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Peptide chemistry: in-silico development tools for an efficient process design

KEYWORDS: SPPS, peptide chemistry, optimization strategy, predictive tools, process design.

ABSTRACT

This article highlights a new way of thinking for the optimization of Peptide Synthesis. The concept is based on numerical tools to assist the peptide chemists for the choice of the manufacturing route. Process development can be shortened without compromising on process performance and robustness, through the development of unique predictive tools and the use of numerical models to better understand the reaction mechanisms.

INTRODUCTION

The field of Solid Phase Peptide Synthesis (SPPS) has boomed over the last decades. This development has been made possible, largely influenced by the chemist's expertise at the laboratory scale, whose philosophy is still embedded in the industrial processes of peptide synthesis. Indeed, the first needs for peptides in large scale lead to a simple scale-up of laboratory procedures. The growing demand in the peptide market, the development and greater availability of new raw materials (e.g. Fmoc-protected amino acids) together with an increasing competition has brought costs down and allowed the synthesis of peptides at an industrial scale. The overall quest remains producing high-quality peptides at lower costs while meeting customers' expectations such as reducing solvent consumption (or other environmental impact) without affecting the product quality (1).

Although SPPS has existed for more than five decades, the available literature still does not provide a clear understanding of peptide synthesis concerning the contributions of different fundamental physical and chemical phenomena like chemical kinetics, resin-liquid equilibria, mass transfer, and others. This lack of precise understanding is hampering a streamlined process development and explains why most manufacturing processes are developed with a high proportion of trial and error.

The PolyPeptide Group is running an innovation program called "Advanced Peptide Synthesis" to get more efficient at developing peptides by reducing the number of trial iterations. Together with YpsoFacto, we elaborate a new way of thinking to facilitate process development and get a deep understanding of the fundamental phenomena.

THE CDMO PARADIGM

There are several synthesis strategies that could yield a desired new peptide. Figure 1 explains the paradigm faced by all Contract Development and Manufacturing Organization (CDMO) organization during the lifecycle of a new API.

At an early stage, the goal is to get a fast access to a sufficient quantity of the API in order to support the preclinical and early clinical phases. Time is generally lacking for the development of optimized processes. The process is ideally locked at this early stage and the impurity profile is more or less controlled, that is, with little variability allowed at a later stage. When the project progresses to a later stage, the end-customer expects from its CDMO to deliver an efficient process, meaning robust and cost-optimized. At this stage, the possibility to significantly modify the manufacturing recipe is however getting problematic without affecting the entire development program. There is therefore a risk to get stuck with poor process performance at commercial stage if the process selection is not optimized at an early stage. Getting to an optimized process at such an early stage is a major challenge for peptide chemistry.

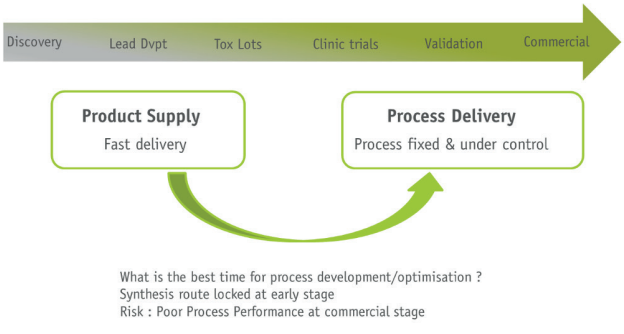

Figure 1. the CDMO paradigm.

A trial-and-error strategy is not the best answer to this demand as we ideally expect to develop the most efficient process at the early stage with limited efforts and adjustments before validation.

The development of the manufacturing process is typically based on an iterative strategy, described by the loop presented in figure 2. The selected strategy for peptide

synthesis (upstream) generates a crude peptide, which is purified to get the expected API purity (downstream). Analytical techniques are used to identify the nature of critical impurities (impurities difficult to get resolved from the target peptide), as they directly impact the productivity and the yield of the downstream process. The upstream strategy is then optimized to remove, or at least reduce these impurities. All efforts should be made to minimize the number of iterations and converge quickly to an optimized process.

