C] Combinatorial chemistry:

Combinatorial chemistry is an innovative method of synthesizing a large array of structurally diverse compounds quickly and at the same time through systematic, repetitive and covalent linkage of various "building blocks". It contrasts with the time consuming and labour-intensive methods of traditional chemistry where compounds are synthesized individually, one at a time. While combinatorial chemistry is primarily used by organic chemists who are seeking new drugs, it is also applied to other fields such as semiconductors, superconductors, catalysts and polymers.

The large pharmaceutical companies have vast collections of compounds synthesized by the traditional slow and consequently expensive methods. They have already screened most of these for pharmaceutical activity. While natural products are a source for new drugs, most of the readily obtainable natural products have already been found. They need another approach to make and test many more compounds easily and inexpensively. Also, new and small pharmaceutical companies without the benefit of such compound collections, need to compete with the established companies, and combinatorial chemistry is a way they can.

Combinatorial Chemistry enables pharmaceutical companies to develop new candidate drugs in months instead of years. Because thousands of new compounds can be created and screened in days, hundreds of millions of dollars have been invested in this technique, and nearly all of the major pharmaceutical companies now have their own combinatorial chemistry departments.

Traditionally, chemists make compounds one at a time, step by step. If the synthesis of a compound requires numerous steps, the intermediate compounds are usually purified after each step. On the other hand, when chemists use combinatorial methods, they will always be making many different compounds at the same time often in the same reaction vessel. The purification steps are usually faster and less complicated compared to the traditional methods.

Here is a simple example of combinatorial chemistry: Chemists often start a combinatorial synthesis with a batch of small plastic beads, each individual bead is about the size of a grain of sand. They attach small molecular building blocks to these beads stepwise. They like to use these plastic beads because they need only wash the beads to purify the intermediate compound between each step. To make this washing easier, the chemist often puts these batches of beads into chemically resistant porous bags resembling tea bags, or puts the beads into columns resembling coffee filters.

In this example we will start with eight of these tea bags in one container, although we could use any number of these bags. Each bag contains a spoonful of these small plastic beads. To keep track of these bags, each bag can simply be labelled with a pencil. The chemist first attaches a small molecular building block to these beads by soaking them in a solution containing this building block. We will call this first building block molecule "a". At this point we have eight individual bags of beads with the first molecule, "a" attached to every bead.

beads-a beads-a beads-a beads-a

beads-a beads-a beads-a beads-a

The beads are then washed to remove any excess "a" and any undesired reaction products. The chemist then puts two of these bags in a vessel containing building block "b", two bags in another vessel with "c", two bags in another vessel containing "d", and finally two bags in a vessel containing "e".

beads-a-b	beads-a-c	beads-a-d	beads-a-e
beads-a-b	beads-a-c	beads-a-d	beads-a-e

Here also, once this set of reactions is finished, the bags are washed, to remove undesired substances. A third round of reactions takes place in eight individual containers. A new and different building block is added to each container.

beads-a-b-f	beads-a-c-h	beads-a-d-j	beads-a-e-l
beads-a-b-g	beads-a-c-i	beads-a-d-k	beads-a-e-m

We now have eight different and clearly defined compounds. After all building blocks have been added, the bond between the plastic bead and the compound attached to it can easily be cleaved. Each of the compounds can now be tested for the desired activity. In this example we used the same building block only in the first position. Chemists always have the option of repeating the same building blocks. Even the compound a-a-a can be made if desired.

This example fulfils the requirement of combinatorial chemistry because the chemist made more than one substance at the same time. However, several reaction vessels were required. Chemists are always seeking to make even more compounds with the same amount of work involved in this example. Wherever possible they want to be able to make many different compounds in the same reaction vessel in a minimum number of steps.

Let us now look at a more sophisticated application of combinatorial chemistry. We will start here with three batches of beads, in three separate containers, one reacted with building block "a", one with "b", and one with "c".

beads-a	beads-b	beads-c
container #1	container #2	container #3

After this first reaction, the beads are washed and then mixed together in one vessel, then split among three separate containers.

beads-a	beads-a	beads-a
beads-b	beads-b	beads-b
beads-c	beads-c	beads-c
container#1	container #2	container#3

Now, building blocks "a" are added to container #1, "b" to container #2, and "c" to container #3. After these are allowed to react, each container has the following:

beads-a-a	beads-a-b	beads-a-c
beads-b-a	beads-b-b	beads-b-c
beads-c-a	beads-c-b	beads-c-c
container#1	container #2	container#3

All of the beads are again washed then mixed together and again split into three containers. Again, building blocks "a" are added to container #1, "b" to container #2, and "c" to container #3. After this set of reactions, each container has the following:

beads-a-a-a	beads-a-b-a	beads-a-c-a	beads-a-a-b	beads-a-b-b	beads-a-c-b
beads-b-a-a	beads-b-b-a	beads-b-c-a	beads-b-a-b	beads-b-b-b	beads-b-c-b
beads-c-a-a	beads-c-b-a	beads-c-c-a	beads-c-a-b	beads-c-b-b	beads-c-c-b
	container #1			container #2	

beads-a-a-c	beads-a-b-c	beads-a-c-c
beads-b-a-c	beads-b-b-c	beads-b-c-c
beads-c-a-c	beads-c-b-c	beads-c-c-c
	container #3	

Here in only three sets of reactions, never using more than three containers, we made all 27 possible combinations of compounds consisting of these three building blocks. In this still rather simple example, we stopped after three steps. Performing just one more round of this mixing and splitting would yield 81 distinct compounds. Note that after each round of reactions the contents were mixed together than split into separate containers. Hence this type of synthesis is called *mix and split* or *split and mix*.

