CHAPTER 1

INTRODUCTION

One of the most rapid growing areas of research in applied enzymology is the practical uses of enzymes in process catalysis, biochemical analyses and chemical diagnosis. This area of enzyme research constitutes an important part of enzyme technology. An essential ingredient in enzyme technology is the immobilization of the enzyme. Enzyme immobilization refers to the attachment of enzymes to a soluble or insoluble macromolecular support. Generally immobilization can be achieved by chemical or physical attachment of the enzyme to a support or by confining the enzyme by means of a semipermeable matrix. Classically, enzymeshave been immobilized by attaching them to a water insoluble material; hence insolubilization is only one of the immobilization techniques. Enzymes that are not immobilized are generally termed 'native' or'scluble'. This includes most commercially available enzymes, analytically and preparatively useful.

Enzyme immobilization technology is a new field which opens a door to a whole range of controlled biochemical transformations. During the recent years a considerable amount of work has been done with immobilized enzymes[1-4] or whole cells[5] in insoluble polymers of various physical and chemical properties.

There are a number of advantages associated with an immobilized enzyme system[6]. Expensive enzymes, when immobilized, can be re-used for many times. Processes can be run continuously; products are enzyme free; product formation can be controlled more closely by rapid introduction and removal of immobilized enzymes. Enzyme properties can sometimes be altered favourably without altering its specificity[7]. The immobilization of an enzyme to a support generally results in an attachment of the enzyme which makes it more resistant to the unfolding of its native structure caused by heat or pH extremes[8-9]. When enzymes are immobilized they are held in an environment more akin to that in which they are found naturally. The immobilized enzymes are also less succeptible to the effect of activators and inhibitors[9] that normally affect the soluble enzyme, thus in a complex analytical sample, such as blood, the immobilized enzyme is much more useful than the soluble one as an analytical reagent[10].

When an enzyme molecule is fixed on a solid support, the environment it experiences may not be the same as the one experienced by the native enzyme. This in turn may lead to changes in the physical and chemical properties of the enzyme such as catalytic activities, pH activity behaviour, temperature activity profile, temperature and pH stabilities, substrate specificity and Michaelis constant[11]. Major factors that affect the properties of an immobilized enzymes are :

(i) unstirred layer of solvent surrounding the immobilized enzyme particle and (ii) electrostatic interactions. In addition, changes may also be brought about by chemical modification of the enzyme during immobilization. The above mentioned properties of immobilized enzymes would be discussed in detail in relation to their soluble counterparts with some experimental results.

The properties exhibited by an enzyme in its immobilized form i.e., when it is fixed on a support, may differ significantly from those of the same enzyme in free solution. This is essentially due to the following reasons. In the first place, the conformation of the enzyme molecule in bound form may not be the same as that of the native soluble enzyme the alteration of which results in intrinsic changes in its catalytic properties[12]. Secondly, the microenvironment which the enzyme experiences in its immobilized form may be entirely different from that of the soluble enzyme, which may lead to apparent changes in the properties of immobilized enzymes. Changes in conformation of the enzyme molecule upon immobilization may occur due to (a) Chemical modification during the coupling reaction (b) modification of the amino acid residues essential for the catalytic activities and multipoint attachment of the enzyme on to the support. Changes in the microenvironment of the enzyme upon immobilization may occur

due to (i) nature of the support material[13] (e.g. degree of hydrophobicity, electrostatic interactions etc) (ii) accumulation of charged products as a result of certain enzyme reactions[14] and (iii) diffusional limitations due to the formation of an unstirred layer around the immobilized enzyme particle[15-16].