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## APPENDICES

## Appendix A: Preparation of reagent

### A. Bradford reagent

#### 1. Bradford stock solution

100 ml 95% ethanol

200 ml 88% phosphoric acid

350 mg Coomassie Blue G-250

stable indefinitely at room temperature

#### 2. Bradford working solution

425 ml distilled water

15 ml 95% ethanol

30 ml 88% phosphoric acid

30 ml Bradford stock solution

Filter through Whatman no.1 paper, store at room temperature in brown glass bottle. Usable for several weeks but may need to be refilled.

### B. Reagent and buffer for purification CGTase

#### 1. Reagent for assay CGTase activity

##### 1.1 0.2 M phosphate buffer pH 6.0

Dissolved 2.27 g of  $\text{KH}_2\text{PO}_4$  and 0.55 g  $\text{K}_2\text{HPO}_4$  in distilled water then adjusted pH to 6.0 with 1 M HCl and adjusted volume to 100 ml with distilled water.

##### 1.2 Iodine reagent

Dissolved 0.2 g  $\text{I}_2$  and 2 g of KI in 50 ml of distilled water by keeping in the dark, then adjusted volume to 100 ml, keep in the dark bottle. Before using, dilution with distilled water for 1:10 was performed.

##### 1.3 Soluble potato starch solution

Dissolved 0.2 g of soluble potato starch in 100 ml of 0.2 M phosphate buffer pH 6.0 for 0.2% starch solution and 2 g of soluble potato starch in 100 ml of 0.2 M

phosphate buffer pH 6.0 for 2% starch solution. After dissolved in buffer the starch solution mixture boil in hot plate for 5 minutes and then adjusted volume to 100 ml.

## 2. Buffer for partially purification step

### 2.1 10 mM Tris-HCl buffer with 10 mM $\text{CaCl}_2$ pH 8.5 (TB)

Dissolved 1.21 g of Tris (hydroxymethyl)-aminomethane and 1.47 g of  $\text{CaCl}_2$  then adjusted pH to 8.5 with HCl and adjusted volume to 1 litre.

## C Reagent for reducing sugar determination

### 1. Alkaline copper reagent

The composition of alkaline copper reagent

Potassium sodium tartrate	40	g/l
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	71	g/l
1 N NaOH	100	ml
Distilled water	700	ml
$\text{Na}_2\text{SO}_4$	180	g/l
10 % $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	80	ml

The 100 ml of NaOH and 40 g of Rochelle salt were dissolved then 80 ml of 10 %  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  was added with stirring. The  $\text{Na}_2\text{SO}_4$  was dissolved in about 500 ml of hot water and boiled to expel air. After cooling, the two solutions were mixed and diluted to volume in 1,000 ml. The reagent was left at room temperature for 48 hours before used. The reagent should be stored in a glass-stoppered brown bottle.