In the first example using the tea bags, each tea bag produced only one compound. In this mix and split example each container has nine different compounds. In the field of combinatorial chemistry, a collection of several or many different compounds in the same container is called a *library*. This rather unusual use of the word library was adapted from the field of molecular biology where the term library defines a mixture that results when a large piece of genetic material (DNA or RNA) is cut into many small pieces, and the piece of genetic material with a sought-after property is identified and isolated from the mixture.

Again, looking at the above example of a combinatorial synthesis, suppose the contents of these three containers are tested for the desired activity, and only the contents of container #3 are active. How does the chemist know which of these nine compounds in this library contributed to the activity? The active compound has the structure ?-?-c, where the question marks represent building blocks "a", or "b", or "c".

Determining the structure of an active compound present in a mixture of compounds is termed *deconvolution*. In the above example, one way to find the active compound is to backtrack, and make all nine possible combinations of compounds that end with building block "c". Deconvolution is one of the major challenges of combinatorial chemistry and often its most time-consuming step. Chemists have developed ingenious and elaborate methods to speed up this process. Sometimes they tag or encode some of the building blocks or the beads with a tracer they can later detect.

Another variation of combinatorial chemistry is known as *parallel synthesis*. Here all the products are synthesized in separate reaction vessels. Typically, a plate with 96 individual wells is used, with beads attached to each well. Building blocks are added individually to the beads in each well. The advantage of parallel synthesis is that the composition of each compound is known, but generally, the split and mix procedure can generate many more compounds in the same amount of time.

These examples illustrate the main limitation of combinatorial chemistry. The simplicity of combinatorial chemistry is also one of its drawbacks. Only certain types of compounds can bind to plastic

beads, and only a fairly limited number of reactions can be used in these repetitive steps. This means that only certain types of substances can be made using combinatorial chemistry. One of the challenges facing combinatorial chemists is to develop new reactions and new classes of substances that can be applied in simple and repetitive steps.

To overcome the limitation of the small number of reactions that can be performed on beads, chemists have developed methods of performing combinatorial chemistry in solution where a vast range of reactions can be performed. Other chemists have devised methods that combine the advantages of both the plastic bead method and the solution method. They use beads that dissolve in some solvents, but not in others. Here they perform a large range of reactions in solution then transfer the reaction mixture to a solvent where the beads are not soluble, so that the impurities can be removed by simply washing the now solid beads.

Still other chemists have combined molecular biology with combinatorial chemistry. They introduce different combinations of genes into microorganisms, turning each batch of microorganism into an individual bioreactor.

After any combinatorial synthesis whether split and mix, parallel or solution, the products need to be tested or screened for the sought-after activity against the intended biological target, which could be an antibody, a receptor or other biological target molecule.

Combinatorial chemists can now create thousands of compounds in just days. They need rapid and inexpensive ways of screening such large numbers of compounds. Consequently, they developed robotics and automated methods to help them with these screenings. This need to be able to rapidly screen huge numbers of compounds has spawned its own discipline known as *high throughput screening*. Combinatorial chemistry would not be manageable or efficient without it. Much automation has also worked its way into the synthesis steps.

Rarely will a combinatorial synthesis produce a useful drug. Instead, a candidate compound will have to be modified by substantial traditional chemistry. The combinatorial product will lead the way to a new drug, hence the term for such preliminary products is *lead compounds*.

Source: https://journals.library.ualberta.ca/istl/index.php/istl/article/view/1875/1786

If a “lead or promising compound” is identified by the drug design group, then a large number of derivatives of this lead are rapidly tested for their efficacy using the combinatorial approach. This philosophy has enabled a large number of substances to be made and their properties assessed without generating a sizable amount of waste and its later disposal. Green chemistry would benefit from the principles of combinatorial chemistry, since the latter approach produces practically very little waste.

Advantages of combinatorial chemistry:

1. Avoids formation of non-biodegradable and toxic waste.

2. High atom economy, less wastage.

3. Solvents, other auxiliaries can be minimized or avoided.

4. Saves time and energy, also other resources.

5. Lowering of production cost, makes use of non-conventional energy sources.

6. Solventless reactions / solid phase reactions possible.

Disadvantages:

i) The size, solubility, and function group of a compound all have a significant impact on its efficiency.

ii) Compounds generated are typically achiral or racemic.

iii) There is a limit to the chemistry you can do when using solid phase synthesis. The resin you use is often affected by the reaction types available and care must be taken so that the attachment of the reagent to the substrate and bead are unaffected. Each reaction step has to be carefully planned, and often a reaction isn't available because the chemistry affects the resin.

iv) While a large number of compounds are created, the libraries created are often not focused enough to generate a sufficient number of hits (Library components whose activity exceeds a predefined, statistically relevant threshold) during an assay for biological activity. There is a great deal of diversity created, but not often a central synthetic idea in the libraries. One can argue that there should be a focus on the type of molecule developed in order to maximize hits.

Examples of combinatorial synthesis: