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APPENDICES

APPENDIX A : Preparation for polyacrylamide gel electrophoresis**1. Stock reagents****30% Acrylamide, 0.8% bis-acrylamide, 100ml**

acrylamide 29.2 g

N,N'-methylene-bis-acrylamide 0.8 g

Adjusted volume to 100 ml with distilled water

1.5 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane 18.17 g

Adjusted pH to 8.8 with 1M HCl and adjusted volume to 100 ml with distilled water

2 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane 24.2 g

Adjusted pH to 8.8 with 1M HCl and adjusted volume to 100 ml with distilled water

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane 6.06 g

Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

1 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane 12.1 g

Adjusted pH to 6.8 with 1M HCl and adjusted volume to 100 ml with distilled water

Solution B (SDS PAGE)

2 M Tris-HCl pH 8.8	75 ml
10% SDS	4 ml
distilled water	21 ml

Solution C (SDS PAGE)

1 M Tris-HCl pH 6.8	50 ml
10% SDS	4 ml
distilled water	46 ml

2. Non-denaturing PAGE**7.0% Separating gel**

30% acrylamide solution	2.33 ml
1.5 M Tris-HCl pH 8.8	2.50 ml
distilled water	5.15 ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50 μl
TEMED	5 μl

For preparative gel, 25 μl of 10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$ and 2.5 μl of TEMED were added.

5.0% stacking gel

30% acrylamide solution	1.67 ml
0.5 M Tris-HCl pH 6.8	2.50 ml
distilled water	5.80 ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50 μl
TEMED	10 μl

Sample buffer**For analytical gel**

1 M Tris-HCl pH 6.8	3.1 ml
glycerol	5.0 ml
1% bromophenol blue	0.5 ml
distilled water	1.4 ml

For preparative gel

0.5 M Tris-HCl pH 6.8	1.0 ml
glycerol	0.8 ml
0.5% bromophenol blue	0.4 ml
distilled water	5.8 ml

One part of sample buffer was added to four parts of sample.

Electrophoresis buffer, 1 litre

(25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3.03 g
Glycine	14.40 g

Dissolved in distilled water to 1 litre. Do not adjust pH with acid or base

(final pH should be 8.3).

3. SDS-PAGE**7.5% separating gel**

30% acrylamide solution	2.5 ml
solution B	2.5 ml
distilled water	5.0 ml

10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50 μl
TEMED	10 μl

5.0% stacking gel

30% acrylamide solution	0.67 ml
solution C	1.0 ml
distilled water	2.3 ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	30 μl
TEMED	5 μl

Sample buffer

1 M Tris-HCl pH 6.8	0.6 ml
50% glycerol	5.0 ml
10% SDS	2.0 ml
2-mercaptoethanol	0.5 ml
1% bromophenol blue	1.0 ml
distilled water	0.9 ml

One part of sample buffer was added to four parts of sample. The mixture was heated 5 minutes in boiling water before loading to the gel.

Electrophoresis buffer, 1 litre

Tris (hydroxymethyl)-aminomethane	3.0 g
Glycine	14.4 g
SDS	1.0 g

Adjusted volume to 1 litre with distilled water (pH should be approximately 8.3).

APPENDIX B : Preparation for phenol-sulfuric acid (PAS) staining solution

Fixative solution

Ethanol	200 ml
Glacial acetic acid	20 ml
Adjust volume to 500 ml with distilled water	

Schiff 's reagent

1. Dissolve 10 g of basic fuchsin in 21 ml of distilled water with heating. Cool in ice-bath to 50°C (not below 40°C).

2. Add 200 ml of 1 N HCl. Mix, and cool to 25°C.

3. Add 17 g of sodium metabisulfite. Mix, and let sit overnight at 4°C in the dark.

4. Add 20 g of HCl washed charcoal, and centrifuge charcoal to avoid contact with filter paper. Filter the supernatant through glass wool to remove remaining charcoal, the filtrate should be clear and colorless.

