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The expansion of the therapeutic applications of **peptides**: drivers and challenges

The notable expansion of peptide therapeutics in the late 1990s and 2000s led to an unprecedented number of marketing approvals in 2012, and has provided a robust pipeline that should deliver numerous approvals during the remaining decade (1). Peptides offer certain advantages as drugs; these include their high biological activity, high specificity and low toxicity. However, challenges exist for the drug development of peptide therapeutics. Obstacle number one: in general, peptides need to be parenterally delivered (via injection) because oral administration would lead to their degradation in the digestive tract. Obstacle number two: they have a short half-life because they are quickly broken down by proteolytic enzymes. Obstacle number three: their chemical nature prevents them to a large extent from getting past physiological barriers or membranes (2). That said, why has there been a renaissance with respect to peptide drugs in the pharmaceutical industry? First of all we should say that peptides often target receptors and enzymes that are difficult or impossible to access with small molecules; thereby, providing drug discovery and development of novel targets to potentially offset the revenue void left by recent drug failures and the loss of patent protection of blockbuster drugs. Moreover peptides can complement biologics as drugs with the hope for greater efficacy, selectivity and specificity. Peptides possess bioactivities that are of major interest for drug discovery; peptides, peptide fragments, or peptidometics can intervene in most physiological processes and pathways. Given therefore the growing importance of peptide-based drugs, Chimica Oggi / Chemistry Today has decided to interview a few key players in this field to provide insights into the current challenges, drivers and regulatory situation (3).

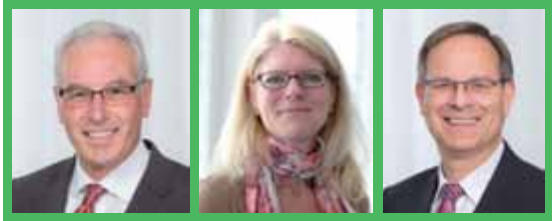
What are some of the current barriers or challenges to the development of peptide therapeutics?

Dr. Alex Fässler, COO Europe, Bachem AG; Dr. Martina Diekmann, Head of Global Marketing Bachem Group; Dr. Gerhard Haas, Vice President QA/RA, Bachem AG

There are a variety of challenges for the development of peptide therapeutics:
First is their intrinsic complexity for manufacturing, characterization and formulation, which increases their COGS. For this reason many peptides are developed for niche markets with smaller Drug product demands, where the typical high potency of peptides offsets the costs of the API.

Secondly, formulation of peptides other than injection is still a challenge and requires extensive development. In recent years, though tremendous advances have been made through active or passive transporters, carriers, transdermal or nasal delivery systems and development of oral formulations. Yet, oral bioavailability is still a major hurdle in mainstream applications to compete with small molecules, which in turn exerts pressure on the peptide manufacturers to reduce costs for the API.
Thirdly, expectations from the agencies for the control of the purity profile of peptidic APIs are becoming increasingly sophisticated and require a thorough understanding of the nature and the fate of impurities. Manufacturing of peptides on solid support for instance does not rely on intermediates which can be crystallized to remove impurities in the course of the synthesis, but rather progresses through so-called

telescope steps with final purification only. Therefore regulators expect a commensurately high level of process understanding and control. Specification for starting materials, e.g. for protected amino acid derivatives should address impurities based on the origin, fate and purge and use validated analytical methods. It is evident that for a peptide of 30 or more amino acids in chain length development time and cost will increase considerably to satisfy such demands.



Dr. Alex Fässler, Dr. Martina Diekmann, Dr. Gerhard Haas Bachem AG

Jordi Pirò, Sales manager, BCN Peptides S.A.

We are glad that the Peptide therapeutics is a very active field, with very hot topics like GLP derived peptides or Therapeutic Vaccines. There are some challenges that we might consider as the biggest difficulties that we are facing these days, we are more and more looking into the long peptides as therapeutic molecules, modified peptides and insoluble peptides. In some cases in one peptide you can find combination of these challenges, so process development becomes a critical aspect in order to have a robust process that yield the desired quality and keeps the COGS as low as possible.

