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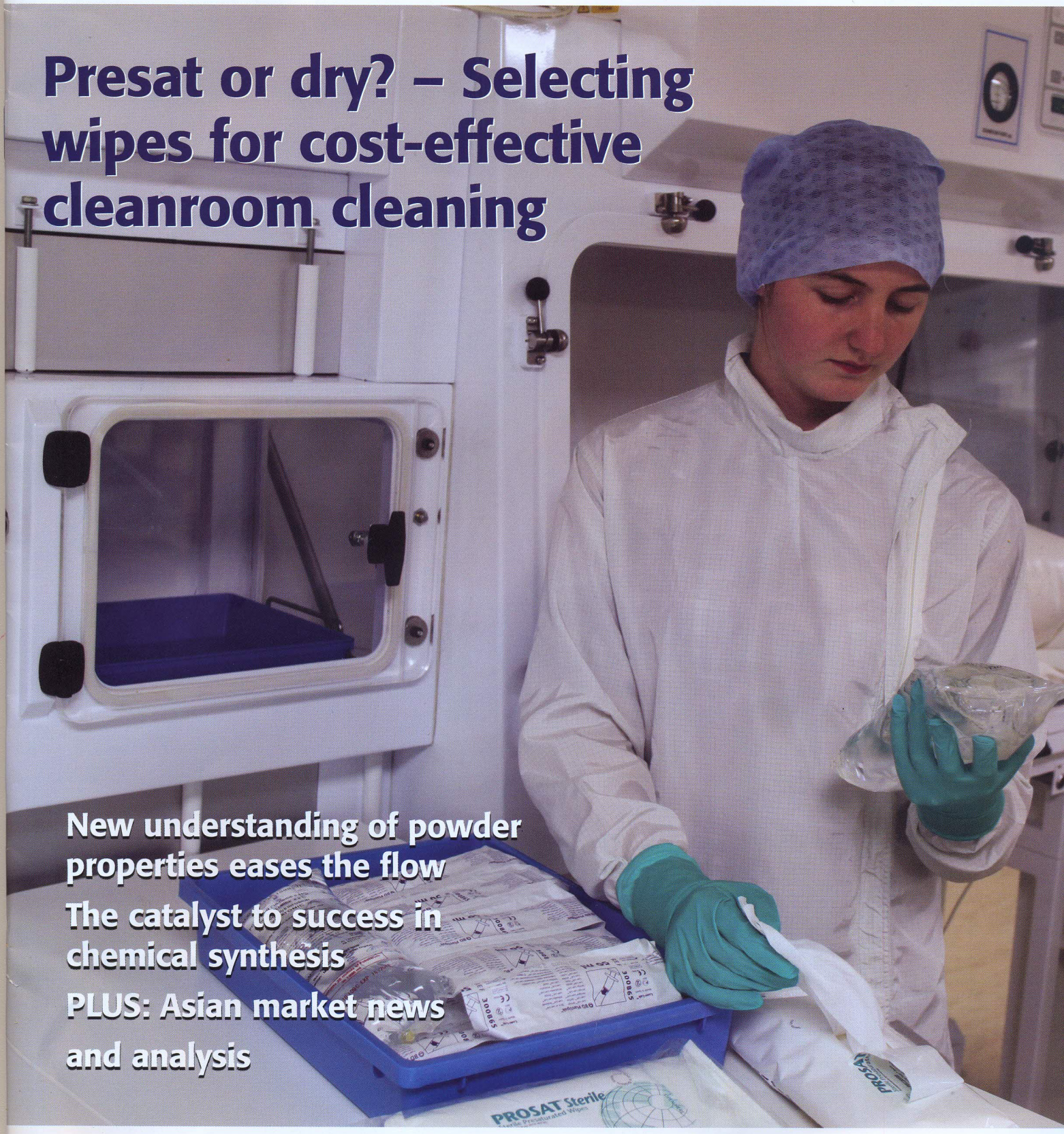
Presat or dry? – Selecting wipes for cost-effective cleanroom cleaning



