

## **APPENDIX A**

### **British Drug House Ltd. Poole, England.**

Sodium hydrogen phosphate heptahydrate

Sodium dihydrogen phosphate monohydrate

(Tris [hydroxymethyl] amino methane)

### **Pharmacia Fine Chemicals, Uppsala, Sweden.**

DEAE Sephadex A-50

Bio-gel Sephadex G-100

### **Merck**

Absolute Ethanol

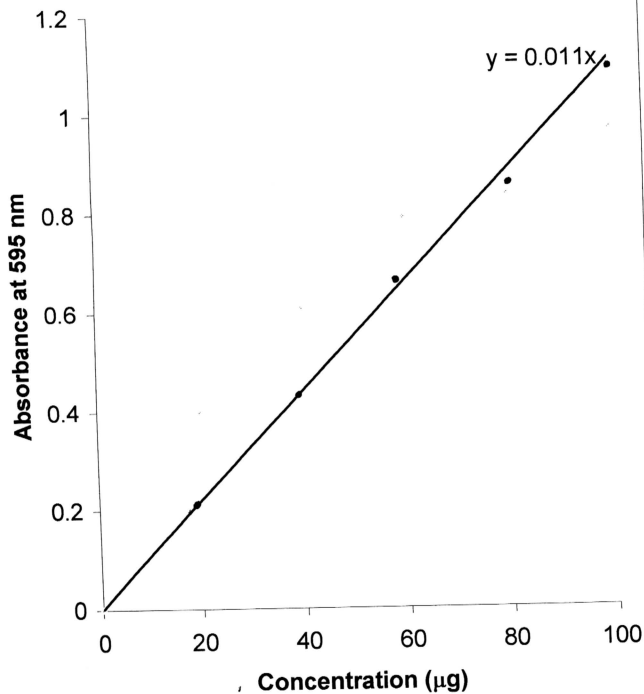
### **Sigma**

Syringaldazine (4-Hydroxy-3,5 - dimethoxybenzaldehyde azine)

### **Preparation of Buffers**

1. 0.1M sodium citrate buffer pH 5.5 : 0.1M Disodium hydrogen citrate solution adjusted to pH 5.5 with 1M NaOH.
2. 0.1M Tris-HCl buffer pH 7.0 : 0.1M (Tris[hydroxymethyl]amine methane) adjusted to pH 7.0 with 1M HCl.
3. 0.1M Sodium dihydrogen phosphate monohydrate adjusted to pH 6.8

Figure 4.0: Standard Curve for Protein Estimation by Protein-dye Binding Method of Bradford (1976)



## APPENDIX C

Raw mean data for thermal stability studies

Temperature ( °C )	Free Enzyme ( Abs/min )	Immo. Enzyme ( Abs/min )
4	0.18	0.18
20	0.18	0.18
30	0.18	0.18
40	0.09	0.10
50	0.03	0.04
60	0.018	0.027

Calculation to enzyme units :  $\frac{(\text{Abs/min}) \times 150 \text{ beads} \times 25 \text{ times dilution factor}}{10 \text{ beads}}$

Example :  $\frac{0.18 \times 150 \times 25}{10} = 67.5 \text{ U} \approx 70 \text{ U}$

Raw mean data for storage stability studies at 4°C

Days	Free Enzyme ( Abs/min )	Immo. Enzyme ( Abs/min )
0	0.18	0.18
2	0.11	0.13
4	0.09	0.108
6	0.07	0.08
8	0.04	0.05
10	0.02	0.04
12	0	0.018
14	0	0

Raw mean data for storage stability studies at 25°C

Time (Days)	Free enzyme ( Abs/min )	Imm. Enzyme ( Abs/min )
0	0.18	0.18
2	0.09	0.1
4	0.04	0.05
6	0.018	0.03
8	0.009	0.010
10	0.003	0.005
12	0.001	0.001
14	0	0

Raw mean data for optimum concentration of enzyme for immobilization

Amount of enzyme ( $\mu$ l )	Abs/min
50	0.04
100	0.18
150	0.31
200	0.36
250	0.72
300	0.90
400	0.85
500	0.81
600	0.78