



**Figure 11.29.** Separation of a binary mixture in a chromatographic column.

vent into the column ( $\Delta V/A\varepsilon$ ), then the resolution ( $R$ ) or the ratio of the distance traveled by solute  $I$  to that of solvent in the space above the column is given by

$$\frac{L_I}{L_S} = R = \frac{1}{\varepsilon + MK} \quad (11.80)$$

where  $K = f'(C_L) = df(C_L)/dC_L$  = partition coefficient. Similarly, we can define  $R_f$  as

$$R_f = \frac{L_I}{L_C} = \frac{\varepsilon}{\varepsilon + MK} \quad (11.81)$$

where  $R_f$  is the ratio of the travel distance of  $I$  to that of solvent in the column.

When component  $I$  is moved out of the column, then  $L_I = L_0$  and  $L_S = V_0/A$ , where  $V_0$  is the volume of solvent required to displace out of the packed column. Then  $L_S/L_I$  becomes

$$V_r = \frac{L_S}{L_I} = \frac{V_0}{L_0 A} = \varepsilon + MK \quad (11.82)$$

where  $V_r$  is the ratio of solvent volume to column volume.

This theory of chromatography is applicable to fixed column adsorption, partition, ion-exchange, and affinity chromatography. The theory of *gel-filtration chromatography* is somewhat different. In gel-filtration (molecular sieving) chromatography, solute molecules diffuse into porous structures of support particles, depending on their molecular size and shape. Small solute molecules get into the fine pore structures of the support particles and remain in contact with the solid for a “long” period, while large molecules are adsorbed on the outer surface and remain in contact with the solid for a “short” period. When the column is eluted, the largest molecules appear first in the eluent solvent, and the smallest molecules last. Different bands of solute molecules are obtained on the basis of their size and shape. Consider the case where a buffer with the solutes of interest is added to the column, followed by sufficient washes to elute the original buffer solution.

The total volume of the buffer solution eluted from the column over a certain period of time is