2nd International Electronic Conference on Synthetic Organic Chemistry (ECSOC-2), http://www.mdpi.org/ecsoc/, September 1-30, 1998

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The "Portioning-Mixing" (Split-Mix) Synthesis

Árpád Furka
Advanced ChemTech Inc.
5609 Fern Valley Road, Louisville, KY 40228, USA

Introduction

The portioning-mixing (PM) procedure is one of the earliest combinatorial synthetic methods. It was introduced 10 years ago, in 1988 [1]-[3]. This method exemplified the first time that it is possible to prepare with high confidence a previously unimaginable large number of compounds. It taught a large number of chemists to think combinatorially. The tenth anniversary of the method offers an occasion to summarize its main advantages, the areas of application and new developments.

The portioning-mixing (PM) method

The PM method is a very simple modification of Merrifield's solid phase synthesis [4]. One coupling cycle of the solid phase procedure is replaced by the following operations:

Dividing the solid support into equal portions
Coupling a different reagent to each portion
Mixing the solid support

The scheme of the procedure outlined in Figure 1 exemplifies the synthesis of tetramers from three different monomers.

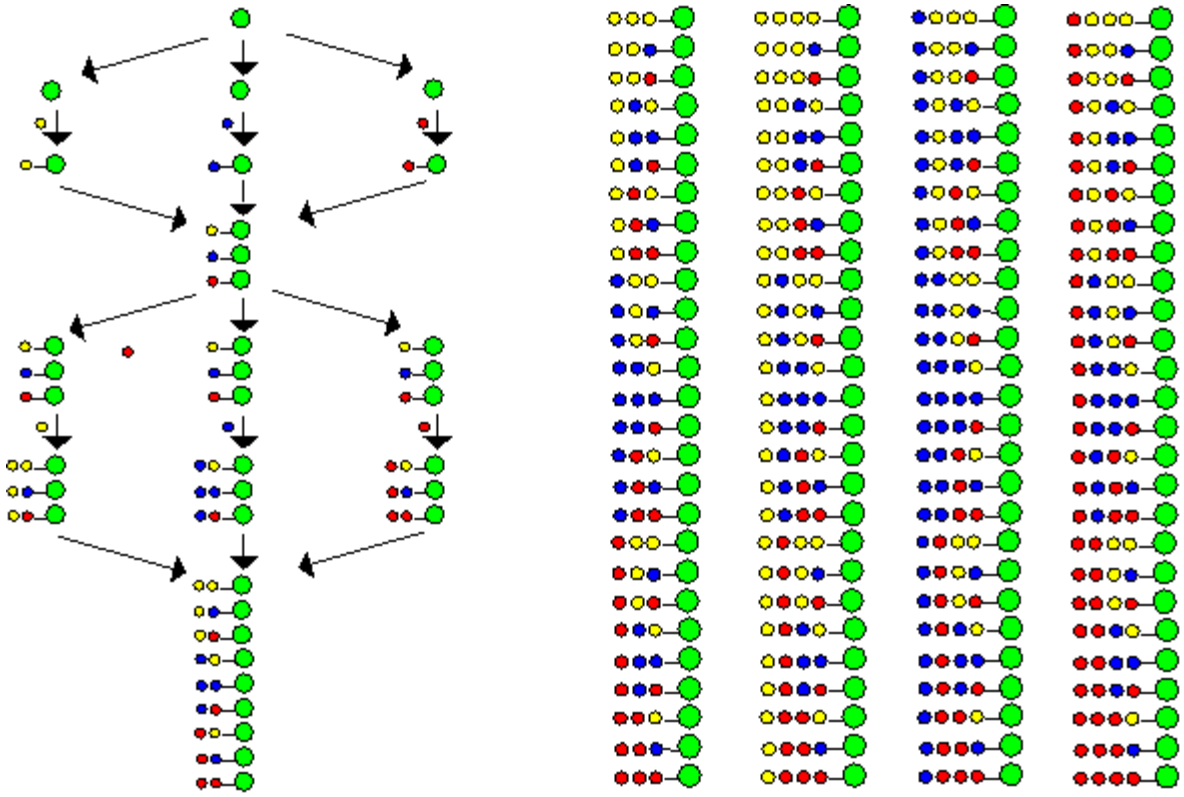

Figure 1. Scheme of the portioning-mixing synthesis

Monomers: yellow, blue, red and green circles. Solid support: larger green cycles. The divergent, vertical and convergent arrows indicate portioning, coupling and mixing, respectively

