

Detergent Removal for Protein Samples Using SDR HyperD[®] Resin and Mass Spectrometry Based Detection

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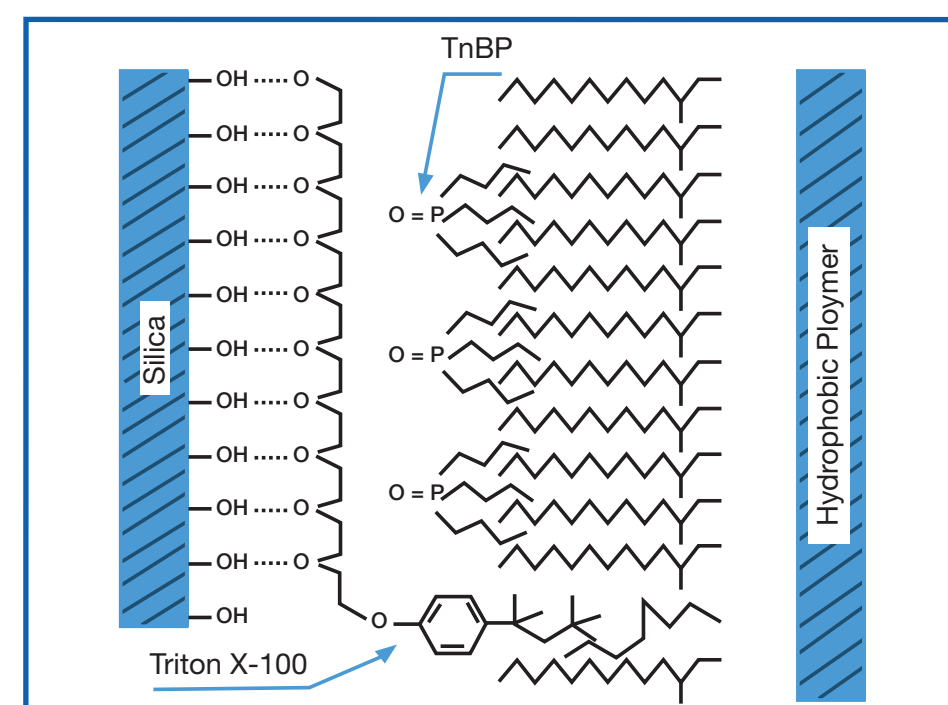
Abstract

Detergents are widely used in protein chemistry protocols and may be necessary for protein extraction, solubilization and/or denaturation. However, the presence of detergents is known to interfere with many techniques including mass spectrometry (MS); hence, their removal is a prerequisite for accurate results. This study evaluates the use of SDR HyperD[®] resin, a unique, mixed mode chromatography media, for the removal of NP-40, Triton[®] X-100, SDS, Tween[®] 20, and CHAPS detergents from protein samples. MS methods are particularly sensitive to detergents and thus can be used to assess the presence of even low levels of residual detergent in protein samples. Use of SDR HyperD resin pre-packed in a 1 mL column and in combination with Nanosep[®] centrifugal devices was evaluated. SDR HyperD resin has high capacity for all detergents tested and SDR HyperD treatment results in substantial improvement in MS signal intensity of proteins.

Background

SDR HyperD chromatography separations rely on mixed mode interactions, specifically a combination of hydrophobic and hydrophilic interactions along with some degree of size exclusion. Efficient binding requires that molecules are hydrophobic, to interact with the long chain aliphatic group, and relatively small, to access the bead interior. Hydrophilic interactions help to increase the strength of binding. This mechanism is illustrated in Figure 1 (shown with Triton X-100 and TnBP binding). The mixed mode nature of this resin is due to the combination of a porous, spherical, silica bead with a hydrophobic polymer. The polymer is uniformly distributed throughout the silica pores allowing the specific interaction of small molecules in solution with silanol and hydrophobic groups. Detergents are hydrophobic and often contain polar groups capable of hydrophilic interactions. These characteristics, combined with some degree of size exclusion, allow them to remove detergent efficiently.

