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Aspects of greening peptide chemistry within the pharmaceutical manufacturing industry

KEYWORDS: Greenchemistry, peptide, pharmaceuticals, API, manufacturing industry, industry perspective, solvent reduction, solvent exchange, solvent replacement, zero discharge, smarter processing.

ABSTRACT

In 2016 a critical need for development of greener alternatives within the peptide manufacturing processes was identified. The move towards achieving this has started on several levels within the industry. The awareness of the need and requests for greener alternatives have reached the peptide manufacturers, who, in response, are working on expansion of the greener processing options. Exchange of harmful solvents to greener alternatives, reduction of overall need for solvent, elimination of processing steps for smarter processing and recycling are all achievable and encompassed by the 12 principles of green chemistry. However, a single effort is not enough, many contributions are necessary to achieve the goal of overall greener manufacturing processes without compromising the product quality and still being conscious of the manufacturing costs.

INTRODUCTION

In a recent review (1), P. T. Anastas *et al.* highlight the significant change that has happened over the past 20 years since the 12 Principles of Green Chemistry came out (2). Scientists now, they remark, have access to conferences and courses, softwares and databases etc., and the green chemistry agenda has moved away from being niche to having concrete impact. It is safe to say that the scientific field has been tremendously prolific.

On the other hand, when it comes to industry and the adaptation of the green chemistry, the picture may look a little different. In the pharmaceutical manufacturing industry, which is notoriously conservative, the advances are perhaps less pronounced for the broader applications and are typically driven by pressure from environmental authorities or by a desire to lower the cost of manufacturing (3).

In particular, within the synthetic peptide manufacturing industry a very high consumption of solvents and materials is the norm rather than the exception. This is exemplified by Kopach noting that 3000-15000 kg of waste is generated to produce 1 kg of synthetic peptide active pharmaceutical ingredient (API) (4). Consequently, in 2016 the American Chemical Society's Green Chemistry Institute (ACS GCI) identified the critical need for development of greener peptide processes and formed a Roundtable team which recently offered a thorough review of the challenges of the peptide chemistry and processes and the advances made so far (5).

From a green perspective, as it is so material intensive to produce synthetic peptide APIs, it could be argued that this class of APIs should be limited or avoided completely.

However, peptide APIs have proven to be a class of products which is unique and structurally extremely versatile due to the modular way of making peptides. As a class of products, the peptide APIs are capable of both unifying and filling the gaps between the small molecules and biologics, and today the success of synthetic peptide APIs is cemented by encompassing more than 70 peptide APIs approved in one or more regions around the world (6).

From an industrial perspective, the enormous use of hazardous materials holds a potential for significant improvements and greening of the chemistry and the processes. While there are many enablers for greener alternatives, there are also restrictions and limitations. We will herein address some of the industrial challenges as well as some of the progress made towards greening the peptide chemistry and processes.

MANUFACTURING OF PEPTIDE APIS

Peptide APIs is a very broad class of compounds ranging from dimers to structures with 40 or more amino acids. In consequence, they can be made in a number of different ways (7). The two classical synthetic approaches are liquid phase peptide synthesis (LPPS) and solid phase peptide synthesis (SPPS), and the combination of the two is referred to as the hybrid approach. For the sake of completeness, there is also a biological (recombinant) path to peptides (8), but this is outside the scope of the present paper.

An important element of peptide synthesis is the modular building up of the peptide. On one hand this modularity is attractive because many motifs can be generated with little effort (automated synthesizers), while on the other hand, the modularity sets some requirements for the versatility of the chemistry, protecting groups, solvents and general processing. The LPPS approach, in which each step is individually developed, the perspectives of greening the chemistry appears to be easier accessible. In the SPPS approach, it has long been a "one size fits all" with only little process development on each step. Naturally, the efforts for developing each step, both with respect to process efficiency and greener alternatives, is larger than just employing a standard chemistry package.

AWARENESS

In recent years, the awareness of the need to go to greener alternatives has increased. This is primarily due to the pending risk of restrictions of the use of the most used solvents, i.e. N,N-dimethylformamide (DMF), N-methylpyrrolidone (NMP) and dichloromethane (DCM) (9). In fact, in some geographical locations, the use of DCM is already heavily restricted. Interestingly, for an increasing number of pharmaceutical, cosmetic and industrial players, the awareness is changing into a real desire to employ the greener alternatives, and requests for green processes and green process research is more frequently seen.

THE GREENER TOOLBOX

To be able to offer a greener solution, the peptide chemistry and processing toolbox must be expanded. In process design, we are taking steps towards better design to eliminate unnecessary processing steps or make a more efficient sequence of steps. In chemistry and solvent choices, we are expanding the knowledge of possible greener substitutes. In general processing the use of less material for both solvents, starting materials and processing aids is investigated as well as lower energy processing alternatives. Finally, the recycling of solvents and materials is also actively on our agenda. The first example shows elimination of unnecessary steps and saving of energy. In this case, the product is a small cyclic peptide, tavilermide. The manufacturing process was originally developed as an SPPS process with classical global deprotection, cyclization, purification and isolation by lyophilization. The revised process achieved complete avoidance of a preparative HPLC purification, evaporation and lyophilization in exchange of a crystallization and a vacuum drying process (10). The general approach is now a part of the toolbox and has already been successfully applied on another short peptide under clinical development.