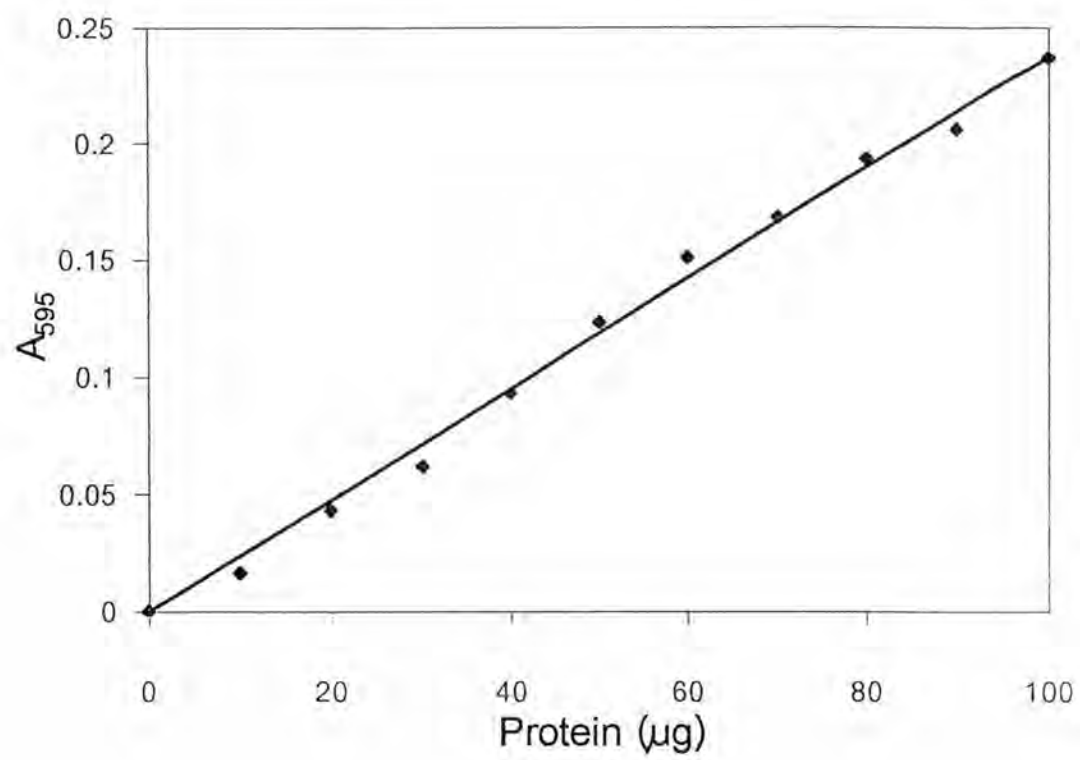
## 2. Nelson reagent

The composition of Nelson reagent

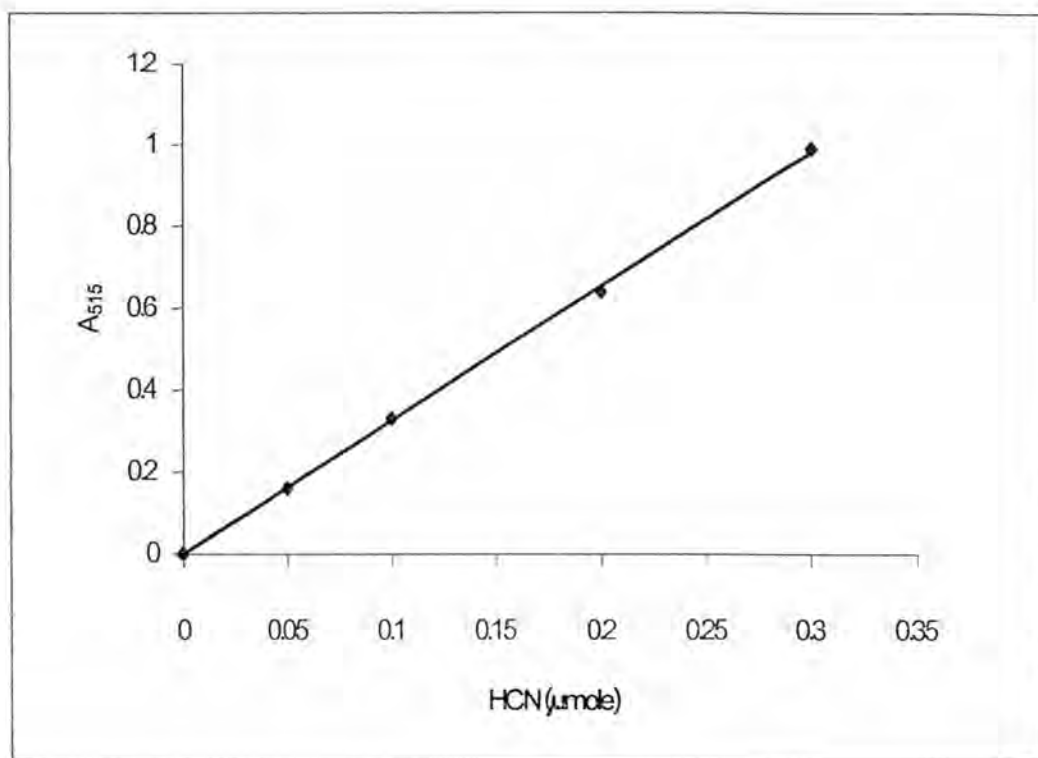
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	53.2	g/l
12% $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$	50	ml
Concentrated $\text{H}_2\text{SO}_4$	21	ml
Distilled water	900	ml

All of chemicals were mixed and stirred. After completely dissolved, the volume was adjusted to 1,000 ml. The reagent was left at room temperature for 48 hours before used. The reagent should be stored in a glass-stoppered brown bottle.

