The ideal peptide plant

Dr Olivier Ludemann-Hombourger of PolyPeptide Laboratories France looks at the latest process trends in peptide manufacturing and key drivers for innovation

Peptide APIs have generated considerable interest during the last few decades and this attention is expected to grow. Chemical synthesis is the most common approach to manufacturing peptides. There is a strong group of established CMOs serving this market, where specific expertise is required and specific equipment is used (solid phase reactors, preparative HPLC, lyophilisers, etc).

The production process however, remains complex, the lead time is often critical and large volumes of solvent and reagents are required to produce the final API. Innovations in peptide chemistry are essential for improving performance and increasing the purity and yield achieved in peptide synthesis.

Nevertheless, efforts must also be made in process engineering to design the most efficient process to transfer and implement the chemistry developed in the laboratory to larger scale and improve the performance of the downstream processes. The aim of this article is to consider the current state of the art and how it can be improved from a process engineering point of view, and how the innovation efforts can be driven by the development requirements.

In order to drive this innovation strategy, let us dream ... and imagine the ideal plant design to manufacture peptides by chemical synthesis. Let us define, first, the criteria of an ideal plant design for a CMO before evaluating the relevant innovations axis to be followed to make this dream become (almost) reality!

What are the key weaknesses of the existing peptide production processes? Lead times are long, due to the multi-step syntheses required to assemble the amino acids into the peptide chain. Preparative HPLC is also time-consuming. The combined process requires large volumes of reagents and solvents, due to the multiple steps involved, and many large-scale processes may still use large volumes of undesirable solvents (DMF, chlorinated solvents, etc.).

An ideal process should drastically reduce the lead time and the human resources required to operate the process, be solvent-free (!) and reduce the quantity of reagents needed.

Managing a manufacturing plant dedicated to peptide manufacturing, several ‘off the wall’ ideas came to my mind when I have tried to imagine what the ideal facility could be.

**Upstream**

For upstream activity, the ideal process would be much quicker, with a short lead time. There would also be a reduction in the volume of reagents and solvents used for the process and fewer manual operations to reduce costs, variability and risks of failure.

Even if the development of the solid phase peptide synthesis (SPPS) has been, in itself, a major step in this evolution by comparison with traditional chemistry, peptide synthesis remains complex, due to the multiple steps involved to produce the desired sequence (loading, coupling and deprotection for each incorporated amino acid, cleavage and deprotection, purification and isolation).

Peptide production remains one of the worst processes in terms of solvent consumption, with an average solvent usage of several tonnes/kg of final API produced. Chemistry can be modified to avoid undesirable solvents, but the process can also be optimised to reduce the volumes.

Large volumes of solvent are required to rinse the resin efficiently between the coupling and deprotection steps. Online monitoring can be used to track the efficiency of the rinsing and end the procedure when it is completed. The design of the reactor can also be optimised to achieve a more efficient flush and minimise back mixing, so that the volume of solvent is minimised.

Solvent recycling is also another approach to consider. Most of the peptides currently used in pharmaceutical products are produced at relatively small (kilos) scale, due to the potency of therapeutic peptides and their typically low therapeutic dose. This is the reason why solvent recycling is far from systematic.
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Solvent recycling should be considered, not only at large scale, but also at smaller scales in the future. The environmental constraints and the desire for sustainability, combined with cost pressures - for the solvent, but also for its destruction - make this increasingly attractive and necessary. Chemistry should also move to ‘greener’ solvents and research efforts should be conducted in this direction.

Peptide synthesis involves a multi-step approach to assemble the different amino acids. Increasingly, most of the recently established production processes for newly approved peptide pharmaceuticals apply SPPS alone or combined with liquid phase fragment assembly.

One dream would be to reduce the molar excess of the incorporated amino acids by means of an online detection of the reaction's progress and the addition of the desired quantity in the shortest period of time. These operations are time-consuming and some GMP operations are still performed manually.

In the context of such a repetitive process, process automation can offer major advantages in reducing production costs and ensuring the best reproducibility of the process. Combining this automation with online detection of the progress of the process, all coupled with online dilution of the exact quantity of reagent, would deliver the required results and meet expectations.

Process automation is widely used at laboratory scale and online detection is also evaluated, but this is challenging with solid phase reactions. These tools should become more systematic at industrial scale, taking into consideration the regulatory constraints associated with GMP manufacturing. Online control is progressively applied in GMP production, in the context of process analytical technology guidance for industry.

