APPLICATION NOTE #103

detergent removal

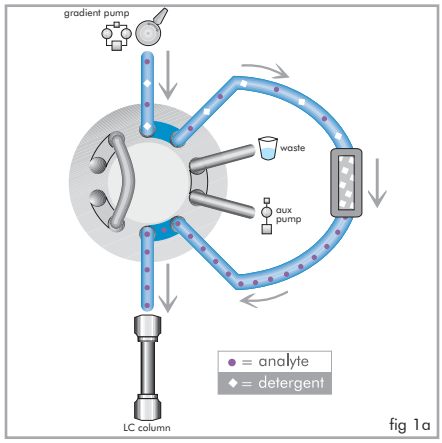
Products like the OPTI-LYNX Quick-Connect Trap System can be used as a convenient means of on-line detergent removal.

DETERGENT REMOVAL VIA TRAPPING

Protein chemists are often faced with the challenge of removing detergents from their protein samples prior to qualitative analysis via LC or LCMS. Detergents may be present as carryover from SDS PAGE analysis, or have been added to help solubilize the sample. These detergents must be removed prior to LC or LCMS analysis.

Many detergent removal methods involve time-consuming off-line procedures that can result in a significant loss of sample. Use of on-line trapping columns is a far more convenient and efficient approach, with considerable potential for automation.

The optimal approach for removal of detergent from protein samples depends upon the type of detergent present in the sample. Generally, there are three types of detergents, ionic, zwitterionic, and non-ionic, and each requires a distinct method. But in all cases, the idea is to use a trap cartridge to either trap the protein, wash the detergent to waste, and then elute the protein, or to trap the detergent while allowing the protein to pass through unhindered.

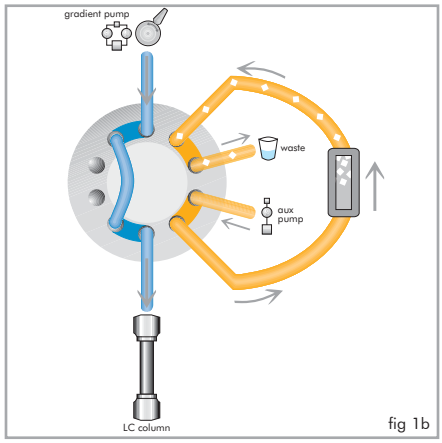


Detergent(SDS) binds to trap while protein/peptide travels to LC column.

SDS & IONIC DETERGENT REMOVAL

Ionic detergents like SDS (Sodium Dodecyl Sulfate) can usually be removed from the flow stream using a simple ion exchange trap. The packing material used in the trap must have affinity for the type of charge on the polar head group of the detergent. For anionic detergents, a strong anion exchange (SAX) phase (e.g. quaternary amine) can be used, while cationic detergents require a strong cation exchange (SCX) functionality (e.g. Benzenesulfonic acid). Because SDS is by far the most common ionic detergent encountered in protein analysis, we will address this application specifically.

The removal of SDS is best accomplished using a polymer-based anion exchanger. In order to provide conditions where the trap has maximum affinity for SDS and minimal affinity for the protein sample, mobile phase pH should be kept at 4.4 or lower. This ensures that a majority of a protein's anionic side chains will be protonated, thus reducing the potential for protein to interact with the packing material in the trap. While a silica-based anion exchanger could certainly be used, polymeric supports are a more resilient option at low pH.

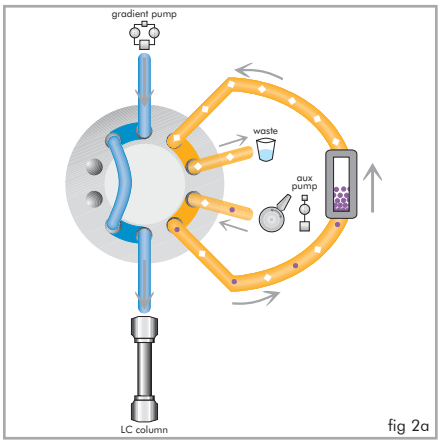


Trap must be regenerated and detergent removed before capacity is exceeded.

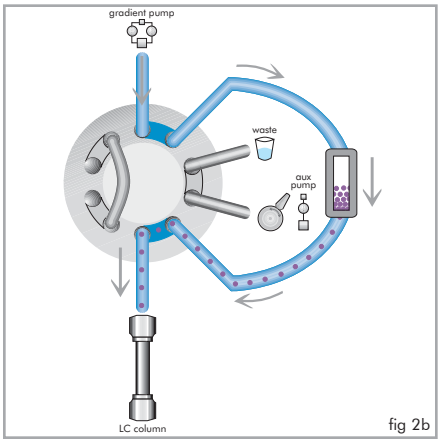
Under the above conditions, pumping sample solution across the trap bed should result in the selective binding of SDS to the trap. (fig 1a). The protein should pass through the trap unretained, and can be sent on for immediate analysis, or subjected to further on-line purification steps (concentration, desalting, etc.).

Since the anion exchange trap will have a finite capacity for SDS, the trap must be regenerated before that capacity is exceeded. SDS can be removed from the trap bed using a mobile phase that is both high in organic and also strongly acidic, a combination that should ensure interruption of the interaction between the detergent and the anion exchange bed. A pH below 2 and an organic content above 90% should be sufficient (fig 1b).

NON-IONIC DETERGENT (NID) REMOVAL



Protein/peptide is retained in trap cartridge while detergent passes through to waste.



Detergent-free protein/peptide is eluted for analysis.

Because non-ionic detergents have hydrophobic character and have no charge, ion exchange approaches are not an option. In the absence of a highly ionized head group on the detergent, the best approach is to temporarily adsorb the protein to a trap bed while detergent is flushed to waste.

The best trap for use in separation of NID from proteins is of course going to be one with affinity for proteins of interest, and little to no affinity for non-ionic detergents. This can sometimes mean a single phase, such as a silica or polymeric SCX, or possibly a "mixed mode" combination of two phases, such as SCX/SAX. Again, while predictions can be made, the ultimate effectiveness of a given chemistry for your protein sample may need to be determined empirically.

Generally, the easiest and most basic sample loading approach is to deliver sample to the trap in a mobile phase with a low percentage of organic modifier. The protein should bind to the ionic packing material, while the NID passes through unretained (fig 2a). However, note that proteins with pI at or near the pH of the mobile phase may also pass through the column unretained. Therefore, if the pI of the protein is known, pH should be below pI for optimal interaction with an SCX trap, and either above or below it for a mixed mode SCX/SAX trap. Once the detergent has completely passed through the trap, the protein may be eluted using a mobile phase containing a salt solution at 0.5 M concentration (fig 2b). If the protein is destined for an MS, a second trapping step utilizing a reverse phase bed can be employed to desalt the protein. Of course, switching valves would be required to keep the desalting trap out of the flowstream during detergent removal.

A second method for getting rid of NID is to use a "normal phase" approach. In this method the protein is loaded in high concentrations of organic (typically 80-95% Acetonitrile) onto a highly polar stationary phase. The protein is then eluted by a gradient of decreasing organic, or increasing salt concentration. By introducing the sample in a highly organic matrix, affinity of the sample for the polar stationary phase will be maximized, ensuring near-complete binding of the sample and elimination of detergent.