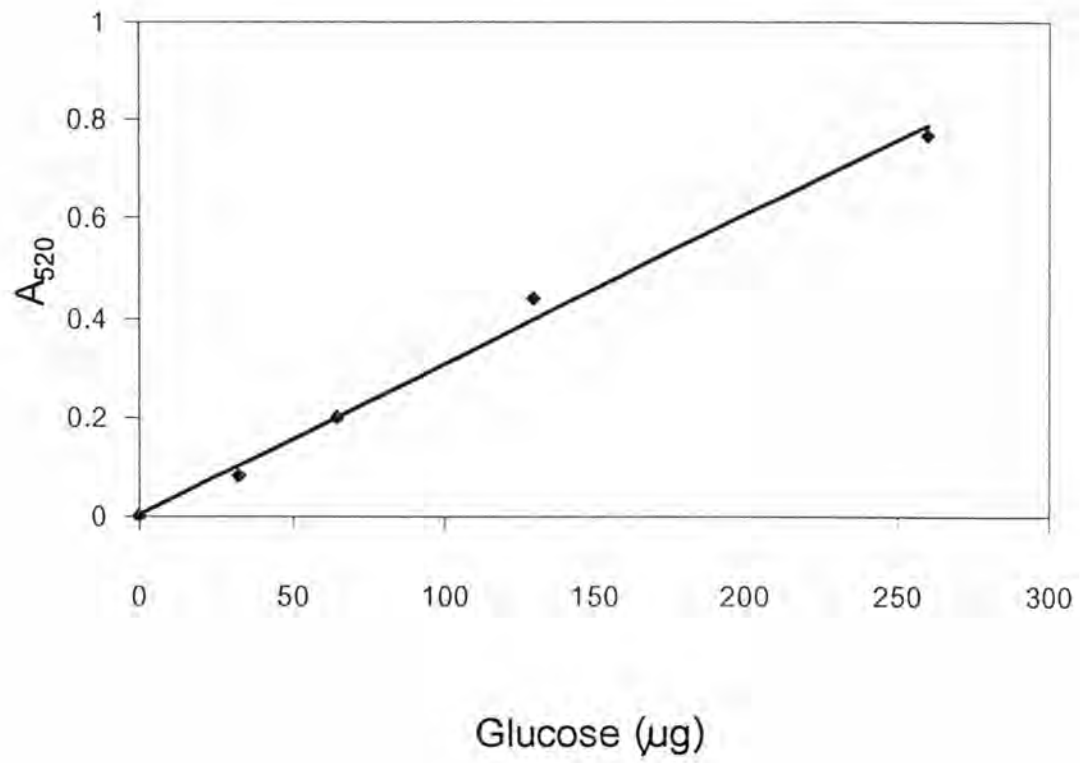
## Appendix B: Standard curve of protein determination by Bradford's method



