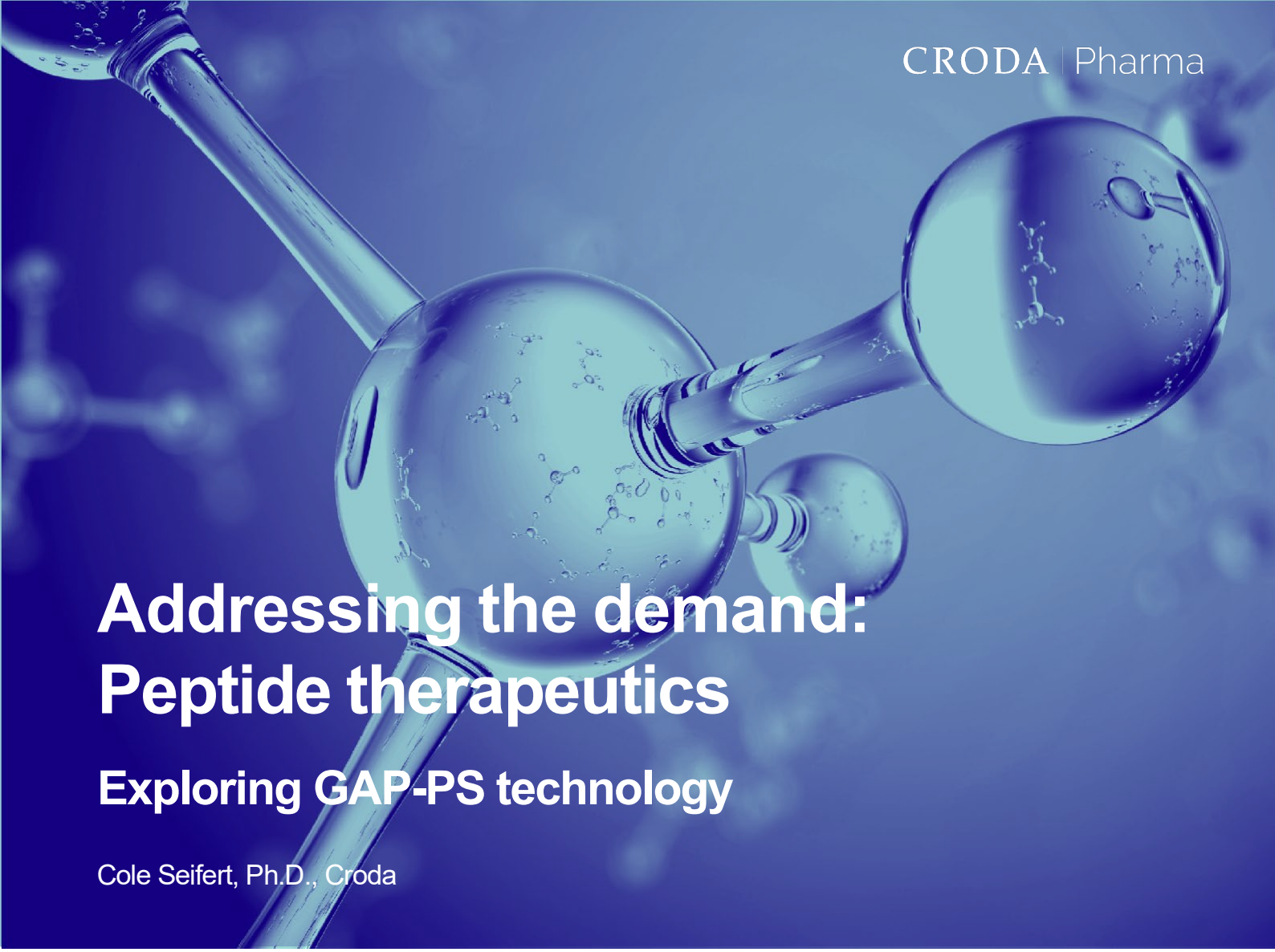

Addressing the demand: Peptide therapeutics

Exploring GAP-PS technology

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Peptide therapeutics are critical therapeutics for a range of pathologies. Their recent uptake as a treatment for obesity has seen a surge in global awareness and demand.

