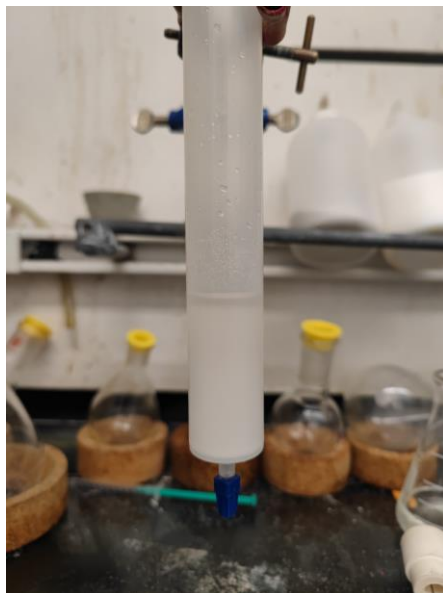
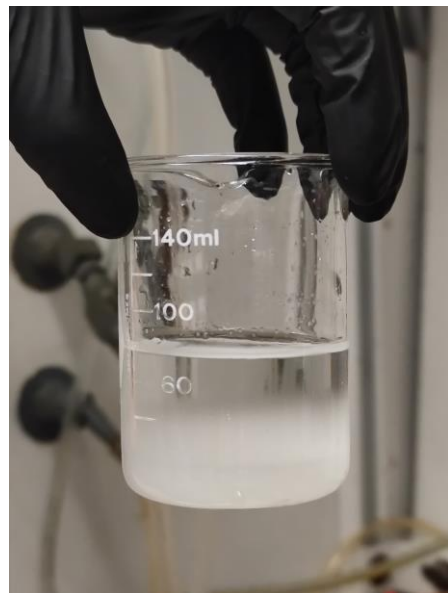


Ion-exchange chromatography is a process that allows the separation of ions and polar molecules based on their affinity to ion exchangers. The principle of separation is by reversible exchange of ions between the target ions present in the sample solution to the ions present on ion exchangers. The separation is influenced by the electrostatic forces of attraction between the charged molecules and the oppositely charged chromatography resin. The separation can be controlled by changing the pH and temperature of the solution.

Ion-exchange Chromatography with Sephadex® C-25



Prepare the Sephadex media:
swell the commercial resin beads (~ 5 g dry resin / 10 mg pre-purified sample) in DI water before packing in the column at room temperature for 24 – 48 h or in boiling water for 2 h.

In my case, the beads were swollen in DI water for ~24 h.

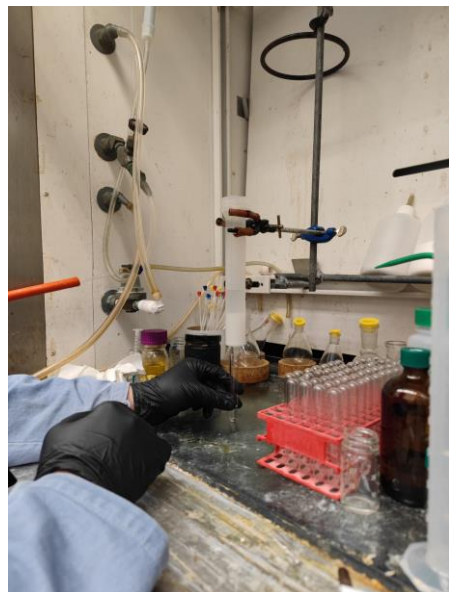
Pack the column carefully, switch the mobile phase to the solvent / buffer for elution, make sure there is no air bubble in the column.

Prepare of the sample:
dissolve the sample in the appropriate media. The volume of sample solution should be less than 25% of the column volume

In my case, the crude aminoglycoside was dissolved in the minimum amount of 10% acetic acid.

Load sample solution onto the column through a syringe filter (for regenerating the resin).

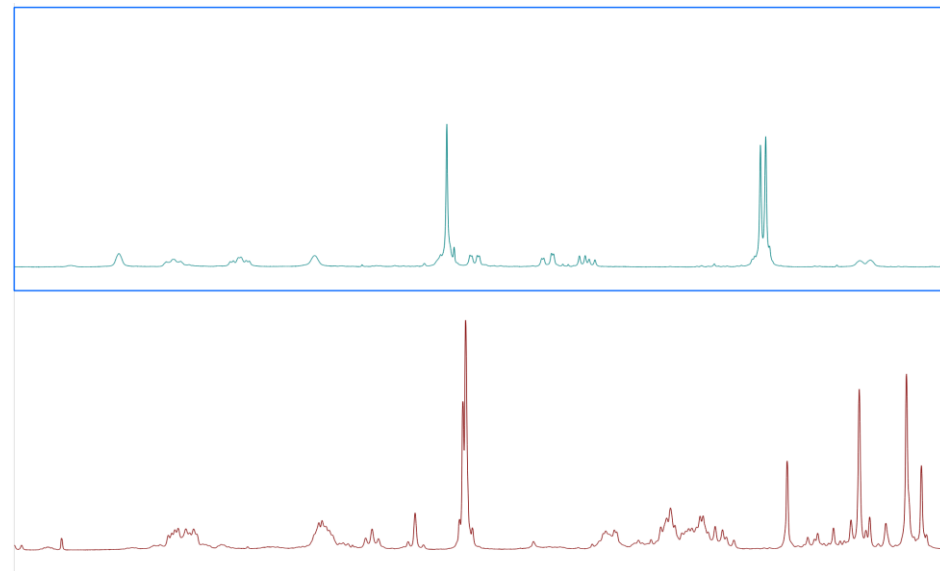
Ion-exchange Chromatography with Sephadex® C-25



Elute the column with buffer and **without** air-pushing, collect the fractions that have the product.

In my case, I eluted the column with DI water -> 0.1% NH₄OH

Clean the column by washing with 2 column volumes of 0.2 M NaOH, then wash with 4-5 column volumes of water, then switch the eluent to 20% EtOH and keep the resin in 4 °C fridge



Top: after ion-exchange chromatography

Bottom: same sample before ion-exchange chromatography

Useful Links

Sephadex ion exchange resins <https://cdn.cytivalifesciences.com/api/public/content/digi-11385-original>

Performing a Separation with Sephadex®

<https://www.sigmaaldrich.com/US/en/technical-documents/protocol/protein-biology/protein-purification/performing-a-separation-with-sephadex>

How Crich group uses Sephadex® C-25 to purify aminoglycosides: https://pubs.acs.org/doi/suppl/10.1021/jacs.7b07754/suppl_file/ja7b07754_si_001.pdf