## Appendix C: Standard curve of hydrogen cyanide

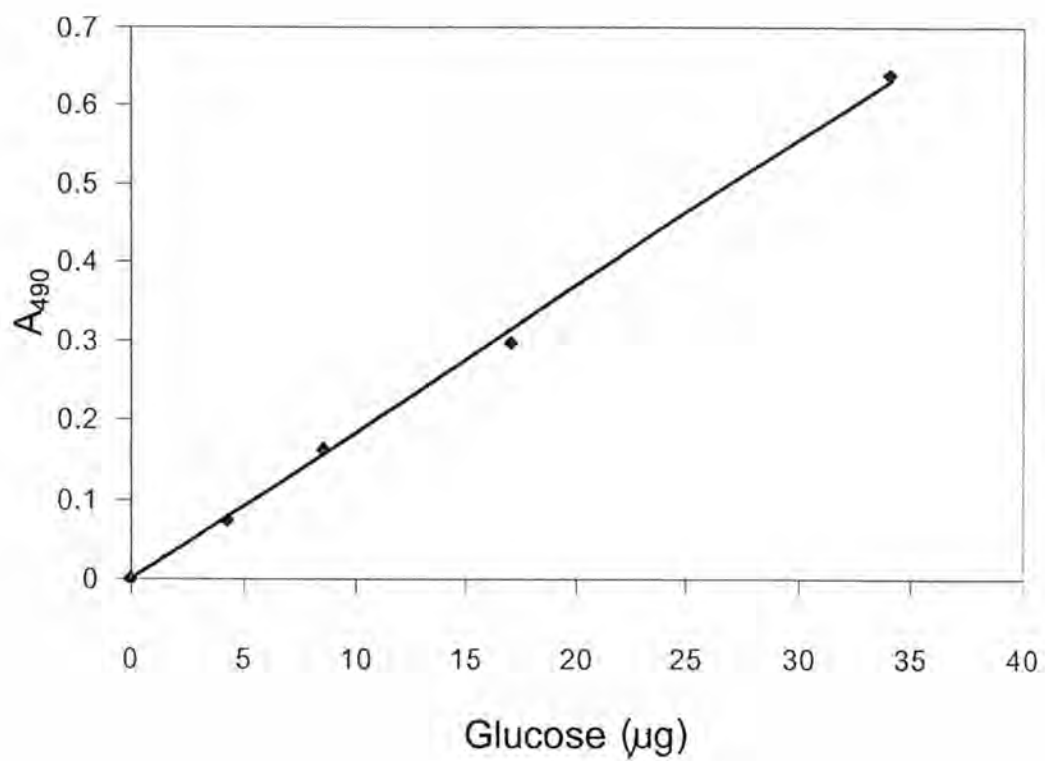


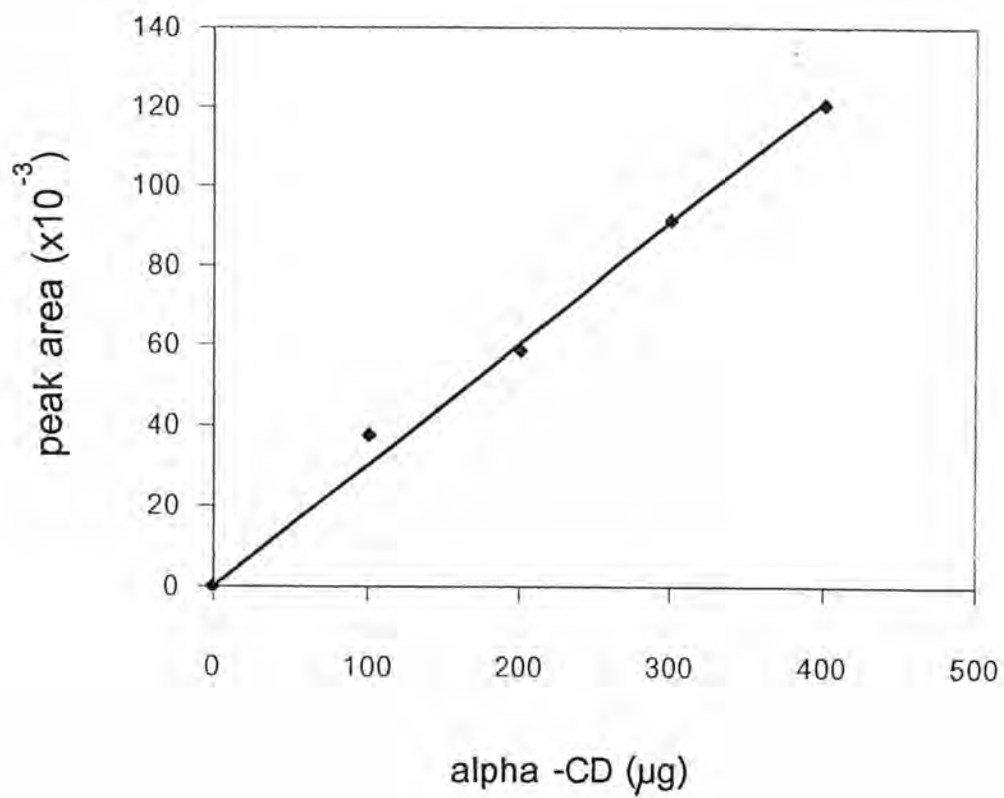
## Appendix D: Standard curve of reducing sugar by Somogyi-Nelson method