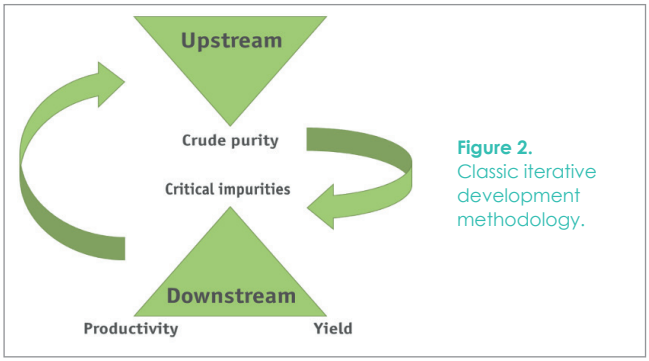

Figure 2. Classic iterative development methodology.

Over the last years, we have developed new tools to assist our development chemists in their work so that they can make the most efficient choices at early stage.

This strategy rests on two complementary pillars:

- The management of the expertise gained over the last decades to develop a predictive toolbox able to preliminarily assess new peptides
- The development of simulation tools able to represent our understanding of the complex peptide chemistry mechanisms and to get access to a new way of developing and optimizing the process *in silico*.

CHEMISTS PREDICTIVE TOOLBOX

Although companies' strategies may differ, a classic development process starts from the identification of the peptide sequence and a comparison with the knowledge available within the company. Very early, some preliminary research such as investigation of peptide stability, identification of synthesis structural difficulties etc. needs to be performed as well as other considerations e.g. on raw material and equipment availabilities. Then, the synthetic route strategy scouting can take place, for which various raw materials and operating conditions are selected and tested. This iterative process depends on the difficulties encountered during the synthesis as well as the difficulties associated with the corresponding purification operations.

To reduce the long lead times, few basic tools exist and are available to the public. For instance, several websites offer the possibility to enter a peptide sequence, sometimes with specific N- or C-terminal modifications as well as description of disulfide bridges. The calculated results provide information on molecular weight, hydrophobicity plot and the evolution of net charge with respect to pH. Some tools can even go further by providing some structural prediction (with propensity to form alpha-helices, beta-sheets, hairpins or random coils) and by giving some warnings on synthesis, cleavage and purification along with suggested solutions. However, structural prediction is only proposed for simple linear peptide sequences consisting of the twenty natural amino acids. Nowadays, synthesized peptides can be far more complex, made of several motifs such as cycles, branches, with a great versatility of building blocks, amino acids but more generally any polyfunctional chemical monomer. One quickly understands that calculations such as e.g. net charge with respect to pH will not be available without information on the various pK_a of each monomer.

Companies building peptides strongly rely, rightly so, on their internal cumulated knowledge. However, the decades of experience are either trapped in the brain of the development chemists or only partly consolidated.

This approach is a first step toward a decrease of the number of iterative steps for the improvement of peptide synthesis. However, this approach, although necessary, does not allow understanding the fundamentals of peptide chemistry. As part of a larger program aiming at improving the process development methodology and manufacturing technology, a novel approach at PolyPeptide Laboratories now consists in going back to the basics of peptide chemistry and looking through the chemist's glasses with the engineer's eyes. This methodology can sometimes challenge the existing practices and open new perspectives.

