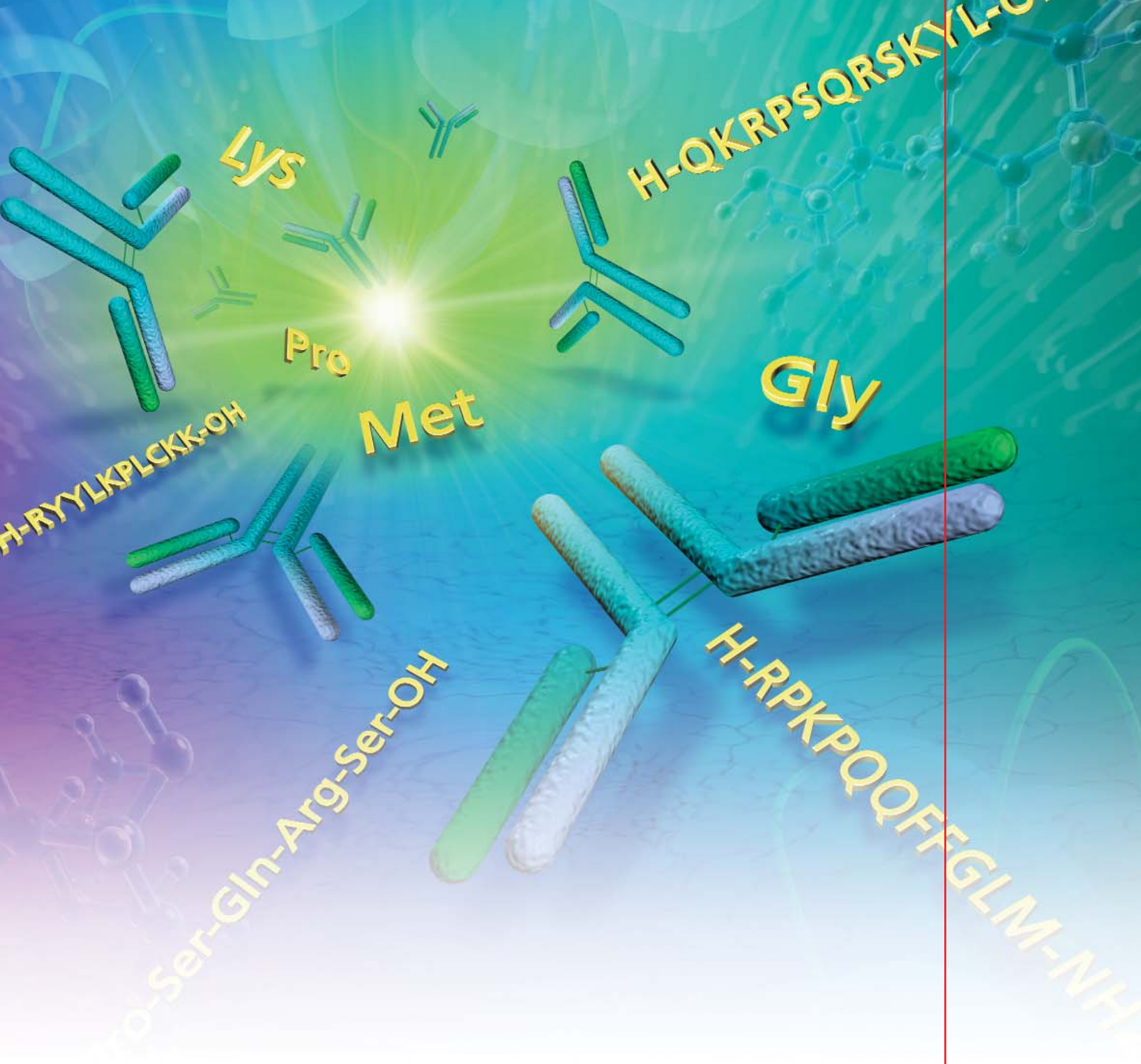

Storage and Handling Synthetic Peptides

GUIDELINES

SIGMA® GENOSYS

Handling and Storage of Synthetic Peptides

Often times the most challenging aspect of working with synthetic peptides is determining the best solvent in which the peptide will dissolve. This document should serve as a general guide for proper storage, handling and dissolving of your synthetic peptide.

