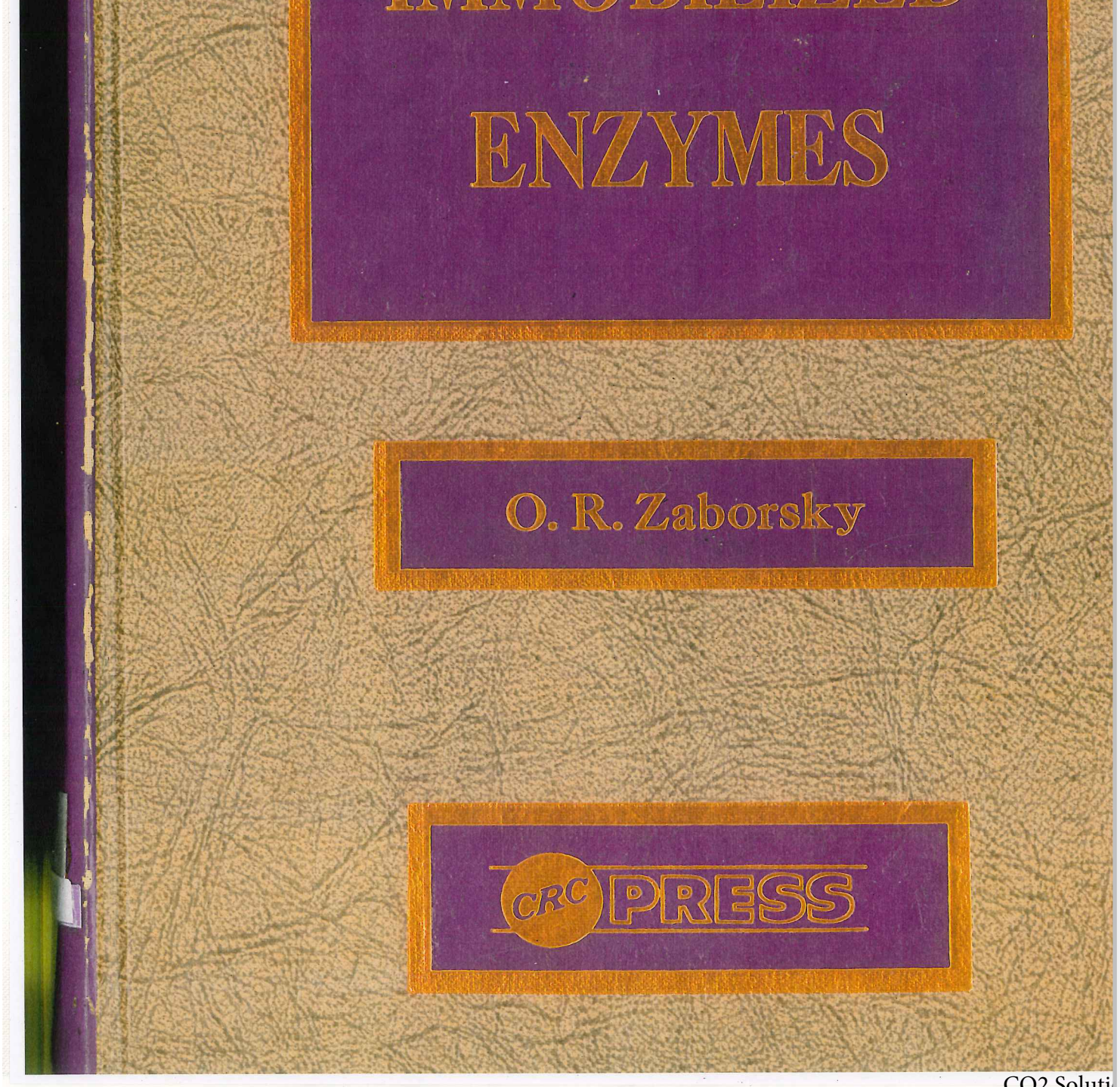

Immobilized Enzymes

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polymer in the presence of an enzyme. Enzyme molecules are physically entrapped within the polymer lattice and cannot permeate out of the gel matrix, but appropriately sized substrate and product molecules can transfer across and within this network to insure a continuous transformation (see Figure 17). Other commonly used names for the method are inclusion, lattice entrapment, and occlusion.

Lattice entrapment was first employed successfully for the immobilization of trypsin, α-chymotrypsin, and other enzymes by Bernfeld and Wan[355] in 1963. Since then, numerous enzymes have been immobilized in this manner, and several polymeric networks have been used (see Table 10). The most commonly employed crosslinked polymer for enzyme entrapment is the well-known polyacrylamide gel system, but silicone rubber (Silastic®), starch, and silica gel have also been used. Dickey[356] reported a partially successful entrapment of urease and catalase in silica gel as early as 1955. Although immobilization of enzymes in

