3.4.2. Gel Exclusion Chromatography

Gel Exclusion Chromatography (also called molecular exclusion chromatography, size exclusion chromatography, or gel filtration chromatography) is a low resolution isolation method that employs a cool “trick." This involves the use of beads that have tiny “tunnels" in them that each have a precise size. The size is referred to as an “exclusion limit," which means that molecules above a certain molecular weight will not fit into the tunnels. Molecules with sizes larger than the exclusion limit do not enter the tunnels and pass through the column relatively quickly by making their way between the beads. Smaller molecules, which can enter the tunnels, do so, and thus, have a longer path that they take in passing through the column. Because of this, molecules larger than the exclusion limit will leave the column earlier, while those that pass through the beads will elute from the column later. This method allows separation of molecules by their size.

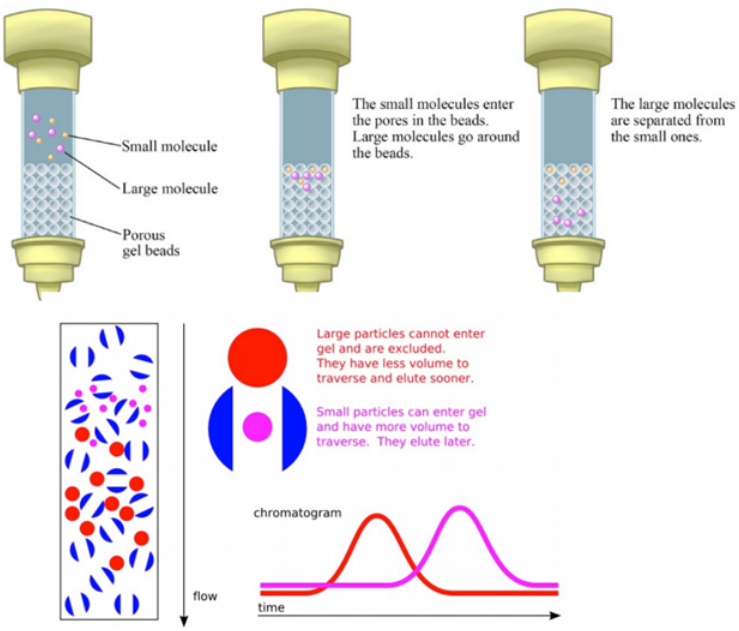

Figure 3.4.2.1: *Gel Exclusion Chromatography*

- Gel filtration does not rely on any chemical interaction with the protein, rather it is based on a **physical** property of the protein - that being the *effective molecular radius* (which relates to mass for most typical globular proteins).
- Gel filtration resin can be thought of as beads which contain pores of a defined size range.
- Large proteins which cannot enter these pores pass around the *outside* of the beads.
- Smaller proteins which can enter the pores of the beads have a longer, tortuous path before they exit the bead.
- Thus, a sample of proteins passing through a gel filtration column will *separate based on molecular size*: The big ones will elute first and the smallest ones will elute last (and "middle" sized proteins will elute in the middle).
- If your protein is unusually "small" or "large" *in comparison to contaminating proteins* then gel filtration may work quite well.

Where will a protein elute in a gel filtration experiment?

- There are two extremes in the separation profile of a gel filtration column.
- There is a critical molecular mass (large mass) which will be ***completely excluded*** from the gel filtration beads. All solutes in the sample which are equal to, or larger, than this critical size will behave identically: they will all eluted in the excluded volume of the column
- There is a critical molecular mass (small mass) which will be ***completely included*** within the pores of the gel filtration beads. All solutes in the sample which are equal to, or smaller, than this critical size will behave identically: they will all eluted in the included volume of the column

- Solutes between these two ranges of molecular mass will elute between the excluded and included volumes

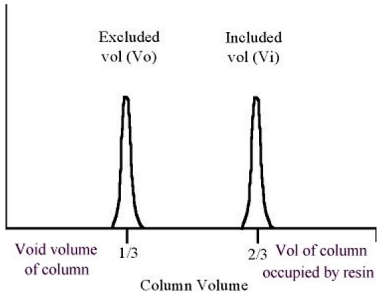

Figure 3.4.2.2: Excluded vs. included volume

As a general rule of thumb, the excluded volume (Vo) is approximately equal to one third of the column volume, the included volume is approximately equal to two thirds of the column volume

- In gel filtration the resolution is a function of column length (the longer the better)
- However, one drawback is related to the maximum sample volume which can be loaded. The larger the volume of sample loaded, the more the overlap between separated peaks. Generally speaking, the sample size one can load is limited to about *3-5% of the total column volume*.
- Thus, gel filtration is best saved for *the end stages of a purification* ,when the sample can be readily concentrated to a small volume.
- Gel filtration can also be used to remove salts from the sample, due to its ability to separate "small" from "large" components.
- Finally, gel filtration can be among the most "gentle" purification methods due to the lack of chemical interaction with the resin.