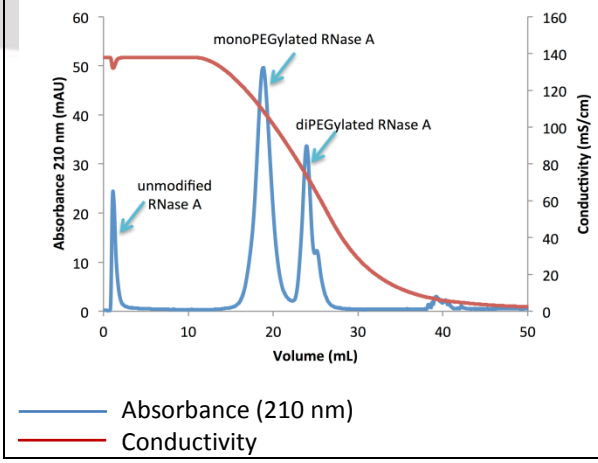
A050 Separation of PEGylated Ribonuclease A using a CIM® C4 A Disk (Butyl)

PEGylation involves the formation of a stable covalent bond between activated poly (ethylene glycol) polymers and polypeptidic drugs and molecules. This process causes a change in protein hydrophobicity and results in variance between the obtained conjugates. Despite this, hydrophobic interaction chromatography (HIC) is used less frequently for separation of PEGylation reaction products than other techniques. Separation of PEGylated conjugates of Ribonuclease A (RNase A) via HIC on monolithic supports was analysed in this work. The protein was PEGylated in the N-terminal amino group with 20 kDa methoxy poly (ethylene glycol) propionaldehyde.



Column: CIM® C4 A Disk (Butyl), V = 0.34 mL
(Catalogue Nr.: 210.8134-1.3)
Sample: 1:3 dilution of an RNase A PEGylation reaction mixture in 20 mM Tris-HCl buffer + 1M (NH4)2SO4.
Loop: 100 µL.
Mobile phase A: 20 mM Tris-HCl pH 7.0.
Mobile phase B: 20 mM Tris-HCl pH 7.0+ 2M $(NH_4)_2SO_4$.
Gradient: Linear gradient from 50% B to 100% A (1.0 M to 0 M $(NH_4)_2SO_4$), 25 CVs.
Detection: 210 nm
Flow rate: 4 mL/min.
HPLC system: Äkta explorer 100

CONCLUSIONS:

The results obtained demonstrate that CIM® C4 A Disk can resolve PEGylation reaction mixtures; the peaks of mono and di-PEGylated RNase A can be clearly distinguished in the chromatographic profiles.

REFERENCES:

K. Mayolo-Deloisa, J. Gonzalez-Valdez, M. Rito-Palomares: PEGylated Protein Separation Using Different Hydrophobic Interaction Supports: Conventional and Monolithic Supports. American Institute of Chemical Engineers Biotechnol. Prog., 32:702-707 (2016).

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