## Continuous flow applied to peptide synthesis -

# Next generation of synthesisers

Peptide manufacturers can bring solid-phase peptide synthesis to a whole new level by following Big Pharma's interest in flow chemistry, says **Dr Manuel Nuño** of **Vapourtec**

Continuous flow chemistry has been a powerful tool for discovery chemistry and process development in the fine chemical and pharmaceutical industries since the early 2000s.

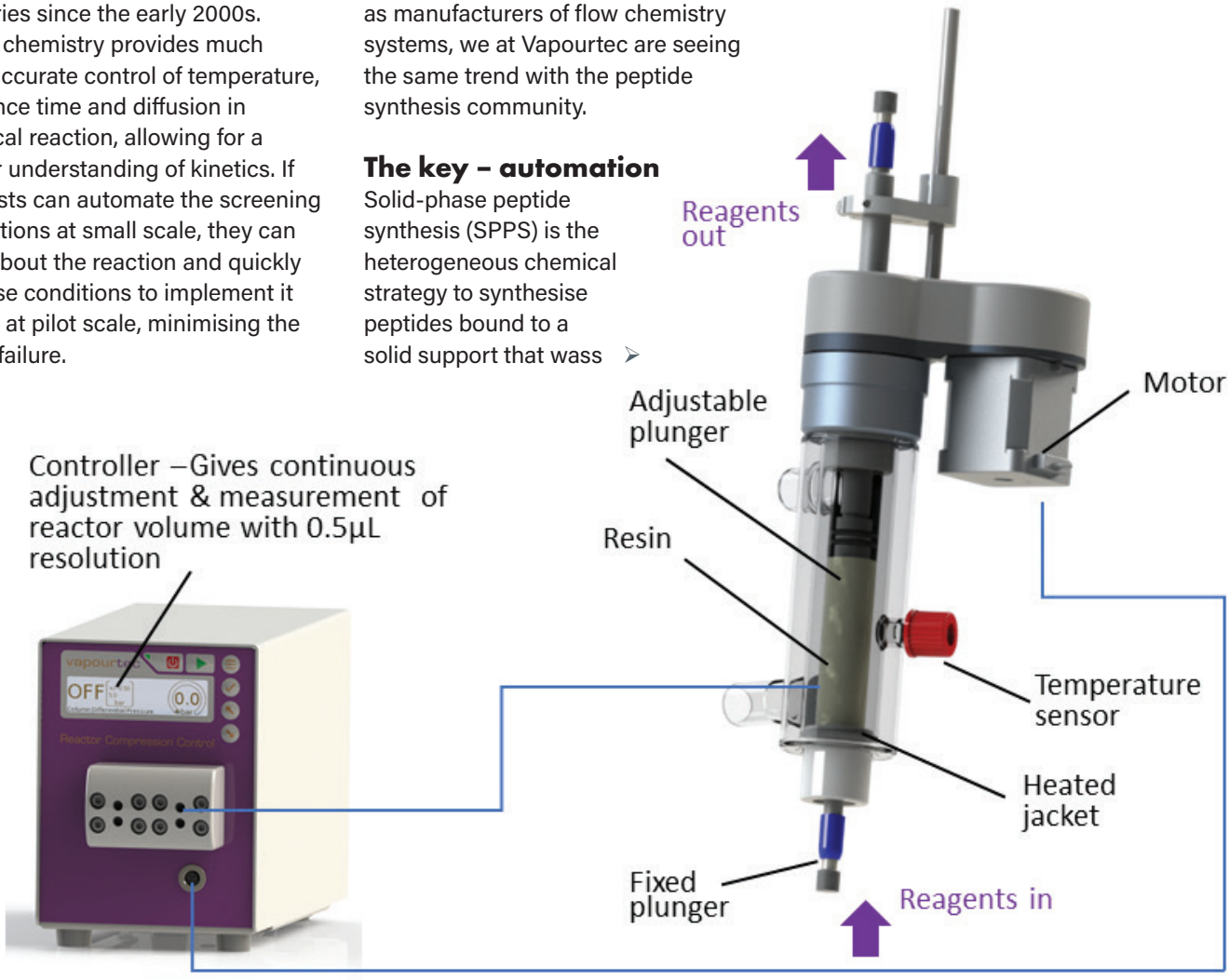
Flow chemistry provides much more accurate control of temperature, residence time and diffusion in chemical reaction, allowing for a deeper understanding of kinetics. If scientists can automate the screening of reactions at small scale, they can learn about the reaction and quickly optimise conditions to implement it rapidly at pilot scale, minimising the risk of failure.

The early adoption of flow chemistry in these industries gave the pioneer companies a key technological advantage over competitors who adopted the technique later. Now, as manufacturers of flow chemistry systems, we at Vapourtec are seeing the same trend with the peptide synthesis community.

