Fast-Flow SPPS of proteins and peptides from optimization to scale-up

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Why Fast-Flow SPPS (FF-SPPS) ?

Vapourtec's Variable Bed Flow Reactor (VBFR) technology[1] inhibits the movement of resin beads while minimising the reactor volume throughout the whole synthesis. This ensures a unique interaction between the reagents and the static solid support, back mixing is eliminated and reaction by-products are 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 **sub stoichiometric conditions**. The volume of **wash solvent is also minimised**. In addition to the chemical advantages, the VBFR can access real-time in-line data never seen before at that level of detail: Reactor volume change, which can help detect aggregation events, and quantitative UV spectroscopy[3] (Figure 2).

The VBFR technology ensures FF-SPPS becomes a **pivotal tool for peptide discovery and pilot scale manufacture** opening the opportunity for:

• **Difficult peptides** • **Short proteins** • **Rapid optimization** • **Fast Scale up**

As one example, a 70-mer peptide was synthesized in continuous flow for Real-time analysis purposes (Figure 3). To further show the advantages of FF-SPPS, this paper shows the linear scale-up of GLP-1, from 50 µmol up to 15 mmol.

Experimental setup

The **Peptide-Explorer™** synthesizer was used for the synthesis at small scale, the **PS-30™** Pilot scale synthesizer for up to 30 mmol synthesis. Protocols comprise of isothermal couplings with Oxyma/DIC activation and piperidine deprotection were completed at 80°C[2].

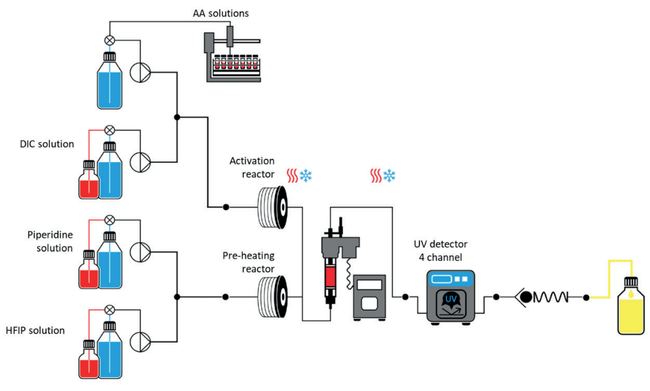

Figure 1 – Flow schematics for FF-SPPS

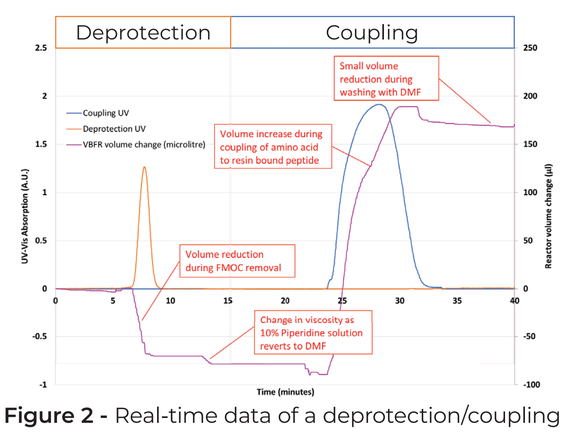

Figure 2 - Real-time data of a deprotection/coupling cycle

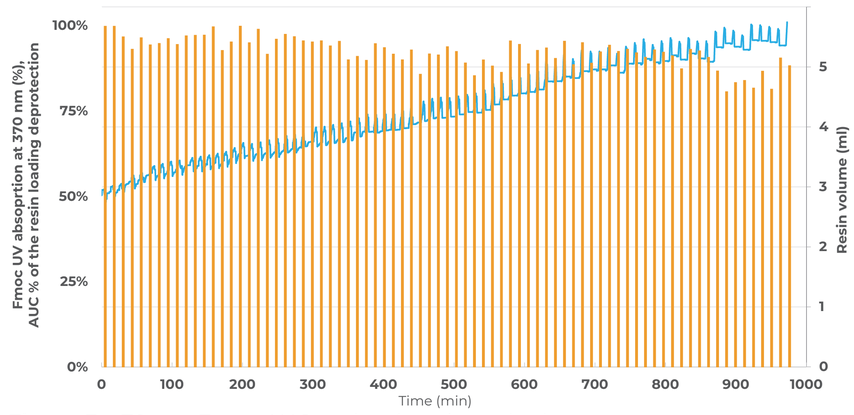

Figure 3 – 70mer Fmoc AUC and resin volume in time

Results & Discussions

Current batch technologies require weeks and many syntheses to scale up a peptide synthesis previously only optimised at lab scale. The synthesises optimisation at 50 µmol scale[1] was performed on a Peptide-Explorer (Figure 4), a fully automated system that delivers amino acid solution, piperidine solution, DIC solution and HFIP solution for side chain deprotection. On this platform a complete deprotection-coupling **cycle time is 7 min**.

The PS-30 Pilot scale synthesizer was developed to match the performance of lab scale FF-SPPS systems (Figure 6). Reactor dimensions, components and pump capabilities were scaled up, the same mixing and heating characteristics were transposed while maintaining the key reaction parameters constant: stoichiometric ratio, residence time, resin minimised volume technology and uniform heating.

