# Improving Peptide Synthesis

Solid-phase peptide synthesis is an effective method to quickly and effectively provide small quantities and a large number of peptides to the market at a respectively low cost. With peptides increasingly growing portfolio including their use as dietary supplements, cosmetics, and therapeutics, these highly customizable molecules need to be supplied in an effective manner so research in these fields is not hindered. It is the responsibility of the peptide manufacturers to continually evaluate and improve peptide manufacturing processes to ensure the continued growth of these fields.

The manufacturing of peptides includes multiple steps in order to deliver a usable peptide product to the market. These steps include synthesis, cleavage/ post modifications, purification, and drying for product stability.

During the peptide synthesis process a single amino acid is attached to the growing peptide chain in two steps. First is the deprotection step followed by the coupling step with washing occurring in between. These steps are repeated until the desired number of amino acids have been attached to create the target peptide. The repetitive nature of the synthesis process naturally has led to the automation of Solid-phase peptide synthesis. There are numerous types of automated peptide synthesizers in the market today, ranging in combinations of scale, chemical delivery methods, reaction vessel volumes and available positions, and whether or not the reactor is an open or closed system.

Available synthesizers can accommodate either large, molar (mol), or smaller, micro molar (µmol), scale synthesis but it is highly recommended to determine the average scale of synthesis you require prior to selecting a synthesizer. Some synthesizers are better tailored to only one end of this spectrum, with a few including options to increase the range of supported synthesis scales. To determine your preferred synthesis scale, you can use the following formula.

$$Average\ Synthesis\ Scale\ (mol) = \frac{Target\ Crude\ Peptide\ Yield\ (g)}{Average\ Peptide\ Molecular\ Weight\ (\frac{mol}{g})}$$

The fluid delivery systems found in synthesizers can include pneumatic vales, mechanical pumps, or any combination of the two with single or multiple fluid channels. The available options of delivery methods will be specific to the synthesizer you select and can have an overall impact on ease of use, reliability, and performance if not properly maintained per manufacture recommendation.

The synthesizer's reactor(s), which will be containing the solid support to attach the growing peptide chain, will have the largest influence on the synthesizer's available synthesis scale range. The volumes of reagents necessary to achieve synthesis in the mol scale will be significantly larger than the volumes required of a synthesis in the µmol scale. Many synthesizer manufactures are aware of the wide range of potential synthesis scales and have designed the synthesizer to be more inclusive. To accommodate the widest spectrum of synthesis scales it is best to select a synthesizer with interchangeable reaction vessels varying in working volumes.

In synthesis situations where more peptide is required than what can be synthesized in a single reaction vessel, multiple reactors will be used to generate additional peptide. For the best synthesis efficiency, it is

best to select a synthesizer which can accommodate multiple reactors, simultaneously, keeping the overall synthesis run time at a minimum while still achieving the final target amount of peptide.

The design of the synthesizer and how it handles the reaction vessel may also impact the performance of the synthesizer. Some synthesizers contain the reaction within a closed system which is generally pressurized with an inert gas, most commonly nitrogen. This reactor design is most common in synthesizers utilizing the pneumatic valve fluid delivery systems previously described, as the pressurize nitrogen is what moves the reagents to the reactor. The pressurized reactor(s) on a synthesizer may either act as a vessel or a flow though cell depending on the synthesizer design. Flow chemistry has become a leading method in performing chemical reaction as it may reduce the time and reagents needed to complete the desired reaction. In flow chemistry, it is possible to continually flow fresh, unreacted reagent over the solid support to help drive the desired reaction to completeness faster. Other synthesizers may leave the reactor open to the outside environment allowing for the reagents and peptide to be exposed to reactive oxygen. If you plan on synthesizing a peptide containing Methionine or Tryptophan and is susceptible to oxidation, it may be best to consider a synthesizer utilizing the closed reactor design.

With peptide synthesis and production being a repeating and multistep process, each step poses its own set of challenges as inefficiencies are cascaded down, often times multiplying in magnitude. For example, if a peptide synthesis is comprised of 30 amino acids and each step is graciously assumed to be 98% efficient, the theoretical maximum percentage of available peptide at the end will be 55%. This calculation can be done using the formula.

$$Theoretical\ Peptide\ Amt.\,(\%) = Coupling\ Efficiency\,(\%)^{\#\ Amino\ Acids\ in\ Peptide}$$

With the assumption that each coupling is just as efficient as the previous. However, many times in peptide synthesis this is not the case. As the peptide chain grows, complications in the coupling step increase due to steric hindrance, adverse side reactions, and incomplete deprotection steps. This puts a high emphasis on peptide chemists to ensure each step has gone as far as possible.