Experimental realization

Both manual and automated methods are applied in the experimental realization of the PM synthesis. In Figure 2 a manual device is outlined [5]. The device is a vacuum manifold (A) mounted on a shaker (B) and connected to a water aspirator pump via a filter flask serving as a waste collector. The reaction vessels are fitted into the holes of the tube with rubber rings to hold them strongly and support vacuum. The reaction vessels are screw cap glass or polypropylene tubes with a frit at the bottom. The aluminum tube can be turned around its axis so the reaction vessels can be fixed in any position between vertical (1) and horizontal (3). Vertical arrangement is used in all operations except shaking, when a tilted position (2) is recommended. The mixer (C) is a polyethylene vessel with a frit and gas inlet tube mounted at the bottom. The resin suspension can be mixed by nitrogen bobbling. Portioning is carried out by pipetting the resin suspension into the reaction vessels.

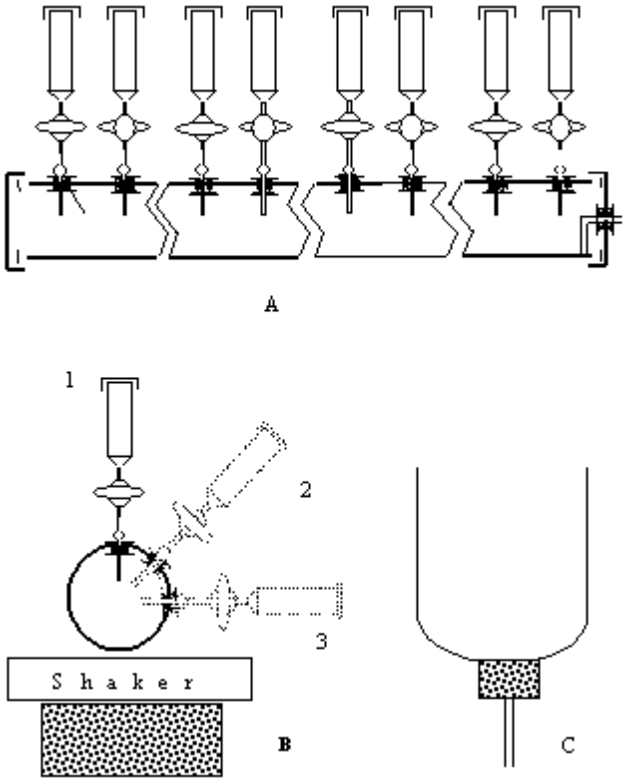

Figure 2. Manual device for portioning-mixing synthesis
A: vacuum manifold, B: shaker with manifold and reaction vessels, C: mixer

An automatic machine has also been developed and commercialized by Advanced ChemTech Inc. [6]. The ACT Model 357 Flexible Biomolecular Synthesizer (Figure 3) is a computer controlled automatic instrument capable of performing the PM library synthesis. Its reaction block contains 36 reaction vessels and a larger collection vessel for mixing the samples. The instrument has two robotic arms for dispensing solvents from system fluid containers (not visible in Figure 3), amino acid or other monomer solutions, from the 32 containers of the amino acid rack, and reagents from the 5 table-top bottles to the reaction vessels and the collection vessel. A resin handling probe is attached to the right arm. This provides the capability to move resin between the reaction vessels and the collection vessel. Each reaction vessel and the collection vessel contains a frit in the bottom to hold the resin during the removal of the liquid. A nitrogen port at the top of each reaction vessel provides a protective atmosphere. Nitrogen can be bobbled from the bottom of the collection vessel. The whole reaction block can be shaken at various speeds. All operation can be preprogrammed using Windows based software.

