



Abstract

Introduction: An important feature of proteins, especially enzymes, is their stability. **Structural stability** is directly related to the quantity and quality of the **molecules' performance**. when the enzymes are in their natural tissue, they have a specific stability that determines their half-life but when extracted from natural tissues, their stability has changed and often declines. Therefore, one of the challenges that is always faced by bio-scientists is to increase the stability of these macromolecules when working with them in vitro or when immobilizing them on unnatural substrates. In this article, we attempt to present studies about the factors that influence the stability of enzymes and novel methods for their stabilization after **immobilization**.

Methods: UV-vis, fluorescence, circular dichroism (CD) and FT-IR Spectroscopy. Transmission Electron Microscope, gel electrophoresis, solubility and osmometry, Molecular Dynamics. The enzymatic kinetics with the Michaelis-Menten or Lineweaver-Burk equations have also been investigated.

Results and discussion: If extraction or immobilization decreases the stability and activity of the enzyme, some conditions or compounds are designed to improve this stability. For example, by converting van der Waals bonds between the enzyme and its substrate into hydrophobic and hydrogen bonds, the enzyme stability and function can be improved in some cases. Occasionally, due to stabilization, the **thermodynamic parameters** of the stabilized enzyme increase relative to the free enzyme or the **kinetic parameters** such as K_m and V_{max} change that depending on the type of immobilization, the type of enzyme, and the performance we expect from it, these changes can be useful or useless.

Conclusion: By performing structural studies on enzymes and predicting their **orientation** by computational methods, prior to the laboratory test, conditions can be designed for the enzymatic test and stabilization process that not only decrease their efficiency but also **increase the activity** of the enzyme by stabilizing it.

Introduction

Proteins have a **unique 3D structure** that is created by **electrostatic interactions** between side chain of amino acids (Fig. 1). These bonds create a **configuration** for the protein through which it can be **active** and **functional**. Proteins in nature are located in a variety of **electrolyte environments** that, due to their **polarity**, stabilize the structure of proteins. In fact, these electrolytic environments dictate the structure of the proteins (See Fig. 2 for example).

The cause of some **diseases** is the **degradation** or **denaturation** of the body's vital proteins structure. In addition, for using proteins in industry, especially enzymes that are very useful, we have to **extract** them from natural tissues. Protein conformation is so **sensitive** and extraction can changes its structure. Therefore, one of the challenges that biologists have always faced is to stabilize the protein structure.

Electrostatic interactions:

