APPLICATION NOTE

3.03 PHARMACEUTICAL & BIOTECH

CHROMATOGRAPHIC SEPARATION

- Real time in-line continuous measurement
- Non-destructive analysis
- Dual-wavelength measurement
- Zero dead volume, no hold up

Application

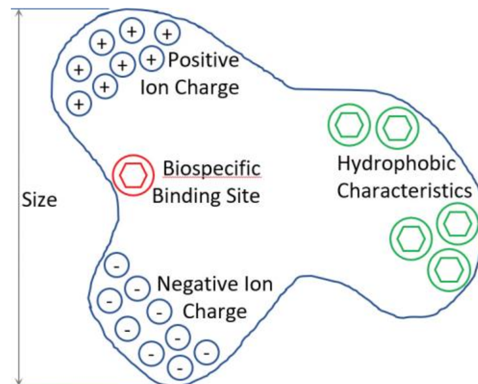
- Process/Yield Optimization
- Quality control
- Protein monitoring
- Solvent monitoring

The versatility and efficacy of chromatographic techniques have made them essential in both large scale and analytical separations. Chromatography is widely used in bioprocessing to separate protein molecules from concentrated process fluids. The center of a chromatography system is a column, filled with a media specific to the separation being carried out.

METHODS OF CHROMATOGRAPHIC SEPARATION

There are several methods of chromatographic separation:

Type	Method
Gel Filtration	Sort material by molecular size
Ion Exchange	Binds material by electrical charge
Hydrophobic Interaction	Separation by hydrophobic character
Affinity	Binds material by attachment to specific binding site



Molecular Properties Utilized in Chromatographic Separation

Gel Filtration

The physical dimensions of the molecule determine the separation because proteins are naturally spherical, the molecular weight of the protein will be proportional to its size and therefor allowing separation based on this property. Larger molecules pass more slowly through column media, while smaller molecules pass quickly.

Ion Exchange

Separation works by opposite charge attraction and like charge repulsion. Using a specific charge within the column media allows for attraction and binding of the molecule(s) of interest.

Hydrophobic Interaction

Separation is based on the fact that polar (hydrophilic) molecules “stick” together and repel those which are non-polar (hydrophobic).

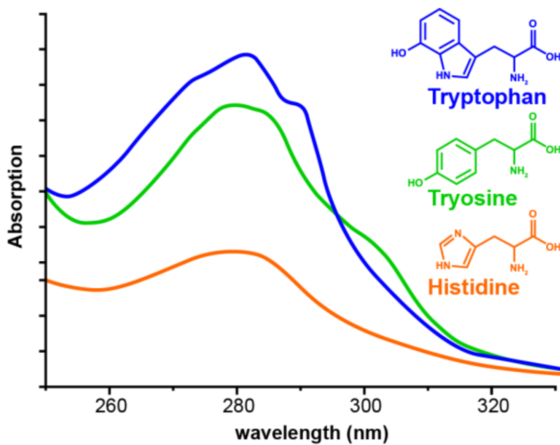
Affinity

This method uses a bio-specific binding site, which is a section of a molecule in which the shape and distribution of charged and hydrophobic groups allow for highly specific binding to a corresponding site on another molecule. The fit between the two sites is analogous to a lock and key.

A chromatography gel is designed to have one half of this lock and key (the ligand), making it stationary within the column. As the product solution is passed through column, the specific mating molecules are bound in place until they are eluted from the column. Examples of affinity interactions include the binding between antibodies/antigens and enzymes/substrates.

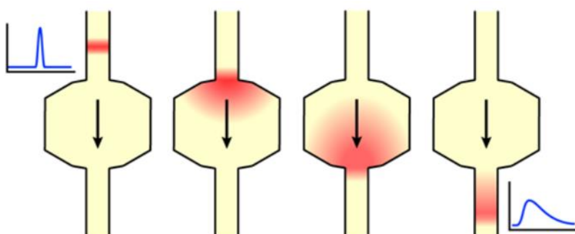
Separation through chromatography requires highly specialized equipment to ensure maximum yield and purity. And a system may include a variety of instruments and sensors for pre and post column control.

Nearly all proteins absorb UV light at 280nm, the primary reason for this is due to the aromatic amino acids, *e.g.* phenylalanine, tryptophan, tyrosine and histidine.

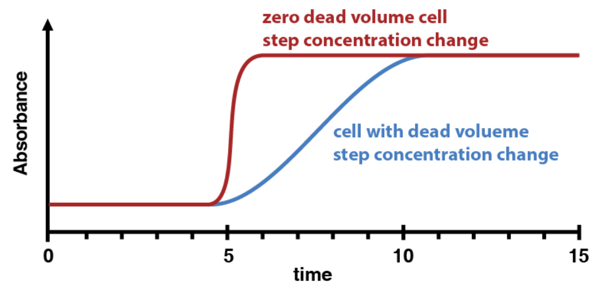


Installing a [Kemtrak DCP007-UV] photometer, measurement cell at column outlet, allows the presence of proteins to be detected, and therefore collection/pooling to begin.

Also, it is important that the UV analyzer has zero dead/hold up volume to ensure crisp, sharp peaks are detected. UV analyzers utilizing measurement cells with internal hold up volumes can lower the purity of the collected protein because of dilution. Dilution blurs sharp peak detection lines and can cause lower yields.



Effect on detection peak of a Cell with dead volume increased hold up time



Effect of absorbance, caused by dead volume/hold up volume on purity and peak sharpness

Using a [Kemtrak DCP007-NIR] photometer, mounted pre-column to determine solvent concentration/composition, allows for feedback control of solvent "mobile media" feed through the column, and increase accuracy and repeatability of the chromatographic system.

With the unique zero dead volume [Kemtrak measurement cell] installed, the Kemtrak DCP007 analyzer can provide single or dual wavelength absorbance analysis, in real time, for improved control of the chromatographic separation process.

The Kemtrak DCP007 is the recommended analyzer for chromatographic separation.