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Efficient Cleanup of Low Molecular Weight Carbohydrate Samples using Gravity Flow Columns

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Introduction

Low molecular weight carbohydrates (oligosaccharides) can be difficult to separate from contaminants such as salts. Methods like ion exchange are not suitable due to interference by the contaminant. Sephadex™ G-10 is a well established gel filtration chromatography medium suitable for group separation of biomolecules with a molecular weight above 700 from smaller molecules such as salts, dyes, and free radioactive isotopes. Here, we show efficient cleanup of an oligosaccharide-containing solution from salt using PD MiniTrap™ G-10, a new pre-packed column format.

Conclusions

- Rapid cleanup of carbohydrates with molecular weights above 700 prior to downstream analysis
- Oligosaccharide recovery of ~95%
- Efficient removal of contaminants such as salts, dyes, and radioactive labels
- High desalting capacity

Trap

Methods

Sample preparation

- ^{3}H-labeled octasaccharides were prepared from heparin by deaminative cleavage, reduced with NaB_3H_4 followed by fractionation on a size exclusion column.
- Concentrated ^{3}H-labed oligo- or polysaccharides in water were diluted with different salt solutions to the final concentrations of salt as indicated.

Salt removal using PD MiniTrap G-10 gravity column

- The column was prepared for use by resuspending the medium, letting it settle, and then the storage solution was removed by gravity flow.
- The column was washed with five column volumes of distilled water.
- A sample of 100 µl ^{3}H-heparin octamer (20 000 cpm) in 0.5 M NaCl was applied on top of the gel bed and allowed to move into the gel. (elution profile start, Fig. 1)
- After sample entry the column was eluted with 20x100 µl-aliquots of water with fraction collection of equal aliquots.

Analysis of eluate

- One milliliter of Optiphase™ HiSafe® 3 scintillation cocktail was added to an aliquot of each sample (50 µl) and analyzed in a Beckman Coulter® LS6500 multipurpose scintillation counter.
- Conductivity was measured by diluting 50 µl of eluate to 1000 µl with distilled water and analyzing on a Radiometer CDM 83 conductivity meter.

Results

Efficient cleanup of the carbohydrate solution was achieved with recovery and salt removal typically over 90%. The elution profile for the octasaccharide showed excellent peak separation from the salt contaminant (Fig. 1).

Corresponding experiments using peptides as the target gave similar results (not shown). Analyses also show that the limits of separation is set by sample volume rather than concentration. For sample volumes exceeding 300 µl, a larger column format is needed, for example PD MidiTrap™ G-10.

Elution profile

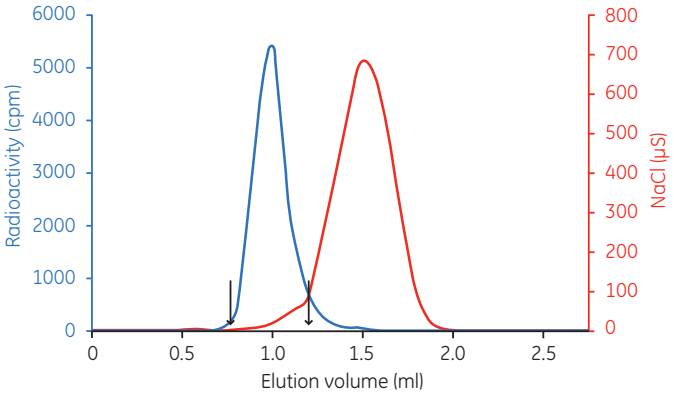

Fig 1. Removal of NaCl from a ^{3}H-heparin octasaccharide solution on a PD MiniTrap G-10 column. The fractions were analyzed with regard to sample content (radioactivity; blue) and salt content (conductivity, red). The oligosaccharide recovery was approximately 95% and the desalting capacity was 94% (between arrows).

Sephadex G-10 characteristics

Matrix	Cross-linked dextran
Separation mechanism	According to size
Particle size range	55-165 µm
Exclusion limit (M_r)	700
Chemical stability	All common buffers
Working pH range	2-13

Characteristics of prepacked columns	MiniTrap G-10	MidiTrap G-10
Vol. prepacked medium	2.1 ml	5.3 ml
Packed bed dimensions	0.97 x 2.8 cm	1.3 x 4.0 cm
Column volume	5 ml	8.5 ml
Void volume	700 µl	1.6 ml
Max. sample volume	300 µl	1.0 ml
Vol. eluted sample	0.5 ml	1.2 ml
Recovery[1]	70% to 90%	70% to 90%
Desalting capacity	>75%	>75%
Column material	PP and PE	PP and PE
Storage solution	20% ethanol	20% ethanol
Storage temp.	4°C to 30°C	4°C to 30°C

[1] Biomolecule dependent

Fig 2. PD MiniTrap G-10 columns are prepacked with Sephadex G-10. The maximum sample volume is 300 µl. For sample volumes up to 1 ml PD MidiTrap G-10 columns are also available.

Ordering information

Product	Quantity	Code No.
PD MiniTrap G-10	50 columns	28-9180-10
PD MidiTrap G-10	50 columns	28-9180-11

Literature	Code No.
Gel Filtration Principles and Methods, Handbook	18-1022-18
PD MiniTrap G-10, PD MidiTrap G-10 data file	28-9267-49

For contact information for your local office, please visit, www.gelifesciences.com/contact

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