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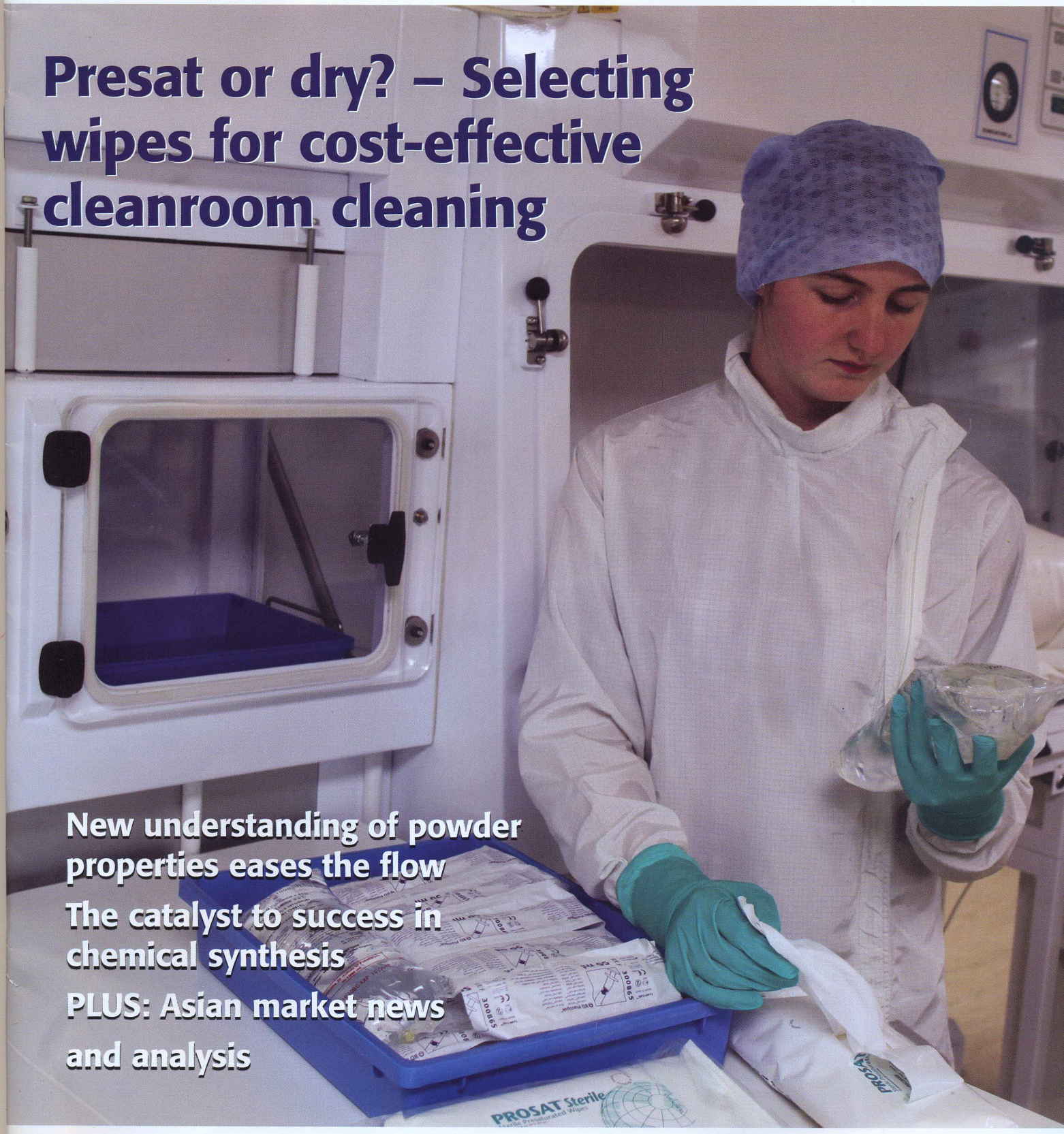

