in a polymer matrix, enzyme solution is mixed with polymer solution before polymerization takes place. Polymerized gel-containing enzyme is either extruded or a template is used to shape the particles from a liquid polymer-enzyme mixture. Entrapment and surface attachment may be used in combination in some cases.

Membrane entrapment of enzymes is possible; for example, hollow fiber units have been used to entrap an enzyme solution between thin, semipermeable membranes. Membranes of nylon, cellulose, polysulfone, and polyacrylate are commonly used. Configurations, other than hollow fibers, are possible, but in all cases a semipermeable membrane is used to retain high-molecular-weight compounds (enzyme), while allowing small-molecular-weight compounds (substrate or products) access to the enzyme.

A special form of membrane entrapment is *microencapsulation*. In this technique, microscopic hollow spheres are formed. The spheres contain the enzyme solution, while the sphere is enclosed within a porous membrane. The membrane can be polymeric or an enriched interfacial phase formed around a microdrop.

Despite the aforementioned advantages, enzyme entrapment may have its inherent problems, such as enzyme leakage into solution, significant diffusional limitations, reduced enzyme activity and stability, and lack of control of microenvironmental conditions. Enzyme leakage can be overcome by reducing the MW cutoff of membranes or the pore size of solid matrices. Diffusion limitations can be eliminated by reducing the particle size of matrices and/or capsules. Reduced enzyme activity and stability are due to unfavorable microenvironmental conditions, which are difficult to control. However, by using different matrices and chemical ingredients, by changing processing conditions, and by reducing particle or capsule size, more favorable microenvironmental conditions can be obtained. Diffusion barrier is usually less significant in microcapsules as compared to gel beads.

3.4.1.2. Surface immobilization. The two major types of immobilization of enzymes on the surfaces of support materials are adsorption and covalent binding.

Adsorption is the attachment of enzymes on the surfaces of support particles by weak physical forces, such as van der Waals or dispersion forces. The active site of the adsorbed enzyme is usually unaffected, and nearly full activity is retained upon adsorption. However, desorption of enzymes is a common problem, especially in the presence of strong hydrodynamic forces, since binding forces are weak. Adsorption of enzymes may be stabilized by cross-linking with glutaraldehyde. Glutaraldehyde treatment can denature some proteins. Support materials used for enzyme adsorption can be inorganic materials, such as alumina, silica, porous glass, ceramics, diatomaceous earth, clay, and bentonite, or organic materials, such as cellulose (CMC, DEAE-cellulose), starch, activated carbon, and ion-exchange resins, such as Amberlite, Sephadex, and Dowex. The surfaces of the support materials may need to be pretreated (chemically or physically) for effective immobilization.

Covalent binding is the retention of enzymes on support surfaces by covalent bond formation. Enzyme molecules bind to support material via certain functional groups, such as amino, carboxyl, hydroxyl, and sulfhydryl groups. These functional groups must not be in the active site. One common trick is to block the active site by flooding the enzyme solution with a competitive inhibitor prior to covalent binding. Functional groups on support material are usually activated by using chemical reagents, such as cyanogen bromide, carbodiimide, and glutaraldehyde. Support materials with various functional groups and the chemical reagents used for the covalent binding of proteins are listed in Table 3.3.