

packed column. Owing to high-pressure liquid (high liquid flow rate) and dense column packing, HPLC provides fast and high resolution of solute molecules.

The choice of the stationary phase and consequently the type of chromatography depends on the nature of the solutes and process goals. Ion-exchange chromatography is widely used, particularly for recovery of proteins. This method offers good resolution of peaks, high capacity, and good speed. Hydrophobic chromatography and LLC share many of these attributes, but are best applied when the aqueous phase is at high ionic strength. Also organic solvents may be needed, which may denature proteins or create environmental problems. Adsorption chromatography is often relatively inexpensive, but resolution is often not very sharp. Gel-filtration chromatography is good for buffer exchange and desalting and offers decent resolution. Because gels are compressible, capacity and throughput are often low; new rigid packings for size exclusion chromatography allow faster flow rates. Affinity methods offer the possibility of very high selectivity and good capacity and speed. However, such methods can be extremely expensive, especially if based on antibodies. For real bioprocesses (e.g., recovery of proteins) multiple types of chromatography are used, since greater purity is possible if more than one basis of separation is used. Ion-exchange and affinity chromatography are the most widely used methods to recover proteins from bioprocesses.

A single chromatographic separation process may include more than one of these mechanisms. For example, adsorption chromatography using silica gel functions properly when the water content of silica gel is less than 15%. The system may operate as liquid–liquid partition chromatography when the water content exceeds 30%. In some cases, electrostatic and weak forces may be simultaneously functional.

A typical chromatographic column is shown in Fig. 11.28, where a solution is flowing downward in the column and a solute is adsorbed on adsorbent solids by forming a

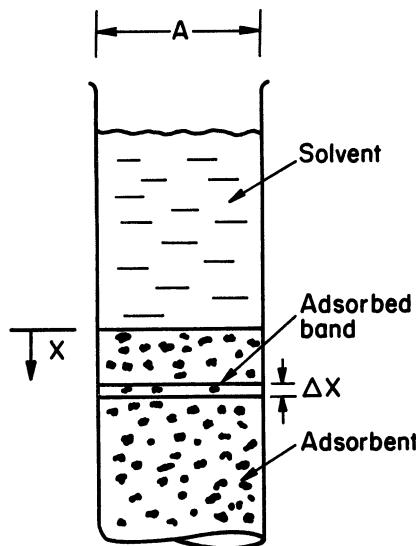


Figure 11.28. Schematic of a chromatography column.