Jonathan Collins, Director of Business Development, CEM

From a synthetic perspective, current barriers include generating crude peptides efficiently with high purity, fast synthesis time, and economical reagent usage. The higher the purity of a crude peptide the easier it will be to subsequently purify. Purification can be extremely difficult and timely for peptides as much difficult to separate impurities can be present. CEM is addressing these hurdles through recently development improvements for the solid phase peptide synthesis (SPPS) process widely used to make peptides. In 2014, CEM published its new High Efficiency Solid Phase Peptide Synthesis (HE-SPPS) process (1), which improves peptide purities with microwave irradiation, reduces cycle times down to only 4 minutes, and eliminates more than 80% of the chemical waste. This method has been automated on a newly released peptide synthesizer, Liberty BlueTM which utilizes this new process and was a winner of a 2014 R&D 100 Innovation Award. Additionally, we recently have exclusively licensed the rights to manufacture an improved resin, SpheriTide. The SpheriTide resin has a unique chemical structure based on the food preservative poly-lysine that provides an ideal environment for building peptides on resin. SpheriTide allows for significant improvements in peptide purities even at high substitution levels > 1.0 mmol/g.
1. Collins, J.M.; Porter, K.A.; Singh, S.K.; Vanier, G.S.; Org. Lett., 2014, 16, 940.

Mimoun Ayoub, Director, Global Peptides / Carbohydrates / Lipids & Injectables Platform, CordenPharma

Peptides constitutes interesting drugs in many ways. However, there are also challenges associated with peptide drug discovery and development. The synthesis challenges can be addressed. The main issues are associated with the plasma half-life and low bioavailability. Furthermore, nearly 95% of the approved peptide drugs are injectable. The drug product sterile manufacturing is an area requiring special expertise. The drug needs to be reconstituted before the injection to the patient, which usually requires intervention from the physician. Fortunately peptide drug delivery has progressed a lot over the past 15 years.

Robert Hagopian, Director Business Development, PolyPeptide Laboratories

The biggest challenge for peptides is the short half-life in the blood stream due to enzymatic degradation.

James Cain, Ph.D., Applications and Account Manager, Protein Technologies, Inc.

While there has been a renewed interest in peptide therapeutics in recent years, some real and perceived challenges remain, including the lack of oral bioavailability, lower in vivo stability, and higher synthesis costs relative to small molecules. A number of approaches are being taken to address the first two issues, including macrocyclization and other synthetic modifications like N-methylation and backbone amide replacements. Large projects like the development of T-20 (Fuzeon) have helped to reduce the costs of peptide synthesis significantly in the past fifteen years. In any case, these make up a very small percentage of drug development costs.

Brady Clark, Ph.D., CEO, Sussex Research laboratories Inc.

Sussex Research is not directly involved in the development of peptide therapeutics. My company supports development of peptide-based therapeutics through R&D collaborations, contract research and supply of glycosylated peptides (glycopeptides).
Having said that, it's well known that peptide therapeutics can have solubility and stability issues, are difficult to deliver and generally can't be taken orally. Due to the effect of proteases, peptides tend to have rather short in vivo half-lives. This tends to limit what would otherwise be very specific and efficacious peptide drugs in a hurry.

Companies source peptides for many therapeutic indications. Is unmet medical need a big driver for peptide therapeutics? In your opinion, why or why not?"

Dr. Alex Fässler, Dr. Martina Diekmann, Dr. Gerhard Haas, Bachem AG

Absolutely. Peptides as therapeutics are very specific for their target and typically highly active. Given the current fragmentation of the market, for instance in oncology diagnostics and therapy, highly active, small volume APIs can easily satisfy a niche market.

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Jordi Pirò - BCN Peptides S.A.

Jordi Pirò, BCN Peptides S.A.

In the past maybe an unmet medical need was clear driver, but we believe that peptides have proven to be very competitive therapeutic ingredients. So, for new developments we see companies working with non-niche indications also.

Mimoun Ayoub, CordenPharma

Peptide drugs cover a wide range of therapeutic indications. Some of the therapeutic targets are better addressed using peptides. There are clearly unmet medical needs for multiple disease areas. However, what drives most of the peptide drug development is more the advantages over the classical small molecules. Peptides differentiate mainly by their:

- High Biological Activity
- Low Toxicity (Less accumulation in organs and drug-drug interaction)
- High Specificity and Aaffinity
- High Biological and Chemical Diversity

Robert Hagopian, PolyPeptide Laboratories

In principle, the general safety and potential efficacy are the drivers for developing peptide therapeutics.
Some peptides may present some unmet medical needs, however the main driver for peptides is the safety factor and at times potency.