For the SPPS solvent selection the primary efforts have been on exchanging the DMF for greener alternatives (11), and several alternatives are suggested in the literature, e.g. γ -valerolactone (GVL) (12,13), N-formylmorpholine (NFM) (12), N-butylpyrrolidone (NBP) (14) and 2-methyltetrahydrofuran (2-MeTHF) (15). However, there are other chemistries performed on-resin which have not yet drawn attention from a greening perspective. To this end, we have started to expand the toolbox by extending green alternatives into including synthesis of more complex peptides by means of on-resin derivatization – a second dimension (2D) of the SPPS. So far, the 2D green SPPS was illustrated by a synthesis of a melanocortin receptor agonist (16) consisting of green SPPS of the peptide resin (NBP/EtOAc mixtures) (17), green on-resin removal of acid labile protecting groups using TFA in EtOAc/acetonitrile (MeCN) and on-resin cyclization (NBP/EtOAc) followed by TFA cleavage/green precipitation (18) of the target peptide in 4-methyltetrahydropyran (MTHP) (19) /n-heptane (Figure 1) (20). In addition, the green peptide synthesis was achieved without notably increasing the costs of the raw materials, and the use of greener solvents was accompanied by attaining valuable chemoselectivities, for example in on-resin deblocking of Lys/Orn(Mtt) and Asp(O-2-PhiPr) which were performed without cleaving the acid labile peptide-linker (Ramage) and Cys-Trt bonds. Finally, a cleavage of a CTC peptide resin (21) using 1% TFA in green MeCN/EtOAc was carried out (20), paving the way for the use of green chemistry in the convergent synthesis of larger peptides (22).

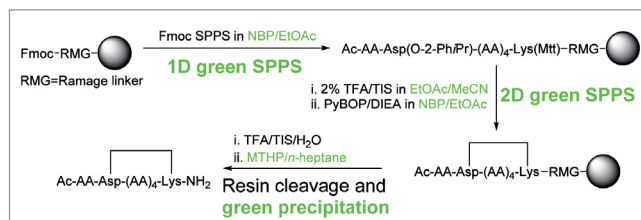


Figure 1. Green synthesis of a cyclic melanocortin receptor agonist.

The use of Nature's own coupling reagent, i.e. the enzymes, has been evaluated and added to the toolbox.

In collaboration with EnzyPep, the chemo-enzymatic peptide synthesis (CEPS) methodology (23) was successfully tested on exenatide, where two unprotected peptide fragments of comparable size were ligated in aqueous milieu with impressive efficiency on up to 18 mmol scale (24). Another example relates to LPPS and illustrates both use of green solvent and reduced volumes. The extraction of a 29-mer peptide with 2-MeTHF as main solvent proved to be significantly more efficient than EtOAc or DCM after its subsequent precipitation in an antisolvent. On volume, the 2-MeTHF extraction was superior by only requiring 50% compared to DCM and EtOAc. On filtration time, the 2-MeTHF-based suspension filtered in less than a minute, while the EtOAc comparator was filtering in minutes and the DCM yielded a gum. Yields of the step were found to be 84% for 2-MeTHF, 69% for EtOAc and 36% for DCM (25). If complete avoidance of the purification step cannot be achieved, there are other alternatives for a greener process. The solvent of choice can be changed from MeCN to the less harmful ethanol or isopropanol (IPA). However, loss of selectivity and increasing back pressure on the column may be limiting the extent of adaptation. When that is said, there are currently multiple large scale processes for approved peptide APIs using ethanol for the HPLC purification. The use of ion exchange chromatography is another greening approach which has been deployed. When the peptide charge distribution is favourable, the use of the high capacity of the ion exchange chromatography resins minimizes the need and solvent consumption for a preparative HPLC step. Finally, recycling of acetonitrile is an established method which has been scrutinized and found acceptable by the US FDA (25).

As a final example, the desirable significant decrease of solvent consumption in the SPPS should be mentioned. The larger part of the solvent consumption in SPPS is used in the washings of the resin between the chemical steps. By employing online monitoring and adjusting the washing regime and methodology, reductions of up to 70% DMF consumption have been achieved. These new practices are now deployed on industrial applications. From a facility point of view, steps to be greener are also taken. The PolyPeptide Group facility in Ambarnath, India, is now recognized as a zero discharge of water facility. All processing water from the operations is handled on the premises. After treatment in the effluent treatment plant (ETP) the water is finally reused in the cooling towers of the plant.

REGULATORY PERSPECTIVES AND EXISTING PROCESSES

For anyone working in the pharmaceutical industry, the regulatory approval and maintenance of a file is a clear key objective. From a green perspective, this can be somewhat of a hurdle for already approved products and processes.

Green changes to a process are not treated differently from any other process change and, depending on the changes, are subject to authority review and approval. The rate of the green change in the established product portfolio will probably be slow unless a ban of a chemical or significant cost savings can be a driver. Consequently, the most efficient green change is with the products that currently are under development or will come under development in the future.

SUMMARY

The need for greening the peptide chemistry and associated processes has been established and there is a recognized large potential. The work ongoing in the field is increasing as is the awareness and requests for greener solutions and options. In the manufacturing industry we do not consider the requests for greener processes as a constraint. It generates new ways of thinking eventually leading to greener and more cost effective solutions. We have taken and will continue to take steps to be able to offer greener solutions without compromising on quality and cost. The solutions lie not in a single technology revolution, but rather in a continued green evolution where many small steps are taken. Put together in new ways of collaborations, these steps form a real, attractive alternative to the existing manufacturing technology. Moreover, by establishing green targets in a process development, we can accelerate the change into a more sustainable peptide manufacturing.

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