Technical Tip

How Sample Solubility Affects Separations

Purification and process chemists are faced with chromatographic challenges every day. How much yield am I willing to sacrifice to obtain the highest purity? Cost of raw materials (resin, solvents, time etc.) vs. amount of product purified, operating considerations and many more. An important factor purification chemists take into account with each project is concern with solubility.

Low solubility of the crude mixture in weak solvents (solvents with weak elution strength) and the mobile phase is probably the largest issue purification chemists face with chromatography. Preferably, loading your sample in a weaker solvent than the chosen mobile phase results in the sample being "trapped" at the head of the column before elution begins. A stronger sample solvent often times sees the loaded sample band travel down the column during sample loading causing loss in resolution.

Another problem purification chemists doing chromatography face is low solubility in the mobile phase. This particular problem results in sample precipitation onto the sorbent along with low re-dissolution. Chromatographically speaking, the sample "smears" through the column with very little resolution as a result.

There are factors and common practices that scientists should follow with each scale up purification project regarding solubility. A good place to start with sample concentration for loading is at or below 15mg/ml in order to minimize the sample zone within the column. If the sample has a high affinity for the stationary phase (is really "sticky"), this will not be an issue. However, for poorly retained samples such as short chain or polar peptides, this is a fairly big problem. Loading too much crude onto the head of the column not only takes a lot of time but will result in the sample traveling down the column before it is fully loaded. The resolution, yield etc. will become compromised. With large biomolecules (large peptides and proteins), high sample concentration could lead to aggregation of these samples. Aggregates can sometimes be a deal killer in protein/peptide therapeutics. These aggregates can initiate an adverse antibody response (neutralizing antibody which can be extremely harmful) or lower the effectiveness of the therapeutic. It is a fine line to meet with regards to sample concentration. You want to load as much onto the column (especially for your poorly retained compounds), but solubility is going to play an important role in this decision. Because of this, chromatographers should try dissolving the sample in the weakest solvents (compared to the mobile phase) possible as well as verifying that the sample is soluble in the starting mobile phase. Solubility can often times be enhanced by acidifying the sample solvent with modifiers such as acetic acid.

Key words: Solubility, peak shape

Author: Brad Turek