Separations Based on Size:

Size-Exclusion Chromatography [SEC] –Gel-Permeation Chromatography [GPC]

In the 1950s, Porath and Flodin discovered that biomolecules could be separated based on their size, rather than on their charge or polarity, by passing, or *filtering*, them through a controlled-porosity, hydrophilic dextran polymer. This process was termed *gel filtration*. Later, an analogous scheme was used to separate synthetic oligomers and polymers using organic-polymer packings with specific pore-size ranges. This process was called gel-permeation chromatography [GPC]. Similar separations done using controlled-porosity silica packings were called size-exclusion chromatography [SEC]. Introduced in 1963, the first commercial HPLC instruments were designed for GPC applications [see Reference 3].

All of these techniques are typically done on stationary phases that have been synthesized with a pore-size distribution over a range that permits the analytes of interest to enter, or to be excluded from, more or less of the pore volume of the packing. Smaller molecules penetrate more of the pores on their passage through the bed. Larger molecules may only penetrate pores above a certain size so they spend less time in the bed. The biggest molecules may be totally excluded from pores and pass only between the particles, eluting very quickly in a small volume. Mobile phases are chosen for two reasons: first, they are good solvents for the analytes; and, second, they may prevent any interactions [based on polarity or charge] between the analytes and the stationary phase surface. In this way, the larger molecules elute first, while the smaller molecules travel slower [because they move into and out of more of the pores] and elute later, in decreasing order of their size in solution. Hence the simple rule: *Big ones come out first*.

Since it is possible to correlate the molecular weight of a polymer with its size in solution, GPC revolutionized measurement of the molecular-weight distribution of polymers that, in turn, determines the physical characteristics that may enhance, or detract from, polymer processing, quality, and performance [how to tell *good* from*bad* polymer].

Conclusion

We hope you have enjoyed this brief introduction to HPLC. We encourage you to read the references below and to study the Appendix on HPLC Nomenclature.