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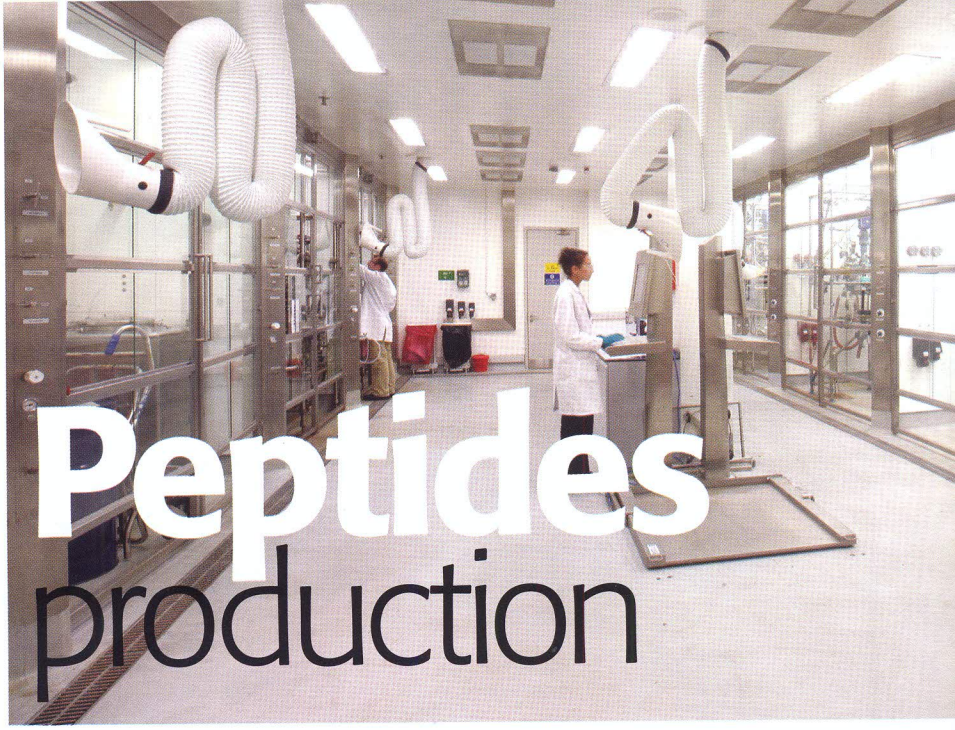


Peptides production

Peptides are of growing interest in therapeutics, not only as actives but also as carriers. **Alastair Hay**, Almac, looks at peptide developments and production challenges

Peptides are considered an attractive class of molecule as therapies because they can offer higher specificity and present lower toxicology issues than traditional small molecule drugs. Interest in peptide therapeutics has grown significantly in the past 20–30 years, with only one entry per year to market in the 1970s, rising to an average of nearly 17 per year in more recent times.[1] Currently, there are around 60 therapeutic peptide products available on the market, with an estimate of several hundred in clinical and preclinical development.

Peptides are used not only as therapeutic APIs, but also as carriers of non-peptidic payloads. For instance, peptides recognised for their specificity for a particular cell surface receptor, or their ability to penetrate cell membranes, are used as transporters of small molecule APIs, cytotoxics or imaging agents in current clinical development programmes.

One of the main challenges to peptides as therapeutics – their lack of oral bioavailability – has led to innovations in product design to enable controlled release, or an increase in the inherent half-life of the peptide entity. These factors are contributing to increasing demand on peptide manufacturing across the industry.

peptide manufacturing

As a manufacturer of peptides, Almac assesses a number of different methodologies available and the ultimate method chosen depends on the nature of the product. Biological methods of peptide production are well established with inexpensive raw materials and have been scaled to hundreds of kilograms a year. On the other hand, such production methods require significant investment in process design, and can result in long lead times for even small quantities of material.