James Cain, Protein Technologies, Inc.

Yes. Evolution has given us peptides with exquisite potency and selectivity that often can't be duplicated by traditional small molecule drug candidates. Furthermore, underexplored drug targets like protein-protein interactions are increasingly recognized for their potential in a variety of disease areas, but are inherently difficult to modulate with small molecules.

Brady Clark, Sussex Research laboratories Inc.

Since Sussex Research is not directly involved in the development of peptide therapeutics, I think that there are many people more qualified than me to talk about this. However, since we are primarily focused on chemistries around carbohydrate-based molecule and glycoconjugated biological molecules, Sussex Research is often called upon to glycosylate peptides and other biomolecules for therapeutics targeting very specific and rare diseases that often have some sort of glycobiological component. So, at least from my vantage point, it does appear that peptides are being developed for numerous unmet medical needs.

Can the goal of orally bioavailable peptides be achieved? Will macrocyclic peptides be the answer?

Dr. Alex Fässler, Dr. Martina Diekmann, Dr. Gerhard Haas, Bachem AG

Metabolic stability of peptides remains an issue, given the very nature of a peptidic amide bond, which is susceptible to cleavage by a number of enzymes, as well as by hydrolysis. A number of approaches are being explored to stabilize peptides without reducing their target affinity. Macrocyclic peptides or polycyclic peptides are one approach to protect the peptides against metabolism or to prolong their half-life. Other approaches include incorporation of unnatural amino acids, which are not recognized by hydrolytic enzymes, or structural modifications to sterically encumber the labile sites. We need to keep in mind that oral bioavailability is also dependent on the molecular weight and physical parameters of the peptide. The goal of orally available peptides can be achieved, predominantly for shorter chains. The effort, cost and complexity to surmount structure-intrinsic hurdles increases with the length of the peptide chain.

Jordi Pirò, BCN Peptides S.A.

This is one of the targets for the future. It has been already achieved for some Peptides, and obviously the use of macrocycles is necessary to obtain a good oral bio-availability. On the other hand there are other delivery systems that might be more interesting than the oral route for a lot of peptides.

Mimoun Ayoub, CordenPharma

Yes absolutely. We are getting very close. Multiple teams and companies across the academia and industry are working on new oral peptide drug delivery tools/platforms. We start seeing in vivo bioavailability data in the 10-15% range or higher, depending on the peptide sequence and length. This development complements the existing technologies consisting of long-acting release formulation using polymer or liposomal encapsulations. Oral peptide drug delivery will substantially change the face of this already interesting business.
Will macrocyclic peptides be the answer? Definitely! By constraining the peptide backbone through cyclisation and introduction of non-natural amino-acids, one increases the peptide bioavailability by decreasing the enzymatic degradation. There are multiple orally delivered peptides in clinical trials. Multiple cyclizations are necessary for a peptide to become orally bioavailable. Linaclotide is a good example. Other similar peptides are in clinical trials phase 2 and 3.

Robert Hagopian, PolyPeptide Laboratories

It is conceivable that some peptides are and may be rendered bioavailable orally especially with the new drug delivery techniques. Will macrocyclic peptides be the answer? This is one exciting avenue to constrain peptides and render them more bioavailable.

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James Cain, Protein Technologies, Inc.

There are a lot of exciting approaches being explored. It remains to be seen whether a general answer to the problem can be found, but clearly cyclization is a modification that can improve many properties, including oral bioavailability. Taking a cue from nature and the example of cyclosporin A, many researchers are looking at the combination of cyclization and other modifications like N-methylation.

Brady Clark, Sussex Research laboratories Inc.

Since Sussex Research is not directly involved in the development of peptide therapeutics, I think that there are many people more qualified than me to talk about this. But I would like to think that this can be achieved either through modifications to the peptides themselves or using drug delivery vehicles.
At Sussex Research, we are working on glycosylation aspects of not only peptides but other types of difficult to deliver molecules. We work on direct glycosylation of the native peptides themselves. We work in a peripheral way with collaborators on delivery systems such as nanoparticles for targeted delivery of biotherapeutics in general. Our interest is in carbohydrate ligand-targeted cell delivery systems. These are systems that exploit specific carbohydrate ligands recognized by specific lectin receptors on specific cell surfaces. The goal is a cell specific drug delivery system.
Many antibiotics are based on macrocyclic lactone rings (macrolides) and, by the way, these tend to be glycosylated with deoxy sugars. So, macrocyclic peptides would seem to hold great promise for oral delivery of therapeutics. I particularly like the idea of glycosylated macrocyclics but haven't seen anyone try to do it yet.

