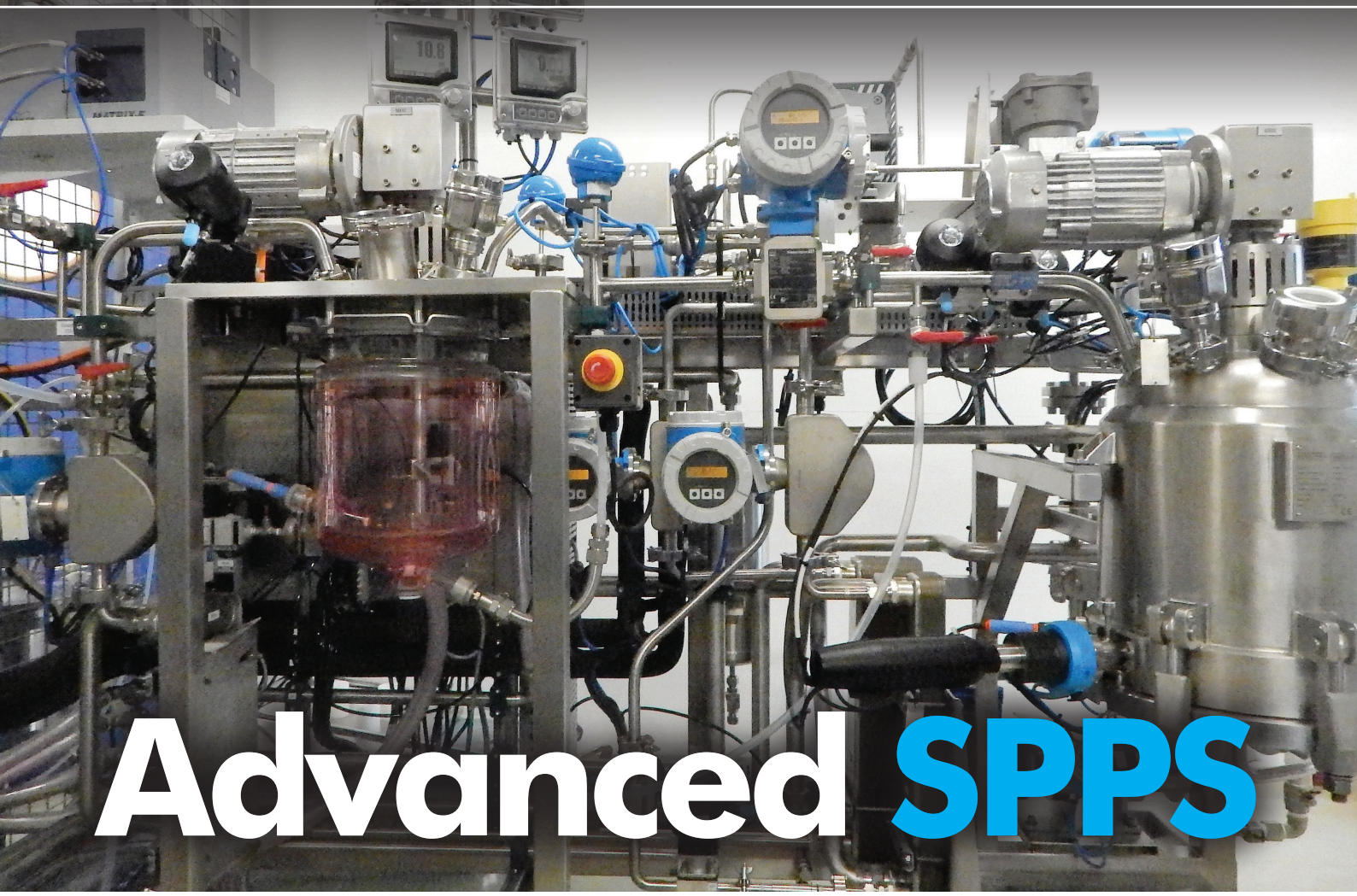


Advanced SPPS

Olivier Ludemann-Hombourger of PolyPeptide Laboratories and N. Petitjean of Ypso-Facto look at the challenge of large-scale peptide synthesis on solid supports*

