are very specific, such as enzyme–substrate or antigen–antibody interactions. That is, an enzyme inhibitor or substrate may be used as a ligand in separating a specific enzyme from a mixture. Monoclonal antibodies (MAb) may be used as a ligand to separate specific antigen molecules by affinity chromatography.

Another type of affinity chromatography is *IMAC* (immobilized-metal-affinity chromatography) which exploits the different affinities that solutes (esp. proteins) have for metal ions chelated to a support surface. A very attractive feature is the low cost of metals and the ease of regeneration of the stationary phase, particularly in contrast to affinity chromatography using antibodies. Typically, iminodiacetic acid (IDA) or nitrilotriacetic acid (NTA) linked to an agarose or silica gel support provide attachment sites for a metal ion. A variety of target proteins have been purified using zinc, copper, and nickel chelates. Water molecules will normally solvate metal ions, but in the presence of a strong Lewis base (e.g., histidine residue in a protein) the water molecules can be displaced. The bonds that form between the immobilized metal and the base (protein) result in an adsorbed protein. Factors such as the accessibility, microenvironment of the binding residue (i.e., histidine, cystein, and tryptophan), cooperation between neighboring amino acid side groups, and local conformations play important roles in the strength of binding and thus protein retention. Subsequent destruction of the bond by lowering the pH or adding a competing ligand in the mobile phase results in the elution of the metal binding protein.

Not all proteins naturally bind metals. However, IMAC can still be used if the genetic information for the desired protein is altered to include a *tail* that has high metal-binding affinity. Typically the structural gene encoding the target protein is extended to code for a metal-binding peptide, resulting in production of a fusion protein of the target protein and tail. The amino acid sequence of the tail is based on a sequence of amino acids known to bind metals. A common choice is six adjacent histidines. Peptide libraries have also been used to generate effective affinity tails. The biggest drawback to the method is that for many applications the tail must be removed. The system can be designed with a linker between the tail and target protein that can be cleaved chemically.

Our discussion of the theory of chromatography is based on many simplifying assumptions. Usually the analysis of real columns is more complicated, owing to dispersion, wall effects, and lack of local equilibrium. Wall effects can be reduced by keeping the column-diameter-to-bead-diameter ratio larger than 10 ($D_c/D_b > 10$). Bed compression can occur under some operating conditions and can further complicate analysis of the system by causing flow irregularities such as viscous fingering.

Understanding these hydrodynamical and kinetic effects is also critical to scale-up of chromatographic processes. These effects alter the shape of the peaks exiting the column. In addition to these flow effects, the step controlling the rate of adsorption will alter peak shape. For a porous support internal diffusion, diffusion through the external film, or external dispersion (Taylor dispersion) in laminar flow can control the rate of mass transfer. In the following we consider a more quantitative description of these ideas.

Figure 11.31 depicts the output from a chromatography column. Refering to this figure, we can define some key characteristics of the output. The *resolution* of two adjacent peaks, R_S, is

$$R_S = \frac{t_{\max,j} - t_{\max,i}}{\frac{1}{2}(t_{w,i} + t_{w,j})} \tag{11.85}$$