Unfortunately, peptide supply cannot keep pace and current production methods are unable to meet the desired volume.

GAP-PS is an emerging synthesis method that could provide a sustainable and cost-effective solution to what is fast becoming one of the major therapeutic supply challenges of our era.

Demand is surging, supply is not

We are now in the peptide therapeutics era. The global market for incretin-based peptide therapeutics is growing rapidly. These drugs, which are based on glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were discovered decades ago but demand for them has recently begun to surge. They help to regulate glucose metabolism and control blood sugar levels, by stimulating insulin secretion from pancreatic beta cells.

Traditionally used for type 2 diabetes mellitus, these drugs have recently been approved for use in obesity, where they are also proving very effective. Their beneficial effects are also being explored across several other potential pathologies, including but not limited to cardiovascular diseases, nonalcoholic steatohepatitis, and kidney disease. The result is a significant demand spike that is proving difficult to meet.

This is partly because these incretins are complex to manufacture (in some cases, molecules of 30 amino acids or more) and expensive when compared to small molecule drugs. This is all exacerbated by challenges around technology, cost, scalability and sustainability that affect the industry's ability to produce these incretins in the required volumes.

Indeed, the market for treating obesity could eventually exceed a billion patients and the effects of this are already being felt by diabetics, who today are experiencing an unreliable supply of these drugs. To a great extent, this production shortfall is down to specialist reactor capacity issues in current production methods but could also be intensified by a lack of source materials and downstream capacity.

As such, these incretins are both scarce and expensive; on both a per-patient basis and for the health service organisations that underwrite their costs. All of this has seen some countries considering bans on the export of such drugs, while others look at limiting their use.

Current production approaches and limitations

Existing production methods are proven at smaller scales, but are unable to meet today's demand, a situation that is likely to deteriorate as incretin demand continues to rise.

Next generation peptide drug manufacturers currently favour chemical synthesis over recombinant manufacturing techniques, with two main production approaches for peptides, solid phase peptide synthesis and classical solution phase synthesis, outlined below.

Solid phase peptide synthesis (SPPS)

Currently the most popular manufacture route, SPPS is a method of chemical synthesis used to construct peptides by assembling amino acids one by one on a solid resin support material.

The process begins with the attachment of the C-terminal amino acid to the insoluble resin support, usually polystyrene beads.

Subsequent amino acids are then added sequentially in a stepwise fashion. Coupling reactions between the incoming amino acid and the growing peptide chain are usually facilitated by coupling reagents and activators.

After each coupling step, unreacted amino acids are washed away using a solvent, leaving the growing peptide chain attached to the resin.

SPPS requires specialised equipment for careful stirring and filtration of the resin suspension. The advantage of this approach is that it is labour efficient - the process of 'wash, filter and repeat' is fast and reliable, once everything is set up.

Yet there are some downsides to this process for the production of longer peptides at the scales required to meet current and predicted incretin peptide demand.

The first is that heterogeneous systems like these can create scalability issues. The specialised equipment required to achieve much larger volumes of peptides via SPPS is not readily available and where it can be obtained it is extremely expensive. Only a small number of peptides have been made at the 100KG+ annual demand, with enfuvirtide as a singular example of 1,000KG+ annual demand.

The second is sustainability. SPPS requires a large excess of amino acids and reagents which inevitably end up in the waste stream. More importantly, repeated resin washing steps require large volumes of petrochemically derived solvents. The main solvent is DMF(Dimethylformamide) which is a CMR (carcinogenic, mutagenic, or toxic for reproduction) agent and classified as an SVHC under REACH, which raises questions for its long-term use.

As a result, the expected sustainability profile for incretin peptides made through this method is poor - around 100 tonnes of CO_2 per KG, compared to roughly 2-5 tonnes for smaller peptides produced through the solid phase production method, even when the same process is carefully optimised.