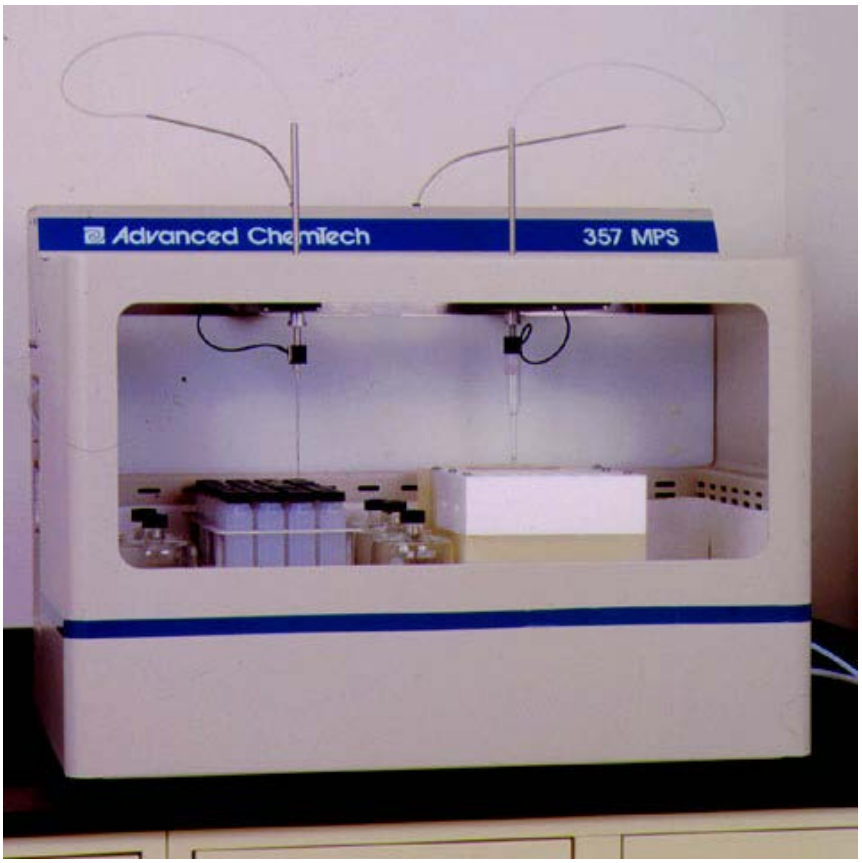

Figure 3. The ACT 357 Flexible Biomolecular Synthesizer

The PM synthesis has several key features that are crucial for the utility of the method in drug discovery or other kind of applications.

Efficiency

Examining Figure 1, it can be seen, that starting with a single substance (the resin, used as solid support), the number of compounds is tripled after each coupling step: first 3x1=3 ($=3^1$) resin bound monomers, then 3x3=9 ($=3^{2)}$ resin bound dimers, then 3x9=27 ($=3^3$) resin bound trimers and finally 3x27=81 ($=3^4$) tetramers are formed. As it can be seen, the number of the synthesized compounds increases exponentially with the number of the executed series of coupling steps. This is one of the most important features of the method. It is the exponential increase of the products that distinguishes the "real combinatorial" procedures from the parallel preparation methods in which the number of substances remains the same in the synthetic process.

If 20 different monomers are used in every cycle of a 5 step synthesis (best exemplified by preparation of peptide libraries), the number of compounds in each step is increased by a factor of 20. The total number of the synthesized compounds can be expressed by a simple formula 20^n, where n is the number synthetic steps. The number of compounds formed in 5 consecutive steps, for example, are 20, 400, 8,000, 160,000 and 3,200,000.

Formation of all possible structure combinations

The PM synthesis has another feature which proved to be very important. Consecutive execution of the three simple operations (portioning, coupling and mixing) ensures - with mathematical accuracy - the formation of all possible structure combinations of the building blocks used in the synthesis. This is clearly shown even in the simple example outlined in Figure 1. No more sequence combinations of the yellow, blue and red circles can be deduced than those found in the figure. This combinatorial principle embodied in the PM synthesis captured the imagination of many researchers and had a profound effect on the development of the field. The combinatorial nature of the product of the PM synthesis is also reflected in its name:

"combinatorial library." The combinatorial principle proved to be so fruitful that even the libraries made by parallel synthesis are designed according to this principle.

