# A New Approach to Automated Solid Phase Synthesis Based on Centrifugation of Tilted Plates

Michal Lebl, Ph.D., D. Sc., Trega Biosciences Inc. and Spyder Instruments Inc., 9880 Campus Point Drive, San Diego, CA 92121

**Abstract**

High throughput solid phase synthesis can be performed with application of the centrifugation based liquid removal. This technique uses readily available standard microtiterplates and eliminates filtration step. It is therefore applicable to simultaneous processing of unlimited number of reaction compartments. Its use is illustrated on the synthesis of an array of 380 tetrahydroisoquinolinones.

## Introduction

Combinatorial techniques (for reviews see e.g. [1]) require new methods for automation of synthetic processes. Solid phase synthesis is optimal for automation, since the complicating factor of unique behavior of different organic molecules is replaced by predictable behavior of the solid support. Instruments available on the market today are relatively complicated and expensive. The instrument that would be rather simple, therefore inexpensive, and would allow each chemist to synthesize 100-1000 compounds in a batch would be welcome by a number of medicinal chemists anxious to discover the next Zantac or Viagra. Such instrument would be used for deconvolution of active compound from biologically active mixtures, synthesis of arrays of compounds for general screening, or for compound optimization, so called "lead explosion".

Even though solid phase synthesis brought about the potential of relatively simple processing of large arrays of synthetic vessels, some problems in realization of machine capable of parallel processing of multitude of samples remain. One of the basic problems is parallel separation of liquid and solid phases. Commercial solid phase synthesizers utilize filtration as the principle for separation of solid and liquid phase (for reviews see e.g. [2]). Filtration can lead to significant complications, especially in the case of multiple synthesizers, since clogging of one vessel can result in overflowing of this particular vessel during the next solvent addition and distribution of the solid support from this vessel into neighboring ones. The principle of "surface suction" for removal of supernatant from the sedimented suspension of solid phase particles [3,4], which we have successfully used in our robotic synthesizer in which up to 72 deep-well microtiterplates can be processed simultaneously [3], is also limited. The surface suction removal of liquid phase is based on the fact, that the flat-end needle lowered against the surface of the liquid while the suction is applied through the needle, does not disturb the bulk of the liquid and only the surface layer of the liquid is removed. In this way, the needle can be lowered very close to the sedimented resin without removing any solid support particles. However, this technique still does not allow to process unlimited number of reaction vessels simultaneously - the number of processed vessels depend on the number of needles performing the suction.

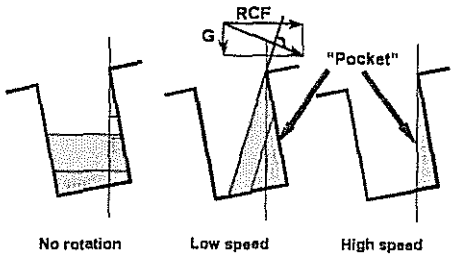


*Figure 1: Formation of the pocket in the well of a tilted plate during centrifugation (direction: left to right). The solid support is collected in the pocket, while the liquid is expelled from the well. The liquid surface angle is perpendicular to the resulting force vector of the relative centrifugal force (RCF) and gravity (G).*

## Results and discussion

We have found the simpler way for simultaneous processing of hundreds of reaction vessels. We call this new technique "tilted centrifugation". The principle of tilted centrifugation is shown in Figure 1.

Resin suspended in the tilted flask placed at the perimeter of the centrifugal plate and spun, will not remain at the bottom of the flask. As the surface of liquid supernatant will move, the solid support layer will move as well. If the speed of rotation is increased, the centrifugal force created by rotation (which depends on the radius of rotation and the speed) combines with gravitation and the resulting force causes