**Downstream**

Downstream, preparative HPLC using reversed phase mode is the purification method of choice for the production of many peptide APIs. Chromatography is currently the principal industrial solution to remove peptide-related impurities. Three key drivers have to be considered to design the ideal process: productivity, yield and solvent consumption.

The global trend is to spend a lot of effort on the upstream portion of the process to improve the chemistry, then accept losses of up to 50% of the target substance in later processing, due to the difficulty of purifying the API. Improving the yield to 75%, even with a less productive purification process, would drastically impact the production cost when the raw material consumption and cost is important, in large-scale campaigns or campaigns employing unusual amino acid derivatives. The process cost should be evaluated in detail to define the criteria for the purification process as productivity and yield are two antagonist factors, as illustrated in Figure 1.

In my 18 years of experience in preparative chromatography, I have observed that many applications still employ a manual mode of injection and collection at industrial scale, even though automatic equipment is widely available. Process automation is still under-utilised, mainly due to a lack of industry acceptance and education on how processes need to be developed to exploit all the benefits of automation.

As an alternative to using long chromatographic beds, large product loads and multiple fractionation, with typically long gradients and recycling of semi-pure fractions, modern approaches tend to use short bed lengths with efficient stationary phases and smaller product loads to achieve good resolution, reduce fraction recycling and ensure better stability of the chromatographic performances with a rapid gradient.

The appropriate development of the purification process can lead to reproducible results under these conditions (Figure 2) and process automation can be implemented easily. The appropriate strategy, however, driven by the purification challenge; a single approach cannot, unfortunately, be generalised for all products.

The purification step is known to be a key contributor to the production cost. The ideal process design should therefore focus on the critical parameters impacting the global production cost: productivity, solvent consumption and purification yield. Innovation should focus on these key criteria.

Multi-column chromatography (MCC) is an attractive technology which can match these expectations. These techniques have received a lot of interest during the last decades when applied to the production of chiral APIs with the implementation of industrial systems.

The well-known advantage of MCC technologies is to improve productivity compared to standard batch technology. This advantage over batch approaches increases with the difficulty of the purification; the benefit of this approach is to achieve the desired purity with an excellent yield even when challenged by poor resolution between the target substance and the critical impurities.

The existing industrial applications are based on the simulated moving bed (SMB) and the Varicola® concept, but these processes are limited to binary separations and isocratic conditions. The separation of central eluting target compounds has been evaluated extensively to overcome the limitation of the SMB concept. Gradient elution on MCC processes has also been evaluated to extend the scope of application to biomolecules.

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**Figure 1 - Impact of target yield on the productivity & solvent consumption of preparative HPLC**

**Figure 2 - Reproducibility of five successive injections on preparative HPLC** (superposed chromatograms)
Recent advances in multi-component gradient separation are offering new opportunities to tackle the demands for peptide purification: high productivity, high yield and low eluent consumption.

The ‘multi-column counter-current solvent gradient purification’ (MCSGP) process is a major breakthrough in the development of this concept. The initial concept was based on a carousel of six columns. This has been rapidly simplified with a three-column concept and the latest evolution proposed design is based on a simple two-column module (the Contichrom concept), which delivers a more practical solution for large scale applications.

The MCSGP process has been successfully applied to the purification of a peptide and showed a 25-fold improvement in productivity and an increase of 5-7% of the achieved yield over batch HPLC. An innovative open-loop multi-column process has also been recently presented for the purification of a crude peptide mixture. These techniques will certainly impact the way of producing synthetic peptides at industrial scales in the near future.

Preparative chromatography is known to be a dilutive process and large volumes of solvent are required in production. Reductions in solvent consumption can be achieved by implementing multi-column processes. Solvents can also be recycled by evaporation or distillation, considering the efficient solvent recycling strategy applied for other large-scale chromatographic applications or using membrane technologies. The latest innovation to produce nano-filtration membranes stable to organic solvents opens up new opportunities to concentrate products and recycle the solvents in the process.

**Dreaming further**

Let us dream of the future steps: a process with no waste of organic solvent; a fully automated process with online monitoring of the progress of reactions; the addition of reagents and amino acid derivatives to reduce the consumption of all chemicals; a reduced process time for each amino acid derivatives incorporation; an automated purification process with quantitative yield without compromising productivity...

There is a huge potential for innovations to improve the existing technologies with wonderful challenges for the research teams. These dreams should drive the future innovation axes to deliver it as a reality.

- * Varicol is a trade mark of Novasep

**References**


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