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BOOK of ABSTRACTS

Combined solid-phase extraction purification method for chondroitin sulfate analysis

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Glycosaminoglycans (GAGs) are long-chained macromolecules that are built up of sugar disaccharide repeating units, and are often attached to proteins as post-translational modifications, thus having an important role in many biological processes. Chondroitin sulfate (CS) is one of the classes of GAGs, and has variable sulfation features. This structural variability results in a wide range of polarity and makes HPLC separation quite complicated. After enzymatic digestion, a purification step is necessary to remove contaminants and salts from the sample. However, due to the diverse physicochemical properties of these disaccharides, developing an all-fit method is still a challenging task.

We aimed to develop a solid-phase extraction (SPE) method that could be used for purification of CS disaccharides extracted from small-size biological samples. As a first step, we developed a method operating with self-packed cotton SPE spin tips (HILIC-type resin) by testing the effect of several elution and loading conditions on the recovery of CS disaccharides. We found that the developed system binds well the components with higher degrees of sulfation, while a significant loss was observed for the non-sulfated and singly-sulfated components during sample loading. In the second phase, we tested three commercial graphite-based SPE systems for GAG purification. All the resins were combined with all the manufacturer's protocols in order to test the effect of these protocols on different resins. The graphite-based resins showed to be ideal for purification of non-sulfated and singly sulfated components, but the more sulfated ones were bound too strongly to the solid phase. As a result, we found that the two optimized methods complement each other, therefore, to achieve high recovery and undistorted sulfation pattern, a combination of the two methods is necessary.

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National Research, Development and Innovation Office (OTKA PD 121187 and OTKA FK 131603) and János Bolyai Research Scholarship of the Hungarian Academy of Sciences

Combined solid phase extraction purification method for chondroitin sulfate analysis

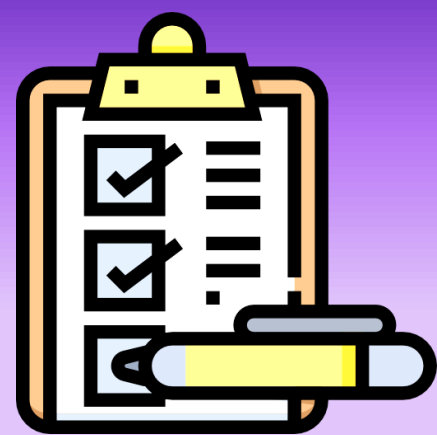


INTRODUCTION

Glycosaminoglycans (GAGs) are long linear polysaccharide molecules built up of repeating disaccharide units. These macromolecules are often connected to a protein core as post-translational modification, exerting immense biological roles. Chondroitin sulfate (CS) building units consist of N-acetylgalactosamine (GalNAc) and glucuronic acid, and they can contain sulfation in different positions.

The disaccharide building block analysis is a widespread technique for CS investigation. Before the HPLC-MS measurements, a purification step is necessary to remove the matrix and residual digestion components. However, due to the wide polarity range of these disaccharides, measurement and purification are still a challenging task.

AIMS



Development of solid-phase extraction (SPE) method for small amount of CS disaccharides