Formation of compounds in one to one molar ratio

Libraries are most often prepared in order to find biologically active substances among the new products. In the identification process, or screening, the goal is to find the biologically most effective component. Serious problems may arise in screening if the components of the library are not present in the mixture in equal quantities. A low activity component, for example, if it is present in a large amount, may show a stronger effect than a highly active component present in lower quantity. It is important therefore, to prepare libraries in which the constituents are present in equal molar quantities. The PM method was designed to comply with this requirement. Before each round of reactions, the resin is thoroughly mixed then divided into homogenous equal portions. This ensures that the previously formed compounds are present in equimolar quantities in each portion. Since couplings with the different reagents are executed on spatially separated samples, it is possible to use appropriate chemistry to drive each reaction to completion regardless of the reactivity of the different reagents. If the reactions are properly optimized, each substrate is almost quantitatively transformed. As a result, both the number of substrates originally present and their equimolar ratio is preserved in every portion at each step.

The parallel nature of the PM synthesis and formation of individual compounds

The PM procedure has an intrinsic feature which plays an important role in screening and gives a unique character to the method: on any individual bead of the solid support, only one kind of substance is formed. Figure 4 makes understandable this phenomenon. The fate of a randomly selected bead is followed in a three step coupling process. Since the bead in every coupling step meets only one monomer, only this single monomer is coupled to all of its free sites.

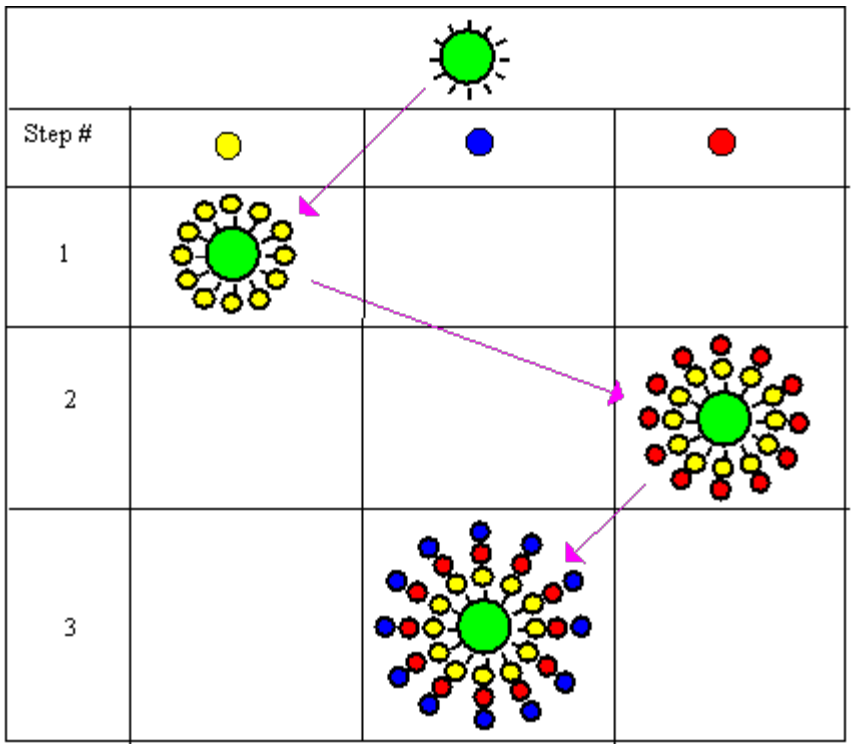

Figure 4. Formation of a single substance on each bead

This monomer in the first, second and third coupling step is the "yellow", "red" and "blue" one, respectively, so the bead ends up with the yellow-red-blue sequence. It is also important to note that in both the Merrifield and the PM synthesis the beads behave very much like tiny reaction vessels which do not

interchange their contents with the other ones. Each of the millions of these reaction vessels preserves its content until the end of the synthesis, when they become containers of a single substance. The identity of the stored compound is not known, however, it has to be determined by suitable analytical methods. This feature of the method means that the PM synthesis is in fact a parallel procedure, with unprecedented efficiency however, leading to individual compounds. The one compound - one substance feature of the PM synthesis proved to be very useful in screening [7].