Intramolecular protein-solvent

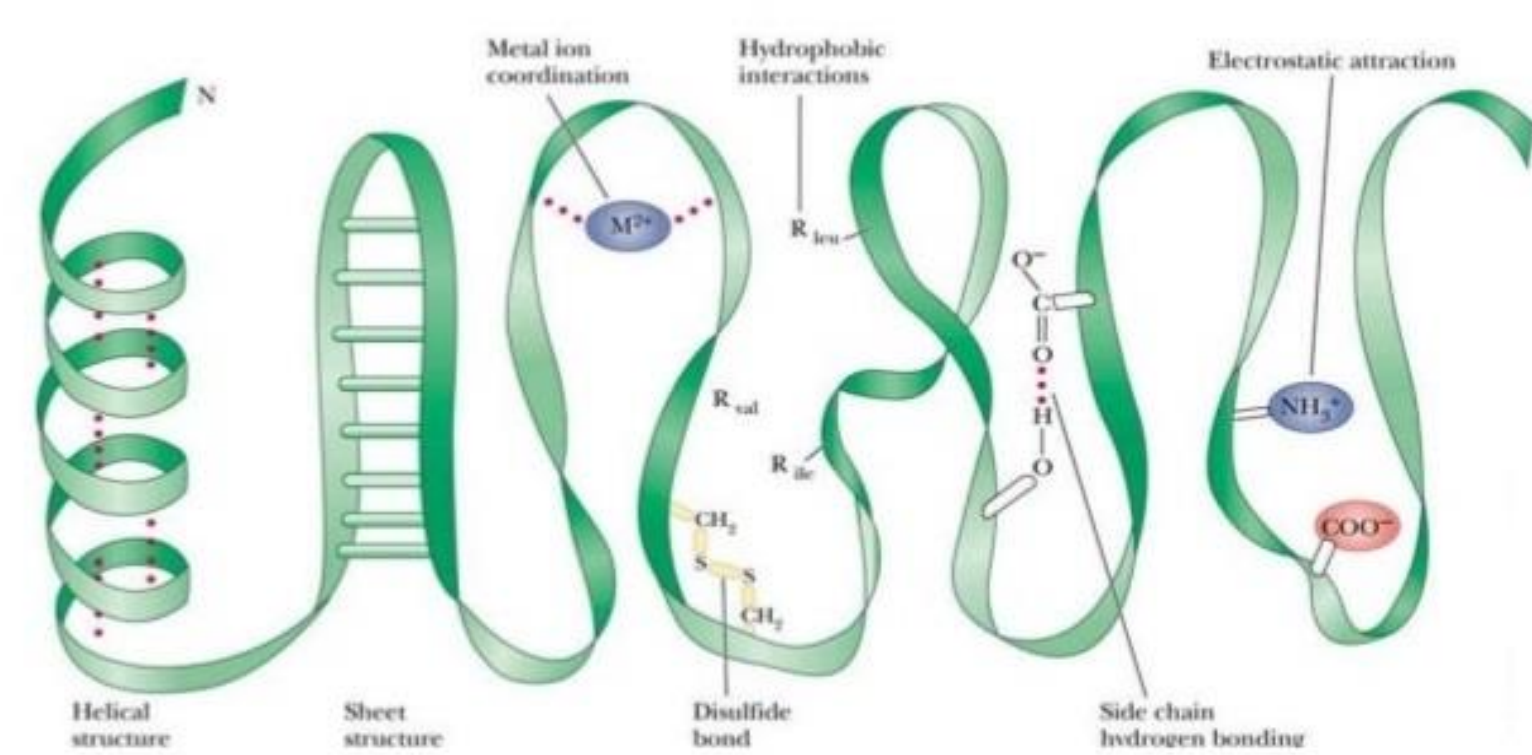


Figure 1: Protein structure is stabilized by electrostatic interactions

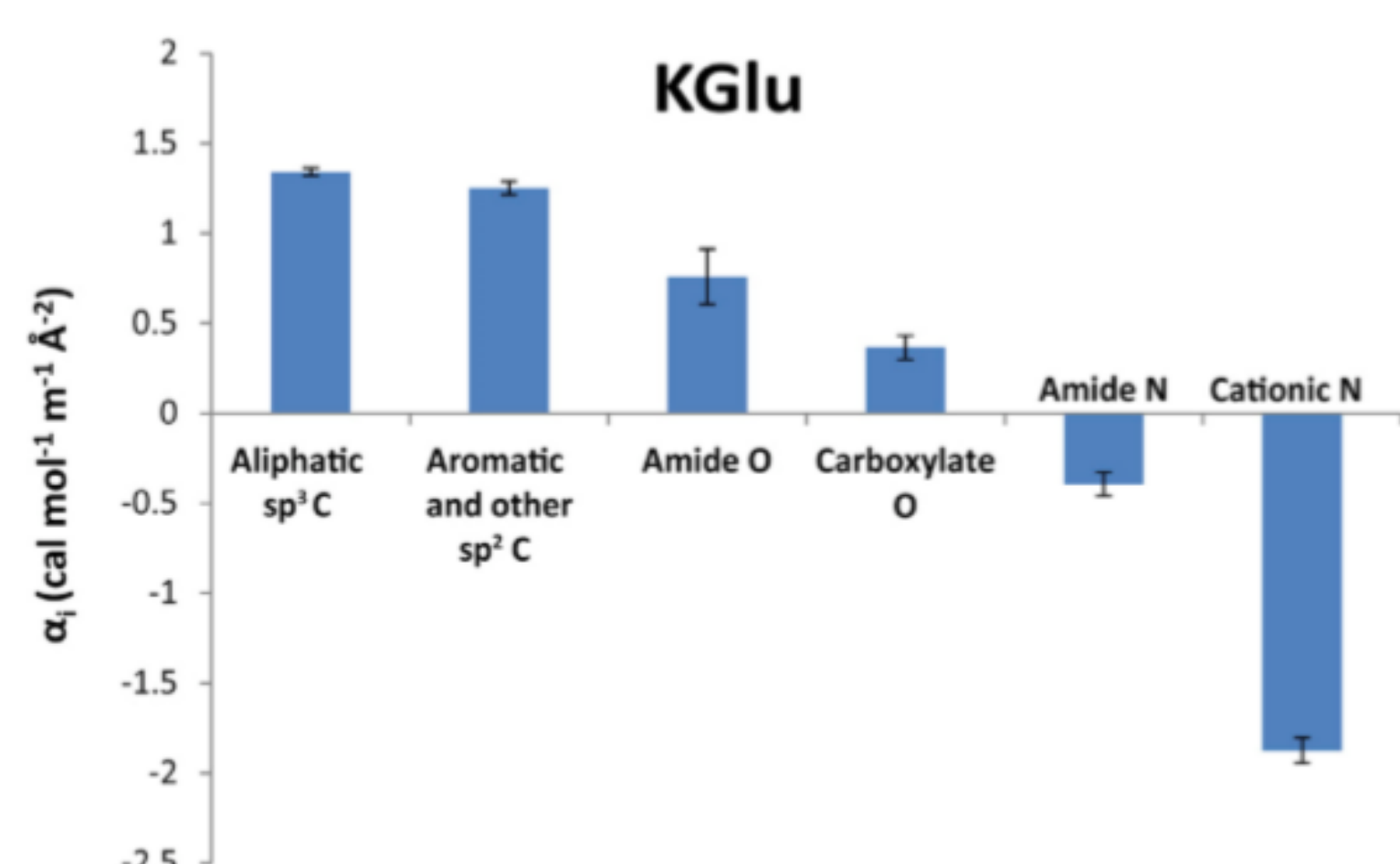


Figure 2: Interaction potentials (a-values) quantifying interactions of KGLu with functional group of proteins at 23–25° C. Unfavorable interactions have positive a-values. Favorable interactions disrupt protein structure. Interaction of KGLu with Hydrophobic Groups is unfavorable.

