APPENDIX A

Reducing sugar determination by DNS method

Chemical reagents

Distilled water	1 l
DNS (3,5-dinitro salicylic acid)	10 g
Na_2SO_3	0.5 g
NaOH	16 g
Na-K tatrate	300 g
Phenol	2.0 g

DNS solution preparation:

1. Dissolve NaOH in 250 ml of distilled water.
2. Add DNS and stir continuously.
3. Add Na-K tartrate, stir until well dissolve.
4. Add Na_2SO_3 and phenol, respectly.
5. Adjust to final volume of 1 l with volume metric flask.
6. Keep DNS solution in brown glass bottle.

Reducing sugar determination procedure

1. Mix 1 ml of sample with 1 ml of DNS solution and boil for 10 min.
2. Cool down the sample by immerse the sample tube into cold water immediately, add 5 ml of distilled water, mix well, and measure A_{540}
3. Convert A_{540} to reducing sugar concentration with standard curve

Standard curve preparation

Xylose standard solution preparation

1. Dissolve 0.100 g of xylose in 50 mM phosphate buffer pH 7.0.
2. Adjust to final volume of 100 ml with the same buffer in volume metric flask.
3. Prepare xylose in various concentrations by using table A1.

Table A1 Xylose Standard Solution Preparation

Xylose concentration (μg/ml)	Xylose solution (μl)	50 mM phosphate buffer pH 7.0 (μl)
1000	1000	0
900	900	100
800	800	200
700	700	300
600	600	400
500	500	500
400	400	600
300	300	700
200	200	800
100	100	900
0	0	1000
Total volume	1000 μl	

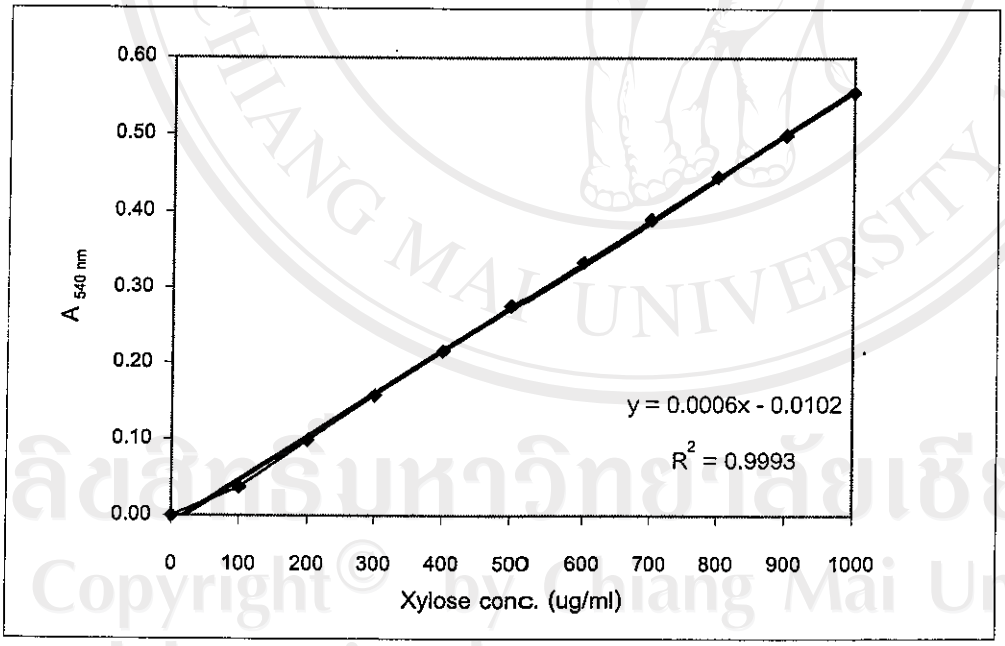

Figure. A1 Standard Curve of Xylose

APPENDIX B

Protein determination by Bradford nethod

Chemical reagents

Bradford Stock Solution

95% ethanol	100 ml.
88% Phosphoric acid	200 ml.
Coomassie Brilliant Blue G-250	350 mg.