Chemical methods of peptide production can be split into solution and solid phase approaches. The former uses classical organic chemistry to couple amino acid building blocks or short peptide fragments, while the latter (usually) requires Fmoc-protected amino acids to build peptides on an insoluble polymeric support, one amino acid at a time. In other cases, a hybrid approach is taken to build peptide fragments on solid support, and then combine one or more fragments in solution. Chemical synthesis has become significantly more attractive in the past decade, as the cost of protected amino acids has significantly reduced, so much so that the solid phase synthesis approach using Fmoc amino acids is recognised as the chemistry of choice.

In many ways, manufacturing methods in solid phase peptide synthesis are highly standardised (Figure 1). Amino acids are added one at a time to the solid support (resin) with a repeated cycle of coupling, Fmoc deprotection and capping failed coupling sites to prevent them from reacting in future cycles. Treatment of the resin-bond peptide with TFA and a scavenger mix simultaneously cleaves the peptide from the resin and removes side-chain protecting groups, which are mopped up by the scavenger mix. The crude peptide is isolated by precipitation and filtration, then purified by reverse phase HPLC. Finally, a second HPLC step will be used to exchange the peptide counterion to the pharmacologically acceptable acetate form, and the final peptide is isolated by freeze-drying.

In devising a process for a particular product for a GMP manufacture, at least a degree of process development is required. Conducting an efficient and effective process development phase is crucial to economically manufacturing material for clinical studies.

Many peptide therapeutics on the market have been manufactured with a variation of the above approach. However, challenges to scale-up remain. At the technical level, the capacity of a solid phase reactor is limited by the level of chemical functionalisation and swelling properties of the resin. The industry has tackled this by developing new solid supports that enable higher levels of functionalisation so that more peptide is obtained from every kilo of resin used.

In tandem with functionalisation, however, is the effectiveness of the coupling chemistry itself. High coupling and deprotection efficiency is required to maximise chemical yield, and with the repeat nature of peptide synthesis chemistry, even 99% efficiency over 40 coupling and deprotection steps required to synthesise a 20mer results in a chemical yield of 82%, and of a 50mer is 37%.

With the increasing interest in long peptides, by the time the calculation is extrapolated to a 100mer, the yield is 13% and so even highly efficient chemistry gives diminishing returns. This has been tackled with inventive ways of intervening in the chemical synthesis, identifying problem areas and implementing solutions. Just how much time is invested in resolving chemistry issues is a point of debate between sponsors and Contract Manufacturing Organisations (CMOs), with sponsors often not financially able or not willing to invest time in the depth of process development that CMOs may advocate.

A combination of approaches can reduce

Fig.1: Unit operations in solid phase peptide manufacture

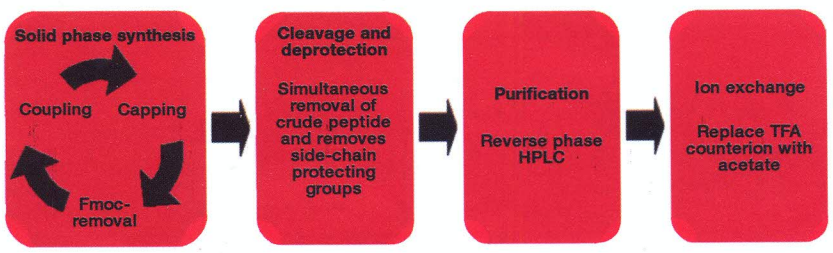
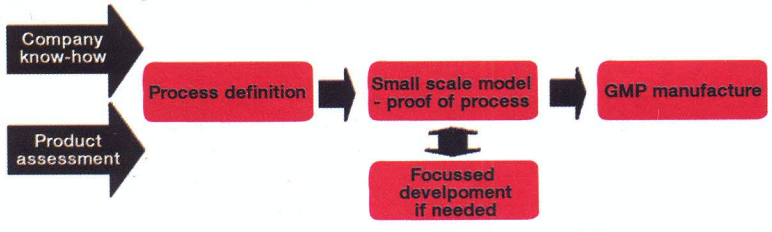