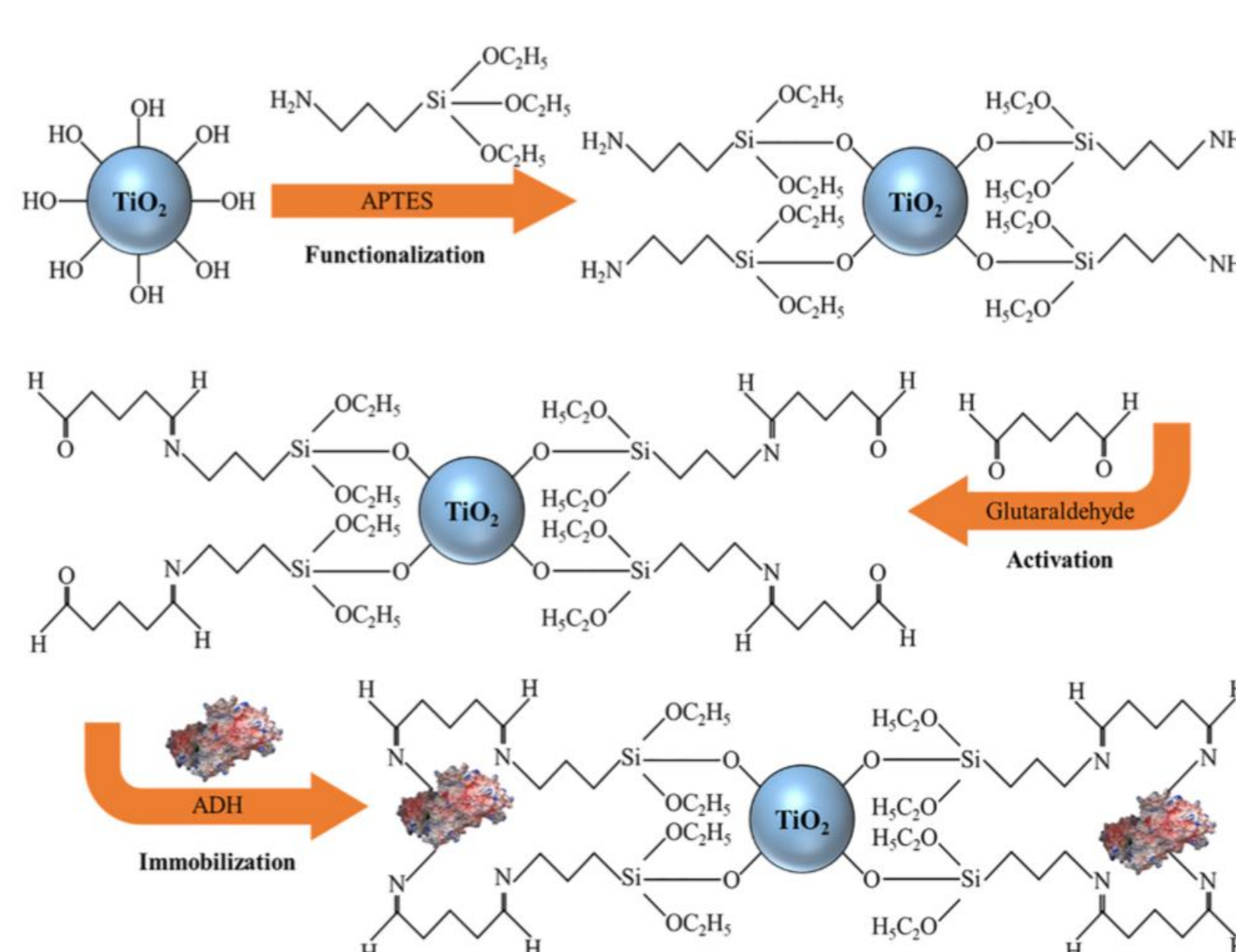


Figure 3: Chemical immobilization of Alcohol Dehydrogenase (ADH) on Titania nanoparticles To enhance enzyme stability