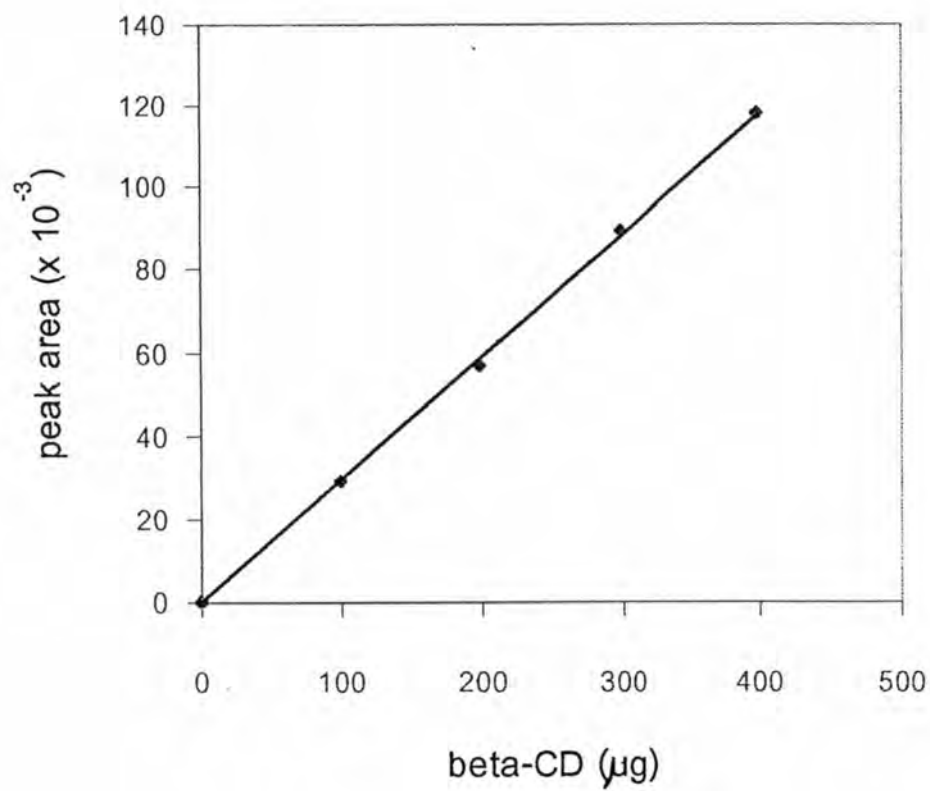


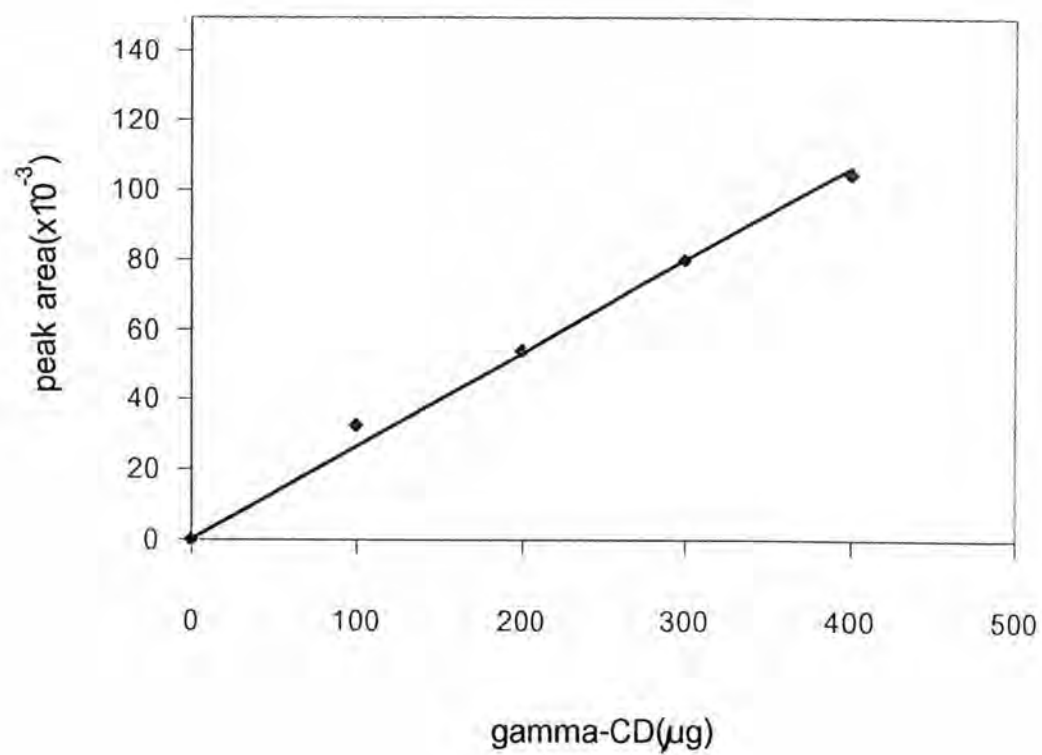


## Appendix E: Standard curve of glucose by phenol-sulfuric acid method



Appendix F: Standard curve of  $\alpha$ -CD by HPLC method

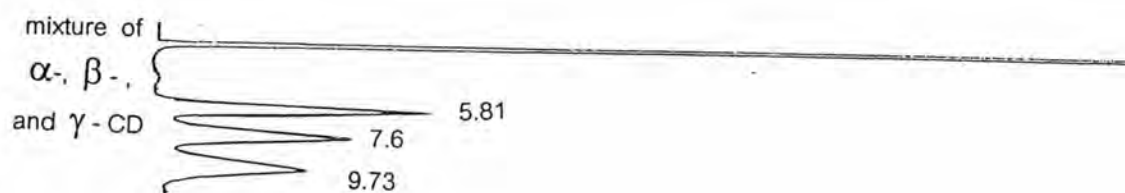