SOLID-PHASE PEPTIDE synthesis (SPPS) is well established and routinely applied on efficient automatic synthesisers, which are available at laboratory scale. However, manufacturing peptides at industrial scale remains a major challenge, considering the complexity of these multi-step syntheses, the long lead times and the large volume of reagents and solvents required to produce the final API. In 2013, we published an article looking at the way to overcome these challenges from a holistic point of view.[1] This article presents the latest practices contributing to making the ideal peptide plant become a reality.

Interest in the large-scale manufacturing of peptide APIs has boomed since 2003, when the FDA approved Enfuvirtide (Fuzeon), a 36-amino acid peptide inhibitor of HIV1 membrane fusion. This offers a promising future to Fmoc-SPPS, based on the Fmoc strategy first outlined by L.A. Carpino in the 1970s.[2] Indeed, since the emergence of SPPS, many resins, linkers, protected Fmoc-amino acids and activating chemistries have been developed. Fewer than ten peptides were commercially available in 1990, against about 70 in 2018, while some 170 are now in various clinical phases and over 500 in the pre-clinical phase. The peptide drug product market was worth $5 billion in 2003 and $25 billion in 2018; it is expected to reach $47 billion by 2025.

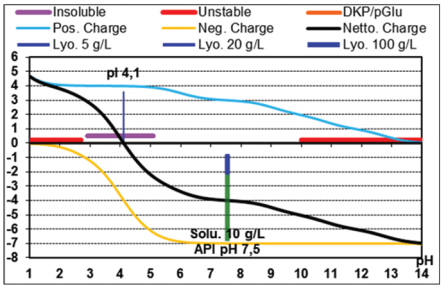
The main challenge of the coming decade will be to cope with the growing demand for peptide manufacturing in an acceptable way. The design of manufacturing processes also needs to take into account the evolution of the ecological factor (waste reduction, green solvent use and technology) of and the economic pressure on drug products. Typically, procedures for industrial SPPS are scaled up directly from the laboratory process: the reactor size is increased and it is operated mostly manually.

To address these challenges, Polypeptide Laboratories is running a programme called Advanced SPPS. This is based on a process engineering approach along several axes: improving the process development methodology to obtain a fundamental understanding of SPPS and develop predictive tools; and improving the manufacturing technology by developing advanced control solutions and fully automating operations.

Figure 1 – Extract of the predictive toolbox used for peptide synthesis

H-His-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp-OH

4 Base, 7 acid. pI ≈ 4.1 Precipitate and aggregate around pI pH (3.0-6.0)



pH >9: Asn-Thr form Asu and Asp, β-Asp.

pH <5: Asp-Gly form Asu and Asp, β-Asp.

Met and Trp oxidizes easily.

Acetate will lyophilize away giving insoluble/fibrillating product close to pI.

Best option is the sodium salt, ≈ 4 eq Na, isolation pH ≈ 7.5

Improving development methodology

Valuable knowledge and data have been gained over years of R&D on several thousand SPPSs. This has been used to build a predictive toolbox to assess any new peptide synthesis and identify critical steps (such as aspartimide, ß-Asp, N-to-O shift, diketopiperazine formation, oxidation and hydrolysis) and elaborate a controlled strategy with a smart selection of raw materials and appropriate operations to minimise impurity formation. It also facilitates the downstream process. Figure 1 illustrates the stability, solubility and charge state of the peptide with regard to pH.

The tool also estimates the potential for aggregation and the foreseeable impurities, to assist developers in selecting the most suitable synthetic route. This can greatly reduce the time required compared to a trial-and-error approach. Moreover, a new methodology has been developed to improve process performances based on a combination of experiments and predictive simulation. This enables us to understand further the mechanisms associated with SPPS and adjust the processes based on this knowledge.