Storage Guidelines for Lyophilized Peptides

Upon receiving the lyophilized peptide, store at 4°C or colder and away from bright light. Lyophilized peptides are stable at room temperature for days to weeks, but for longer-term storage, it is safer to store at -20°C or colder.

Exposure to moisture will greatly decrease long-term stability of lyophilized peptides. Before using the peptide, remove from cold storage and allow the peptide to equilibrate to room temperature before removing the lid of the container, in order to reduce the uptake of moisture that is present in the surrounding atmosphere.

A Strategy for Dissolving Single Peptides

There is no universal solvent for solubilizing all lyophilized peptides, while also maintaining their integrity and compatibility in biological assays. Different solvents may need to be tested until an appropriate solvent is found. Selecting the best solvent for your particular peptide may be a result of a "trial-and-error" process. Wherever applicable, it is advisable to first try solvents that are relatively easy to remove by lyophilization, in case the initial solvent does not work. Therefore, it is necessary to test a portion of the peptide first before dissolving the entire peptide sample. The steps below provide a general guide for peptide solubilization.

Determining Solubility Characteristics

Before adding any solvent to the lyophilized peptide, it is important to evaluate the amino acid composition of the peptide as a preliminary tool in understanding the solubility characteristics of your peptide. The number and types of ionic charges in the peptide determine its solubility in aqueous solutions. In general, the more charged residues the peptide possesses, the more soluble it is in aqueous solutions. In addition, peptides generally have more charges at pH 6-8 than at pH 2-6. It is for this reason that peptides are better dissolved at near neutral pH. Among the many exceptions to the rule are peptide sequences that are very hydrophobic and those that tend to aggregate. While the hydrophobicity of the sequence is the primary cause of aggregation, peptides can also aggregate or "gel" through extensive hydrogen bonding network. The guidelines below are used to determine if the peptide is basic, acidic or neutral.

1. Assign a value of -1 to each acidic residue (D, E, and C-terminal COOH)
2. Assign a value of +1 to each basic residue (K, R and the N-terminal NH_2)
3. Assign a value of +1 to each H residue at pH<6 and zero at pH >6.
4. Count the total number of charges of the peptide at pH 7 (all D, E, K, R, C-terminal COOH, and C-terminal NH2).
5. Calculate the overall net charge of the peptide.

Dissolving Approach for Charged Peptides

Based on the above guidelines, proceed to test the solubility of the peptide using the following strategies:

1. If the overall net charge of the peptide is negative, the peptide is considered acidic. If the peptide is acidic, and/or if the total number of charges of the peptide at pH 7 is greater than 25% of the total number of residues, add a small amount of 0.1M ammonium bicarbonate to dissolve the peptide and dilute it with water to the desired concentration. Make certain that the resulting pH of the peptide solution is about 7 and adjust the pH as needed.
2. If the overall net charge of the peptide is positive, the peptide is considered basic. If the peptide is basic and the total number of charges of the peptide at pH 7 is between 10-25% of the total number of residues, add a small amount of 25% acetic acid to dissolve the peptide and dilute it with water to the desired concentration.
3. If the overall net charge of the peptide is zero, the peptide is considered neutral. If the total number of charges is greater than 25% of the total number of residues, use the strategy described in section 1. If the total number of charges is between 10-25% of the total number of residues, use organic solvents as recommended elsewhere in this document.
4. If the total number of charges of the peptide is less than 10% of the total number of residues, the use of organic solvents is recommended.