Figure 1
Illustration of the SDR HyperD Resin Surface Interacting with Triton X-100 and TnBP



Materials and Methods

- SDR HyperD F Resin, PN 20033-065
- Nanosep Centrifugal Devices, 0.45 µm GHP Membrane, PN ODGHPC35
- AcroSep[™] 1 mL Pre-packed Columns with SDR HyperD F Resin, PN 20033-C001
- Triton X-100, CHAPS, and Tween 20 (Sigma-Aldrich), Nonidet[®] P40 (NP40, Roche), and SDS (Fluka)
- SDS Detection Kit (G Biosciences)

Detergent Removal Efficiency using AcroSep[™] 1 mL Pre-packed Columns with SDR HyperD F Resin

To assess detergent removal efficiency and non-specific protein loss, protein solutions containing 5 mg/mL Bovine Serum Albumin (BSA, Sigma) plus 0.5 or 2.0% detergent (Triton X-100, NP40, SDS, or Tween 20) were tested. PBS was used for binding and equilibration steps except in the case of Tween 20, which required the use of 150 mM NaCl. A syringe pump was used to ensure consistent and accurate flow rates during the sample run. Columns were equilibrated with 10 mL PBS or saline prior to use. 2.5 mL of test sample (BSA + detergent) was loaded onto the column at 2 mL/min. 1 mL fractions were collected for analysis.

BSA concentration was determined with BCA Protein Assay (Pierce) before and after SDR HyperD resin exposure. The protein concentration was used to determine portion of OD₂₈₀ resulting from protein versus detergent. SDS assay relies on a dye-based interaction and is read at OD₆₀₀. BSA does not interfere with this assay.

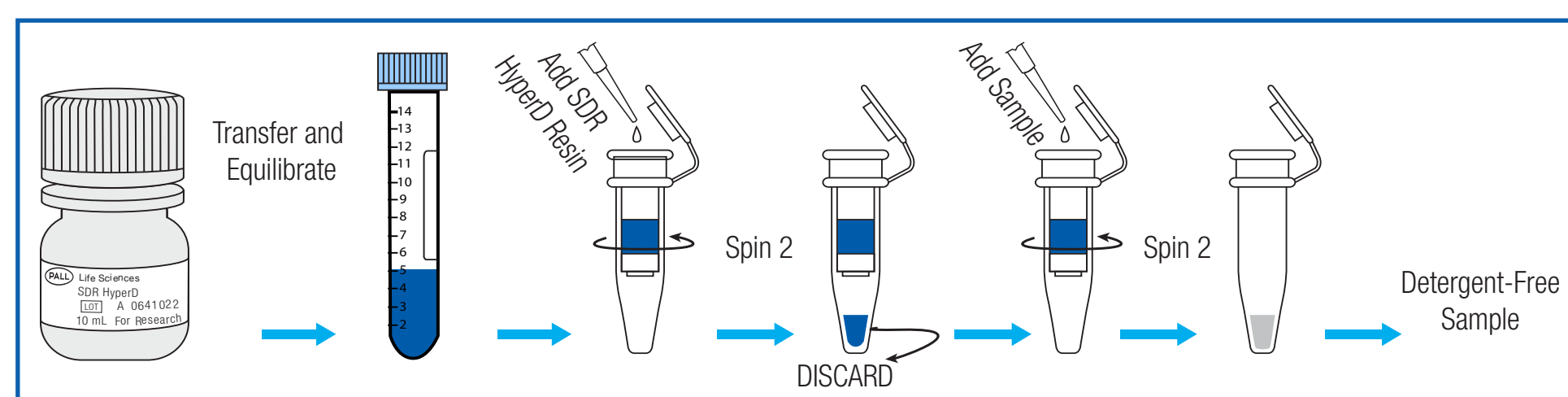
Detergent Removal Using SDR HyperD Resin in Nanosep Centrifugal Devices

Approximately 5 mL SDR HyperD resin were equilibrated with buffer (10 min.) in a 15 mL conical tube. After removal of second wash, 2 mL buffer were added to make a 70% resin slurry (~70% settled resin + 30% fluid above resin). 300 µL of the slurry (210 µL resin) were added to the Nanosep device and centrifuged at 800 x g for 2 min. 200 µL of sample (detergent + BSA 5 mg/mL) were added and incubated for 3 min. at RT. The device was centrifuged again and the flow through fraction was analyzed. The method is illustrated Figure 2.

