CHROMATOGRAPHIC SEPARATION

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

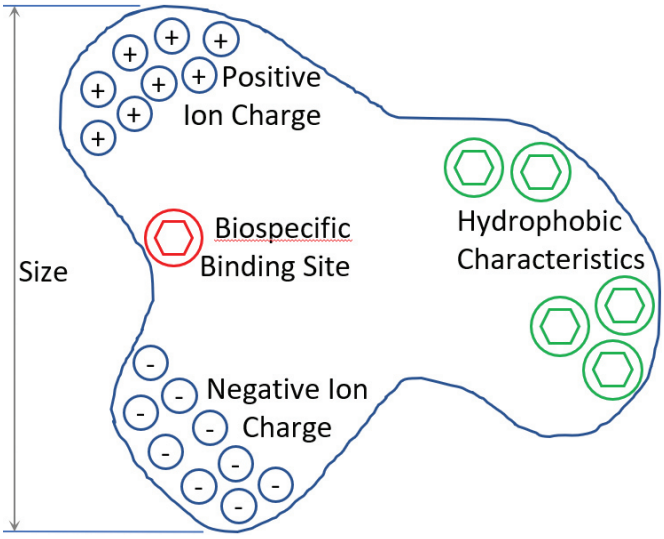
With **Gel Filtration**, size refers to the physical dimensions of the molecule. Because proteins are naturally globular, the molecular weight of the protein will be proportional to its size, allowing separation based on size. Larger molecules pass more slowly through column media, while smaller molecules pass quickly. In applications where several different molecule sizes are collected, they tend to exit the column in "waves," with the smallest exiting first.

Ion Exchange separation depends on opposite charges attracting one another, while like charges repel each other. Using a specific charge within the column media allows for attraction and binding of the molecule(s) of interest.

Separation by **Hydrophobic Interaction** can be utilized for specific fragile molecules through gentler binding and elution conditions, which help prevent denaturation of the final product. This separation mode is based on the fact that polar (hydrophilic) molecules tend to stick together and repel those which are non-polar (hydrophobic).

The **Affinity** method uses a **biospecific 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. 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 the

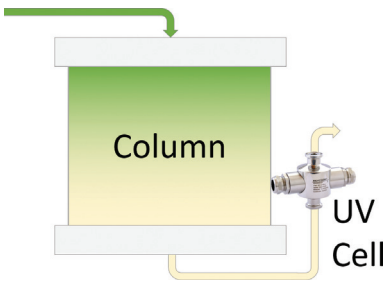
column, the specific mating molecules are bound in place until they are eluted from the column. Examples of affinity interactions include the binding between antibodies/antigens and enzymes/substrates.



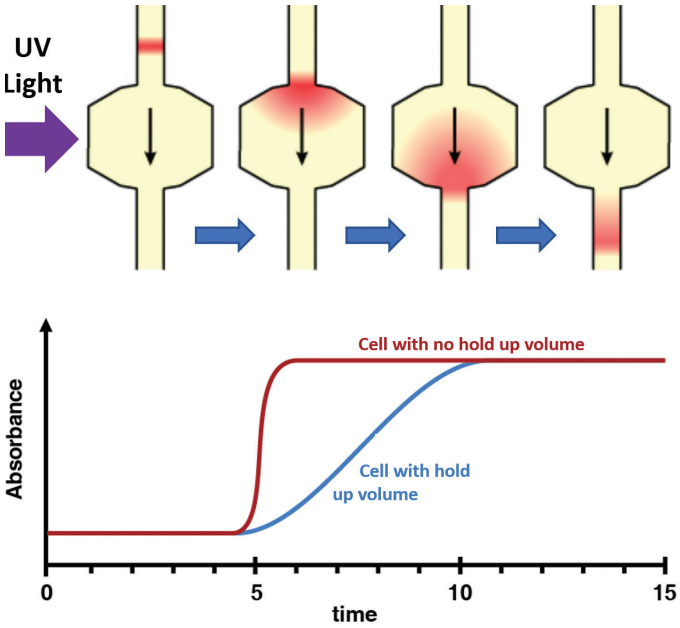
Molecular Properties Utilized in Chromatographic Separation

EQUIPMENT REQUIREMENTS FOR CHROMATOGRAPHIC SEPARATION

Separation through chromatography requires highly specialized equipment to ensure maximum yield and purity, and a typical system will include a variety of instruments and sensors. A UV absorbance analyzer at the column outlet detects protein coming off the column (this works because nearly all proteins absorb UV energy at or around 280nm). The analyzer measurement cell is fitted in the outlet line of the column, and as protein concentration increases, the absorbance output "peaks" up, allowing the protein rich solution to be diverted for collection.



It is important that the UV analyzer has zero 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 of UV detection cell hold up volume on purity and peak sharpness

THE KEMTRAK DCP007 ANALYZER MEETS A GROWING NEED FOR MEASURING HIGHER PROTEIN LEVELS

Advances and improvements in bioprocessing methods have led to protein products being expressed and concentrated to higher and higher levels. Higher protein concentrations mean higher absorbance measurements. Therefore, UV analyzers need the ability to measure to deep absorbance. While absorbance measurement is essentially linear, many light sources generate light at wavelengths other than 280nm; this can enter the measurement path as stray light, which creates artificially low absorbance readings and non-linear responses to protein concentration, particularly at high levels.

Furthermore, these light sources are noisy, hot, and constantly drift—this makes them less than ideal, particularly for fragile protein molecule work.

Accurate, reliable, and repeatable post column UV measurements are a minimum requirement during chromatographic separation to ensure good protein fraction purity and maximum yields. With a unique Kemtrak zero volume hold-up cell installed at the column outlet, the Kemtrak DCP007 analyzer can provide single or dual wavelength UV absorbance analysis, in real time, for instant visibility and improved control of the separation process. With this, the need for offline testing and manual analysis is greatly reduced. Furthermore, the use of solid-state light sources provides the ability to measure to 4.5A at 280nm and to monitor high concentration processes up to 90 OD linearly without “peak clipping” and the undesirable effects of hot and powerful UV light sources on the process. Because of this, the Kemtrak DCP007 is the best analyzer for chromatographic separation.

GET IN TOUCH

South Fork Instruments
3845 Buffalo Road
Auburn, CA 95602

southforkinstruments.com

CALL US TODAY!

(866) 953-4668