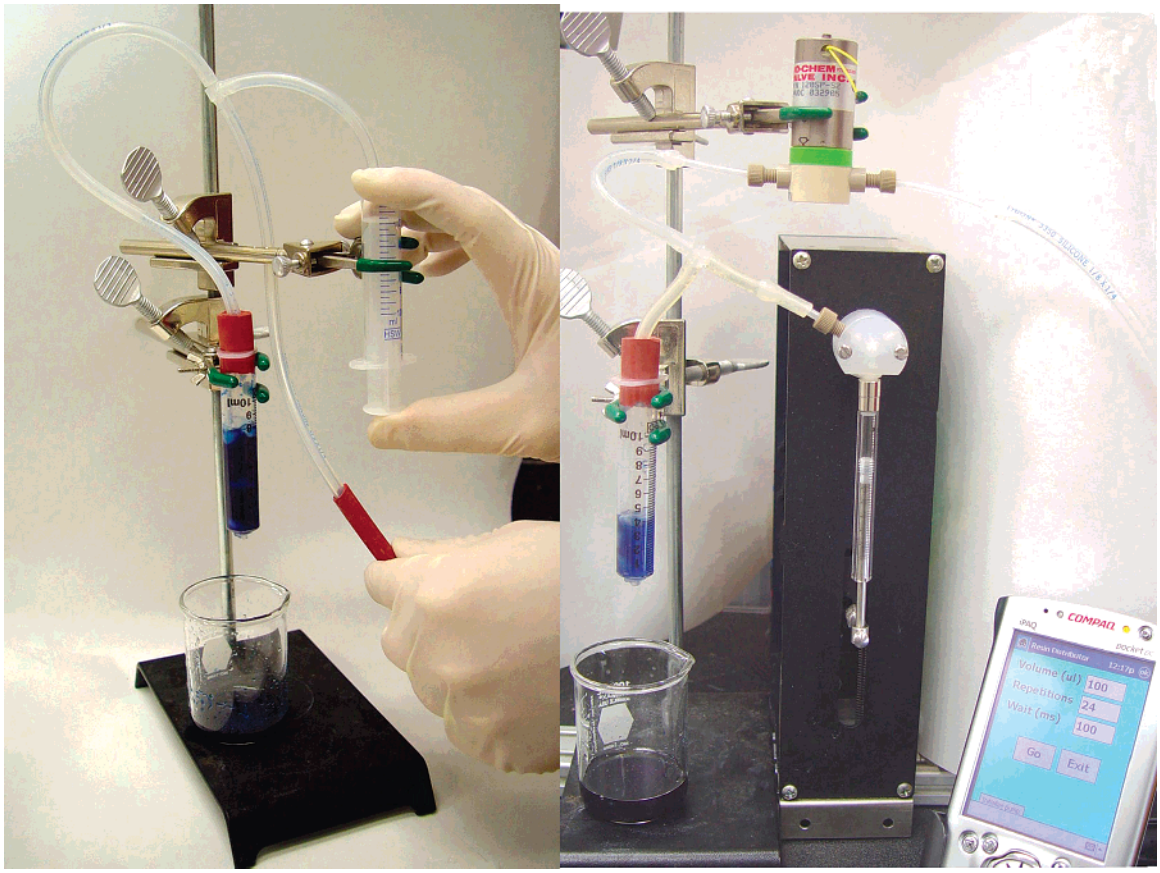

Figure 3. Manual (a, left) and semiautomatic (b, right) version of the universal resin distributor.

to 5 mL, and another 10 cycles of distribution were performed (0.5 mL each). Plungers were inserted into the syringes, and each syringe was charged with 0.6 mL of 0.3 M solution of Fmoc-protected amino acid in 0.3 M HOBT in DMF freshly premixed with 0.3 mL of 1 M DIC. Syringes were stoppered by inserting the needles into a rubber block and were placed on a shaker. After the disappearance of the blue coloration (10 to 40 min), the content of the syringes was expelled, the plungers were removed, and resin was transferred to one 20-mL plastic syringe in which washings (DMF 3×), deprotection (25% piperidine, 2 + 10 min), washing (DMF, 5×), and preparation for monitoring of the next coupling (DMF with 0.1 M HOBT and 0.01% bromophenol blue) were performed. Resin was suspended in DMF and transferred into the distributor for the next cycle of distributions. The process was repeated six times with distribution into various number of aliquots (see Table 1), depending on the number of amino acids coupled in that particular step. At the end, the library was deprotected by 95% TFA 5% water, washed extensively with water, and an aliquot was submitted to the binding test with streptavidin. Several positive beads were sequenced (ABI 477, Applied Biosystems, Foster City, CA, http://www.appliedbiosystems.com/) and (in addition to proving quality of the synthesis) novel binding motif for streptavidin binding was observed (to be published elsewhere).

Table 1. Amino Acids Used in the Construction of the Combinatorial Library

step 1	step 2	step 3	step 4	step 5	step 6
Gly	Gly	Gly	Gly	Gly	Gly
D-Arg	D-Lys	D-Arg	D-Lys	D-Arg	D-Lys
2-Nal	2-Nal	2-Nal	2-Nal	2-Nal	2-Nal
Tic	Tic	Tic	Tic	Tic	Tic
Tic(OH)	Tic(OH)	Asp	Glu	Asp	Glu
Asp	Glu	Pro	Pro	Pro	Pro
Pro	Pro				Phe
Cit	Cit				Val
Dab					Leu
His					Ala

Conclusion

The described simple personal chemistry tools do not match well with the 21st century high-tech trend. However, we decided to share our experience with a broader audience, since we found the technique of practical value in daily laboratory life.

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