These samples were subjected to laser desorption ionization (LDI) MS methods. Saturated solutions of Sinapinic acid (SPA, for BSA detection) and α-Cyano-3-hydroxycinnamic acid (CHCA, for detergent detection) in 50% acetonitrile + 0.05% TFA were used for detection. Detergent-containing samples were diluted tenfold to reduce ion suppression enough that detergent peaks could be detected. None of the other samples required dilution.

Materials and Methods

Figure 2
Schematic of SDR HyperD Resin Use with Nanosep Centrifugal Device



Results

Efficient Detergent Removal Using AcroSep[™] 1 mL Pre-packed Columns with SDR HyperD F Resin

Table 1
Detergent Removal and Protein Recovery

Detergent Concentration	Detergent Removal (%)		BSA Recovery (%)	
	0.5%	2%	0.5%	2%
Triton X-100	100	> 97	> 96	> 93
NP40	> 99	> 98	> 86	> 94
SDS	> 86	> 46	> 94	> 99
Tween 20	100	> 25	> 98	> 74

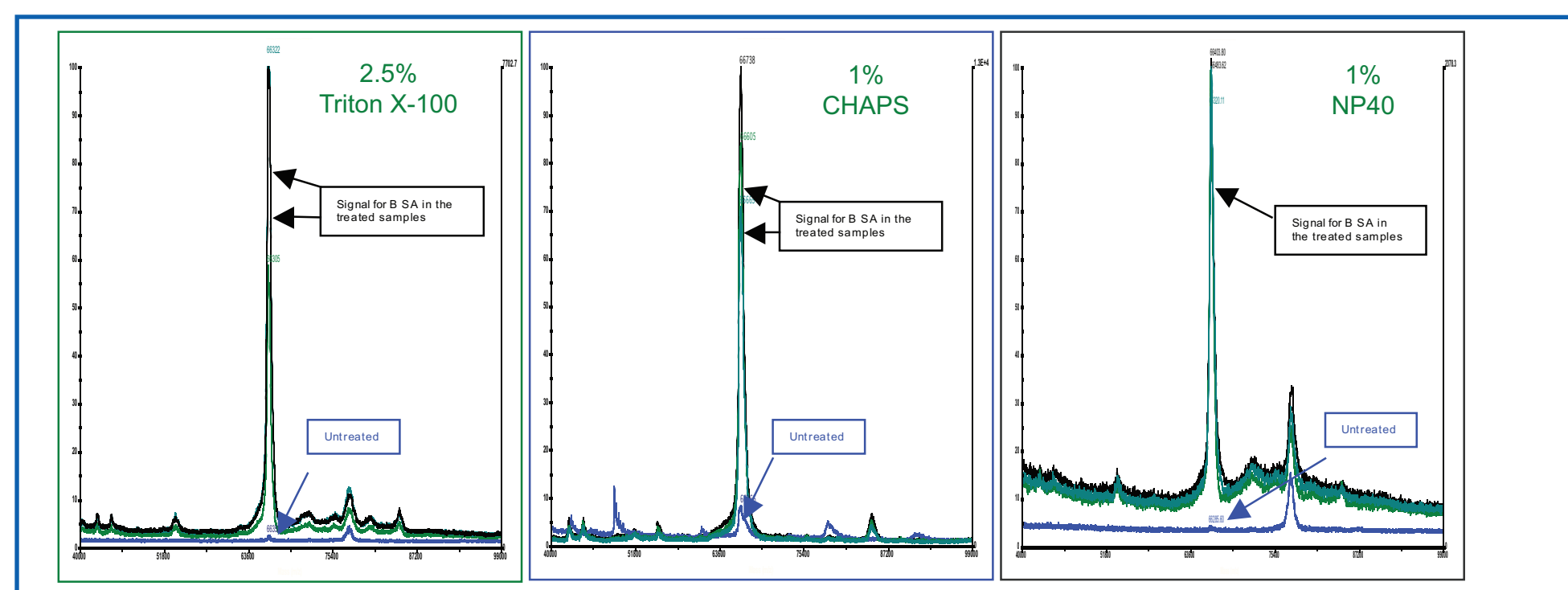
2.5 mL BSA + detergent solution was passed through the AcroSep[™] 1 mL pre-packed column with SDR HyperD F resin. Residual Triton X-100 and NP40 measured by OD₂₈₀, Tween-20 measured at OD₂₃₀. SDS measured with dye at OD₆₀₀. BCA assay used for protein determination (BSA). 3 columns, run in triplicate, used for each condition.