Bradford Working Buffer

Distilled water	425 ml.
95% ethanol	15 ml.
88% Phosphoric acid	30 ml.
Bradford Stock Solution	30 ml.

Bradford stock solution preparation

1. Mix 95% ethanol with 88% Phosphoric acid.
2. Add Coomassie Brilliant Blue G-250, stir until well dissolve.
3. Keep Bradford stock solution in brown glass bottle.

Bradford Working Buffer preparation

1. Mix 95% ethanol with 88% Phosphoric acid and distilled water.
2. Add Bradford stock solution and stir continuously.
3. Filter through Whatman No.1 paper , store at room temperature in brown glass bottle. Usable for several weeks, but may need to be refiltered.

Protein determination

1. Pipet protein solution (maximun 100 μl) in to tube.
2. Add experiment buffer to make total volume of 100 μl.
3. Add 1 ml. Bradford Working Buffer and vortex.
4. Read A_{595} after 2 minutes but before 1 h.

Standard curve preparation

Protein standard solution preparation

1. Dissolve 0.100 g of bovine serum albumin fraction V (BSA) in 50 mM phosphate buffer pH 7.0
2. Adjusted to final volume of 100 ml with same buffer in volume metric flask.
3. Prepare BSA in various concentrations by using table B1
4. Determination of protein by the procedure mentioned above.

Table B1 Protein Standard Solution Preparation.

BSA concentration (μg/ml)	BSA solution (μl)	50 mM phosphate buffer pH 7.0 (μl)
300	300	700
175	175	825
150	150	850
125	125	875
100	100	900
75	75	925
50	50	950
25	25	975
0	0	1000
Total volume	1000 μl	

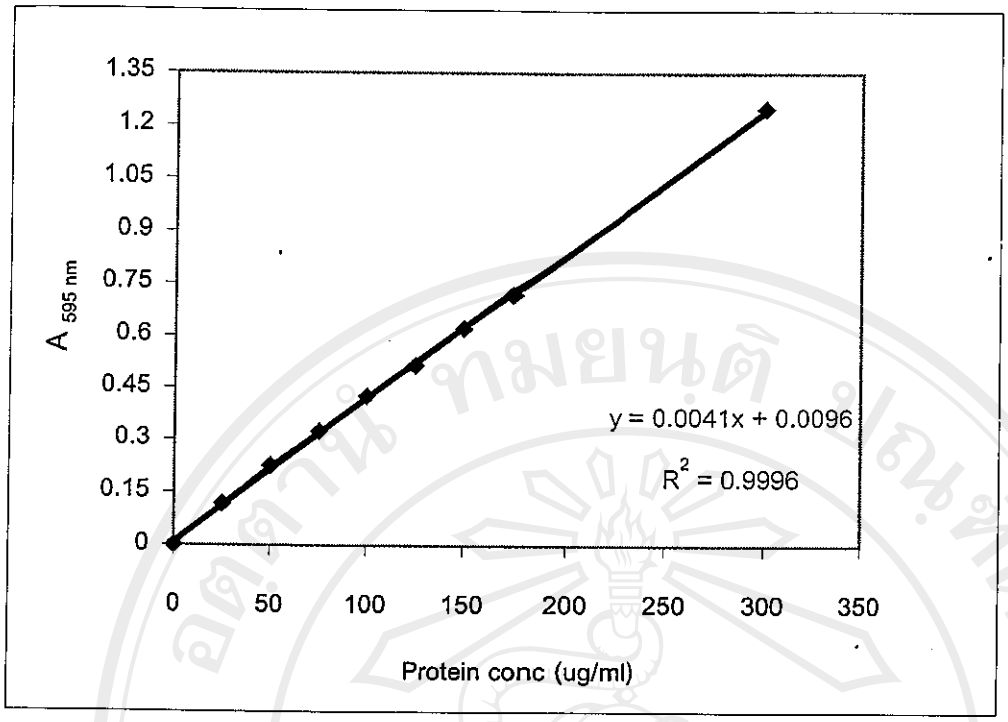

Figure. B1 Protein Standard Curve.

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