Are peptide therapeutics getting more complex (un-natural amino acid substitutions, modifications like PEGylation or lipidation)? What challenges will that pose to manufacturing (chemistry, sourcing raw materials, scale-up)?

Dr. Alex Fässler, Dr. Martina Diekmann, Dr. Gerhard Haas, Bachem AG

We do see a trend towards more complex peptides, either by modifications of the peptide chain or the incorporation of unnatural custom amino acid derivatives. Such peptides require very specific chemistries, for example with orthogonal protecting groups to differentiate reactivity between side chain function for site specific derivatization. Since such starting materials are no longer commercially available from various vendors like the common amino acids, sourcing of specific and increasingly complex starting materials is a time critical factor on the API development path. Frequently the only source is in-house development and scale-up of multi-step syntheses with the concomitant lead time.

Jordi Pirò, BCN Peptides S.A.

Yes, we can see this is a general trend, there are more peptides under development and they are getting more complex also. Obviously, it means that the customers need that the CMOs have the necessary Know-How, not only in chemistry but also in sourcing in order to achieve the targets.

Jonathan Collins, CEM

Non-natural modifications represent a significant area of interest due to the unique properties they can add to potential peptide therapeutics. Non-natural modifications can definitely represent challenges for incorporating them into peptides with SPPS. First, they are often expensive and therefore desirable to couple at low excess amounts. Additionally, non-standard derivatives (ex. N-methyl, Aib derivatives) can also contain high steric bulk that can dramatically increase coupling difficulties. Last, non-natural modifications can also be susceptible to side-reactions that may require modifications to the overall synthesis process.
CEM's HE-SPPS methodology is useful for non-natural modifications in that it can drive difficult reactions to completion without large excesses. This can allow for significant cost savings in the synthesis process for expensive non-standard derivatives. In some cases it can also provide peptide purities that are very difficult to achieve with conventional methods at room temperature conditions.

Mimoun Ayoub, Director, CordenPharma


Mimoun Ayoub
CordenPharma

Yes, there is a clear increase in product complexity. The chemistry has made it possible for discovery to consider very complex molecules as their target leads. Peptide modifications such as the more commonly used conjugations, (i.e. lipidation, PEG-ylations, glycosylation) and cyclizations, as well as the introduction of un-natural amino-acids in the sequence, are usually meant to increase product stability in the plasma and decrease the dose and side effects.
Sourcing complex raw materials is always difficult. In this context of peptide modifications, sourcing building blocks or intermediates can be a challenge. Usually the sourcing company has to provide a production process to allow the supplier to make the said building block or starting material.

Robert Hagopian, PolyPeptide Laboratories

Peptide modification in order to extend the half-life is becoming more popular, especially by adding a larger molecule to the smaller peptide. What challenges will that pose to manufacturing (chemistry, sourcing raw materials, scale-up)? There are some classical manufacturing challenges that will result from emerging of any new class of compounds, however the bigger challenge and depending on the type of modification, the final product characterization and analytical testing, will become an even larger challenge.

James Cain, Protein Technologies, Inc.

While the practice of introducing unnatural modifications isn't new, almost all peptide R&D groups now seem to be examining complex and modified peptides. Whether at discovery scale or in scale-up, the tools used by these researchers, including automated synthesis instrumentation, need to be flexible enough to accommodate unusual monomers, special reactions, and variable protocols. Introducing certain modifications can make a synthesis more challenging, so enabling technologies like rapid infrared (IR) heating become important. When more expensive building blocks are involved, it also becomes especially important that these reagents are used frugally, with no waste. These are all considerations that we've had in mind when designing instruments like the Prelude and Symphony X, with maximum flexibility in programming, many extra amino acid positions, and prime-free deliveries.

Nate Cosper, Protein Technologies, Inc.