Thus, allowing the user to use the same protocols (Figure 5), transposing the reaction conditions to the PS-30 and applying a 300 x scale up factor to synthesise GLP-1 or any other 30 mer peptide up to **30 mmol scale in less than a day**.

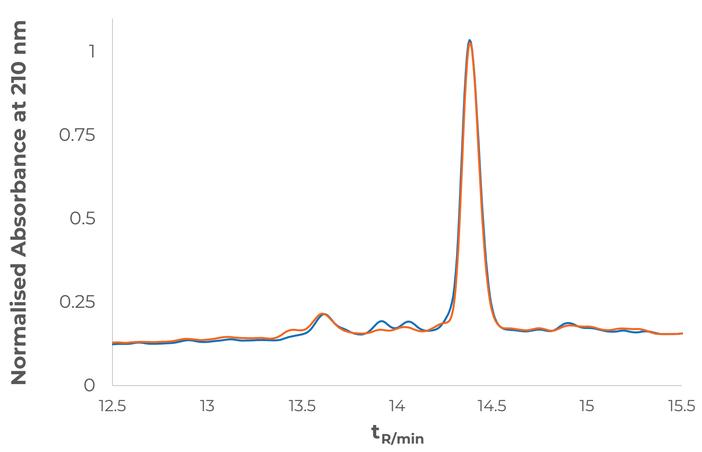

Figure 4 – Identical GLP-1 HPLC traces of 50 µmol (orange, 80 % purity) and 15 mmol (blue, 80 % purity) scales syntheses

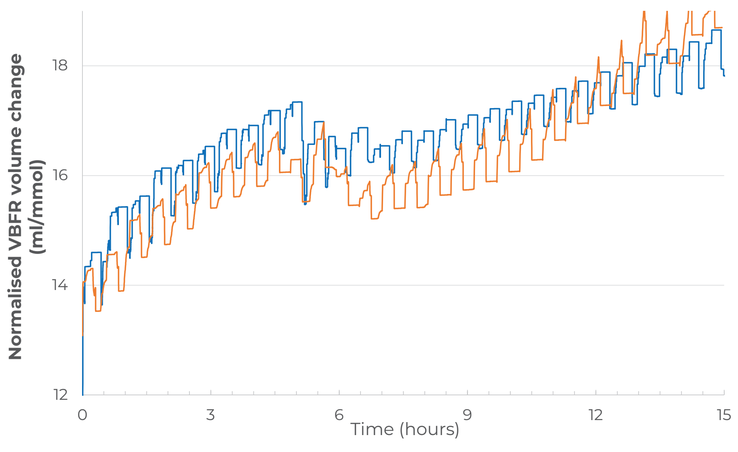

Figure 5 – Resin volume comparison of 50 µmol (orange) and 15 mmol (blue) scales of GLP-1 synthesis (partial aggregation after coupling 11)

Figure 6 - PS-30 pilot scale synthesizer for 2 - 30 mmol scale

The remarkable advantage is not only the **synthesis time at this scale** but also, **delivering identical crude purity (Figure 4)** when comparing the lab scale at 50 µmol and the scale up at 15 mmol completed within 15 hours.

When analysing the real-time data, the level of aggregation was also scalable (Figure 5), indicating it is a purely chemical phenomenon. When the VBFR volume change is normalised to the reaction scale, we can compare relative volume growth, showing the same level of aggregation, which starts after the 11th coupling and partially recovers.

Conclusion

FF-SPPS has shown multiple advantages over conventional batch technology at both lab and pilot scale. The advantages of FF-SPPS have been demonstrated in the synthesis of long peptides as well as difficult sequences yielding high crude purities.

GLP-1 has been successfully synthesized in under 4 hours, key protocol parameters brought to the PS-30 at 15 mmol in less than a day, and over 80 % purity was achieved without further refinement.

Other sequences, such as a 70-mer protein have been synthesized using the same protocols. The use of the VBFR will highlight aggregation events, as well as difficult couplings throughout the synthesis. The uniform heating of the resin beads keep peptide sequences solvated and reduces synthesis time often to less than a day. As all the platforms use the same protocol, it eliminates the need to further refine reaction conditions as the process scales up.

Finally, the DMF usage is as little as **60ml/mmol per cycle regardless of the scale**, providing a final crude purity of over 80 % and a yield of 71 % for a renowned peptide sequence, known to be difficult due to aggregation, GLP-1.

References
[1] E. T. Sletten, M. Nuño, D. Guthrie, and P. H. Seeberger, "Real-time monitoring of solid-phase peptide synthesis using a variable bed flow reactor," Chem. Commun., 2019, doi: 10.1039/C9CC08421E.
[2] Vapourtec application note 69 - Automated CF-SPPS and evaluation of GLP-1 peptide. 2021
[3] Laude, V, Nuño, M, Moses, RC, Guthrie, D. Evaluation of unexpected protecting group removal in solid-phase peptide synthesis: Quantified using continuous flow methods. J Pept Sci. 2022;e3441. doi:10.1002/psc.3441

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