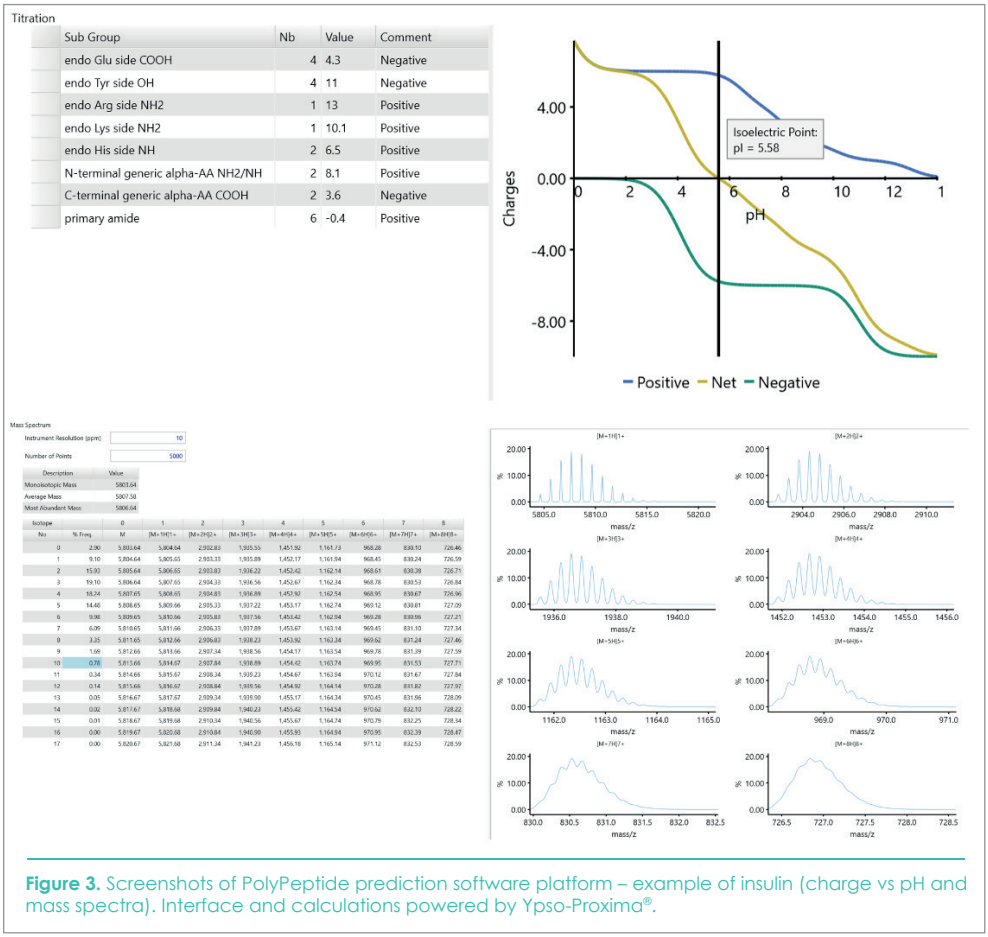

Figure 3. Screenshots of PolyPeptide prediction software platform – example of insulin (charge vs pH and mass spectra). Interface and calculations powered by Ypso-Proxima®.

The PolyPeptide Group has implemented a structured database, where the experimental data are aggregated in standardized formats aiming, for instance, at identifying similarities between a new peptide to be produced and peptides that have already been synthesised. Trends can be observed through the various substeps of deprotection, washing and coupling and lead e.g. to a better selection of raw materials, estimations of coupling times for given target peptide yield and purity, etc. Working with Ypso-Facto and its software Ypso-Proxima®, the PolyPeptide group is improving the consolidation of its knowledge in a series of dedicated tools including peptide sequence edition, predictions of charges vs pH, mass spectra, conformation potential, solubility of peptides, impurity profile, solvent consumption for downstream processes, etc. Confidence in the predictive results is increasing over time as more and more cases are fed to the database.

SPPS SIMULATION TOOL

Although the collection of experimental data and the development of statistical tools are extremely valuable, it is often difficult to find a satisfying answer to specific questions chemists and engineers might ask. For example, what are the rate-determining steps within the set of reactions involved in a peptide synthesis, how to boost the reaction kinetics, how to optimize the volume of solvent used, how to minimize the excess of reagents, how to control the kinetics of an undesired side reaction...?