For any solvent used, the maximum concentration of the initial solvent will depend on the tolerance of your assay against that particular solvent. Before trying stronger solvents, it is necessary to sonicate the peptide solution to confirm that the peptide is insoluble in the solvent. Sonication enhances solubilization, breaking the solid peptide into smaller particles. If, after sonication, the solution has gelled, appears cloudy, or has visible particulates, the peptide has not dissolved completely but is suspended. At this point, a stronger solvent is necessary. If the peptide does not dissolve, lyophilize and remove the volatile buffer solution. Once the sample is dry, alternative solvents can be tried on the same sample.

Dissolving Approach for Hydrophobic/Uncharged Peptides

The above recommendations based on the charged nature of the peptide will likely be inadequate for dissolving peptides containing more than 50% hydrophobic residues in their sequence, neutral peptides with less than 25% charges, and/or peptides that has less than 10% charges. Under these conditions, the use of organic solvents is recommended, such as acetonitrile (ACN), dimethylsulfoxide (DMSO), or dimethylformamide (DMF). Addition of chaotropic compounds such as guanidine hydrochloride or urea can facilitate in breaking up hydrophobic interactions or reduce the "gelling" of peptides by disrupting hydrogen bonding network. Again, the concentration of the initial organic solvent or chaotropic reagents will be dependent on the tolerance of your assay system. Note also that peptide sequences containing Cys (C) and Met (M) are unstable in DMSO.

It is important to dissolve the peptide completely in the initial solvent (such as acetic acid, acetonitrile, DMSO or DMF) because the rate of dissolution of the peptides into these solvents is usually higher than in a water/solvent mixture. If the water/solvent mixture is used first to dissolve the peptide, you may end up adding a much larger than necessary amount of nonaqueous solvent to your peptide sample. Sonication may also be necessary to facilitate complete dissolution of the peptide.

After the peptide is dissolved in the initial solvent, especially those dissolved in organic solvents, dilute the peptide by slowly adding (dropwise) the peptide solution into the buffered solution with gentle but constant agitation. This is to prevent localized concentration of the peptide in the aqueous solution, which can potentially result in precipitation of the peptide. The added benefit of this strategy is that the possibility of precipitation can be visually monitored and acted upon accordingly.

Preparing a Working Stock Solution (General approach)

Make a stock solution that is at a higher concentration than required for the experimental assay by dissolving the peptide in sterile distilled water or sterile dilute acetic acid (0.1%), where applicable. The stock solution peptide can be diluted further with the assay buffer. If the assay buffer is used initially to dissolve the peptide and fails to dissolve, recovery of the peptide, free of nonvolatile salts and/or organic solvents can be a challenge. If the peptide does not dissolve in water or acetic acid, the peptide solution can be lyophilized without any nonvolatile residues. Once the peptide is lyophilized, other stronger solvents can be tried.

Guidelines for Dissolving Several Peptides

A recommended strategy for redissolving sets of peptides containing varying properties is described below. This procedure may result with the final working stock solution of each peptide possessing different volume levels.

1. Add 0.1% acetic acid/water to yield a target concentration of 1-5mg/mL and sonicate the sample.
2. For any insoluble peptides add pure acetic acid to bring the concentration of the acetic acid to 10%(v/v), and sonicate the sample.
3. If peptides are still insoluble, add acetonitrile to 20%(v/v), and sonicate the sample.
4. Lyophilize any remaining insoluble peptides to remove the water, acetic acid and acetonitrile. When the sample is completely dry, add neat DMF or DMSO (dropwise) until the peptide dissolves. Slowly dilute the solution with water to approximately 10%(v/v) DMF or DMSO. If precipitation occurs at any stage during this step, stop adding water and add more DMF or DMSO (dropwise) until the peptide completely dissolves. These peptides may be too insoluble in water to be used at the same concentration of the others in the set.
5. Dilute each solubilized peptide with the most effective solvent to bring the stock solutions to the same peptide concentration. This will simplify working with the peptides in your experimental assay. Further dilutions can be made in the assay buffer. Diluting insoluble peptides with buffer at this step may eliminate the occurrence of precipitation because it is now below its solubility limit.
6. All solutions, except those containing DMF or DMSO, can be lyophilized. This will return the peptide to a suitable state for optimal long-term storage.