- Self-packed cotton-HILIC SPE method
- Commercial graphite-based SPE methods

CONCLUSIONS

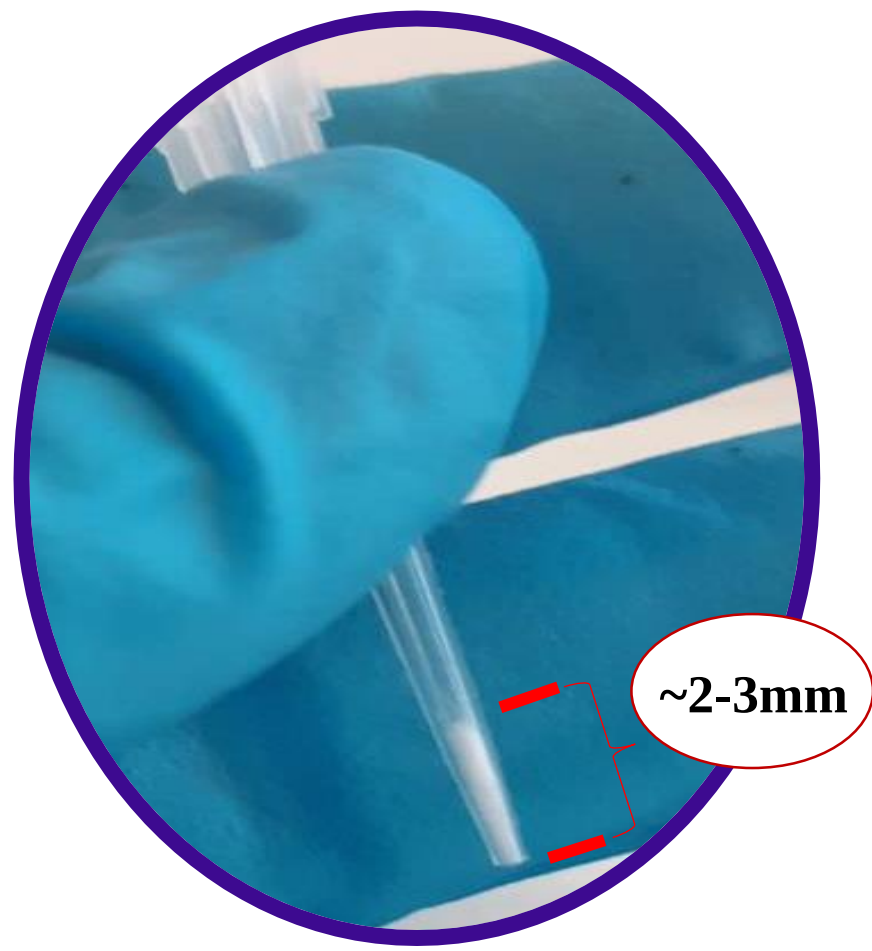


- Cotton-HILIC: for more polar components
- Graphite: for non-polar components
- Combined method: ultimate for all CS disaccharides
- Application on biological samples is feasible

Self-packed cotton-HILIC method

Commercial 100% cotton pads.

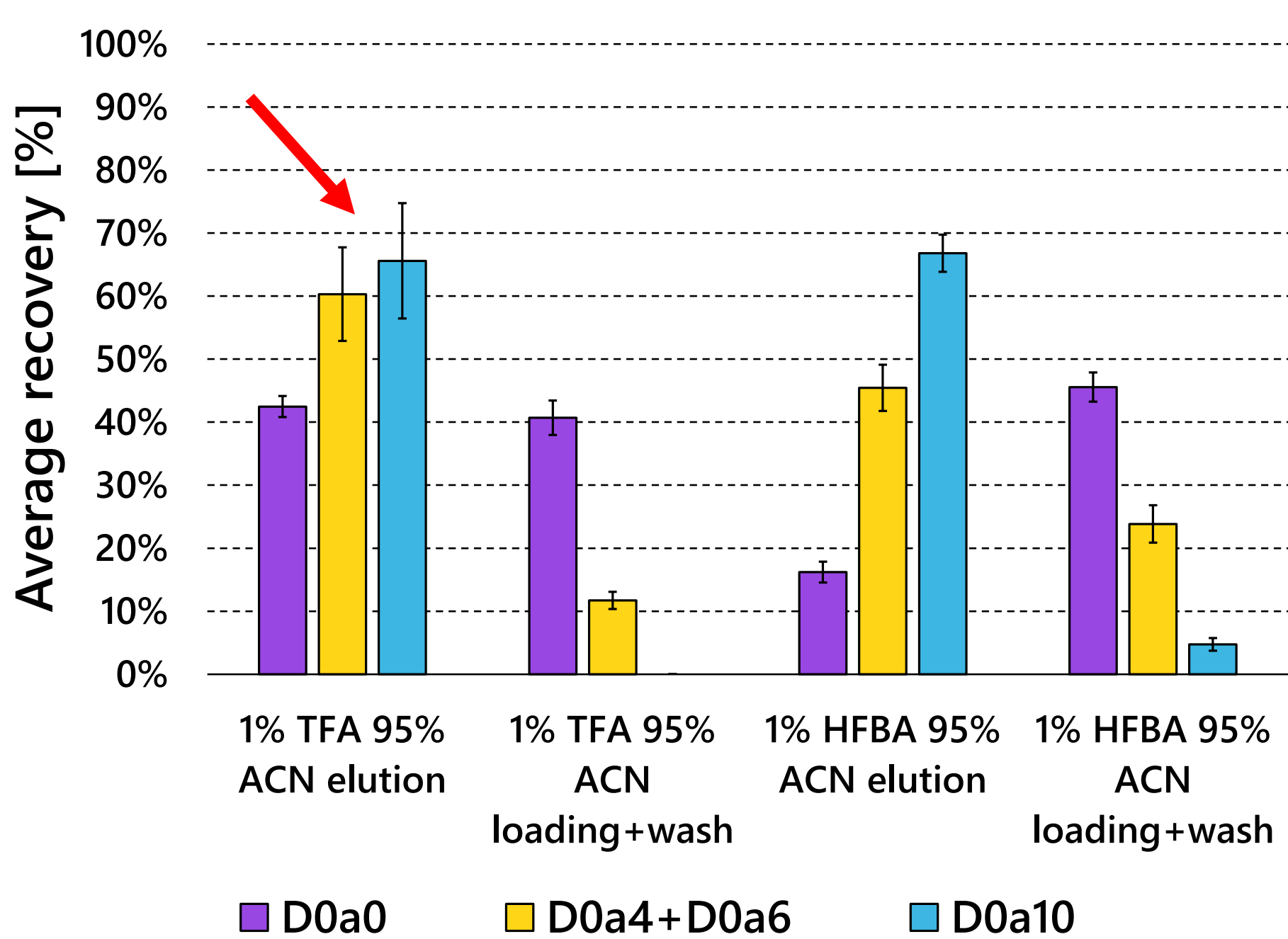
HILIC-type resin
Loaded into pipette tips into 2-3 mm height