In the past, and up to now, these questions have mostly gotten default answers that work in most cases and cannot be optimal without defining objective criteria for such a complex system. One has a liquid phase and the resin composed of its skeleton and pores filled with liquid. For a given step, there are many species involved and many reactions that could occur during the sub-steps of deprotection, washings, coupling and acetylation. The choice of activation agents can vary a lot: carbodiimide, uronium, phosphonium chemistry etc.

Although analytical methods will not be discussed here, it is important to recall that the capacity to design a model and feed it with high-quality data also relies on the ability to measure the evolution of the system over time: concentrations of key species, volumes of resin, temperature, pressure ... The experimental tools must be adapted to the phase in which the species are followed. For the measurement of concentrations in the liquid phase, UV spectrometry and HPLC have been found suitable for most measurements. However, offline monitoring is cumbersome for a routine basis. Instead, online monitoring tools are suitable and have been adapted to SPPS reactors (2, 3).

The complexity of a SPPS system and the means to study it explain why, in the literature, one struggles to find a model capable of describing SPPS (for a given chemistry) which justifies the efforts needed to build a reliable mechanistic model.

To gain a greater understanding of this system, one first needs to build the foundation of the model. The methodological approach employed was (1) build a reaction scheme representing the main species involved during SPPS; (2) with a top-down approach, represent most of the underlying general physico-chemical principles such as mass and heat balances, fluid phase equilibria, heat and mass transfer, stoichiometries and kinetic laws, fluid dynamics; (3) refine the model when needed by representing specific phenomena in greater detail.

Instead of trying to represent all the reactions and species reported in the peptide literature, one can reduce the numbers significantly. By taking into account only main reactions and important side reactions, one is left with about 40 species and 30 reactions per step.

Tools based on statistical fitting of experimental results can potentially generate large mistakes and rarely help at gaining scientific understanding. On the other side, the development of a numerical model, able to represent the reaction mechanisms, opens new perspectives to extrapolate how the system would behave under unexplored conditions.

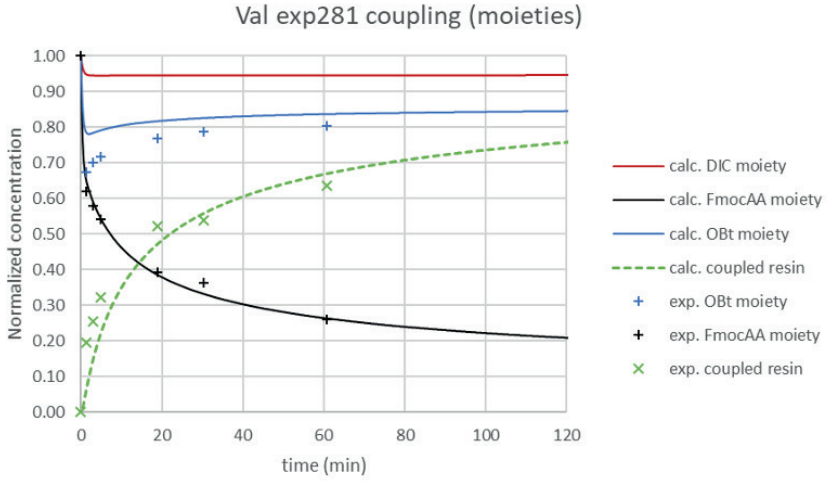

Figure 4. Evolution of key species involved during a given coupling step over time – Experimental versus model agreement.

OUTLOOK

There is no doubt that SPPS performances as well as process robustness will be improved thanks to a better process understanding. With those new in-silico development toolboxes, peptide chemists will be a step closer to be Right First Time in their iterative development strategy.

Having these tools allows to answer some key questions for those willing to assess unexplored domains in the field of SPPS. Such tools also have great capabilities for training. They are a first step before answering even more complex topics or issues like the influence of using a different resin, optimal coupling time for minimizing Endo or Des product, influence of heating on coupling time and impurity formation and many others.

The development of such tools demonstrates that chemists and engineers contribute the best from their area of expertise, by interacting together. This will be seen more often in the future.

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