### The key – automation

Solid-phase peptide synthesis (SPPS) is the heterogeneous chemical strategy to synthesise peptides bound to a solid support that wass ➢

**Figure 1**– Vapourtec VBFR & reactor controller



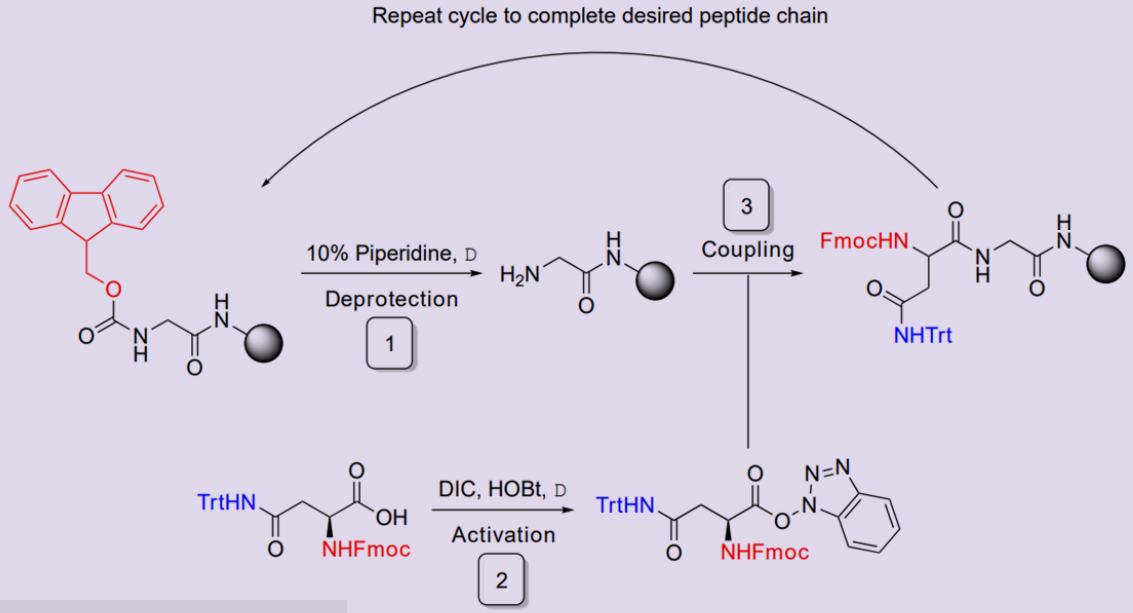



**Figure 2–** Overview of chemical reactions in SPPS

developed by R.B. Merrifield in the early 1960s. Each peptide bond is formed in a cyclic sequence, based on Fmoc- or Boc-protected N-terminal amino acids.[1]

For solid support, a variety of resins can be used. They consist of a polymer matrix with linkers attached to it, where one peptide will be built for each linker. The polymer matrix swells with solvent, which enables reagents to flow through their micropores. Excessive compression of the resin beads will reduce the yield in coupling reactions.

Firstly, the amino acid linked to the resin needs to be deprotected. The next one in the sequence needs to be converted into a more reactive species, an amino ester, for the reaction to complete. Once this is coupled, the solid supported peptide needs to be washed to remove any excess reagent. This minimises cross-contamination between couplings.

Figure 2 shows the sequence. This is repeated for as many amino acids as the peptide sequence contains. Once the peptide has been built, the cleavage and removal of the side protection groups is the last step to obtaining the desired product.

Flow chemistry is an ideal platform for automated synthesis and repeated SPPS cycles have already been implemented successfully in continuous flow.[2-7] The benefits of continuous flow are vital for the development of SPPS in industrial laboratories.

In continuous flow, the solid resin beads (supported media) are usually packed in a cylindrical reactor. Because the beads are spheres, they will occupy the minimum possible volume when packed. This reduces the amount of required solvent on the wash steps, as well as the excesses of reagents needed for the reaction to complete.[4,8]

The explanation for this phenomenon is simple: as the plug of amino ester flows through the packed resin, each active site on the resin will be exposed to a more concentrated solution over time than in a typical batch reactor.

In addition to a more efficient synthesis, another benefit of flow chemistry is the ease of connecting inline detectors (i.e. FTIR or UV-Vis), which provide more accurate insight into the chemical reactions. Experimental inline data allows scientists to evaluate, for example, whether a deprotection step has gone to completion or if the pumped reagents have reached steady state conditions.

One of the drawbacks of traditional flow chemistry is the limited ability to accommodate volume changes in solid reagents. This becomes apparent in SPPS; as the sequence advances, more and more amino acids are coupled to the peptide, adding mass and volume. Thus, in the synthesis of a 30-mer peptide, the final volume of the resin is usually double the initial volume.[9]

When a peptide is being built, each coupling reaction adds mass and therefore volume to the reactor packing materials, which compresses the resin matrix against the reactor's walls, creating high back pressure.

