

# CHAPTER ONE

## INTRODUCTION

### 1.1 General

Enzymes are the catalysts of living organisms. Virtually all the chemical reactions occurring in plants, micro-organisms and animals proceed at a measurable rate as a direct consequence of enzymic catalysis.

Biological catalysis has been known for nearly 150 years. In 1837, Beozelius recognized that there were naturally occurring 'ferments' which promoted chemical reactions and which fulfilled the criteria of catalysis he had proposed a few years earlier. The first significant advances were made more than a century ago by W. Kiihne through his investigations into the nature of trypsin catalysed reactions. He was also responsible for introducing the 'enzyme', translated from classical Greek 'in yeast', to describe the naturally occurring catalysts present in the absence of intact cell ferments. The basic conceptual and molecular basis of enzymology had been laid down in the 1960's with the resolution of the three-dimensional structure of enzymes.

More recently, attention has increasingly been directed to the application of enzymes. Their high efficiencies make them potentially valuable as catalysts in the industries ( Palmieri *et al.*, 1994 ). In most cases crude enzymes ( bulk enzymes ) are preferred, on economic grounds, to the purer preparations of higher specific activity. But to some

extent the application of the latter has been expanded and the handling of enzymes is made easier by immobilization.

Immobilized enzymes are defined as enzymes physically or chemically confined or localised to insoluble supporting material ( carriers ) in a certain defined region of space with retention of their catalytic activities ( Mosbach, 1987 ). In general, they are stable and easy to handle compared to their free counterparts. One of their most important feature is that they can be used repeatedly in a long-term series of batch - wise reactions or continuously in flow systems.

The molecular structure of enzymes that is essential for their catalytic activity can be destroyed under conditions such as high temperature, high or low pH and presence of organic solvents. Immobilization is one way of eliminating some of these disadvantages inherent to enzymes. The success of any immobilization relies on the proper choice of the carrier. Some of them are developed specifically for a special type of immobilization technique ( carrageenan or alginate for entrapping ) while others are universal and may be used in all methods ( agarose and polyacrylamide copolymers ). A suitable combination of supporting material and immobilization for an enzyme is necessary. No systematic concept is available at present for design of the most appropriate method of immobilization for various biocatalysts. Optimization is carried out by trial and error.

## 1.2 Objectives

In the present investigation laccase was chosen as the representative enzyme for its immobilisation in copper alginate by entrapment.

The specific aims were :

- ( a ) to develop optimum conditions for entrapping the enzyme in copper alginate and to optimize parameters involved in immobilization.
- ( b ) to compare the various properties of soluble and immobilized enzymes such as optimum pH for activity, optimum temperature for activity, thermal stability profile and storage stability.