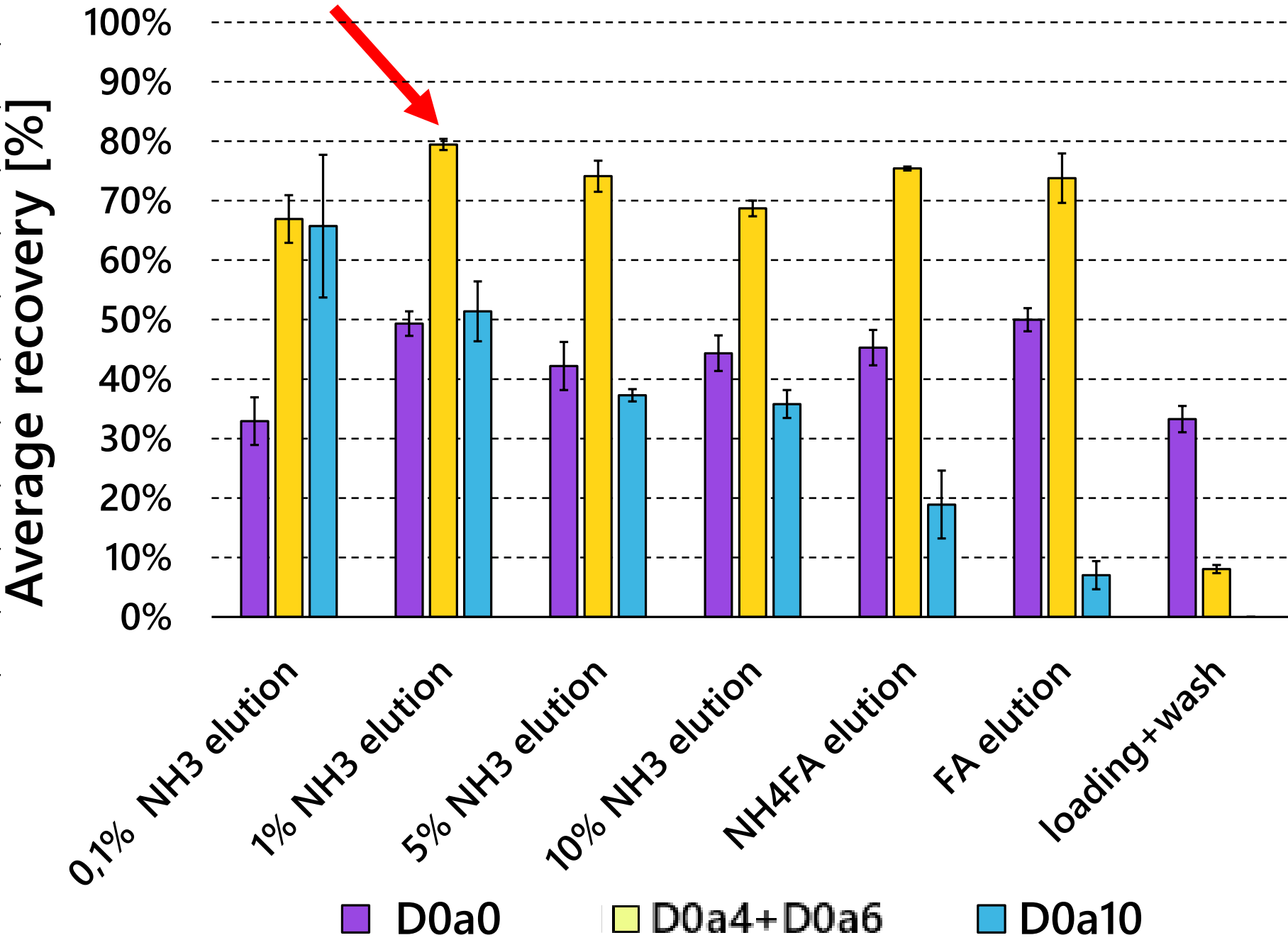
Optimized parameters:

- composition of loading
- acid type
- acid concentration
- composition of elution

Loading solvent composition



Elution solvent composition



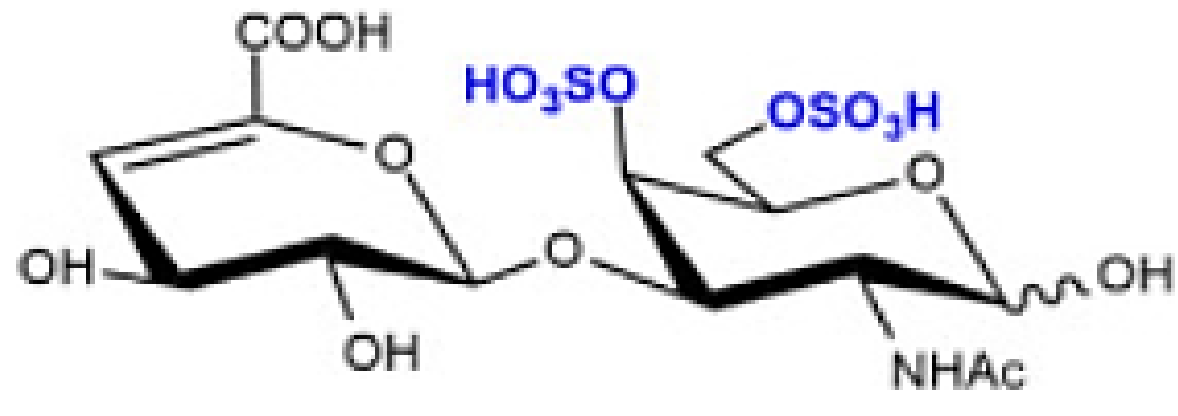
Optimized protocol

Centrifugation parameters: 2500 rpm, 1 min

1. Activation with 50 µl 40% H₂O 60% ACN
2. Conditioning with 50 µl 1% TFA, 95% ACN x 2
3. Loading with 30 µl 1% TFA, 95% ACN
4. Reload x 2
5. Washing with 50 µl 1% TFA, 95% ACN
6. 40°C heated elution with 10 µl 1% NH₃ 2x