Our collaborators and customers are increasingly interested in complex peptides with modified side chains and backbone structures. These un-natural substitutions address both scientific and commercial interests in that they improve in vivo stability and half-lives, while also providing for stronger intellectual property protections. Our efforts at Protein Technologies are centred on providing instruments and reagents that enable these complex molecules to be more readily prosecuted.

Brady Clark, Sussex Research laboratories Inc.

Based on my experience at Sussex Research, there is no doubt that people are attempting to discover, develop and implement more complex therapeutic peptide systems with a view to delivery as well as increasing in vivo half-lives. PEGylation of course is something that has been successfully exploited for quite some time now. Peptide modification via lipidation has also been shown to have beneficial effects on therapeutics by prolonging pharmacokinetics, not to mention enabling peptide interaction with cell membranes where many receptors reside.
From my vantage point as a carbohydrate chemistry specialist at Sussex Research, I'm seeing increasing interest in derivatizing peptides with carbohydrates (glycopeptides). As ligands, carbohydrates attached to endogenous peptides, proteins, antibodies and lipids are known to mediate various physiological processes in nature. So it's not a surprise that glycocosylation of a synthetic peptide, protein or antibody can confer increased therapeutic efficacy via superior stability, higher aqueous solubility, increased bioavailability, enhanced target resolution and longer in vivo half-lives.
At least from the perspective of glycopeptide manufacturing, every case will be different.

Brady Clark - Sussex Research laboratories

People are often very surprised to find that addition of a saccharide to their peptide can actually make it easier to process, particularly when it comes to purification. The challenges will mainly be in sourcing certain carbohydrates (take N-Acetyl galactosamine for example). In support of glycopeptide manufacturing, the manufacture of the glycoamino acid building blocks in large scales is a challenge that we are also currently working on.

How are you improving peptide properties, such as stability and half-life? What about innovating routes of administration?

Dr. Alex Fässler, Dr. Martina Diekmann, Dr. Gerhard Haas, Bachem AG

The selective chemical glycosylation of peptides increases their potential to improve in physicochemical properties, such as binding, half-life, stability and homogeneity. Our partnership with Glytech Inc. offers the chemical development and manufacturing of glycopeptides as a service. Generation of well-defined glycosylated structures through chemical synthesis allows optimal choice of the glycosyl moiety and its position for tailoring drug properties. We have a comprehensive library of glycans available, containing human glycoproteins linked to asparagine. For the lead compound that does not have ideal drug like properties, it can have their derivatives redesigned, which then are expected to have the desired properties and can be synthesized from milligram to multigram scales for further testing. Most chemically synthesized peptide drugs can be easily adapted to chemical glycosylation, a technique which allows

attaching any glycan at the desired position, to specify the number of attached glycans and to control the glycan structure. This results in a homogeneous product with potentially improved physiochemical properties. As a result, this can have a positive effect on pharmacological properties of a lead candidate, such as increased bioactivity, improved and modified receptor selectivity, and prolonged half-life.

Jordi Pirò, BCN Peptides S.A.

As peptide experts we help our customers when they want to improve certain properties of their peptides, we also provide to them information about alternative routes of administration and collaborate into the formulation development.

Mimoun Ayoub, Director, Global Peptides / Carbohydrates / Lipids & Injectables Platform, CordenPharma

As said before, peptide constraints helps to increase stability and plasma half-life. These constraints can be of different natures such as cyclization, conjugation, and non-natural amino-acids in the sequence.

As regards innovating routes of administration the most commonly used route is clearly the injection. Inhalation and patch release have not seen expected success but there is some activity in this area. Inhaled peptides cause multiple regulatory questions that are not always straightforward to address.

Robert Hagopian
PolyPeptide Laboratories

Robert Hagopian, PolyPeptide Laboratories

As a contract manufacturer, we can only comment generally on this based on what we have seen as trends. Currently several avenues exist to extend the half-life or bioavailability, including short acting release using advanced injectable formulations or needle free administration such as patches, longer acting release including but not limited to chemical modification and nanoparticles, or continuous release by using devices such as external pumps. As regards innovating routes of administration, this is a constantly evolving topic and the advances in drug administration will be applicable for peptides as well.

James Cain, Protein Technologies, Inc.

