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# Peptide Purposes and Peculiarities

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## Overview

The use of peptides in compounded preparations is gaining popularity, primarily due to their ability to treat a wide variety of maladies that often cannot be treated successfully any other way. Among these treatments are; anti-aging, body building, sexual disfunction, various cancers, bed wetting, macular degeneration, weight loss, promoting ovulation and fertility, wound healing, osteoarthritis, and much, much more. Most peptides have high specific activity, thus require very low amounts to accomplish their intended purpose. In many cases, these peptides are produced naturally in the bodies of humans and animals, but because they are present in such low amounts, they are seldom obtained and purified from these tissues. Therefore, it makes more since to make them synthetically.

While the process of synthesis can be straightforward, it is time-consuming and typically only produces a few grams at a time. Since peptides are made up of a specific sequence of amino acids, they are synthesized in a way that resembles box cars on a train, one amino acid added to the "train" at a time. Some contain only a handful of amino acids while others are more complex containing 40+ amino acids. Many are essentially linear trains with some folding, some are circular where the chain ends connect, while others are branched to form highly complex shapes with bends, helix coils, and disulfide bridging to maintain their structures. Some are even more complex where other moieties such as sugars, lipids, phosphates or acetates are also part of their structures.

Some peptides can be produced through genetic modification of bacteria, then purified through various processes to obtain an essentially pure peptide, while many others are produced by means of solid phase peptide synthesis (SPPS). During the SPPS process, the initial amino acid is attached to resin beads, then through a series of chemical synthesis cycles each successive amino acid is added one by one to complete the chain. Lastly, the now-finished peptide is cleaved from these resin beads by reaction with trifluoroacetic acid (TFA). Since there are several peptide chains being synthesized in the batch at the same time, there will be occasions in which one or more amino acids fails to couple as intended. These are called "failure sequences" and must be removed during a purification process. Some of these are so similar to the intended peptide that they can be very difficult to remove. To account for this, the resulting certificate of analysis (CoA) from the supplier will often report less than 100% purity even though the total reported peptide content can be 100%.

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At Compounder's International Analytical Laboratory (CIAL), we have tested the following peptide-containing formulations:

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| **AOD9604** – 16 amino acid peptide | **Ipamorelin**: 5 amino acid peptide |
| **BPC-157**: 15 amino acid peptide | **Kisspeptin 10**: 10 amino acid peptide |
| **Bremelanotide (PT-141)**: 7 amino acid peptide | **Leuprolide**: 9 amino acid peptide, |
| **CJC-1295 (GRF 1-29)**: 29 amino acid peptide | **Liraglutide**: 32 amino acid peptide |
| **Corticotropin (ACTH 1-39)**: 22 amino acid peptide | **Melanotan II**: 7 amino acid peptide |
| **Cosyntropin (ACTH 1-24)**: 24 amino acid peptide | **MOTS-c**: 16 amino acid peptide |
| **Deslorelin**: 10 amino acid peptide | **Oxytocin**: 9 amino acid peptide |
| **Desmopressin**: 9 amino acid peptide | **Semaglutide**: 29 amino acid peptide |
| **Elamipretide (SS-31)**: 4 amino acid peptide | **Sermorelin**: 29 amino acid peptide |
| **Epitalon**: 4 amino acid peptide | **Sincalide**: 8 amino acid peptide |
| **GHK Cu (Copper peptide)**: 3 amino acid peptide | **Tesamorelin Acetate***: 44 amino acid peptide |
| **GHRP-2**: 5 amino acid peptide | **Tirzepatide**: 39 amino acid peptide |
| **GHRP-6**: 6 amino acid peptide | **Thymosin Alpha 1**: 28 amino acid peptide |
| **Glutathione**: 3 amino acid peptide | **Thymosin Beta 4***: 43 amino acid peptide |
| **Insulin***: 51 amino acid peptide | |

* At the time of this writing, the Food and Drug Administration (FDA) will not allow compounders to prepare formulations classified as "biologics", which are defined to be those exceeding 40 amino acids.

Beyond these peptides there are many others which, over time, will be found useful in treating a wide array of medical issues. Whatever the next ones will be, CIAL is confident in our ability to develop methods for testing their potency, beyond use dating, sterility, endotoxin levels, and more.