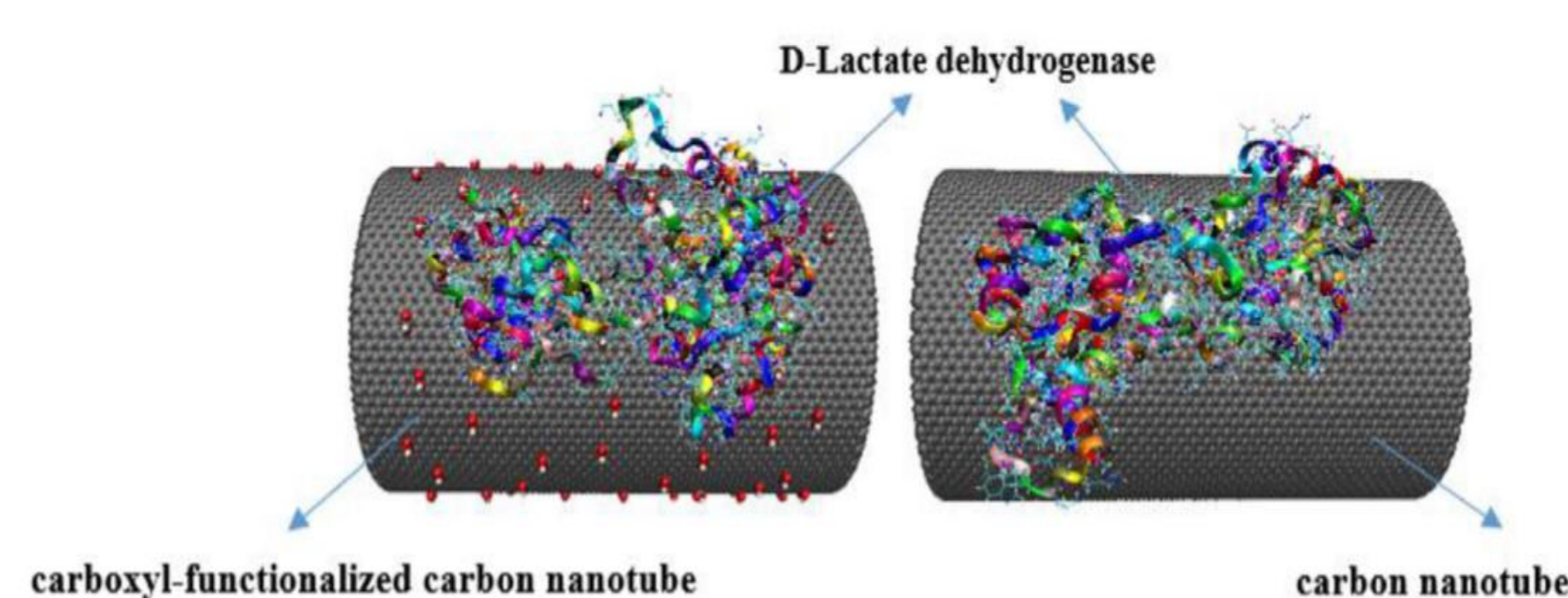


Figure 4: D-LDH enzyme immobilization on two kind of Carbon Nano Tubes (CNT); simple CNT and carboxyl-functionalized CNT (fCNT). D-LDH is better stabilized on fCNT with hydrogen bonding (MD simulation).

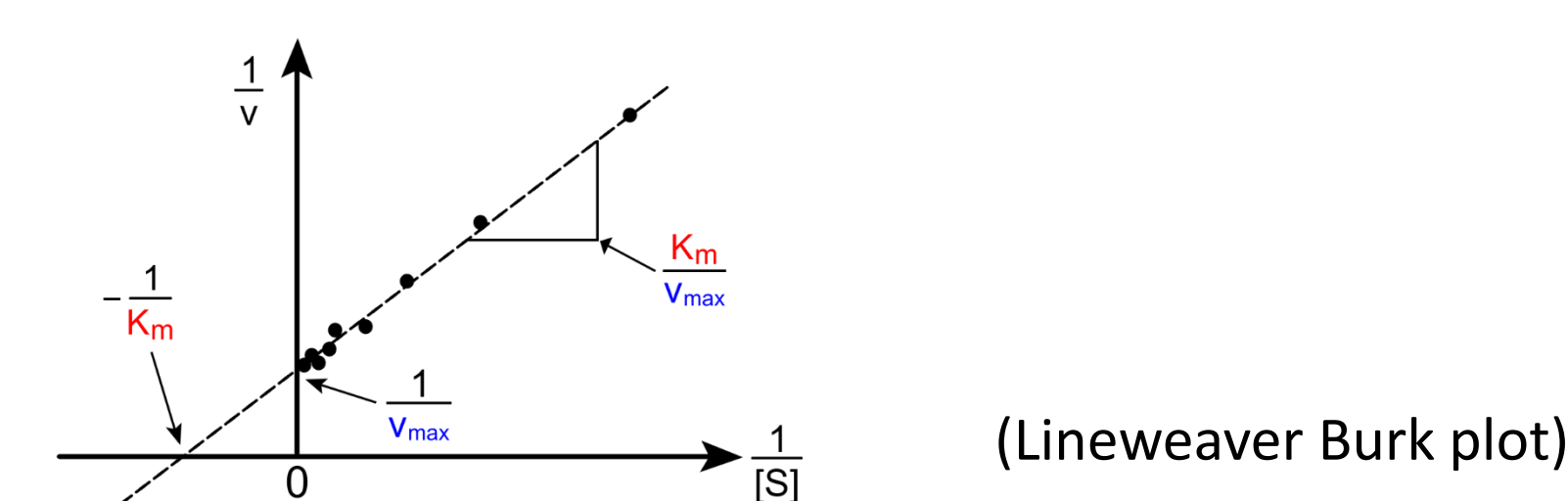
Methods

Nowadays, various solutions and methods have been developed to stabilize proteins. From stabilizing membrane proteins using **detergents** to immobilizing enzymes on biocompatible solids. **Immobilization** is really one of the most attractive ways to stabilize enzymes for industrial use. In Fig. 3 you can see an example of enzyme immobilization processes and Fig. 4 shows a visual MD simulation result related to this issue.