We provide tools that allow scientists in the peptide community to rapidly explore improvements in these properties, via molecular design, efficient synthesis, and screening. We've built instruments that are flexible and convenient for adding unnatural modifications and performing special chemistry. Our instruments have been used to make cyclic peptides by a variety of methods, peptidomimetics including peptoids and PNAs, N-methylated peptides, mirror image peptides, peptides that are PEGylated and lipidated, and many other examples with improved pharmacokinetic properties in mind. We also translate chemistry that might traditionally have been performed off the instrument, like cyclization, into automated methods.

Brady Clark, Sussex Research laboratories Inc.

Again, from my perspective as a carbohydrate chemist, I have been involved in projects where glycosylation of a native peptide can have profound effects on biological properties. Interestingly, in vitro studies of the effects of peptide glycosylation often do not hint at this and so glycosylation advantages normally go completely undetected unfortunately. The effects of glycosylation are often only witnessed in *in vivo* studies. I have seen very significant increases in stability, *in vivo* half-life and efficacy when compared to the non-glycosylated analogue. I suspect that there are a lot of reasons why this happens and that the protection from proteases that glycosylation offers is just one aspect of what is likely an extremely complex series of interactions in sera and at cell surfaces.

As far as improving peptide properties such as stability and half-life, although we have been working indirectly with collaborators on therapeutic peptides over the years, within the company we have been directly developing glycopeptides for topical use on skin. In our case, in vitro studies on skin cells have shown great potential in stimulating production of beneficial structural skin proteins such as elastin, fibronectin and collagens when compared to the non-glycosylated peptides. To the best of our knowledge, this is the first systematic study of glycopeptides on skin. Our in vitro studies have been supported independently by a 3D skin model at another company. We have a patent application for the work. Based on the improvement to native peptides via glycosylation, we plan to clinically test one of our candidates on human subjects this spring. We hope to have the glycopeptide active on the market and available to skin care companies by autumn 2015.

My work does not involve development of new routes of physical administration.

The current challenges in peptide manufacturing are impurities especially in peptide APIs. How are you handling this problem? What about regulatory issues?

Dr. Alex Fässler, Dr. Martina Diekmann, Dr. Gerhard Haas, Bachem AG

We combine thorough process and analytical development with our existing knowledge. Based on this knowledge we limit the critical impurities in raw and starting materials and minimize the potential for side reactions (e.g. the formation of epimers) by choosing appropriate preparative techniques. Then we identify the relevant impurities in the manufactured batches and try to avoid or reduce these by further process development. However, the worldwide

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harmonized thresholds for identification and qualification of impurities according to the ICH Q3A guideline are extremely challenging for peptides. Therefore, peptides are exempt from the scope of this guideline but only the European Pharmacopoeia defined more feasible impurity thresholds for peptides instead. Outside the EU it is unclear which limit for unspecified impurities should be targeted during development. Another severe regulatory problem for peptide CMOs is that authorities may require pre-approval for changes to suppliers, specifications, and analytical methods of the starting materials (amino acid derivatives). Since the amino acid derivatives are normally sourced and tested in common for all peptides manufactured by a site, any change would affect many peptides, customers, and authorities. We estimate that just one change to one of our amino acid derivative would easily lead to more than 90 change submissions. Obviously, these regulatory expectations are in striking contrast to the undisputed need for improving supply, quality, and control of amino acid derivatives.

Jordi Pirò, BCN Peptides S.A.

It is very well known that ICH guidelines excludes peptides when they talk about impurities. USP more recently and the EP earlier published some general rules for Peptide Impurities, that we use a starting point when we see a new peptide. These guidelines are also a reference in order to approach the regulatory aspects but at the end of the day, this is a very important topic for which you will typically receive the input of the Authorities.


Jonathan Collins
CEM

Jonathan Collins, CEM

CEM's HE-SPPS methodology improves the quality of peptides made by SPPS which in turn reduces impurities.

Mimoun Ayoub, Director, CordenPharma

Chemistry Manufacturing and Control (CMC) of peptides can be very challenging. The analytical part is absolutely key for a robust CMC. Impurity detection and monitoring, for stability studies for example, usually require more than just an HPLC/UPLC method. Identifying and characterizing the impurities require special skills in mass spectrometry.
As regards regulatory issues, Batch to batch consistency requires both a robust manufacturing process and robust analytical methods to demonstrate that the impurity profile and impurity level is reproducible. Analytical process technology and understanding the rational for impurity formation is therefore key.