And because the projected demand for incretins could be multiple orders of magnitude larger than the peptide industry has been able to produce to date - tens of metric tonnes a year – using SPPS could create millions of tonnes of CO_2 every year.

Classical solution phase synthesis

A fundamental technique in organic chemistry, here reactions occur in a liquid solvent, typically in a flask or reaction vessel. In this approach, starting materials are dissolved in a suitable solvent, and various reagents are added sequentially or simultaneously to initiate chemical transformations. This method is appropriate for short sequences, 3-5 amino acid long peptides, and at a larger scale, where material costs dominate labour costs. However, incretin peptides fall well outside the size limitation of this classical technique.

It is important to note that classical solution phase techniques offer efficiency and scalability, as a result of their homogenous character. However, they don't provide process control over the peptide molecule in the same way as a solid phase resin. In SPPS, the resin dictates the physical properties of the material being handled, making the process more predictable, something that is absent in classical solution approaches. Consequently, workups using classical solution phase synthesis can be longer and more difficult, requiring more development and labour per cycle.

A liquid solution

Group Assisted Purification Peptide Synthesis (GAP-PS) technology - an approach Croda has recently invested in through the acquisition of GAP Peptides - is fast emerging as an exciting alternative to conventional methods of peptide production. This method is appealing due to its potential to help meet the world's growing need for incretins, in a more sustainable way.

It works by attaching a small anchor molecule to the peptide during production, controlling the solubility properties of the peptide molecule in solution, in a predictable fashion. This control is what leads to the advantages of the technique.

Here is how it works in more detail.

How GAP-PS works

Built on the principles of Group Assisted Purification (GAP) chemistry and developed by Dr. Cole W. Seifert and Dr. Guigen Li at Texas Tech University, GAP-PS combines some advantages from both SPPS and classical solution phase synthesis.

The specially designed GAP anchor molecule is attached to the C-terminus of the starting amino acid and simultaneously acts as the C-terminal protecting group. The anchor controls two key aspects of peptide solubility: first, it allows for clean and rapid aqueous workup after Fmoc deprotection, by preventing water solubility and loss of the peptide in the aqueous wash. This allows replacement of the solvent washes used in SPPS with water washes in GAP-PS, without yield loss. This change from solvent to water washes is the primary driver of the sustainability benefits of GAP-PS - reducing the carbon footprint. It is a major benefit in moving from SPPS to GAP-PS.

Second, GAP-PS has a unique advantage over competitor technologies in that it can control (and significantly increase) the solubility of the peptide in the organic solvent. This is critical to ensure process relevant concentrations during scale up, but is also critical to properly and predictably dissolve longer peptide sequences of the type required for incretin peptide synthesis. Controlling the solubility of the peptide, by both favouring the organic phase and disfavouring the aqueous phase, allows for a consistent liquid-liquid extraction, which, in turn, enables the process to be labour efficient and consistent - from one peptide sequence to the next - unlocking the possibility of fully automating the process, in the same way that SPPS is.

The technology is specifically designed to work with the Fmoc/tBu protection strategy, and supports various coupling chemistries, including carbodiimide, uronium, aminium, and Oxyma-based reactions. This versatility makes it suitable for a broad range of peptide synthesis applications.

The advantages

Scalability

Meeting incretin peptide demand challenges by scaling SPPS reactors will be very expensive, and maintaining expected product yields and quality at these scales is a serious challenge.

Solution phase methods scale much more efficiently and cost-effectively. 10-15kg batches of peptide are a typical output from production SPPS reactors today - not enough to meet current or projected incretin peptide demand. Even if reactor size was not a barrier to scale, the financial implications are. Scaling solid phase reactor technology to the sizes required to meet growing demand will become financially prohibitive to all but the largest entities.