A simulation tool was developed in collaboration with Ypso-Facto, a company with experience in chemical process simulation. This was developed based on information gathered from experiments; the model was validated by comparing existing experimental results with their simulated counterparts. The tool combines a reaction model (about 40 reactions and 50 species/step), a mass transfer model and adsorption models that describe the distribution of species between the liquid and the solid phases, and its influence on the different reaction rates.

This makes it possible to simulate the successive steps of deprotection, washing, amino acid activation, coupling on the resin and acetylation. The evolution of the concentration of species versus time in the liquid phase and solid phase are calculated and can be compared with experimental data. It is thus possible to determine the best conditions to minimise critical impurities, such as deletion, insertion or racemisation, guanidylation products and others, while maintaining maximum resin conversion.

The evolution of species versus time and process performances can also be calculated for batch and continuous stirred tank reactors and plug flow reactors. This tool also facilitates the exploration of new ideas *in silico* and running only a minimum of experiments for validation (Figure 2), which considerably reduces experimental efforts to propose solutions toward an optimised process.

The tool is constantly improved by making the most of the strong experimental knowledge of the chemist, the data accumulated over the years and the approach of the process engineer. For example, it can identify working conditions for which the solvent consumption during the washing steps is reduced by a factor of two to three compared to common practices. Coupling times have been reduced by a factor of two to ten by adjusting reaction-operating conditions, while keeping the same reagents and chemistry.

Advanced process control & automation

Increasingly stringent standards, increasingly challenging customer demands and intensifying competition have forced industries to develop more reasoned and controlled syntheses. To help with this, online monitoring methods have been developed. Thanks to the large quantity of data collected and the reduction of the analysis time, they can be used at every stage of product development, offering a better understanding of the chemical and physico-chemical phenomena affecting the reaction to establish links between process and product during manufacturing and ensuring control and smooth running during manufacture.

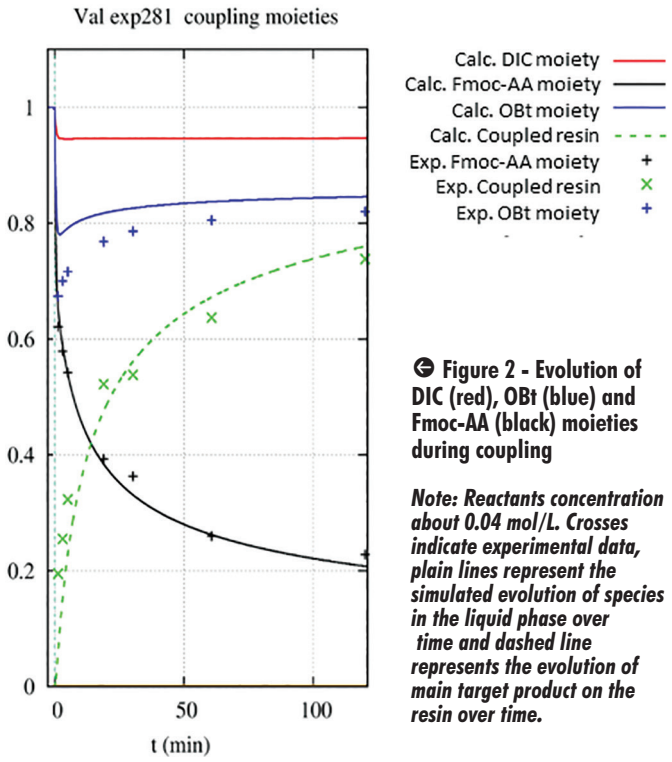
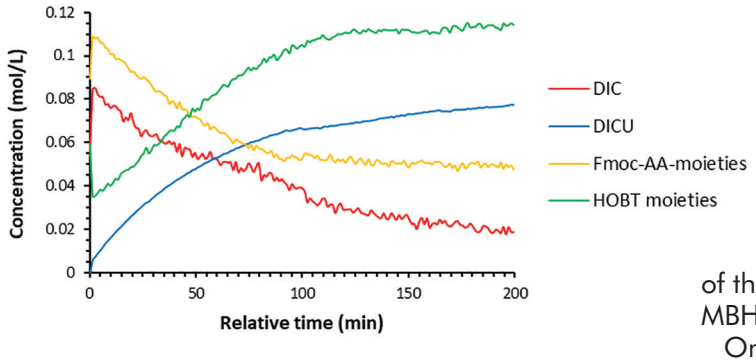


