

CHEM 4114: Size-Exclusion Chromatography (SEC)

Objectives: In this laboratory you will use Size-Exclusion Chromatography (SEC), also called Gel-Permeation Chromatography (GPC), to determine the molecular weight of an unknown sample.

Reference: Strobel and Heineman, *Chemical Instrumentation: a systematic approach, 3rd Ed.*, chapter 26, Basic HPLC operating notes and HPLC instrument manuals are in Davidson 123A.

Instrumentation: Perkin Elmer Series 200 HPLC pump with absorbance detector, computer integrator, and Zorbax Bimodal (60Å - 1000Å) SEC column.

Chemicals: THF mobile phase; THF solutions of polystyrene standards with molecular weights of 670,000, 95,800, 29,440, and 3,700; a THF solution of all standards and xylene; styrofoam cup sample.

WARNINGS: THF is a very reactive solvent. Keep containers of THF covered and work in the fume hood when possible.

HPLC Procedures:

- Allow the HPLC instrument to warm up for ~30 min. Set the absorbance detector to 254 nm. Sparge the mobile phase with helium in the fume hood. Prime the pump, set the flow rate to 0.5 mL/min, and flow mobile phase through the column for 10 min.
- Clean the syringe with THF before and after each injection. Between runs, place the injector in *load* position and inject ~40 µL of THF to flush the 20 µL injector loop.
- Autozero the detector before beginning each run.
- To inject a sample: place the injector in the *load* position, insert the syringe, and inject enough sample to overfill the 20 µL injector. Do not remove the syringe or return the injector to *load* position until the end of the run. Turn the injector handle to *inject* to insert the injector loop in-line with the flowing mobile phase. The computer will begin acquiring data automatically.

Measurements:

- Record the chromatograms of each of the polystyrene molecular-weight standards, the mixture of all standards, and xylene. (The xylene requires an injection of only 2 µL.)
- Weigh a 25-50 mg piece of the unknown, dissolve in THF, and make up to 10.0 mL. Record the chromatogram of this sample.

Data Analysis:

1. Tabulate the detector response of the standards and the unknown.
2. Calculate the concentration of each polymer in the mixture.
3. Tabulate and plot the log of the molecular weight of the standards versus the elution volume.
4. Determine the peak molecular weight and the molecular-weight distribution of the unknown. A working definition of the molecular-weight distribution is the range between the lower and upper molecular weights that are at 10% of the maximum intensity on the chromatographic peak.

Discussion:

1. Discuss any differences in the detector response for the standards and the unknown.
2. Explain the separation mechanism of SEC. Define total exclusion, total permeation, and selective permeation, and label them on the chromatogram of the mixture.