Applicability of the PM method in the synthesis of organic libraries

Originally, the PM synthesis was developed to prepare peptide libraries. Peptides are not preferred drug candidates, however, because of their high susceptibility to enzymatic degradation. The ideal drug leads are small organic molecules. The PM method is fully applicable in the synthesis of such organic libraries. Both sequential type and cyclic libraries can easily be prepared if the reaction conditions for solid phase are well developed. The synthesized libraries are often screened as individual substances released from beads. Since determination of the structure of the various organic compounds is not as simple as sequencing peptides, the beads are usually encoded. The building blocks of the encoding tags are attached to the beads in parallel with the organic building blocks of the library. Three basic types of encoding were described in the literature.

Encoding with sequences [8]-[12]
Binary encoding [13], [14]
Electronic encoding [28], [29]

When encoding by sequences, the encoding tags are either peptides or oligonucleotides. Their sequences encode both the identity of organic reagents coupled to the bead and the order of their coupling. In Figure 5a the white, black, gray squares encode the yellow, blue and red organic monomers and their yellow-blue-red-yellow coupling order. The code can be read by determining the amino acid sequence of the peptide or the nucleotide sequence of the oligonucleotide tag.

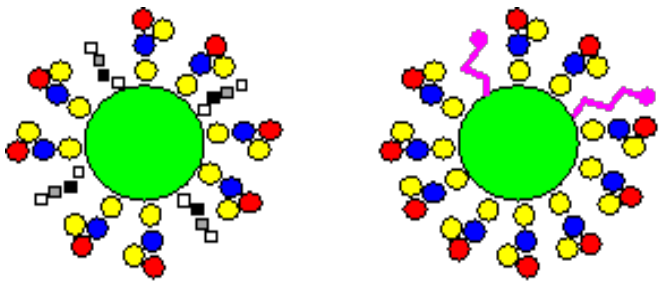

Figure 5. Beads encoded by sequence (a) and binary code (b)

In the binary encoding system the coding units are halobenzenes carrying a varying length hydrocarbon chain (pink structures in Figure 5b) attached to the beads through a cleavable spacer. It is characteristic for this labeling technique that the coding units do not form a sequence. It is simply their presence which codes for the organic building blocks and their position.

Deconvolution

The deconvolution strategies developed in different laboratories had a significant effect on the applicability of the PM synthesis. Deconvolution methods have been developed for both soluble mixtures and tethered libraries. Two methods are most often used for soluble peptide libraries.

The iteration method
Positional scanning

The principle of the iteration method was developed in different laboratories [15]-[18]. Its practical applicability in screening mixtures prepared by PM synthesis, however, was first demonstrated by Houghten et al. [16]. Both the iteration method and positional scanning is based on the use of special partial libraries. In positional scanning, the partial libraries can be pre-prepared. The principle of the method was developed in two laboratories [19], [20]. The practical application was introduced by Pinilla et al. [21]. Two different methods have also been developed for determination of the amino acid composition of the bioactive components of peptide libraries [22]-[25].

The method for screening tethered peptide libraries prepared by the PM procedure was developed by Lam et al. [7]. In the procedure used extensively at Selectide, the beads were mixed with the solution of the target protein and the beads binding the target could be distinguished by their color. The colored beads were picked out manually then sequenced after removing the attached protein. In alternative methods developed for deconvolution of tethered libraries, the substances are released and screened as discrete compounds [26], [27]. The gradual release of compounds from the beads, determining their activity in solution and identifying the structure of the bioactive components of of small organic libraries by reading the binary codes of the beads constitutes the core technology at Pharmacopeia [13], [14], [30]. Considering the role of organic libraries in pharmaceutical research as well as the tendency of decreasing quantities of substances required for screening, the importance of the Pharmacopeia procedure is becoming more and more emphasized.

The IRORI version of the PM synthesis

The nowadays practice favors the use discrete compound libraries in screening, since in this case no deconvolution is needed to determine the structure of the bioactive component of the library. The IRORI version of the PM synthesis produces such libraries. The compounds are prepared using resin as support enclosed in capsules [29]. Each capsule also encloses an electronic chip on which the synthetic history of the capsule is encoded. The structure of the substance can be identified by reading the electronic code. The procedure follows the usual PM scheme (Figure 1). The number of capsules is exactly the same as the number of compounds to be synthesized. The capsules are pooled after each synthetic step then sorted as required by their electronic tags. The key operation is sorting. The automatic machine of IRORI sorts 1000 capsules per hour.
The scheme of the sorting machine is demonstrated in Figure 6.