*continued*

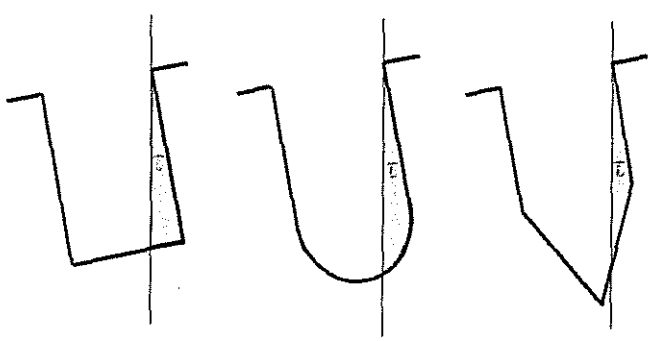

*Figure 2: Pocket formed by centrifugation in vessel of different geometry.*

*Michal Lebl, Ph.D., D.Sc., is Director of Robotics and Synthesis Automation at Trega Biosciences, Inc., San Diego, CA. He is also President, Spyder Instruments, San Diego, CA. He is a graduate of the Technical University of Chemical Technology, Prague (MS, 1974, Organic Chemistry), the Institute of Organic Chemistry and Biochemistry, Prague (Ph.D., 1978, Organic Chemistry), University of Arizona, Tucson (postdoc 1983, Solid Phase Chemistry) and the Institute of Organic Chemistry and Biochemistry, Prague (O.Sc., 1992, Organic Chemistry). Dr. Lebl has more than 200 publications and more than 30 patents to his credit.*

liquid surface to stabilize at the angle perpendicular to the resulting force vector. At the ratio of relative centrifugal force (RCF) to G of 3, the angle of the liquid surface will be about 61 degrees. If the speed is increased so that the ratio of these forces is more than 50, we will be getting close to the situation where RCF is infinity - therefore the liquid (and resin layer) angle will be close to 90 degrees. The pocket created by the tilt should allow only solid phase to remain in the pocket and all of the liquid should be expelled. The pocket can be created in the vessel of basically any shape (see Figure 2) - flat bottom, U bottom, or V bottom vessel, as well as in the array of vessels, e.g. in the commonly used microtiterplates.

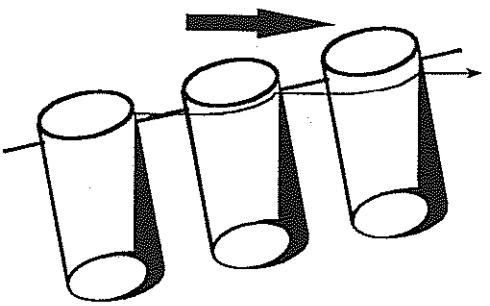

*Figure 3: Trajectory of liquid removed by centrifugation from the well of the tilted microtiterplate. Liquid and/or resin expelled from the wells cannot contaminate neighboring wells, but is caught in the interwell space.*

Situation of wells in microtiterplates placed on the perimeter of the centrifuge depends on the distance of the individual well from the axis of rotation. The volume of the "pocket" created by centrifugation in the wells closer to the axis is bigger than the volume of the pocket created in the wells more distant from the center of rotation. The volume of the pocket is not as important as the ratio of volumes of pockets in different wells of the microtiter plate. This ratio depends on the dimension of the centrifugal rotor, speed of the rotation, and the tilt of a plate. Wells placed on a rotor of very large diameter, or rotor spun very fast, will have insignificant difference between forces exerted onto "inside" and "outside" wells. In the example given here, we were working with the tilt of 9 degrees, 350 rpm, and the diameter of centrifugal rotor of 48 cm. Under these conditions the volume of the pocket in inner and outer wells differed by 8%, which we found to be an acceptable difference.

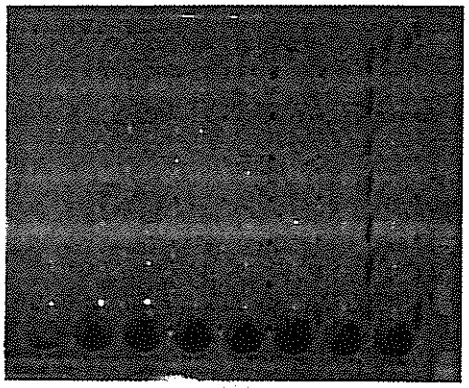

*Figure 4: Image of the section of plate which contained various amounts of the resin in each well of the first row (from the left: 3, 3, 4, 5, 6, 7, 8, 9 mg) after centrifugation (resin was colorized by bromophenol blue for easier observation).*