More recently, some peptide synthesizers are now equipment with added reaction detection technology to provide the user with real time information on how the peptide synthesis is progressing. By incorporating a spectrophotometer and equipped software, the Fmoc protecting group removed in the deprotection step can be quantified. This technology provides the user with much greater control over the synthesis process. At each deprotection step if the starting amount of Fmoc is found to be the exact same as the prior deprotection step, it can be inferred that the coupling step that occurred in between was 100% complete. Another example, in the event that the Fmoc amount was found to be 50% of the prior deprotection step amount, it is inferred that only 50% of the oncoming amino acid was coupled. This technology can also be used to determine if the deprotection step itself has come to completeness. By periodically checking the amount of Fmoc in solution throughout the deprotection reaction, you can determine if anymore Fmoc is being removed from the peptide chain. Once a stabilization is seen in the spectrophotometer, the deprotection step can be considered complete and the software will advance the synthesis to the next step. In this way the synthesis protocol is determined by aspects of the synthesis and not just a pre-determined time which may prove to be inadequate.

Prior to executing solid-phase peptide synthesis, the peptide chemist will need to address some key factors to ensure a successful synthesis. Common aspects considered are target peptide yield, process inefficiencies inherent to peptide synthesis, and starting raw materials.

In synthesis design, it is paramount, to begin with, quality raw materials, such as amino acids and resin, as these will mitigate potential adverse reactions and proved better control when determining required peptide yield. Allocate time to ensure your raw material supplier is providing you with the highest quality starting materials and supplying them consistently. Consider Advanced ChemTech Louisville, KY for raw materials needs, www.advancedchemtech.com.

Many times, chemists are forced to overcome peptide synthesis inefficiencies simply by using more materials. An increase in scale of the overall synthesis may be necessary to increase peptide yield at completion. This may require a higher concentration of starting materials to drive reactions to completion. These strategies by no means should be considered feasible options as this increases production cost and waste generation.

Increasing starting material quantities has been a blanket approach applied to peptide production in an attempt to overcome inefficiencies which may be addressed by adjusting less costly parameters such as time and temperature. A great way to determine the feasibility and characteristics of peptide synthesis is to run smaller peptide scouting runs on pilot systems. This approach provides insight into how the peptides handle the synthesis process and if some parameters can be adjusted for a better result. Having the ability to better understand peptide assembly characteristics can have a large impact on overall material usage and cost.

It is important for peptide manufacturers to keep the time spent at the pilot-scale phase short but effective. Many automated peptide synthesizers in the market today provide adjustable parameter controls, such as reaction time, temperature, and excess material usage, which help in determining optimum peptide synthesis conditions. These automated systems can run multiple peptides simultaneously with limited operator interference keeping required run time and resources minimal.

Some examples of how these parameters may affect the peptide synthesis,

# Time

A crude peptide purity may be increased, for example, from 70% to 80% simply by adding 10min to a step cycle time. Determining the appropriate amount of time for each reaction can increase peptide product yield for nothing more than additional time. Moreover, a reaction may only take 10min to complete, so why run this reaction for any longer? Analyzing reaction time may allow a decrease in step time without impacting overall peptide quality.

# Temperature

By adding heat to the reaction, either thermally or energetically through microwave, amino acid coupling and deprotection times could be reduced. This method increases the energy to the reaction in hopes to increase reaction speed. However, it is important to note that not all the reactions taking place during peptide synthesis are favorable. Some side reactions such as racemization, aspartimide formation, and/ or amino acid oxidation may be increased due to added heat. The potential risks associated with temperature adjustment should not be overlooked. However, temperature control can be a great parameter to control when developing a peptide synthesis process.

# Material Excess

When preparing the necessary reagents, amino acids, bases, activators, etc. the ratio at which these reagents are used will have an impact on the success of any peptide synthesis. At a small scale, using reagents in excess may not pose a serious issue to resources but can be detrimental when scaling up. Understanding if and how much excess starting material is needed without impacting peptide quality is another great method to ensure efficient peptide synthesis practices.

When examining peptide synthesis, it is important to understand this is the first step in the production process with cleavage/ post modification, purification, and drying to follow. Taking the time to make sure peptides are being synthesized effectively and successfully is the key to creating an efficient peptide manufacturing process from start to finish.

Sources


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