



# Peptide Formulation: Challenges and Strategies

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The properties of peptides make them particularly difficult to formulate but, with the right approach, they can be developed into effective therapies.

Peptides are fast becoming an important segment of the pharmaceutical industry. Although there have been tremendous advances in production of the active pharmaceutical ingredient (API), production of peptide-based drug products is still a significant challenge. One often-overlooked aspect of these peptide products is formulation development. Although much has been written about protein formulation, relatively little literature exists about how to formulate peptide drug products. Even though they are smaller, they do present significant challenges for the formulation scientist.

Peptides are defined as polypeptides of less than 50 residues or so and lacking any organised tertiary or globular structure. Some do adopt secondary structure, although this tends to be limited, for example a single turn of an  $\alpha$ -helix. While their smaller size makes them easier to deliver across biological barriers than larger proteins, their formulation can be problematic. This is the focus of this review.

## PEPTIDE PROPERTIES

Peptides present four particular challenges to the formulation scientist:

- ◆ They exhibit maximal chemical instability
- ◆ They adopt multiple conformers
- ◆ They tend to self-associate
- ◆ They can exhibit complex physical instabilities, such as gel formation

### Chemical Instability

The most common chemical degradation mechanisms for peptide and protein degradation are deamidation and oxidation (1). Oxidation rates of Met residues appear to correlate with the degree of solvent exposure (2). As peptides do not possess a globular structure that can sequester reactive groups, the side chains of nearly all of the residues in a peptide are fully solvent exposed, allowing maximal contact with reactive oxygen species. Often, the only viable formulation strategy for reducing oxidation in a liquid formulation of a peptide is to use a sacrificial additive – such as free Met – which will become oxidised instead of the API. Oxidation of parathyroid hormone [1-34] and brain natriuretic peptide [1-32] is slowed by the addition of

sucrose, although the mechanism for protection has not been determined (3). Sucrose also allows deamidation and dimer formation in these two peptides.

Deamidation involves the hydrolysis of the amide side chain of Asn and Gln. Above pH 6, the reaction proceeds through a cyclic imide intermediate formed by intramolecular nucleophilic attack by the succeeding amide nitrogen on the carbonyl group of the amide side chain. Since this intermediate forms a five-membered ring in the case of Asn, deamidation of Asn tends to proceed more quickly than for Gln, although both have been observed (1). For peptides, the high degree of flexibility of the peptide chain leads to maximal rates of deamidation, although it is important to note that the nature of the succeeding amino acid (the one following Asn) has a drastic impact on deamidation rates. Lack of steric bulk and the ability to hydrogen bond to the Asn side chain will speed up the reaction. Typically, only Asn-Gly, Asn-Ala, Asn-Ser and Asn-Asp sequences display reaction rates that are important on a pharmaceutical time scale.

The greatest control over hydrolytic reactions, including deamidation, is exerted by pH and buffer composition (1,2). This was recently demonstrated in the formulation screening for calcitonin, a marketed peptide product, where the proper choice of pH and buffer resulted in a marked improvement in stability (4).

Another formulation approach to slow hydrolysis is to develop a lyophilised or freeze-dried formulation. All of the rules for rational development of lyophilised products applies equally as well to peptides as they do to proteins. In other words, the aim is to embed the peptide in amorphous glass with a stabiliser that replaces many of the hydrogen bonds usually provided by water. This glassy state should have a high glass transition temperature (well above room temperature) and a relatively low moisture content. The only real difference is that, while it is critical to maintain the native structure of the protein in a lyophilised formulation, with peptides this is much less important, for reasons described below.

### Multiple Conformers

Unlike proteins, which adopt a 'native' globular structure (actually an average of many conformers within the native state ensemble), peptides rarely possess any

**Table 1: Peptides found in approved pharmaceutical products**

| Class                       | Peptide           | Number of residues |
|-----------------------------|-------------------|--------------------|
| Insulins                    | -                 | 51-56              |
| LHRH Agonists               | Leuprolide        | 9                  |
| -                           | Gonadorelin       | 10                 |
| Somatostatins               | Somatostatin      | 14                 |
| -                           | Octreotide        | 8                  |
| HIV Fusion Antagonists      | Enfuvirtide       | 36                 |
| Calcitonins                 | Human Calcitonin  | 32                 |
| -                           | Salmon Calcitonin | 32                 |
| -                           | PTH (1-34)        | 34                 |
| Atrial Natriuretic Peptides | Nesiritide        | 32                 |

organised higher order structure. Those that do are often in rapid equilibrium between the different structural forms, suggesting that a shallow energy minimum landscape exists. Therefore, the aqueous solution conformation may have little similarity to the 'active' structure found when bound to a receptor. Either the receptor induces a conformational fit, or the peptide binds to a membrane and diffuses in two dimensions to the receptor, making the lipid-bound form of greater importance with respect to bioactivity (5).