Fig.2: Streamlining process development for early phase GMP peptide manufacture



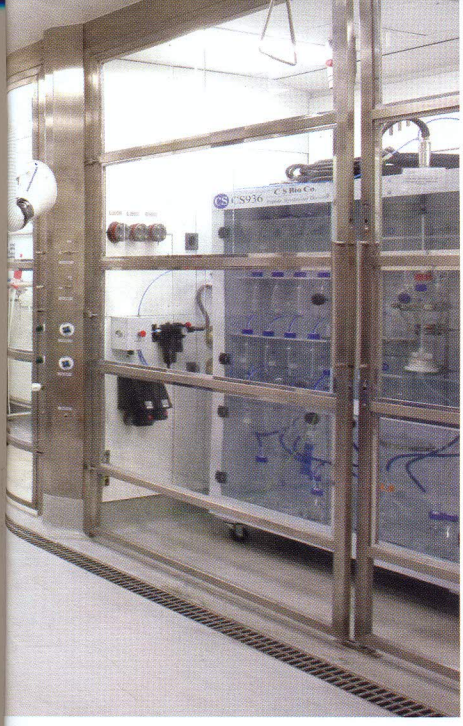


Fig.3: TAG-based peptide purification

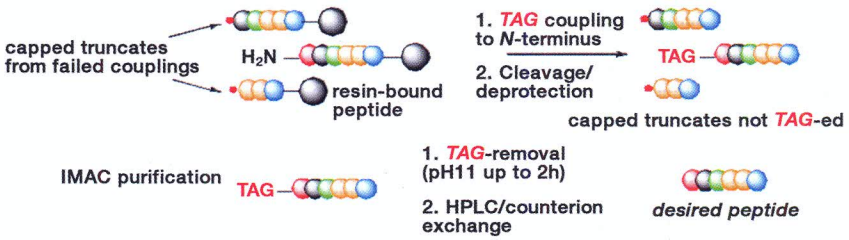
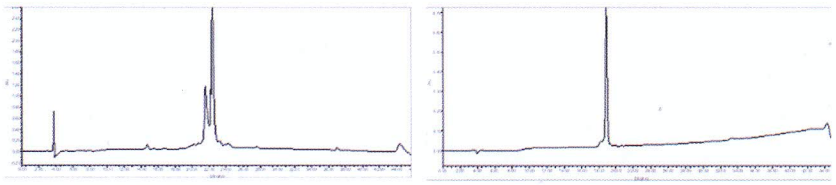


Fig.4: HPLC analysis of 36-amino acid peptide purified using cleavable TAG



Crude HPLC of *TAG*-peptide

Purified peptide after IMAC and HPLC

process development time in the early clinical phases where short supply times are crucial. In moving to cGMP manufacture, the process must be sufficiently locked, such that the outcomes are predictable. Almac has taken a combined intellectual and practical approach to defining and implementing process development for early phase GMP manufacture (Fig. 2).

Assuming there are no existing manufacturing protocols beyond those used in the lab to produce the first 100mg or gram, the peptide in question is assigned to a 'family', based on the amino acid sequence or structural motifs. Using proprietary rules Almac then defines a synthetic approach designed to overcome predicted difficulties in the peptide chain assembly.

The practical implementation of the defined approach is critical, with small-scale models of the manufacturing equipment ensuring the smooth transfer of the process to manufacturing scale. Where required, limited scope development is performed on key problem areas exposed by the small-scale model.

