Limiting factors

- The presence of high amounts of primary amines or saline concentrated buffers can interfere with some step of Edman degradation. Tris and glycine are common in samples recovered from SDS-PAGE/electroblotting. Tris interferes with the Edman chemistry and glycine interferes with the interpretation of sequence data.

- Glycerol and sucrose often added to buffers designed for the storage and handling of proteins are not volatile and leave a highly viscous residue.

- Large quantities of SDS can cause instrument malfunction and may lead to the loss of sample.

- Free amino acids, peptides or molecules with high UV absorbance or that migth react with PITC (primary amines) give interferences that generate a high background in the initial cycles, making assignment of the sequence in these cycles difficult.

- Glycosilation, phosphorylation or other modifications may result in blank cycles, reduced peaks intensity or altered retention times.

- Cysteine residues will give a blank result unless reduced and alkylated. Therefore the sample has to be modified before sample submission for detection of Cys.

- **N-terminal blockage**. If the amino terminus is blocked the protein or peptide cannot be sequenced using Edman degradation. Proteins may be blocked naturally (e.g. by pyroglutamyl, acetyl or formyl groups), as in eukaryotic cells (at a rate of 70% or greater), or they may be blocked during the process of manipulation, purification and storage. In the latter case, modification to the N-terminus may result from the following manipulations: handling steps at elevated pH (>9.0), using inferior grade reagents and water (use the highest quality available), exposure to elevated temperatures (if glutamine is the N-terminal residue), exposure to protease inhibitors (some inhibitors may react with amino groups), use of formic acid (e.g. in CNBr cleavage) may formylate the N-terminus (use 50-70% TFA as an alternative), and exposure to urea that has not been deionized on an ion exchange resin (e.g. Amberlite) prior to use (cyanate ions will carbamylate the proteins).

  Several different methods are available for deblocking modified N-termini, although these methods usually require relatively large amounts of protein and are not consistently successful, especially if the nature of the blocking group is not known.

  Debloquing protocols: Joseph W. Leone, Brian Hampton, Elizabeth Fowler, Mary Moyer, Radha G. Krishna, and Christopher C.Q. Chin. Removal of N-Terminal Blocking Groups from Proteins. *Current Protocols in Protein Science* 11.7.1-11.7.20, Published online February 2011 in Wiley Online Library. DOI:10.1002/0471140864.ps1107s63 http://onlinelibrary.wiley.com/doi/10.1002/0471140864.ps1107s63/full