These observations illustrate an important difference in formulating peptides and proteins. With proteins, maintaining the native structure is essential. Analytical methods, usually spectroscopic techniques, need to be available to monitor the retention of native structure. With peptides, the solution conformation may not be related to the active form in any way. For this reason, the use of spectroscopic methods for structural characterisation, which are important in protein formulation development, have a lesser role with peptides. However, this behaviour can be used to an advantage. If one conformer is much less reactive, then shifting the equilibrium towards that form will slow chemical degradation. This has been shown for the peptide, VYPNGA, an often-used model for peptide deamidation. By adding sucrose – a preferentially excluded solute that causes the peptide to adopt conformation with smaller surface area – the deamidation rate can be slowed appreciably (6).

#### Self-Association

Many peptides are amphiphilic, forming amphipathic  $\alpha$ -helices and  $\beta$ -sheets. These structures tend to associate, usually through interaction of the more hydrophobic regions. For this reason, many peptides tend to be monomeric at very low concentration, but self-assemble as the concentration is increased. In doing so, they often display an increase in secondary structure, especially for amphipathic  $\alpha$ -helical peptides (7). A number of peptides appear to form amphipathic  $\alpha$ -helices that assemble into

tetramers; this behaviour has been the basis for the *de novo* design of four-helix bundle proteins.

Peptides can form larger micellar structures as well. For example, peptide  $A\beta(1-40)$ , thought to be an essential component of Alzheimer's plaques, forms micelles above a concentration of 0.1mM. Therefore, one must consider that the API in the formulation may not behave as an independent monomer, but in a highly associated state. If it forms micelles, this could also affect the ability to pass through membranes, similar to the behaviour of surfactants and detergents.

In theory, this self-association behaviour could lead to diminished solvent accessibility, thereby increasing chemical stability. While this may be true for micelles, in our experience, oligomeric peptide structures (for example, trimer, tetramers, and so on) do not display any preferential protection of reactive Met groups. Either the structures are too transient or too fluxional to provide any increase in long-term stability.

This fluxional behaviour, where the peptide converts easily from one structure to another, must be considered in designing peptide formulations. It means that maintaining some specific structure is relatively unimportant. This is true for both liquid and lyophilised formulations. In rare cases, peptides have been reported to be isolated in specific conformations, each with its own solubility and activity profile. However, this is certainly the exception to the rule.

#### Physical Instability

The propensity of peptides to self-associate is connected with their physical instability – that is, their likelihood of forming aggregates. While self-association of peptides – like melittin and corticotrophin-releasing factor (CRF) – has been widely investigated, the relationship between these metastable oligomeric species and larger aggregates is unclear.

Aggregation can lead to either amorphous or ordered forms. Ordered aggregates usually take the form of fibrils; these fibrillar structures are the basis for the most common type of aggregation seen for peptides, namely gelation. Gelation is the last step in a pathway that starts with the formation of peptide protofibrils that exhibit  $\beta$ -sheet structure. The protofibrils then associate to form mature fibrils, which propagate and intertwine, resulting in gelation.

Gelation is the process that converts a fluid solution into a semi-solid mass. Microscopic examination reveals that the gel is composed of multiple peptide fibrils, intertwined in a complex mesh. It is known that pH, temperature and ionic strength all affect the rate of gelation, as well as the physical properties of the gel – for example, transparency, gel stiffness, reversibility and so on. These factors all suggest that colloidal stability plays an

important role in gel formation. Colloidal stability determines whether peptide molecules are attracted to each other or repelled. Low colloidal stability means that the net forces between peptide molecules are attractive overall, which leads to decreased solubility and increased likelihood to assemble into larger structures, such as fibrils. Conversely, increased colloidal stability indicates net repulsive forces between peptides, which improves solubility and diminishes growth of organised fibrils. Fibril formation has been described for approved peptide products, such as calcitonin and glucagon. Another indicator of aggregation propensity is surface tension (8).

Given this type of complex associative behaviour, one must be sure to limit exposure of the peptide to elevated temperature or pH extremes. This may not always be possible, as with acidic deprotection during synthesis and some purification schemes. Colloidal stability of a peptide can be modulated by adjusting solution conditions – such as pH, buffer composition and ionic strength – and the addition of other excipients (9). Cyclodextrins have been shown to improve the physical stability of peptides by shielding hydrophobic amino acids. For glucagon, the addition of a cyclodextrin was found to delay the formation of insoluble aggregates. Similarly, the addition of sucrose has been shown to improve the physical stability of bioactive peptides (3). Additional information has been reported about the gelation of peptides to provide some guidance for the formulation scientist regarding conditions that do not favour this common degradation pathway.

## CONCLUSION

Peptides are smaller polypeptides that do not adopt a globular structure, as proteins do. In addition, their conformational fluxionality and propensity to self-associate make them more difficult to formulate than proteins in many cases. The most vexing problem tends to be that they display maximal chemical instability due to their high degree of conformational flexibility and complete exposure to solvent (and dissolved reactive species). Fortunately, there are some formulation approaches that can reduce degradation in solution. If instability continues to be problematic, then lyophilised formulations can be devised and prepared, in much the same way as is done for proteins.

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