Lawrence-code

ΔHexA-GalNAc	D0a0
ΔHexA-GalNAc4S	D0a4
ΔHexA-GalNAc6S	D0a6
ΔHexA-GalNAc4S6S	D0a10



Better applicable for the more polar disaccharides

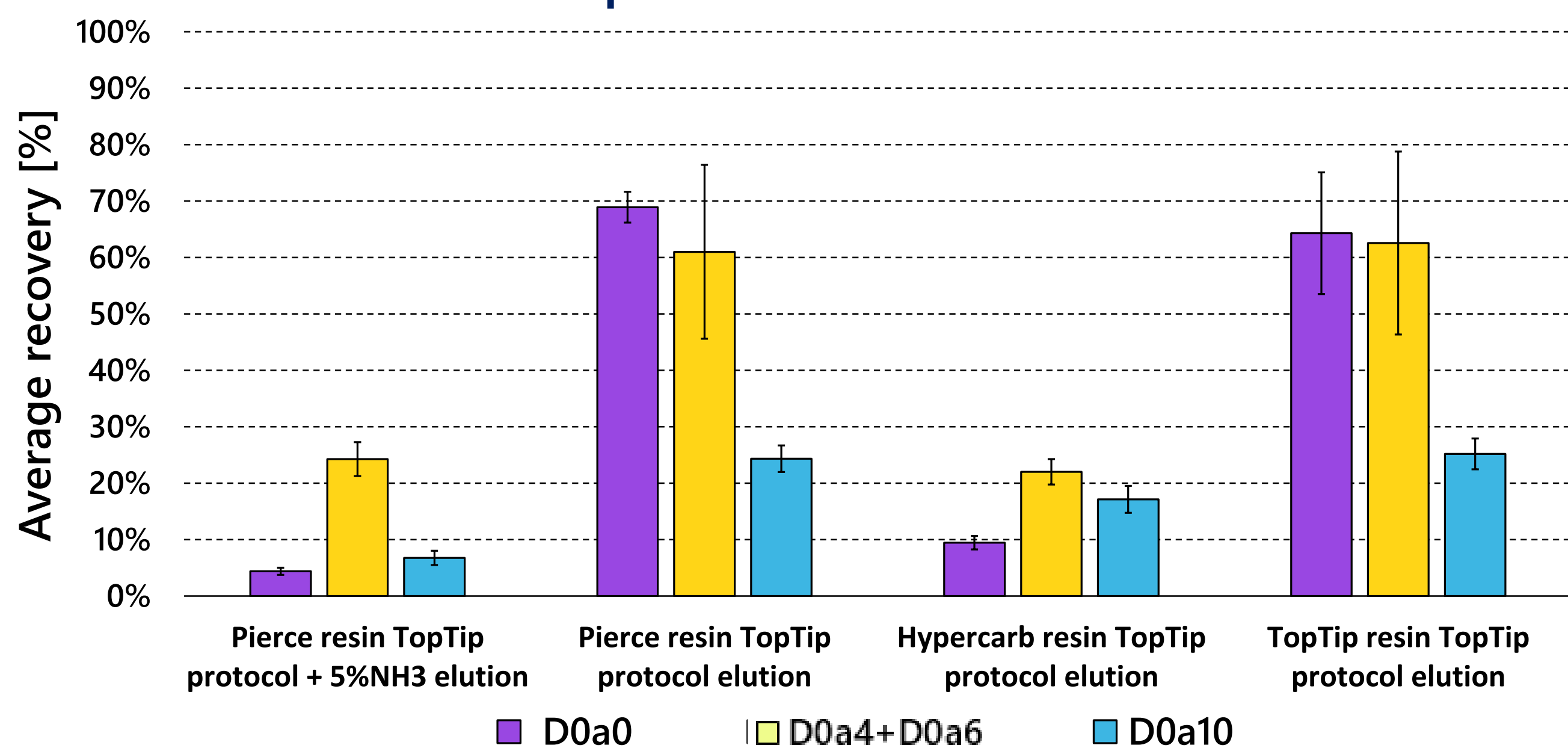
Graphite-based methods

Three type commercial graphite base solid phase extraction resins



TopTip protocol by the manufacturer

All possible combinations
Graphite-based methods



Optimized protocol

Centrifugation parameters: 2500 rpm, 1 min