In contrast, the solution phase reactors needed for GAP-PS are easily available off the shelf to peptide manufacturers up to significantly larger sizes - 20,000 – 40,000 litres are easily obtainable. And many pharmaceutical companies and contract manufacturers already possess large scale solution phase capability and thus are ready to start using GAP-PS with a few adjustments. With GAP technology, it is projected that manufacturers could produce a 20kg batch of incretin-based peptides in a 1000L reactor, and 100kg in a 5000L reactor using readily-available equipment.

Sustainability

GAP-PS also adheres to green chemistry principles, focusing on waste reduction, energy efficiency, and safe solvents - which significantly reduce hazardous waste compared to existing conventional processes. The efficiency of the method allows for highly efficient reactions in minimal solvent volumes, reducing waste by up to 80%.

GAP-PS uses more environmentally friendly solvents like 2-MeTHF, which significantly reduce carbon use when compared to SPPS solvents. This, combined with its need to use less solvent and more water overall, can reduce CO_2 from the synthesis by 80-90%. Therefore, we could see roughly 100 tonnes of CO_2 per KG of peptide via SPPS brought down to 20 tonnes via GAP-PS.

Cost

Unlike many other sustainable technologies, GAP-PS does not come with a cost premium. Conversely, it can cut 30-50% on a manufacturer's COGS.

Risk mitigation

GAP-PS also provides more control over the production process. Manufacturing the large batches of incretin peptides required to meet current and future levels of demand comes with a considerable cash risk. In-process checks can help to ensure batches aren't lost and GAP-PS allows for rapid and accurate in-process checks using HPLC and LC-MS - enabling more detailed, real-time understanding to help prevent losses or optimise production.

This is unlike SPPS, which requires time consuming chemical separation of the peptide from the resin to achieve the same level of analysis, which can either significantly slow the process or motivate the manufacturer to accept an element of chance – a dangerous proposition considering the heightened financial risk.

This additional level of awareness offered by GAP-PS is particularly important during experimental production phases - e.g. pilot, development and scale up, where improved in-process checking can lead to better optimisation, and ultimately more reliable, better-performing production processes.

Fragment optimisation

For those companies that have already looked to optimise their SPPS assets through deploying a fragment and associated fragment condensation strategy, the GAP-PS technology is applicable here too as side chain attachment of the GAP Anchor confers solubility to the peptide fragments post resin cleavage enabling simpler and more efficient fragment condensations.

Conclusion

GAP-PS offers a very real prospect of increased product yield, with greater quality and reliability, in less time from a much smaller operational footprint, and with a considerably better sustainability profile. Through collaborative development efforts between Croda and its partners in the pharmaceutical sector, it could help address the world's growing incretin peptide demands with fewer resources, fewer costs, greater safety, and less hazardous waste than existing approaches.

To this end, Croda has conducted an incretin peptide proof of concept study on the GAP-PS synthesis of one of the major market relevant peptides. If you would like to know more about GAP-PS or the outcome of the proof of concept, please get in touch and help play an important part in tackling one of the major drug supply challenges of our era.

Croda Pharma

Croda's Pharma business is a leading partner for the development of excipients and the supply of high purity materials for pharmaceutical formulations, committed to enabling the next generation of drug delivery systems. The business is focused on empowering biologics drug delivery, through its adjuvant systems, small molecule, protein, and nucleic acid delivery platforms. With a wide range of solutions for both human and animal health markets, our pharmaceutical portfolio is unsurpassed in its excellence for drug and vaccine delivery.

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Dr. Cole Seifert obtained his Bachelor's and PhD in Chemistry from Texas Tech University. During his PhD research, Dr. Seifert invented Group-Assisted Purification (GAP) Peptide Synthesis Technology and secured investment to commercialize the technology via the startup company, GAP Peptides, LLC. From 2017 – 2022 he served as GAP Peptides' President and Chief Scientific Officer, and led the development of scalable, sustainable methods for peptide and biopolymer synthesis. Following acquisition of GAP Technology by Croda, Inc. in 2022, Dr. Seifert now leads peptide development activities at Croda.

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