Being a cycle of repeated operations, solid phase peptide synthesis lends itself to automation. Taking advantage of automation can also result in shorter development and manufacture times, so long as the development model can be reliably reproduced at scale. A manual approach, in which every amino acid coupling is reviewed and a decision taken on whether or not to proceed, may result in as few as one or two couplings/day. Alternatively, a well defined synthesis protocol can be fully automated to couple 4–6 amino acids/day. Whatever approach is taken, the quality of the crude peptide obtained after the synthesis step is an important factor in choosing approaches to purification.

purification challenges

The standard work-horse in the purification of peptides is reverse phase HPLC. In the past 10–15 years, advances have been made in the range and quality of media available for bulk preparative HPLC, boosting the effectiveness of HPLC as a purification technique. Typically, a two-stage chromatographic purification is required. Step one, using trifluoroacetic acid (TFA) as an ion-pairing agent performs the vast majority of purification work, separating the desired peptide sequence from the mix of related substances, e.g. shorter peptide sequences from failed couplings. The result is a TFA salt of the peptide that, for toxicological reasons, is not an acceptable final form of the product, and so an exchange of the counterion to acetate is the standard approach taken. This is usually achieved with a second HPLC step, which may or may not be designed to enhance product purity as well as enable the exchange.

Almac's development of the purification method once again starts with an empirical assessment of the peptide sequence. Different amino acids impart different characteristics to the behaviour of the peptide in solution. For example, basic residues lysine and arginine help solubility in the acidic conditions preferred for HPLC purification, whereas a high ratio of hydrophobic amino acids could indicate difficulty in purification by standard means. Assessment of this information helps define the starting point of any purification development programme, which examines choice of solid phase and mobile phase to resolve the desired product from impurities.

Preparative HPLC is a time-consuming process with multiple injections required to process a single batch, and lengthy analysis to support fraction pooling decisions. Analysis time can be reduced by devising methods based on UPLC, which achieve short run times compared with HPLC. Devising the most cost-effective process depends on the scale of manufacture. In early clinical phases, fixed costs are the main contributor to overall costs, while later phases and commercial production are dominated by variable costs. So, low volume campaigns may be best served by taking tight cuts on a single pass HPLC approach, whereas larger scale processes will include a second pass purification of front and tail cuts to maximise yield.

Non-reverse phase HPLC techniques, such as ion exchange or size exclusion chromatography, can be useful in assisting purification, although these are not commonly used for synthetic peptides. Almac has been investigating a truly orthogonal method to HPLC to purify synthetic peptides, which uses Immobilised Metal Affinity Chromatography (IMAC). This method uses a small molecule TAG that has been designed to be able to bind reversibly to the N-terminus of peptides, and chelate to metal ions, such as copper and nickel. Functionally, the TAG behaves in an analogous manner to poly-Histidine[2] tags used to help purify recombinant proteins, but with the added advantage that the TAG is chemically cleavable, thus after the purification has occurred, the target peptide sequence is recovered (Fig. 3).

Although used so far in lab scale research projects, the capacity of the IMAC method is around 10 times that achieved with a 104-mer synthetic peptide that was purified by size exclusion chromatography, and around four times that achievable by reverse phase HPLC. An example where TAG has been successfully used is in the lab scale purification of a 36 amino acid peptide (Fig. 4). In this case, the desired peptide was obtained in 22% weight recovery from crude peptide, compared with 10% by HPLC alone. The use of IMAC on manufacturing scale is well documented, and the method could be applied accordingly.

In conclusion, as the number of peptide clinical candidates continues to increase, and more sophisticated drug delivery methods are developed, challenges remain to ensure that manufacturing methods keep pace with product development. To stay ahead of the curve, Almac combines robust process definition with accurate small-scale models, ensuring that process development is completed efficiently and effectively, allowing smooth passage to manufacture. **mc**

Reference

1. Reichart, J. et al (2010), Peptide Therapeutics Foundation, CA. USA.
2. Block, H. *et al*. Methods in Enzymology. 2009 **463**, 439-473

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