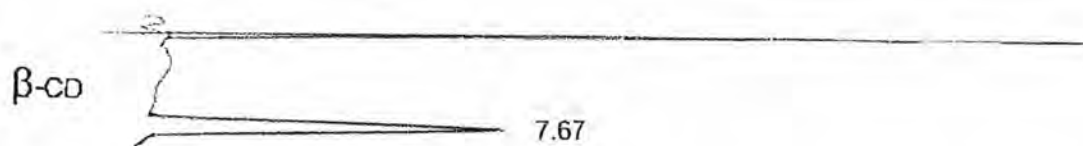
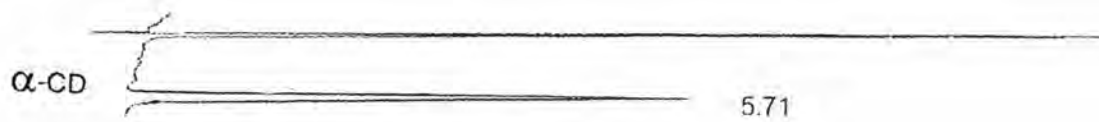
Appendix G: Standard curve of  $\beta$ -CD by HPLC method

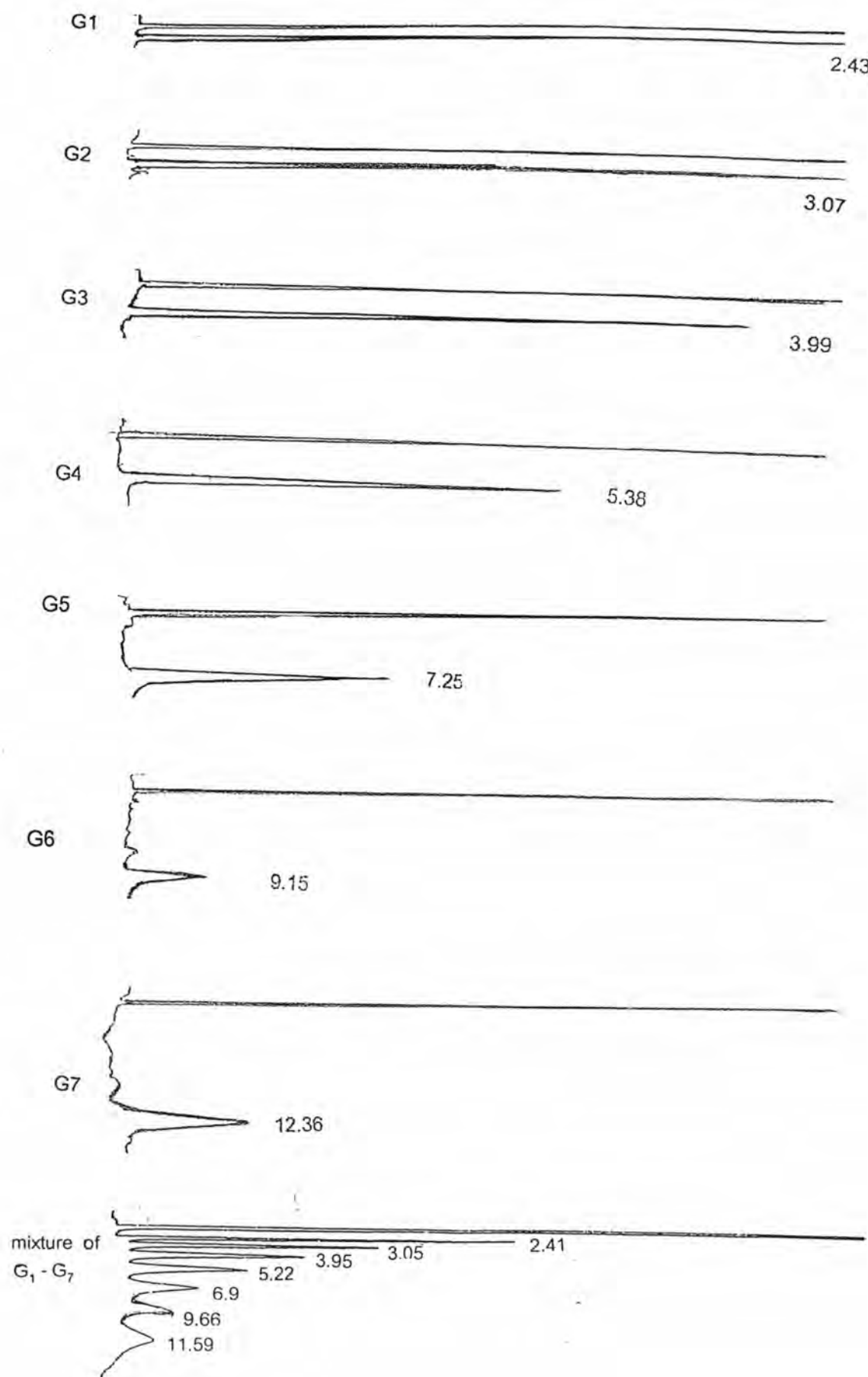
Appendix H: Standard curve of  $\gamma$ -CD by HPLC method

