

# CHAPTER FIVE

## CONCLUSIONS

Urease find uses in industrial, agricultural and clinical application. These enzymes if used in the form of free enzymes can be used only once in any commercial process. This is uneconomical, as active enzyme is lost after each batch reaction. It is therefore necessary to immobilize these enzymes on some inexpensive solid support for continuous reuse, improved stability and economic advantage. Use of an inexpensive carrier matrix is therefore of great importance. Developing a cost effective and a simple coupling method is also desired.

Immobilized urease was chosen for study because the kinetics and thermochemistry of the free urease system have been well studied. The presented study shows that urease can be successfully immobilized on vermiculite . The urease-vermiculite system offers various advantages:

- 1) vermiculite is a relatively inexpensive material ;
- 2) the proposed immobilization procedure is simple to carry out ; and
- 3) the properties of the immobilized enzyme offer potential for use in waste treatment processes. The obtained material has a comparatively high enzymatic activity. The physico-chemical properties of urease were changed after immobilization, but whilst the kinetic parameters are slightly

less favourable than those of free urease, the excellent storage stability, thermal stability and reusability of the immobilized enzymes demonstrates the superior potential of the immobilized enzyme for practical application.

---

## APPENDIX A

### Preparation of standard curve for the estimation of urease activity

A pure ammonium sulphate solution (47.15 mg  $(\text{NH}_4)_2\text{SO}_4$  in 250 ml water) was prepared. Different aliquots of it (0.025 to 0.1 ml) are taken, and the volume was made up to 3 ml with water. To this, 1 ml of the Nessler's reagent was added and after mixing, the colour intensity was measured at 500 nm with spectrophotometer. The absorbance values of the standards against their corresponding ammonium concentration (mM) were plotted to prepare a standard curve (Table A).

**Table A: Calculation of ammonium concentration of standard curve with the corresponding observation values.**

<b>Ammonium sulphate solution (ml)</b>	<b>Concentration of Ammonium in the final solution (mM)</b>	<b>O.D. (at 500 nm)</b>
0.025	0.011895	0.048
0.05	0.02379	0.061
0.1	0.04758	0.170
0.2	0.09516	0.321
0.3	0.14274	0.462
0.4	0.19032	0.610
0.5	0.2379	0.749
0.6	0.28548	0.925
0.7	0.33306	1.071
0.8	0.38364	1.208
0.9	0.42822	1.361
1.0	0.4758	1.54

### **Protein estimation**

Protein determination of soluble urea was performed using Sigma assay kit. The procedure performed was based on Peterson's modification of the Micro-Lowry Method (1951).

---

Protein estimation of the soluble urease is necessary, to estimate the amount of protein recovered in the combined washings of the support urease complex from the protein used for immobilization. The protein concentration of the protein used for immobilization and those recovered in the washings are determined from the standard curve drawn with protein standard solution using the Sigma protein assay kit comprising of Lowry Reagent, protein standards and Folin & Ciocalteu's phenol reagent.

An alkaline cupric tartrate reagent complexes with the peptide bonds of the protein (urease) and forms a purple-color when the phenol reagent is added. The amount of protein in the samples urease measured colorimetrically, using a spectrophotometer.

#### **Preparation of a standard curve**

Standard Tubes were prepared by diluting protein standard solution in water to a volume of 0.1 ml in labeled test tubes. Appropriate dilutions were made to construct a standard curve.

**Table B: Preparation of protein standards**

<b>Protein standard solution (ml)</b>	<b>Water (ml)</b>	<b>Protein concentration (<math>\mu\text{g/ml}</math>)</b>
0.125	0.875	50
0.250	0.750	100
0.500	0.500	200
0.750	0.250	300
1.000	0.000	400

Blank was prepared with 1.0 ml water added to a test tube. 1.0 ml of Lowry reagent solution was added to Standard and Blank tubes and mixed well. The solution were allowed to stand at room temperature for 20 minutes. Then with rapid mixing, 0.5 ml Folin Ciocalteu's Phenol Reagent Working Solution was added to each tube and allowed colour to develop for 30 minutes. Finally, the solution were transferred to 3 ml cuvettes and absorbance of the Standards against the Blank at wavelength 750 nm were measured. The absorbance values of the Standards against their corresponding protein concentration were plotted resulting in a standard curve.

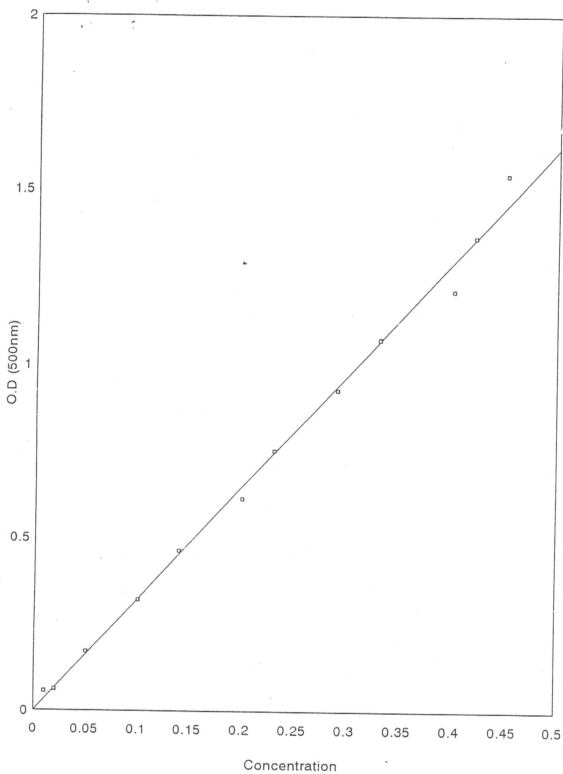


Figure A: Calibration curve of absorbance value for ammonium standard versus their concentration

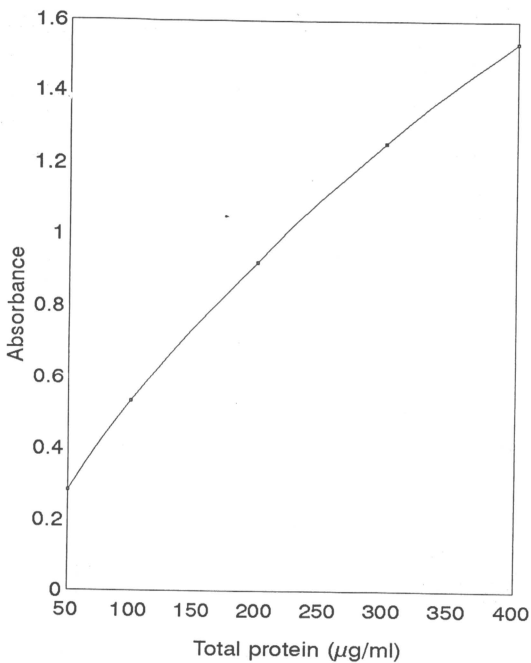


Figure B: Calibration curve of absorbance values for protein standard (p7656) solution versus their protein concentration using Sigma protein assay kit no. p5656