As we turn our attention from a general understanding of peptides to more practical considerations related to compounding, we have found the following information to be helpful guidance to our customers…

**Solubility**

Peptides which are formulated as aqueous injections can demonstrate solubility difficulties, especially those which are more hydrophobic. This is because they contain many aromatic amino acids such as phenylalanine, tyrosine, or tryptophan, or those having non-ionic side chain amino acids such as leucine, isoleucine, or lysine. These must be given more time for dissolution and checked carefully for any undissolved powder before undergoing sterile filtration. Those which are more hydrophilic will readily dissolve in water or saline. Generally, mixing in a beaker on a magnetic stirrer should work fine but it is best to avoid vortexing, vigorous shaking, or placing in a sonic bath. These actions can break down those larger peptides (10+ amino acids).

**Lyophilization and Freeze/Thaw Cycles**

As with many higher molecular weight active pharmaceutical ingredients (API's), there is the possibility that during freezing steps, ice crystal formation of aqueous solutions will cause mechanical breaking of peptide molecules. It can be expected that a loss of potency (~5%) of the peptide to occur for each freeze/thaw cycle. Many of our compounders have found it useful to add extra API to compensate for this loss. Multiple freeze/thaw cycles are not recommended.

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If the compounded product will be delivered as a lyophilized cake in an injection vial, it is recommended that some water remain in the cake (1 – 2%). It should not be thoroughly dried in a hard vacuum, but rather the freeze-drying vacuum process be slower and gentler as often used for small molecules.

**Storage**

Whether dry, lyophilized, or as an injection, most peptides do best when the final dosage form is stored under *refrigeration* (2º - 8ºC).

**pH**

As a general rule, peptides tend to be more stable in neutral to slightly acidic solutions. Although individual examples may vary slightly, some examples CIAL has found include; Semaglutide appears to be more stable at pH 7.4, Tirzepatide can precipitate under acidic conditions, and Liraglutide and Semaglutide can break down in lower pH solutions. In an aqueous solution, buffering the pH is recommended. Solid, non-aqueous forms do not typically break down as easily, thus, should be less pH sensitive. With very few exceptions, nearly all peptides are sensitive to heat and oxidation.

**Peptides and other API's**

Surprising things can happen when adding other actives to a peptide preparation. Some examples we have found; the addition of Methylcobalamin, Hydroxocobalamin, Pyridoxine HCl, or Pyridoxal 5 Phosphate to Semaglutide can negatively affect the stability of aqueous formulations. The combination of these API's appear to bind or react with Semaglutide in a way that reduces its potency. Without laboratory test data, these interactions would not have been known.

**Calculations**

As many peptides do not have USP monographs, reviewing the peptide CoA can be confusing. Assay values and other information affecting potency can be highly varied because the information is inconsistent between manufacturers. For instance, some report Purity % or Related Substances % while others do not. In short, the goal in determining the Potency value should be based upon the amount of the bare peptide and should remove values from, but not limited to; water, solvents, salts, and acids.

**Sterile Filtration Issues (Plastic vs Glass)**

It is common for peptides to absorb onto the surfaces of microporous membrane filters. The type of filter membrane can also make a difference. While CIAL prefers to use polypropylene or wettable PTFE membranes in polypropylene housings, others can also work but may absorb more of the peptide. A good technique during sterile filtration step is to waste the first 1-2 mL, then begin collecting the solution in glass vials. This technique works well because during the wasting step, you are allowing the filter to bind whatever amount of peptide that it will. After this has been accomplished, it should not retain more, so the remaining solution should be fully potent.

In addition, most all peptides will cling to plastic surfaces, to some extent. While the amount is generally minimal, we have seen some that will retain significantly more (>5%). Use of glass beakers and vials has not yet been found to be a problem, but use of plastic (ie: syringes), can often reduce the peptide concentration in an aqueous solution. Adding more peptide to the preparation to compensate, can be a helpful remedy.

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While on this topic, CIAL utilizes biocompatible UHPLC instruments which are specially modified for potency testing of peptides. This is done by eliminating use of all stainless steel in the instrument tubing which can potentially bind up many proteins and peptides. If not accounted for, a peptide's value could be skewed resulting in a subpotent recovery when in fact it is not. As a result, CIAL is very cautious in each analytical step taken to ensure the CoA's we release are as accurate as possible.

**Conclusion**

CIAL's primary focus is to assist compounders in making successful and accurate (peptide) products that will safely, and effectively, provide the desired result(s) to their patients. We understand that our success stems from your success. Due to the highly variable "personalities" among peptides, we strongly suggest compounders have their initial preparations tested to be sure they are made as intended. Having successfully tested a wide array of peptides in various formulation types, CIAL hopes the information provided in this White Paper will prove helpful.