Figure 2 - Evolution of DIC (red), OBt (blue) and Fmoc-AA (black) moieties during coupling

Note: Reactants concentration about 0.04 mol/L. Crosses indicate experimental data, plain lines represent the simulated evolution of species in the liquid phase over time and dashed line represents the evolution of main target product on the resin over time.

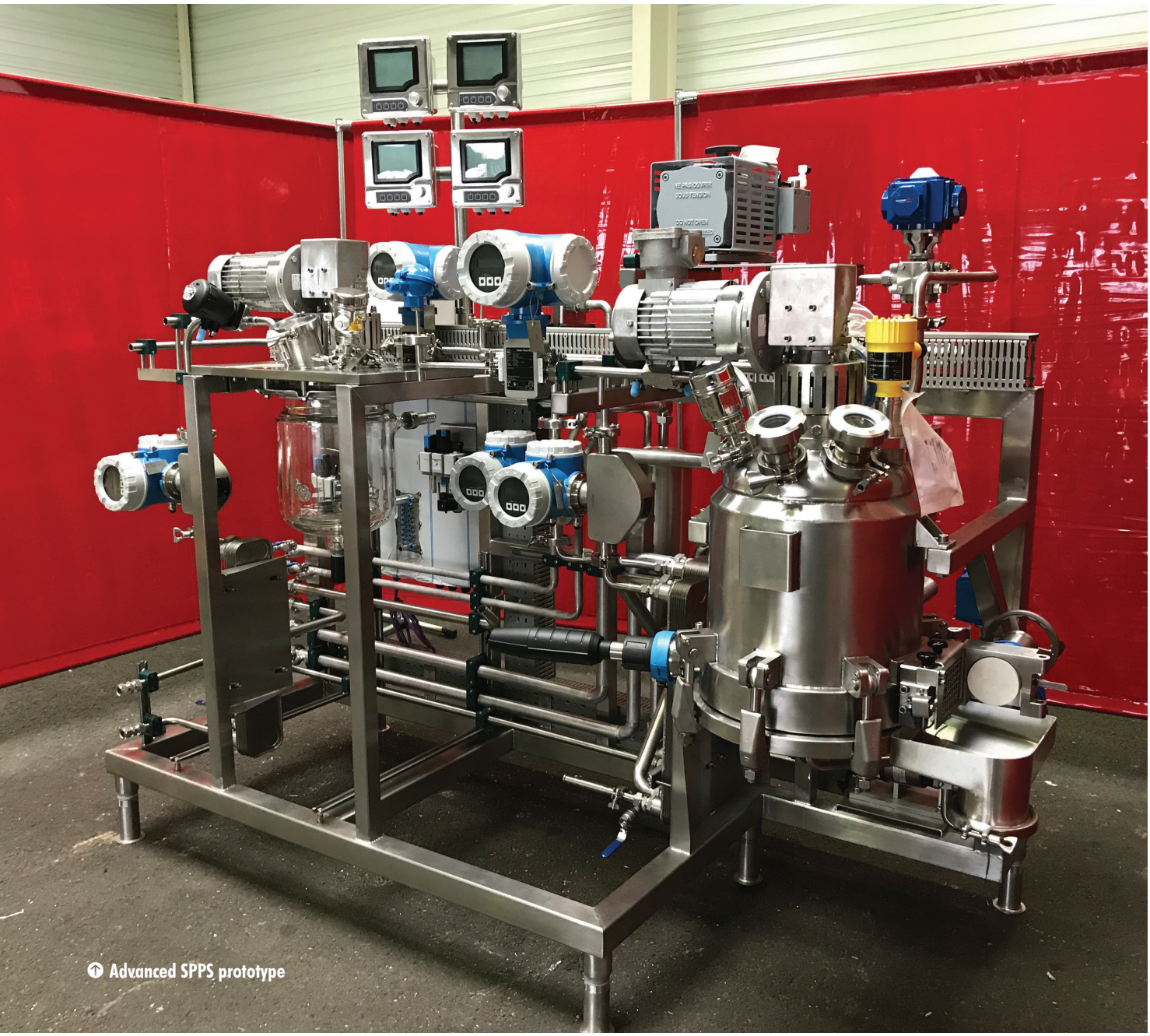
Figure 3 – Online monitoring of the coupling reaction of a lysine on a Ramage-MBHA resin



