BIOCHEMICAL ENGINEERING FUNDAMENTALS

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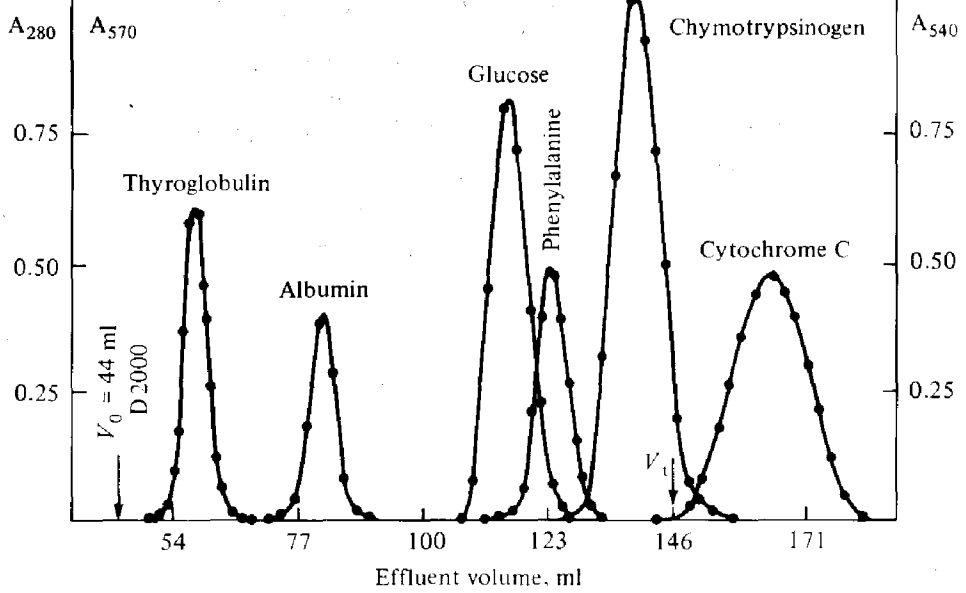

Figure 4.15 Chromatogram resulting from molecular-sieve chromatography of a solute mixture (packing is 6 percent cross-linked desulfated agar. Buffer is pH 7.5, 0.05 *M* tris-HCl). (*Reprinted by permission from J. Porath, Chromatographic Methods in Fractionation of Enzymes, in L. B. Wingard, Jr. (ed.), "Enzyme Engineering," p. 154, John Wiley & Sons, New York, 1972.*)

needed to ensure protein stability, for example) the composition of the *dialysate fluid* in which the dialysis bag containing the sample is placed must be such that no finite concentration difference of these particular dialyzable components exists across the dialysis membrane, e.g., the use of 10^{-3} *M* phosphate buffer as dialysate in step 7 of Example 4.1. When there are many chemicals which we wish to retain in the sample (such as blood), the cost of the necessary dialysate fluid may be very large. If a low concentration of certain dialyzable compounds is ultimately needed in the sample, relatively larger volumes of dialysate fluid must be used, for example, 6 l of phosphate buffer to dialyze 50 ml of sample (Example 4.1). This is necessary so that the concentration in the dialysate fluid will remain very small and there will always be a diffusive driving force. Dialysis is essentially a diffusion-controlled process.

Ultrafiltration is based upon the ability of a membrane, under a hydrostatic pressure head, to reject relatively high molecular weight components while passing both solvent and low molecular weight solutes. Thus, while dialysis generally removes low molecular weight molecules and ions from the original solution volume, ultrafiltration *concentrates* the original protein solution by removing much solvent as well as small solutes.

With ultrafiltration, the fluxes of solvent and of solute (protein) are often reasonably represented by

$$\mathcal{N}_1 \text{ (solvent)} = L_p(\Delta p - \sigma\, \Delta\pi) \tag{4.9}$$

$$\mathcal{N}_2 \text{ (solute)} \approx \bar{C}_2(1 - \sigma)\mathcal{N}_1 + P\, \Delta C_2 \tag{4.10}$$

Both microencapsulation methods have the potential of offering a very large surface area (for example, 2500 cm^2 per millimeter of enzyme solution) and the possibility of added specificity: the membrane can be made in some cases to admit some substrates selectively and exclude others. In principle these methods should be applicable to a large variety of enzymes. However, the membrane is a significant mass-transfer barrier, so that the "effectiveness factor" for the enzymes may be quite small. Also, these techniques are not applicable when the size of the substrate molecule approaches that of the enzyme.

We have already discussed (Sec. 4.2) how semipermeable-membrane filtration devices can be used to contain enzyme while allowing interchange of smaller molecules with a neighboring solution. A continuous flow ultrafiltration concept illustrated schematically in Fig. 4.24, also contains the enzyme as in microencapsulation. The surface areas separating the two solutions are substantially lower when ultrafiltration is used, and shear denaturation may occur. The mass-transfer rates available through membranes are small and may limit the overall rate. However, almost any enzyme or combination of enzymes can be immobilized in this way. It is advantageous when the substrate has very high molecular weight or is insoluble, situations where polymer-bound enzymes typically may have a small efficiency. Also, the semipermeable membrane will not permit relatively large product molecules from polymer-hydrolysis reactions to escape from the enzyme solution. This provides an interesting method of controlling the molecular-weight distribution of the products which ultimately leave the reactor.

In Sec. 4.6, we shall return to immobilized enzymes and examine some of their present and possible future applications. In current technology, however, enzymes in solution enjoy far greater use, as outlined in the next two sections.

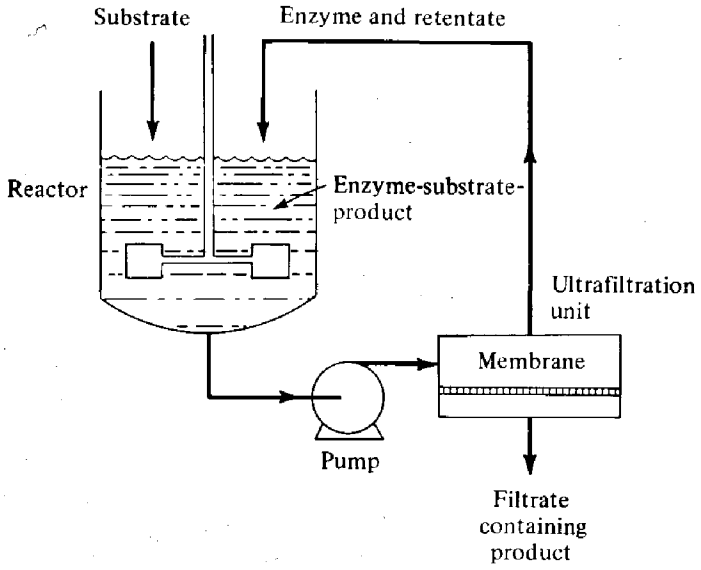

Figure 4.24 Enzyme entrapment on a macroscale. An ultrafiltration membrane is used to retain enzyme and other large molecules in the reactor.