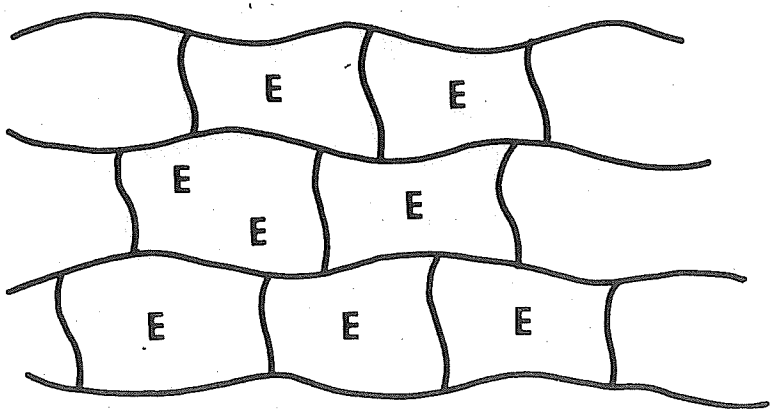

FIGURE 17. Cross-sectional view of lattice-entrapped enzyme conjugate showing polymer chains and occluded enzyme molecules.

polymers such as silica gel.

Nature of Crosslinked Polymeric Matrices

The polyacrylamide gel system is produced the reaction of acrylamide and *N,N'*-methyle bisacrylamide. The polymerization reaction can initiated in several ways and that used most of for enzyme immobilization is shown in Equat 52. The procedure for the formation of the cr linked polyacrylamide-enzyme conjugate identical to that used for the preparation polyacrylamide gels employed commonly separation and isolation of enzymes, except tha this case the protein is present during the p merization. A recent review of polyacrylamide electrophoresis by Chrambach and Rodbard provides a quick survey of the more impor parameters of the reaction and the structure of produced gel. Several studies dealing with p acrylamide gel-immobilized enzymes have described some pertinent details about

procedure. The total concentration and relative ratio of acrylamide and *N,N'*-methylenebisacrylamide determine the pore size of the interstitial space within which the enzyme molecules are entrapped and the physical nature of the water-insoluble material produced.

Hicks and Updike[358] examined the characteristics and activity of lactate dehydrogenase immobilized in different compositions of acrylamide and *N,N'*-methylenebisacrylamide and observed that the best mechanical rigidity was obtained at higher gel concentrations (total acrylamide and *N,N'*-methylenebisacrylamide). However, at any one total concentration, an increase in the relative amounts of crosslinking reagent decreased the mechanical rigidity of the gel but gave a higher yield of immobilized enzyme activity per unit of soluble enzyme activity introduced before polymerization. For example, at a total concentration of 5% (acrylamide and *N,N'*-methylenebisacrylamide), a 5% *N,N'*-methylenebisacrylamide concentration (95% acrylamide) produced a gel of "excellent" mechanical rigidity which showed a relative activity of 60%. The polyacrylamide gel composed of 10% *N,N'*-methylenebisacrylamide exhibited only "fair" rigidity and showed a 66% relative activity. According to Hicks and Updike, the most suitable gel material requires both a relatively high concentration of the monomer (acrylamide) to give mechanical rigidity and a high concentration of crosslinking reagent (*N,N'*-methylenebisacrylamide) to achieve the highest possible yield of immobilized enzyme activity. Immobilization of enzymes can be achieved by polymerization only of the crosslinking reagent as was done initially by Bernfeld and Wan,[355] but the gel so produced is very soft, sediments slowly, and is unsuitable for use in flow system applications. Moreover, the degree of concentration of the crosslinking reagent is severely limited by its solubility in water, which is approximately 3%. The appearance of polyacrylamide gels varies from clear to opaque, depending on the exact composition.

Hicks and Updike[358] also examined the nature of the polymerization catalyst. They noted that mixtures with a high percentage of monomer polymerize more effectively with persulfate, and that solutions with a higher percentage of crosslinking reagent polymerize better with riboflavin and a photocatalyst. Often, it is best to have both catalytic systems present. Other catalytic systems employed for polymerization during the immobilization of enzymes have been TEMED and persulfate,[107,355,359,360] β-dimethylaminopropionitrile and persulfate,[106,361–364] and x-ray radiation.[365] The latter initiation method has, in principle, the inherent advantage of being nonchemical in nature, allowing better heat control and permitting quick termination and control of the initiation step. The polymerization of acrylamide and *N,N'*-methylenebisacrylamide is usually conducted at room temperature or somewhat lowered temperatures and in the absence of atmospheric oxygen. The oxygen molecule, being a paramagnetic species, is a very potent polymerization inhibitor.

The trapping efficiency of polyacrylamide gels of varying composition was examined by several investigators. Degani and Miron[364] observed that the maximum yield of activity trapping (56%) occurred with cholinesterase at a crosslinking concentration of 5% and with a total and constant monomer concentration of 15%. The activity of the water-insoluble conjugate reached its highest value at the same 5% *N,N'*-methylenebisacrylamide concentration. Higher percentages of crosslinking reagent decreased both the activity of the conjugate and the trapping efficiency. The effect of the total monomer concentration (at a constant *N,N'*-methylenebisacrylamide concentration of 5%) on the activity of the conjugate and the trapping efficiency was likewise investigated. With increasing concentrations of total monomer, the

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