For each step, a suitable instrumentation must be chosen to match the goals and constraints.[4] In any case, it should be compatible with the reaction medium and provide accurate and robust data at a frequency adapted to the kinetics of the reaction.[5]

With these challenges in mind, Polypeptide studied the feasibility of online monitoring of SPPS. By selecting relevant sensors – for instance, for pH, conductivity and absorbance – we have demonstrated that the key parameters of each step can be monitored qualitatively and quantitatively, looking at both the solid and the liquid phases. This is illustrated in Figure 3, displaying the progress of the coupling reaction of a lysine on a Ramage-MBHA resin.

Online monitoring helps to improve the SPPS technology: it facilitates better control of the reaction (resulting in an improved impurity profile) and optimised productivity (optimal duration of each process step). It also helps to reduce the environmental



Advanced SPPS prototype

impact of the process by minimising the solvent volume used. In addition, the use of this data with the prediction models and advanced control models previously described will significantly increase the gains already achieved.

Peptide synthesis involves many repetitive steps, which brings a high risk of human error when performed manually. Impurities can be created at each step, which increases the need for purification and hence the cost of the final product. Process automation aims to reduce the operator's influence. His mission is then to plan, monitor and control the process, and handle any situation that has not been anticipated by the control system. This leaves the operator more time to focus on more important tasks. It can also collect more information and make corrections more precisely, faster and more frequently than the operator.[6]

To obtain a fully automated process, it is necessary to implement an advanced control system at each stage of the process, based on data obtained in real time, such as resin or liquid height, temperatures, pressures, flows, concentrations and others. A fully automated SPPS prototype has been designed to implement the automation of the process, integrating the new online monitoring tools.

For example, an advanced control system in the reactor was implemented. The performances obtained during washing steps with such a system agree with the predictive models, that is, reduced solvent consumption and washing time compared to common practices. In the near future, process automation at industrial (GMP) scale will be essential and will lead the race to competitiveness in the peptide segment.[7]

Perspectives

Based on the better process understanding and process automation, Advanced SPPS has been developed to get closer to the 'ideal peptide plant', featuring fully automated and greener processes with a drastic reduction of the manufacturing time as imagined in 2013.[1] The overall process cost was reduced, while its robustness and reproducibility were improved. Seizing innovation opportunities remains important to further boost the performances, through a joined research effort of peptide chemists with process engineers.

References can be found on ***www.specchemonline.com***

**Also contributing to this article were E. Dropsit and R. Ravetti of PolyPeptide Laboratories*

CONTACT

Olivier Ludemann-Hombourger
General Director
PolyPeptide Laboratories France

oliver.ludemann@polypeptide.com
www.polypeptide.com