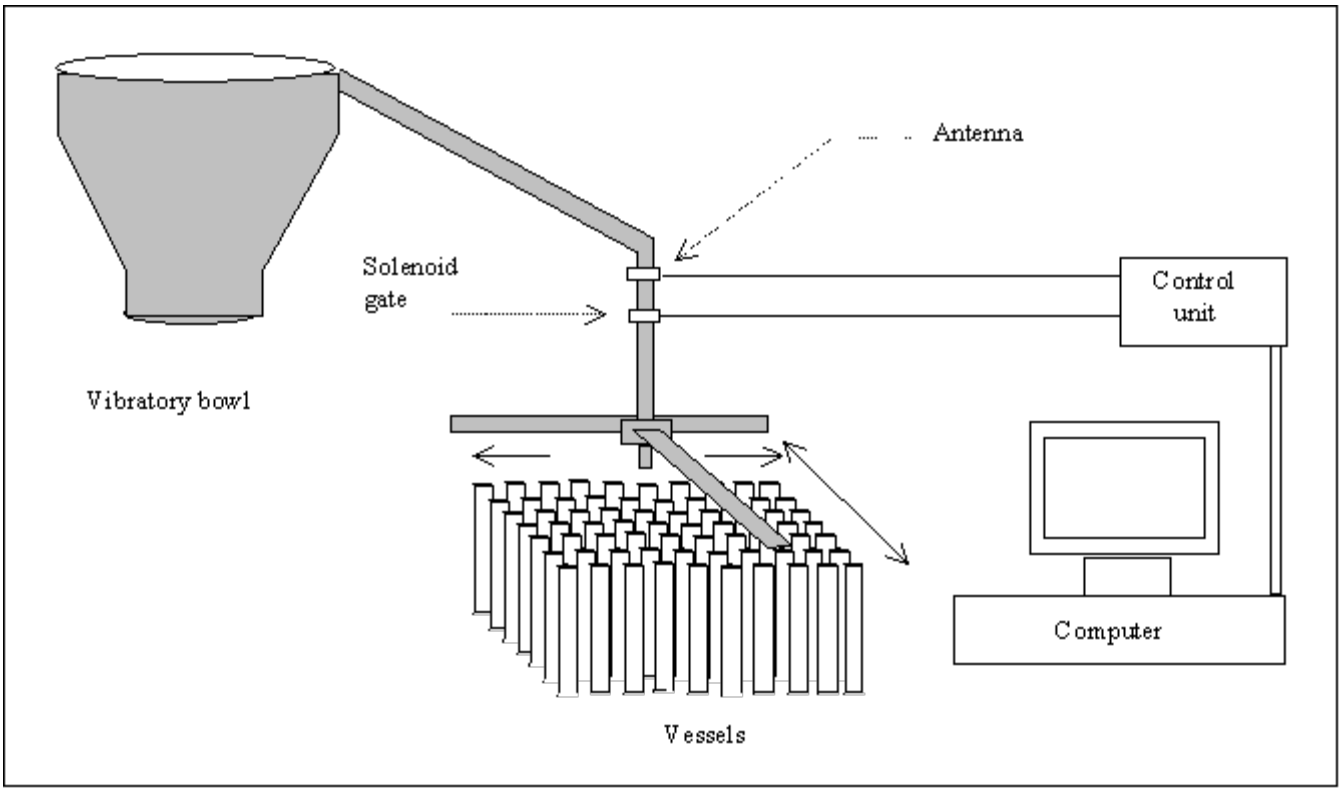

Figure 6. Sorting machine

The pooled capsules are aligned in the vibratory bowl then delivered into the required vessels through the flexible tube. The code of the capsules are read by the antenna. The antenna transmits radiofrequency radiation into the electronic chip which responds by radiating back its code. Based on the code, the computer determines the X-Y coordinates of the target vessel. By appropriate X-Y movement, the end of the tube is positioned over the target and, by opening the gate, the capsule is deposited into the vessel.

Introduction of electronic encoding and the IRORI sorting machine is a significant development. The method retains the high productivity of the PM procedure while produces discrete compounds in relatively high quantities like the parallel methods.

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