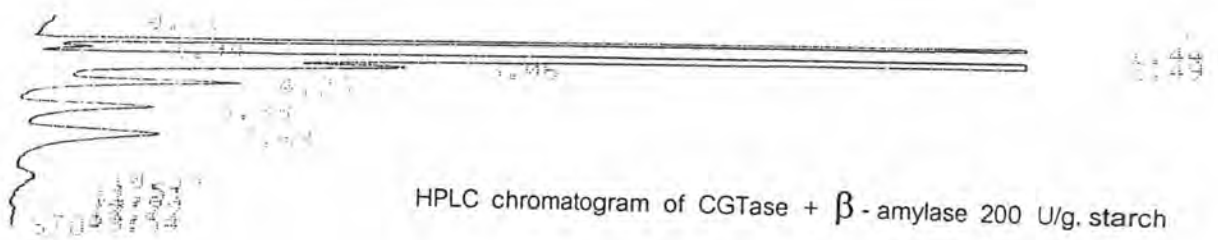
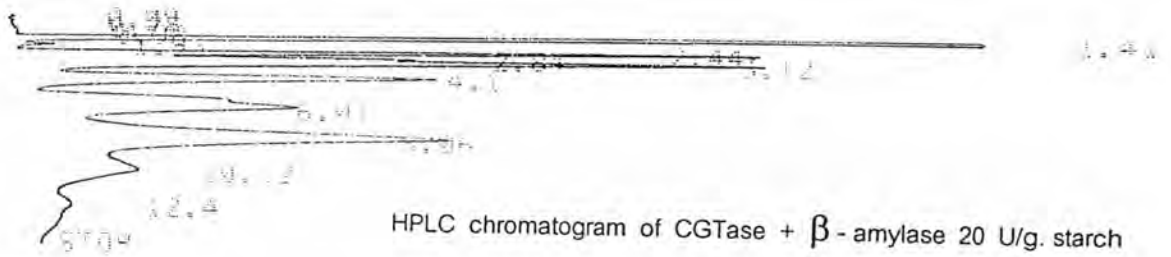
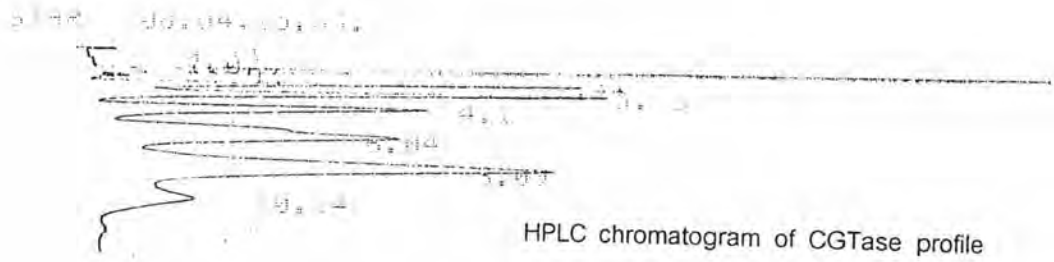
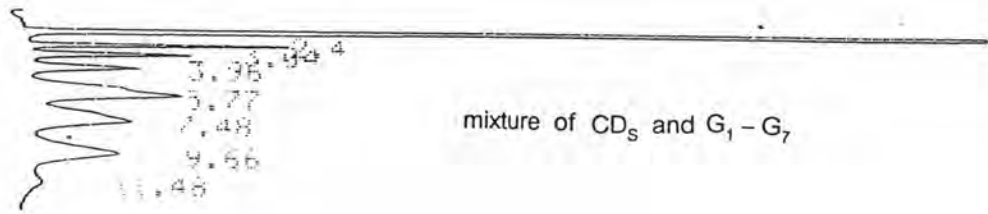
Appendix I: Retention time of standard CD<sub>s</sub> and linear oligosaccharides on HPLC Spherisorb-NH<sub>2</sub> column