If drilling of holes into inert material would create the array of wells, the liquid expelled from one well would inadvertently enter another well placed closer to the perimeter of the centrifuge. However, 96 well shallow microtiterplate is actually composed of 96 small cylinders attached to a flat polypropylene sheet and connected by a thin "rib", creating thus an array of 96 round wells plus 117 interwell spaces. The liquid expelled by centrifugal force fro well comes into the interwell space, flies across this space and up on the outer wall of the adjacent well (see Figure 3). Then it along the well until it detaches and flies across another interwell eventually ending at the edge of the plate from where it flies on well of the centrifuge drum. We have tested the transfer of liquid or solid material from one well into another in several ways. W loaded the wells with the amount of colorized solid support which exceeded the capacity of the pocket and observed the fate of the resin expelled from the well. As can be observed on Figure 4, overflow of the resin ended in the interwell space and we have never observed any transfer of the resin beads into adjacent wells. In another experiment we have analyzed products synthesized in all wells of the microtiterplate by HPLC and mass spectroscopy. We have not found any traces of contamination by liquid or solid transfer between wells in our model experiments. Figure 5A shows HPLC traces of products synthesized in adjacent wells and Figure 5B shows the mass spec the products from the same wells.

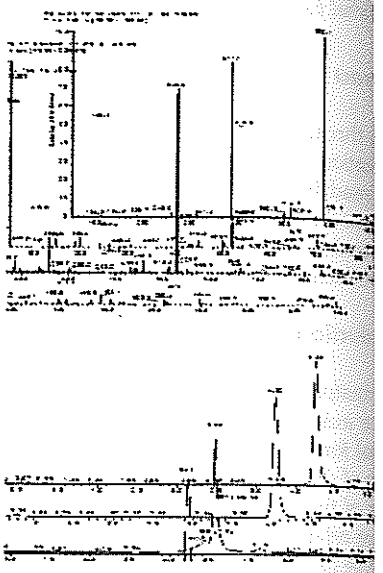

*Figure 5: HPLC traces (A) and mass sp (B) of compounds synthesized in adjace wells in the microtiterplate.*

The first experiments using tilted plate centrifugation were perfo in the Savant centrifuge, which we have equipped with custom rotor. Later we have built the dedicated centrifuge with 8 pos for microtiter plates. This centrifuge is driven from the compute all centrifugation parameters can be flexibly changed. 96-channe tributor (Figure 6) connected to 6 port selector valve perform delivery of washing solvents and common reagents. We have grated the centrifuge with Packard Multiprobe 104 liquid dist tion system for the delivery of individual building blocks and reag Inclusion of the pipetting system allows us to perform the whole thesis in completely automatic regimen. Figure 7 shows the vi this instrument. This compact system can be easily enclosed in atmosphere.

The synthesis is performed in the following way. Microtiterplate slurry of solid support distributed into it is placed on the perime a rotor with a permanent tilt of 9 degrees. The rotor is rotated speed required for complete removal of the liquid portion of the content. After stopping the rotation, microtiterplate is placed (ro turned) under the multichannel (96 channel) liquid delivery head ure 6). The solvent selector valve is turned into the appropriate tion and the washing solvent is delivered by actuating the sy pump. This operation is repeated until all plates are serviced. rotor is spun at the speed at which the liquid phase is just reac the edge of the well, wetting thus all solid support in the "poc and after reaching this speed, rotation is stopped. The cycle of rotation and stopping is repeated mixing thus the slurry of solid

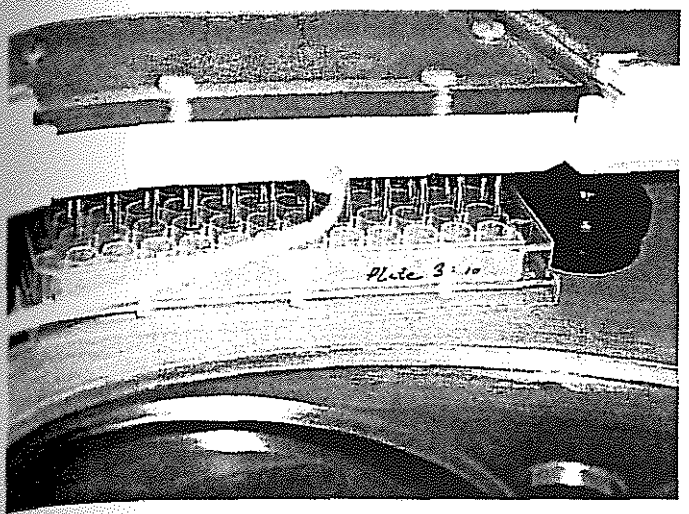


*Figure 6: Detail of 96 channel liquid distributor with microtiterplate placed under it. Note the black knob serving as the attachment of the plate.*

port in the liquid phase. After shaking for the appropriate time, the plates are spun at the high speed. The process of addition and removal of washing solvent is repeated as many times as many washes are required. The plates are then consecutively placed under the array of 96 openings in the centrifuge cover and appropriate building block solutions and coupling reagents are delivered by pipetting (Multiprobe 104) through the openings from the stock solutions placed on the centrifuge cover.