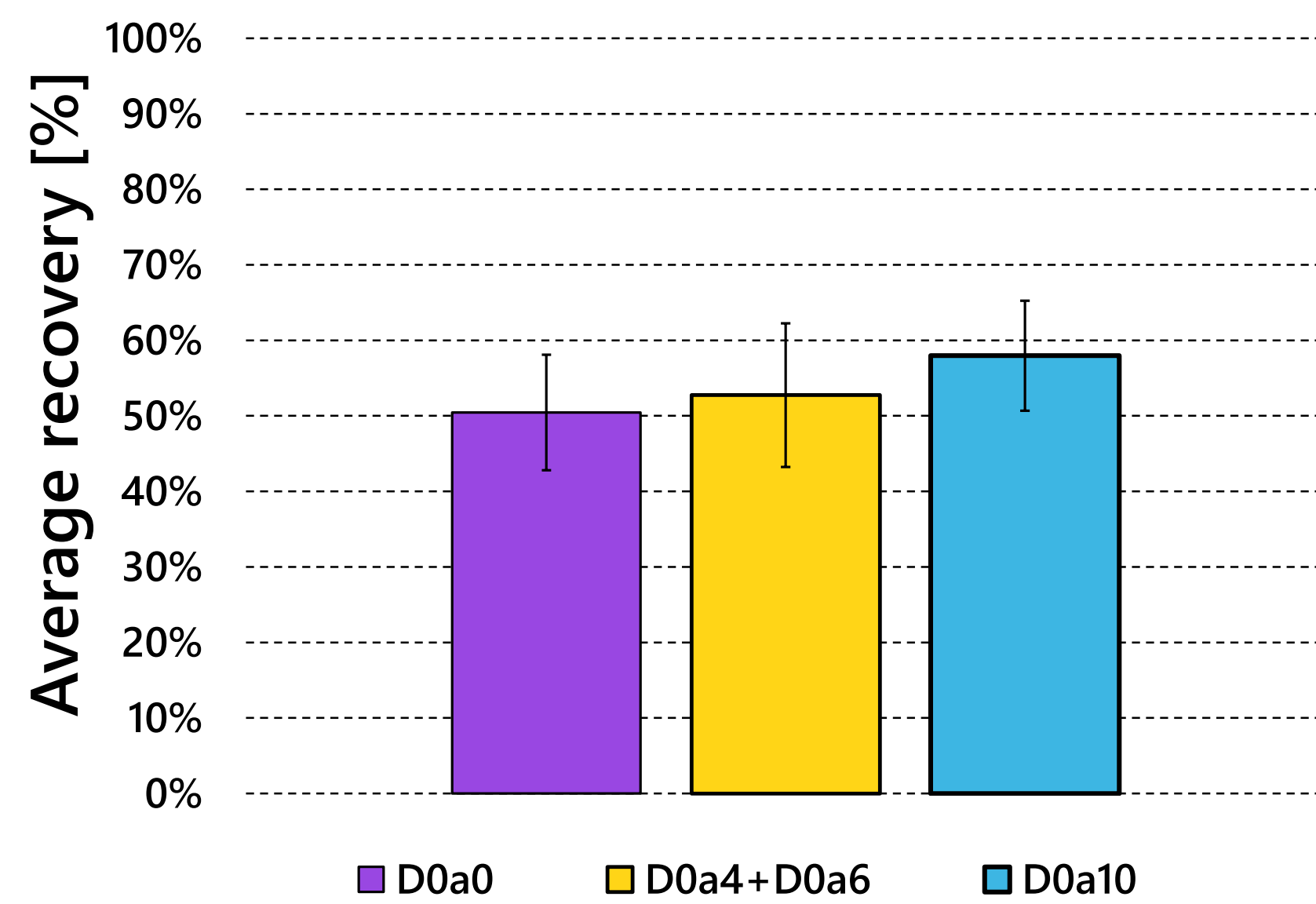
1. Activation with 100 µl 80 % ACN 0,1% TFA 2x
2. Conditioning with 100 µl water 2x
3. Loading in 50 µl water. Incubation 2 min before centrifuging.
4. Reload 1x
5. Washing with 100 µl water 3x
6. Elution with 50 µl 40% ACN 0,05% TFA 3x

Better applicable for the less polar disaccharides

COMBINED PROTOCOL



Standard mixture



RECOVERY ~ 50%

NO SIGNIFICANT BIAS IN SULFATION PATTERN