Standard	Retention time (minute)
Glucose (G1)	2.43
Maltose (G2)	3.07
Maltotriose (G3)	3.99
Maltotetraose (G4)	5.38
Maltopentaose (G5)	7.25
Maltohexaose (G6)	9.15
Maltoheptaose (G7)	12.36
$\alpha$ -CD	5.77
$\beta$ -CD	7.61
$\gamma$ -CD	10.08

## Appendix J: HPLC chromatograms









## Appendix K: Description of enzyme

### 1. $\alpha$ -Amylase (BAN 240 L)

BAN –Bacterial Amylase Novo- is an alpha-amylase produced by submerged fermentation of a select strain of *Bacillus amyloliquefaciens*. The systematic name is 1,4- $\alpha$ -D glucano-hydrolase (EC 3.2.1.1).

BAN is an endo-amylase. It hydrolyzes 1,4- $\alpha$ -D glucosidic linkages in amylose and amylopectin at random, which results in a rapid reduction of the viscosity and of gelatinized starch. The breakdown products are dextrans of differing chain lengths, and oligosaccharides.

Specification: Liquid BAN 240 L                      240 KNU/g

### 2. Promozyme<sup>®</sup>

Promozyme is a heat-stable debranching enzyme obtained from selected strain of *Bacillus acidopullulyticus*. It belongs to the group of debranching enzymes known as pullulanases (EC 3.2.1.41, pullulan 6-glucano-hydrolase).

Promozyme catalyzes the hydrolysis of the 1,6-alpha linkages in amylopectin which has been partially hydrolyzed by alpha-amylase and in pullulan, provided that there are at least two glucose units in the side chain. Promozyme is therefore well suited for debranching starch after liquefaction.

Specification: Liquid Promozyme 400 L                      400 PUN/ml

#### Activity determination

One Pullulanase Unit Novo (PUN) is defined as the amount of enzyme which hydrolyzes pullulan, liberating reducing carbohydrate with a reducing power equivalent to 1 micromole glucose per minute under the following standard condition:

Substrate:	0.2%pullulan
Temperature:	40°C
pH:	5.0
Reaction:	30 minutes



### Appendix L : Viscosity of starch/flour

Viscosity of 5 g% of starch/ flour gel in distilled water was determined by Brookfield viscometer.

Kind of starch/flour	Viscosity (cp)
Potato starch	38,600
Amylopectin starch (com)	18,540
Sago starch	9,320
Thai glutinous rice	3,332
Cassava starch	1,920
Arrow root flour	532.2
Com flour	163.2
Rye flour	117.6
Wheat flour	96.8
Rice (short grain) flour	70.4
Rice (long grain) flour	52.6
Soluble starch	18.8
Amylose starch	10.5

## BIOGRAPHY

Mr. Somponk Nilmanee was born on April 24, 1972. He graduated with the Bachelor Degree of Agro Industry from Prince of Songkhla University in 1995 and then entered the graduate program for M.Sc. in Biotechnology at Chulalongkorn University in 1997.