To investigate the quality of protein stabilization, it is necessary to check its **kinetic** and **thermodynamic** parameters before and after immobilization.

Kinetic parameters obtain by **Lineweaver Burk plot** most of the time;

$$\frac{1}{V_o} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{[S]} \quad (\text{Lineweaver Burk equation})$$



Denaturing agents such as temperature, acidic or alkaline pH, chemicals, etc. are used to determine the stability of the enzyme in terms of thermodynamics by receiving the **spectroscopic** spectrum of the enzyme during the **denaturation process** (like Fig. 5).

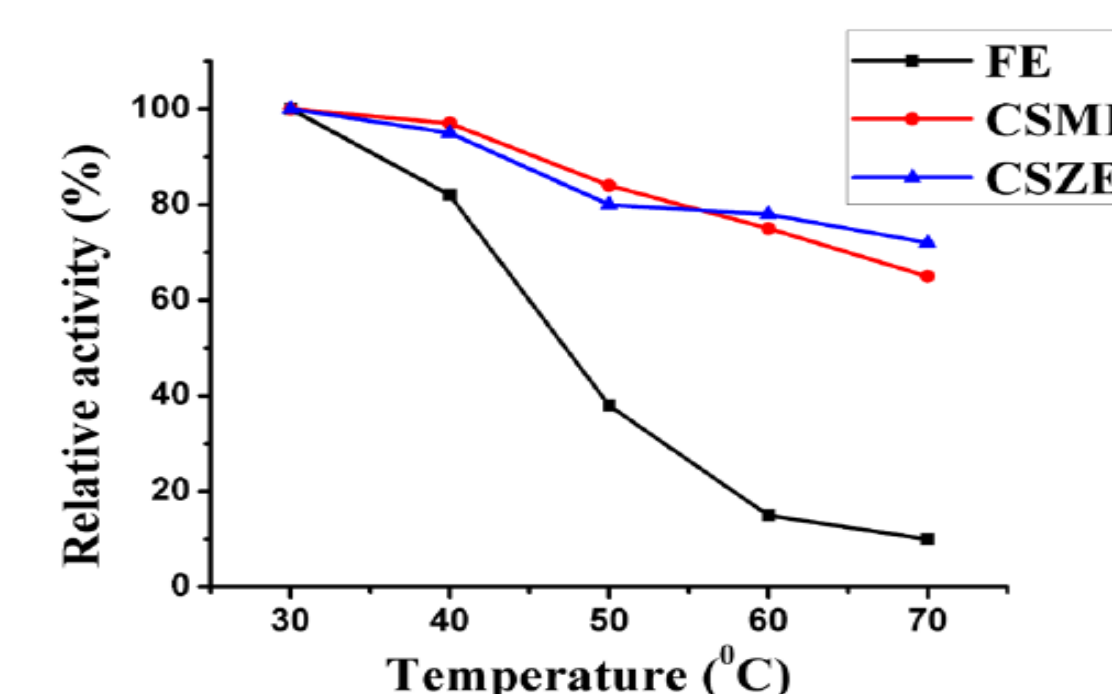


Figure 5: Scanning the temperature over the range of 30–70°C by pre-incubating the free (FE) and immobilized α -amylase on two kind of nanoparticles (CSME & CSZE) in buffer for 60 min followed by enzyme reaction at optimum temperature. Relative activities show that immobilized enzymes exhibited enhanced heat resistance.

Results & Discussion

Enzymes have become the most widely used biological macromolecules, and maintaining structural stability is the most important factor for their optimal performance.

Obviously, enzyme stabilization has many clinical and industrial applications. Recently, immobilized enzymes have been used in the production of a variety of biosensors; from blood sugar biosensors to water pollution detection biosensors. Accordingly, the design and production of functional enzyme nanocomposites is an interesting and useful topic to increase the stability and thus optimize the activity of extracted enzymes.

The scientific community hopes to be able to stabilize the body's vital proteins in future studies to prevent diseases caused by misfolding or protein degradation under stress.

References:

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