Advertorial

customer's publication

GOING WITH THE FLOW FOR GAME-CHANGING PEPTIDE SYNTHESIS

With over 20 years of expertise in flow chemistry and a technology trusted by hundreds of chemists around the world, UK-based instrument manufacturer Vapourtec is changing the peptide synthesizer market. Already adopted by several pharmaceutical and biotechnology companies, as well as academic institutes, Vapourtec's peptide synthesis product portfolio is based on Fast Flow Solid Phase Peptide Synthesis (FF-SPPS). So why should anyone move their peptide synthesis to FF-SPPS?

1) **Eliminating randomness, increasing crude purity**
One of the key characteristics that differentiates FF-SPPS from a batch synthesis, is the arrangement of the solid media. In batch, solid media is stirred in a vessel, continuously mixing. Reagents are then added to the vessel for the reaction to occur. The coupling process is random, and with every coupling the impurity profile increases to give a normal distribution of deletions (Figure 1). In continuous flow, the solid media is packed in Vapourtec's proprietary Variable Bed Flow Reactor (VBFR) while reagents single pass the reactor. The VBFR is a perfect packed bed flow reactor that will automatically vary in volume to accommodate changes in the volume of the resin (increasing as amino acids are coupled, decreasing as Fmoc deprotection occurs). This inhibits the movement of resin beads while minimizing the reactor volume throughout the whole synthesis. This ensures a unique interaction between the reagents and the static resin, back mixing is eliminated, and reaction by-products are continuously removed. High reaction efficiency is achieved, but more importantly, by constraining both the resin and the direction of the reagent flow, the target peptide is preferred even at substoichiometric conditions.

2) **Heated reactions to minimize aggregation and access difficult peptides**
Heating has two important benefits: preventing β-sheet structures that can lead to aggregation events and increasing reaction kinetics which can be particularly beneficial for sterically difficult couplings. Using flow chemistry, it is straightforward to pre-heat and pre-activate the amino acids before they contact the resin. This ensures fast, more efficient coupling cycles as only activated amino acids will flow through the VBFR. However, not all heating is the same. Our experience supporting continuous flow applications over the last 20 years has taught us that uniform and constant heating is the key to reproducibility. Hot spots or uncontrolled temperature spikes cause racemization and can lead to loss of the ligand, particularly with chlorotrityl type resins.

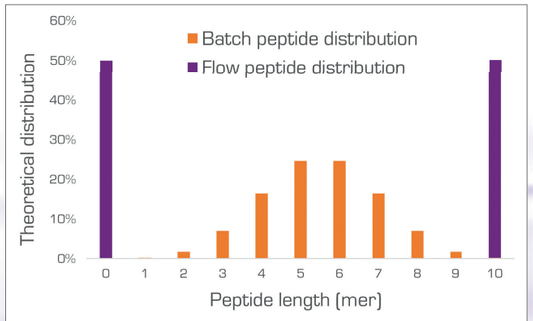

Figure 1. Theoretical peptide distribution in batch vs flow with 0.5 equivalents amino acids

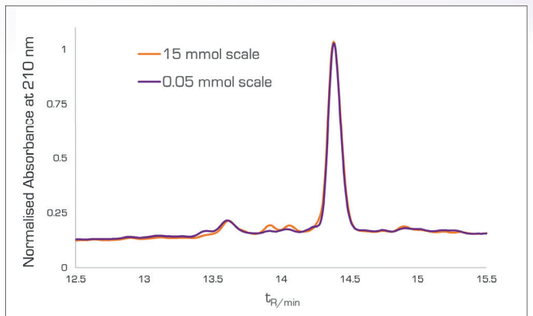

Figure 2. HPLC traces for the product of the GLP-1 synthesis at 50 µmol vs 15 mmol scale.

Peptide-Builder
- **50 µmol - 1 mmol**
- GLP-1 synthesis in 3.5 hours
- Up to 16 different peptides unattended
- Small footprint

PS-30 pilot scale peptide synthesizer
- **2 mmol - 30 mmol**
- GLP-1 synthesis in < 1 day
- No further optimization required
- Supports GMP and non-GMP peptides

3) **Effortless scale-up with identical crude purity**
Current batch technologies can require weeks of optimization and scale up of a peptide synthesis to produce large quantities for clinical studies.

Vapourtec developed the PS-30™ pilot scale peptide synthesizer to significantly reduce the time to market for development of new peptide-based therapeutics. This is achieved by using large scale reactors with identical heating and mixing characteristics as the laboratory scale platforms. Scientists can now optimize a synthesis at 50 µmol scale, and bring the same reaction conditions (equivalents, temperature and residence time) to a 30 mmol scale without further optimization.

As an example, we used the PS-30 to scale up the synthesis of GLP-1 to 15 mmol in less than a day. The remarkable advantage is not only the synthesis time at this scale but also the identical crude purities achieved at both scales (Figure 2).

Figure 2. HPLC traces for the product of the GLP-1 synthesis at 50 µmol vs 15 mmol scale

4) **In-line data for easy reaction optimization**
In addition to the chemical advantages, we can access real-time in-line data never seen before at that level of detail. Thanks to the VBFR, changes in the reactor volume can be plotted, which can help detect aggregation events, with precise timing. Also, rather than using UV spectroscopy to monitor a reaction sample or the waste stream as it would be done in batch, flow SPPS allows for quantitative real-time in-line analysis of Fmoc deprotection. Both sets of data can help with the immediate identification of any non-standard coupling events, allowing the user to recognize and optimize difficult coupling events at a small scale, before scaling up.

5) **Further benefits**
The VBFR technology together with the uniform heating allow for effective couplings with as low as 1.2 equivalents of amino acids. This translates into reduced costs, especially when using unnatural amino acids. FF-SPPS also benefits from an inherent advantage of flow chemistry, reducing solvent usage compared to batch techniques with only 60 ml/mmol per cycle.

The Vapourtec peptide synthesizer range
We offer a range of fully automated FF-SPPS peptide synthesizers covering scales between 50 µmol and 30 mmol.

At the lab scale, the Peptide-Builder™ (coming soon) will increase throughput within a small footprint with up to 16 automated peptides synthesized sequentially, the first peptide available within 2 hours (16-mer). At the pilot scale, the PS-30™ pilot scale peptide synthesizer can potentially speed up the development of peptide drugs, with linear scale-up and synthesis times under a day.

vapourtec
fast-flow for peptides

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