Robert Hagopian, PolyPeptide Laboratories

Related impurities, especially when manufacturing longer than a 10 residue peptide, presents some challenges. We cannot claim that we can resolve all challenges related to production and associated impurities, but our approach is to constantly improve our manufacturing steps. This includes better coupling reagents in the Synthesis step in order to achieve the best possible peptide chain assembly, improving the peptide deprotection or cleavage conditions to generate the highest quality of crude peptide, using better media to purify the manufactured products, and finally better and early analytical detection of impurities. What about regulatory issues? This is strictly dependent on the reviewer at hand, on the peptide length, the related indication. We recommend our clients to interact with the regulatory agencies at an early stage in order to get a general feel of the expectations.

James Cain, Protein Technologies, Inc.


James Cain
Protein Technologies

Impurities can originate with poor quality starting materials or be introduced during a synthesis. On the first point, it's crucial that materials be acquired from trusted suppliers with rigorous quality control standards. On the second, our customers in the peptide manufacturing sector are carefully screening multiple reaction conditions, using simultaneous synthesis on instruments like the Symphony X, in conjunction with Design of Experiments (DoE) programs to identify the best methods to give the highest purity for their synthetic peptides. Of course the purity of the crude peptide affects the time and methods required for purification and analysis.

What emerging analytical technologies are you currently using or plan to use to resolve these challenges?

Dr. Alex Fässler, Dr. Martina Diekmann, Dr. Gerhard Haas, Bachem AG

We use UHPLC purity methods in order to achieve optimal separation for release analysis and faster throughput for the monitoring of preparative HPLC fractions. The excellent resolution of UHPLC mostly allows the selection of an elution system that is MS compatible. Then we employ UHPLC-MS to establish "peak purity" confirming that there are no unresolved impurities (except stereoisomers) hidden underneath the product signal. Furthermore, UHPLC-MS and UHPLC-MS-MS techniques are used for impurity identification and for peptide sequencing (proof of structure). We confirm stereo-chemical purity using amino acid analyses by chiral GC-MS with pre-column derivatization and deuterated reagents for hydrolysis. The latter allows us to disregard epimerization caused by the hydrolysis. Either the same technique or chiral HPLC is used to test amino acid derivatives for optical purity.

Mimoun Ayoub, Director, CordenPharma

Peptide based impurity can be process-related or associated with peptide degradation. Their characterization involves

HPLC-MS, UPLC-MS and MS-MS method for structure elucidation. In some difficult cases the impurity must be isolated and sequenced using standard methods such as Edman degradation. Impurities coming from the racemization during the coupling steps can be determined by GC-MS. In this case the peptide is fully hydrolysed, and the resulting amino acids derivatized (amine as trifluoroacetate, carboxylic function as Isopropyl-ester) and tested by GC-MS.
One of the challenges that require atypical analytical methods for classical peptides is aggregation or gelling. In this case there are multiple methods that can be used such as size exclusion chromatography (SEC), dynamic light scattering, asymmetric field-flow fractionation, and light obscuration.

Jonathan Collins, CEM

In our laboratory we recently adopted Ultra High Performance Liquid Chromatography (UPLC) technology. This has improved our separation and analysis capabilities over conventional HPLC. This has been useful for us to better understand impurities and provide direction for improving our synthetic methodology.

Robert Hagopian, PolyPeptide Laboratories

Better analytical techniques utilized during the in-process testing throughout the manufacturing steps will lead to better overall quality of product.

James Cain, Protein Technologies, Inc.

We have customers that are using fast UPHPLC, sometimes with parallel analyses of different stationary and mobile phases, and in some cases again applying DOE to help determine the best separations methods. In some instances a two-dimensional purification scheme may be employed to select for different impurities.

Brady Clark, Sussex Research laboratories Inc.

Since Sussex Research is primarily focused on the glycosylation process rather than the peptide process, we routinely use High Resolution Nuclear Magnetic Resonance (NMR) Spectroscopy to characterize our molecules. While NMR can hardly be considered an emerging analytical technology, it does not appear to have widespread use in peptide synthesis. We have extended use of NMR to glycopeptide synthesis where warranted.

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