Peptide chemists have taken two different approaches to this problem: starting with a packed reactor and running at increasingly high pressures every cycle, or starting with a headspace in the reactor, so the resin can grow. Although they are working alternatives, these are not actual solutions to the problem that needs to be resolved if we want flow chemistry to be the leading technology for SPPS.

## VBFR

Over the last three years, Vapourtec has worked towards a mechanical solution for a chemical problem in the flow arena. The reactor we have developed has the ability to measure and control the packing density of a given solid media.

In this case, resin for SPPS can be packed with high precision throughout the individual coupling reactions, allowing packing to the minimum possible volume. This is achieved by means of a movable plunger that can

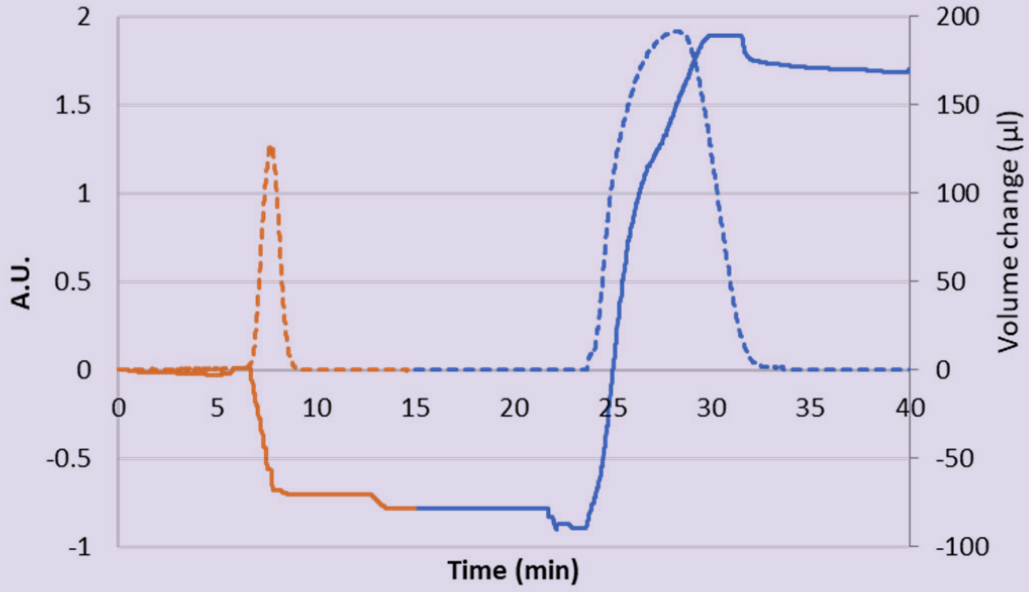



**Figure 3–** Deprotection & coupling cycle

***Note:*** *Dotted line - UV absorption; Solid line -VBFR change in volume*

dynamically adjust the internal volume of the reactor.

We have developed three different reactor volumes that make it possible to scale up SPPS from 100 mg to gram-scale. Figure 2 shows the Vapourtec Variable Bed Flow Reactor (VBFR), along with its reactor controller unit, which adjusts the reactor's volume, with a resolution of ±0.5 ±l, to deliver a constant packing density.

By combining the control that flow chemistry possesses and keeping the resin to its minimum volume throughout the sequences, a more efficient synthesis is achieved. This is of particular relevance when non-natural amino acids are used, as they are more expensive than standard protected amino acids.

Our collaborators at the Max Planck Institute in Germany have successfully demonstrated the potential of the VBFR for SPPS. This ranges from synthesising a 26-mer peptide with 91% purity to synthesising a difficult peptide, JR10-mer, also with high purity.

The implementation of smart reactors does not only lead to an improvement in the synthesis and final crude purity. It is also a tool for the peptide chemist to learn about that synthesis.

As the reactor adapts its volume to accommodate the mass changes on the resin, it records the evolution of internal volume over time. When this set of data is then overlapped with UV data, a much clearer analytical picture is formed.

Figure 3 shows a typical set of data for a deprotection and coupling cycle. This valuable inline dataset makes it possible to evaluate when an aggregation event occurs or when a reaction has not performed as it should.

## Conclusions

Both industry and academia now have years of expertise in flow chemistry. As their knowledge has further expanded, so has their field of application.

Transferring batch processes into flow protocols is a daily routine for any experienced flow chemist but remains a challenge for researchers with less experience in flow. This is harder still with SPPS, as transferring a batch protocol into flow still remains a challenge.

When combining flow systems and dynamic reactors, SPPS not only becomes a reality, it opens the door for a deeper understanding of the chemical processes involved in peptide synthesis. With the wide range of synthesis scales, it enables the optimisation of protocols at 100 mg-scale, saving reagents and reducing scale-up risks, with no need for dedicated equipment, other than a Vapourtec flow chemistry system. ●

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