Storage Guidelines for Peptide Solutions

The shelf life of peptide solutions is limited. Peptides containing N, Q, C, M and W are unstable when stored in solution. Using sterile buffers (pH 5-6) and freezing the aliquots will prolong the storage life of the peptide. Storage at -20°C or colder is optimal. Avoid repeated freeze-thaw cycles, as this can degrade the peptides.

Peptide Stability and Potential Degradation Pathways

The stability of peptides varies with the amino acid composition. A peptide can degrade if appropriate storage conditions are not used. In addition to the risk of degradation from proteolytic enzymes, other chemical changes can occur. The section below outlines some possible degradation pathways that can arise.

1. Hydrolysis - This is generally a problem in peptides containing Asp (D) in the sequence, which is susceptible to dehydration to form a cyclic imide intermediate. If the sequence contains Asp-Pro (D-P), the acid catalyzed formation of cyclic imide intermediate can result to cleavage of the peptide chain. Similarly, if Asp-Gly (D-G) is present in the sequence, the cyclic intermediate can be hydrolyzed either into the original Asp form, which is harmless, or into a potentially inactive iso-aspartate analog. Eventually, all of the aspartate form can be completely converted into the iso-aspartate analog. To a lesser extent, sequences containing Ser (S) can also form cyclic imide intermediate that can result in cleaving the peptide chain.
2. Deamidation - This base-catalyzed reaction occurs in sequences containing Asn-Gly (N-G) or Gln-Gly (Q-G) and follows a mechanism analogous to the Asp-Gly (D-G) sequence. The deamidation (loss of amine) of the Asn-Gly sequence forms a cyclic imide intermediate that is subsequently hydrolyzed to form the aspartate or iso-asparate analog of Asn. In addition, the cyclic imide intermediate can lead to racemization into D-Asp or D-iso-Asp analogs of Asn, all of which can potentially be inactive forms.
3. Oxidation - The Cys (C) and Met (M) residues are the predominant residues that undergo reversible oxidation. Oxidation of cysteine is accelerated at higher pH, where the thiol is more easily deprotonated and readily forms intra-chain or inter-chain disulfide bonds. Disulfide bonds can be readily reversed by treatment with dithiothreitol (DTT) or tris(2-carboxyethylphosphine) hydrochloride (TCEP). Methionine oxidizes by both chemical and photochemical pathways to form methionine sufoxide and further into methionine sulfone, both of which are almost impossible to reverse.
4. Diketopiperazine and pyroglutamic acid formation - Diketopiperazine formation occurs when Gly (G) is in the third position from the N-terminus, and more readily if Pro (P) or Gly (G) is in position 1 or 2. The reaction involves nucleophilic attack of the N-terminal nitrogen on the amide carbonyl between the second and third amino acid, which leads to the cleavage of the first two amino acids in the form of a diketopiperazine. On the other hand, pyroglutamic acid formation is almost inevitable if Gln (Q) is at the N-terminal position of the sequence. This is an analogous reaction where the N-terminal nitrogen attacks the side chain carbonyl carbon of Gln (Q) to form a deaminated pyroglutamayl peptide analog. This conversion also occurs in peptide containing Asn in the N-terminus, but to a much lesser extent.

Conclusion

The most effective way to prevent or minimize peptide degradation is to store the peptide in lyophilized form at -20°C or preferably at -80°C (if available). If the peptide is in solution, freeze-thaw cycles should be avoided by freezing individual aliquots. Exposure to pH>8 should be avoided. However, if it is necessary to dissolve peptides at pH>8, the solutions should be chilled. Finally, prolonged exposure of lyophilized peptides and solutions (especially at high pH) to atmospheric oxygen should be minimized.