Introduction to DSP – Chromatography

Your Objectives:

At the end of the lesson, you should be able to recall the process of Chromatography from a high level and to explain what separations will occur.

One of the most important aspects of Biogen's manufacturing processes is the recovery and purification of a single target protein molecule. The molecule typically exists in solution and must be isolated from the other components in the solution.
Chromatography provides a method for separating molecules from complex solutions. By analyzing a target molecule's characteristics, it can be differentiated from other molecules in a solution. The target molecule can then be separated using the appropriate chromatography processes.

The chromatography methods and the number of chromatography steps depend upon the chemical makeup of the molecule as well as on the degree of purity needed for the final product. In general, chromatography methods involve a specific type of binding interaction that is reversible. Chromatography methods are so powerful and precise that they can often distinguish proteins that differ in composition by a single amino acid or a single atom, even. Moreover, because the conditions and processes of chromatography are gentle, it allows for a reliable way of separating delicate highly fragile components.
Chromatography has two phases: stationary & mobile, each moving in a definite direction, and are typically required for the regulatory approval of protein drugs.

The simplest way to explain chromatography is by comparing it to a raging river:

A raging river can carry a lot of debris along with it. The speed at which floating debris is moved will depend upon (1) the types of floating debris (i.e., grains of sand are transported faster than pebbles), and (2) the nature of the riverbed (i.e., rough surfaces increase the friction of the floating debris, thus slowing or stunting the speed of removal) on the flow velocity.

In chromatography, various substances (= floating debris in the 'river') are transported in the so-called mobile phase (= 'water that is the river') on a stationary phase (= the ''riverbed'). Due to the interactions* between the sample, stationary phase and mobile phases, individual substances are transported at different speeds and are, for that reason, separated and distinguishable from one another when a mixture of 'sand', very small and slightly larger 'pebbles' is introduced at a certain point in time into the 'river'; according to the analogy, after 100 metres all the sand will arrive first (spread over a few metres), and, after a certain waiting period, all the smaller pebbles follow and, much later, any larger particles, each strewn across a certain distance.
The river analogy is indeed illustrative enough for an initial understanding of the process of chromatography, (though the actual process in chromatography is perhaps more evocative of a digital process known as "stop-and-go traffic,") whereby the sample molecules are either carried

along with in the mobile phase (analogous to a 'light raft' that is passively carried in a current) or adhering to the stationary phase (at 'zero speed'). The molecules switch back and forth (vacillate) between these two possibilities very rapidly and heat waves act like 'shocks'.

The analogy of the riverbed nevertheless differs inasmuch as the delays that the various sample molecules suffer (through the chromatographic system) do not account for the friction phenomenon. The basis for understanding this difference is in the distribution of the different types of molecules (types A, B, C, etc.), which correspond to differences in the average proportion of time the individual molecules spend in the mobile phase). Chromatography allows for a conversion of such differences into 'speed differences', thus making them truly effective for a separation, without which, these minute (tiny) differences could not be used, neither for separation and cleaning processes nor for proper analysis/analyses.

* (For interactions, see the "division under separation" principles.)

The Process

Four phases of chromatography are applied:

1. Establishment of the flow of the mobile phase,
2. Injection of the sample to be separated,
3. Actual separation, and
4. Detection of components

The flow of the mobile phase is achieved by any one of three means: by pressure (e.g. hydraulic pump, gas pressure), by capillary force, or then by applying an electrical voltage.

The injection (i.e., introduction of the substance mixture into the chromatographic system) takes place either before the flow of the mobile phase is established (thin-layer chromatography) or while the mobile phase is already in flow. With a large number of samples, so-called autosamplers are used with automatable types of chromatography (together with their own data acquisition systems), which inject the samples fully automatically.

The actual separation of the substance mixture follows on the separating section.
Finally, (as with the separation or separate phase) chromatography would be inconceivable without detection (∴making visible when a substance passes a certain section of the chromatography system or where a substance comes to a standstill after the process has ended). Different detection systems are applied for each type of chromatography, either by using physical properties (e.g., absorption of light, fluorescence, light scattering, and thermal conductivity) of the substances, or then by obtaining a signal through chemical reactions by means of chemical reactions; for example, a coloration achieved in **planar chromatography** (e.g., amino acids using ninhydrin), or then through reactions carried out before separation (pre-column derivatization) or otherwise after separation (post-column derivatization), in **column chromatography**.

In the case of **preparative chromatography**, a fraction collector is additionally required to collect the separated substance.

Due to design chromatographic purification processes are always batch processes. This means that only a certain amount of substance can be applied (injected) and separated before proceeding with the next (equal) amount, making it particularly problematic when working up large amounts. Therefore, specific methods, all of which could not be otherwise be done by a simple column purification, have been developed so as to operate chromatography continuously:

Continuous annular chromatography (CAC), **true moving bed** chromatography (TMB) and **simulated moving bed** chromatography (SMB).

CAC lends itself to the separation of multi-component mixtures as well as of bi-component ones.
TMB is used as a cost-effective process.
SMB is a theoretical concept

Notabene:
A **precolumn derivatization** method was developed for the high-performance liquid chromatographic (HPLC) determination of penicillin using fluorescence detection. ... The resulting reaction mixture was injected directly onto a reversed-phase **column** and analysed by HPLC.

Post-column derivatization, also known as **post-column** reaction, renders visible certain compounds that are normally invisible. This trick is accomplished **after** the separation by performing a chemical reaction on the substances that gives them an easily detectable physical property.