Data in Table 1 illustrate the efficient removal of several commonly-used detergents with limited loss of BSA. Binding of Triton X-100 and NP40, both non-ionic detergents, is very high. Removal of Tween 20 at 0.5% is very good, but drops significantly at the higher 2% concentration. Binding of SDS, a strong anionic detergent, is good, and somewhat less than Triton X-100 and NP40. These results are consistent with known binding capacity of SDR HyperD resin for these detergents (data not shown). The actual detergent removal and protein loss results are dependant upon the sample itself, with total protein and salt concentrations as contributing factors. To achieve greater detergent removal efficiency, a higher volume of resin or reduced sample volume should be used. Increased salt concentration may also improve detergent removal. The high recovery of BSA indicates limited loss of protein with the SDR HyperD resin under these conditions.

Effect of SDR HyperD Resin Treatment in a Nanosep Device on Mass Spectrometry Data Quality

Detergent peaks are often detected by mass spectrometry instruments. As a result, detergents interfere with the detection of the analyte due to overall suppression of ionization or by directly masking the signal. Although detergents interfere with many analytical methods, MS is one of the most sensitive to this effect. Thus, MS-based methods are a good way to assess detergent removal. Laser desorption ionization (LDI) MS methods, both MALDI (Matrix Assisted LD) and SELDI (Surface Enhanced LD), show a clear improvement in protein detection and corresponding decrease in detergent signal after SDR HyperD resin treatment.

Figure 3
MALDI Analysis of BSA Solution Before and After SDR HyperD Resin Treatment