To demonstrate the simultaneous processing power of the tilted centrifugation technique, we have prepared an array of 380 substituted tetrahydroisoquinolinones. The protocol for the synthesis was developed earlier in our laboratories[3] and therefore we could compare our results with the results obtained in the solid phase synthesis performed in so called "tea bags" (technique developed earlier by Houghten[6]). Synthesis was performed in four shallow-well polypropylene microtiterplates using Tentagel resin (0.24 mmol/g, Rapp Polymere, Tubingen, Germany). Products of the synthesis were cleaved by trifluoroacetic acid and extracted by Multiprobe 104. Extracts were transferred to deep well polypropylene microtiterplates and evaporated in SpeedVac. All wells were analysed by LCMS. Purities of prepared compounds were ranked into four categories and the results are given in Table 1. In general, the results of centrifugation synthesis were found superior to results obtained earlier in "tea bag" synthesis.

Table 1. Analyses of crude products. Purities were evaluated by HPLC on the reversed phase (Vydac C-18, 10 x 0.2 cm, gradient 0 to 70% acetonitrile in water/0.05% trifluoroacetic acid in 7 min, HP 1060) with total ion current trace generated by mass spectrometer (Finnigan Mat LCQ) as detector.

| Product Quality Category | Number of Cases | Percentage of Total (%) |
|---|---|---|
| Single peak (>95%) | 201 | 52.9 |
| Major peak (85-95%) | 129 | 33.9 |
| Product present (50-85%) | 14 | 3.7 |
| Minor peak (<50%) | 21 | 5.6 |
| Not present | 15 | 3.9 |

## Conclusion

We have found tilted centrifugation to be the most effective and simplest method for liquid removal from multiplicity of vessels and polypropylene microtiterplates ideal reaction vessels for tilted centrifugation based synthesis. The fact that tilted centrifugation is the only way for removal of liquids from unlimited number of reaction vessels simultaneously is suggesting its application in ultraminiaturized synthesizers.

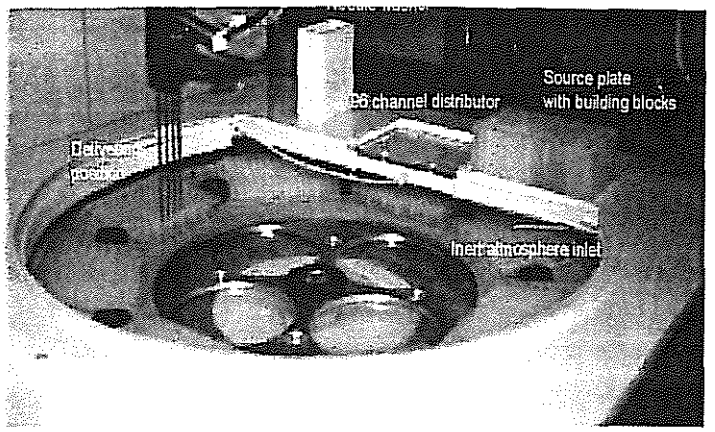


*Figure 7: Centrifuge integrated with robotic liquid distributor Packard Canberra Multiprobe 104.*

## References


1. Leblova, Z.; Lebl, M. Compilation of papers in molecular diversity field. Internet: http://www.5z.com/divinfo.
2. Cargill, J.F.; Lebl, M. *Curr.Opin.Chem.Biol.* **1997**, *1*, 67.
3. Lebl, M.; Krchnák, V. in: *Innovation and Perspectives in Solid Phase Synthesis & Combinatorial Libraries*; Epton, R. Ed. Mayflower Scientific Limited: Birmingham, **1998**, in press.
4. Krchnák, V.; Weichsel, A.S.; Lebl, M.; Felder, S. *Bioorg.Med.Chem.Lett.* **1997**, *7*, 1013.
5. Griffith, M.C., Dooley, C.T., Houghten, R.A. and Kiely, J.S., in: *Molecular Diversity and Combinatorial Chemistry. Libraries and Drug Discovery*; Chaiken, I.M. and Janda, K.D. Eds. American Chemical Society, Washington, DC **1996**, p. 50.
6. Houghten, R.A. *Proc.Natl.Acad.Sci.USA* **1985**, *82*, 5131.