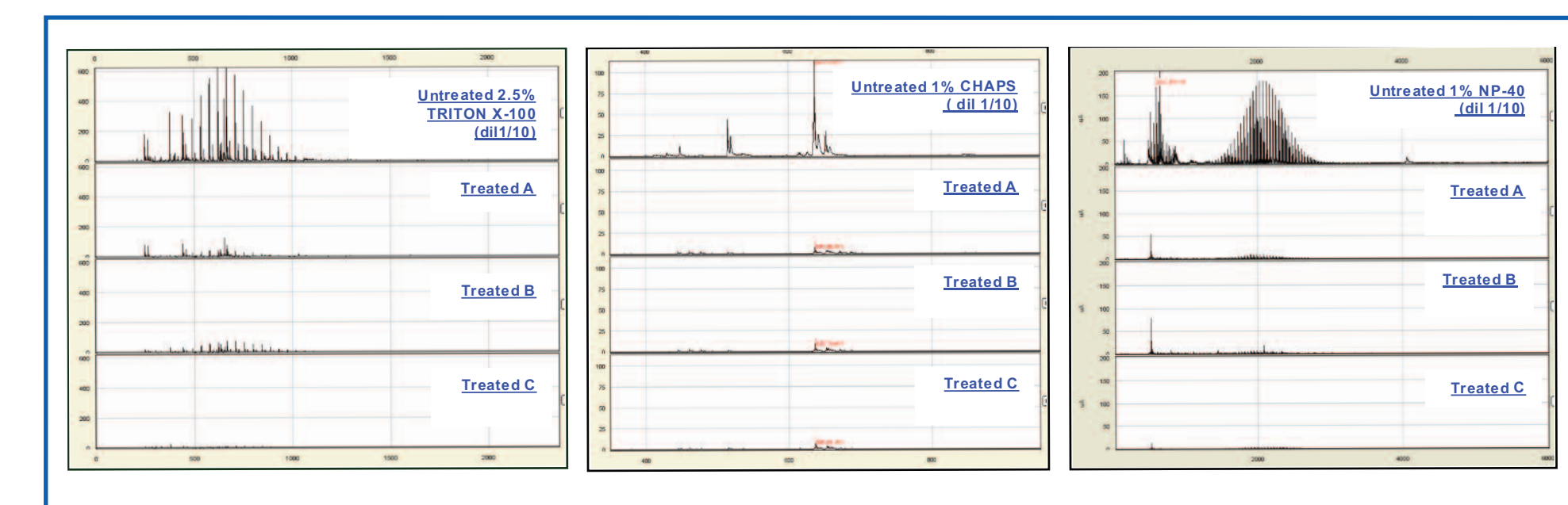


BSA mixed with indicated detergent and detected at 66 kDa. Untreated sample (blue trace) and SDR treated samples (black, aqua and green traces). The Nanosep device method used for detergent removal (Figure 2). 1 µL of sample (SPA dried droplet method) analyzed on MALDI[®] (ABI Voyager-DE[®] PRO Workstation) with automatic data acquisition.

Results (continued)

An overlay of the MALDI data from the removal of three different detergents (Triton X-100, CHAPS, and NP40) is shown in Figure 3. In all cases, the presence of the detergents dramatically interferes with the detection of BSA (blue trace). However, after treatment with SDR HyperD resin to remove the detergent, the BSA signal is very strong (black, aqua, and green traces) and looks essentially the same as the BSA sample without any detergent (data not shown).

Figure 4
Detergent Detection with SELDI Analysis Before and After SDR HyperD Resin Treatment



Analysis of BSA mixed with indicated detergent. Detergents are detected in the low MW region of untreated samples (top panels). SDR treated samples are shown below untreated samples (triplicate tests). Detergent removal using Nanosep device method (Figure 2). 1 µL of sample (CHCA detection) analyzed on SELDI (BioRad PCS4000).

Detergents are typically detected with LDI MS analysis methods in the lower MW region (< 10 kDa). SELDI was used for the detection and quantitation (data not shown) of detergent in the BSA sample before and after treatment with SDR HyperD resin. Data in Figure 4 clearly show the presence of detergent peaks in untreated samples. (Untreated samples diluted in water to reduce ion suppression effect.) After treatment, there are very few detergent peaks detected in undiluted samples. Using this very sensitive detection method and a high detergent load (200 µL solution with 1 or 2.5% detergent + 210 µL resin), > 93% of detergent is removed (data not shown).

The ability to use the SDR HyperD detergent removal resin in multiple formats provides the flexibility to accommodate a wide variety of samples. Table 2 shows some general recommendations regarding the resin and total volumes for various devices used extensively for protein separations.

Table 2
Resin Volume and Maximum Total Volume Recommendations for Devices

Device Type	Packed Resin Volume	Maximum Total Volume (Resin + Fluid)
AcroSep 1 mL Pre-packed Column	1.06 mL	
Nanosep Centrifugal Device*	10 - 200 µL	400 - 450 µL*
AcroPrep [™] 96-well Plate, 350 µL	10 - 100 µL	300 µL
AcroPrep 96-well Plate, 1 mL	20 - 350 µL	900 µL

*Maximum volume recommendations for the Nanosep centrifugal device are limited by the maximum volume of the collection vessel and not by the spin device itself. The Nanosep device can readily accommodate up to 650 µL total volume.

Conclusions

Detergents are a problem for many of the downstream methods utilizing protein samples including analytical (e.g., MS), cell culture based, and protein labeling. As a result, a fast and efficient detergent removal method could prove very useful. SDR HyperD resin has a very high affinity and high capacity (mg detergent/mL resin) for most detergents, and good capacity for others (e.g., SDS). SDR can be used in a pre-packed column or with batch mode devices (Table 2) to readily accommodate a variety of experimental needs. Additionally, large proteins (> 60 kD) cannot penetrate the pores of this resin, so protein losses are minimal. For moderately sized proteins (20-60 kDa) the potential for protein loss is dependant on individual protein characteristics and sample composition, and thus should be determined on a case-by-case basis.

- SDR HyperD resin provides a means for efficient detergent removal including:
 - Non-ionic, e.g., Triton X-100 and Nonidet P40
 - Zwitterionic, e.g., CHAPS
 - Anionic, e.g., SDS
- SDR HyperD resin protocols are simple and fast.
- The format for SDR HyperD resin use is highly flexible and includes pre-packed columns, Nanosep centrifugal devices, and AcroPrep plates.