5. Stored in a brown bottle at 4°C.

0.7% Periodic acid solution

Periodic acid	1.4 g
5% Acetic acid	200 ml

0.2% Sodium metabisulfite

Sodium metabisulfite	0.4 g
5% Acetic acid	200 ml

APPENDIX C : Preparation for isoelectric focusing gel electrophoresis**Monomer-ampholyte solution**

30% Acrylamide solution	0.9 ml
1.0% Bis-acrylamide solution	1.25 ml
Ampholyte pH 5-7	0.243 ml
Distilled water	1.39 ml
50% Sucrose	1.186 ml
TEMED	2 μ l
0.02 M $(\text{NH}_4)_2\text{S}_2\text{O}_8$	39.5 μ l

Fixative solution, 100 ml

Sulfosalicylic acid	4 ml
Trichloroacetic acid	10.5 g
Methanol	30 ml

Immerse gels in this solution for 30 minutes.

Staining solution, 100 ml

Ethanol	27 ml
Acetic acid	10 ml
Coomassie brilliant blue R-250	0.04 g
CuSO_4	0.5 g
Distilled water	63 ml

Dissolve the CuSO_4 in water before adding the alcohol. Either dissolve the dye in alcohol or add it to the solution at the end.

Immerse the gel in the stain for approximately 1-2 hours.

Destaining solution**First destaining solution**

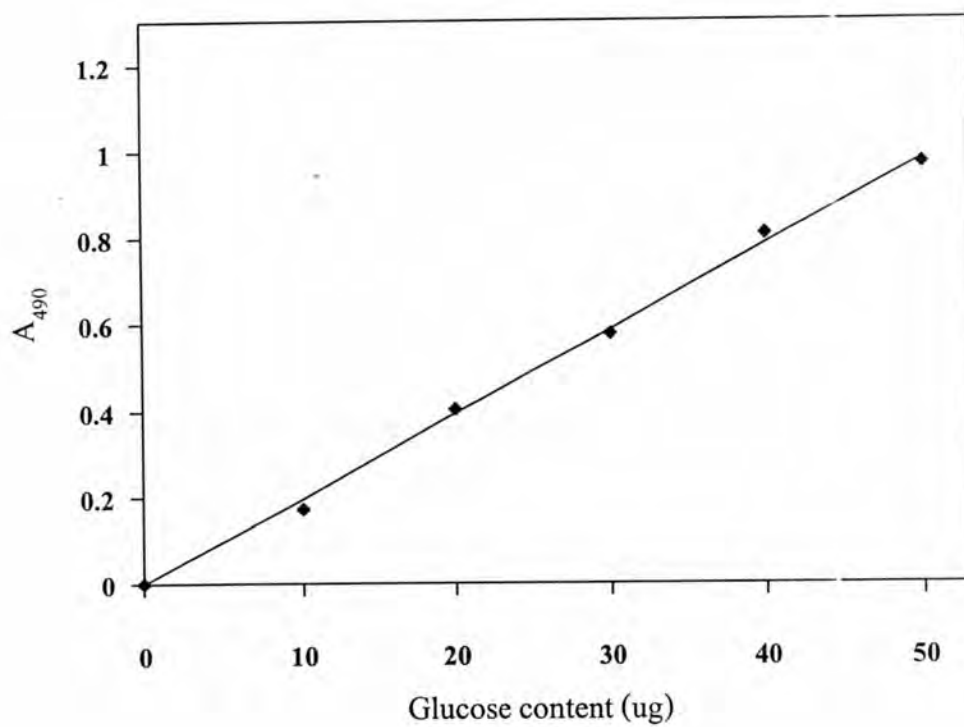
Ethanol	12 ml
Acetic acid	7 ml
CuSO ₄	0.5 g
Distilled water	81 ml

Dissolve the cupric sulfate in water before adding the alcohol. Immerse the gel in two or three changes of this solution until the background is nearly clear.

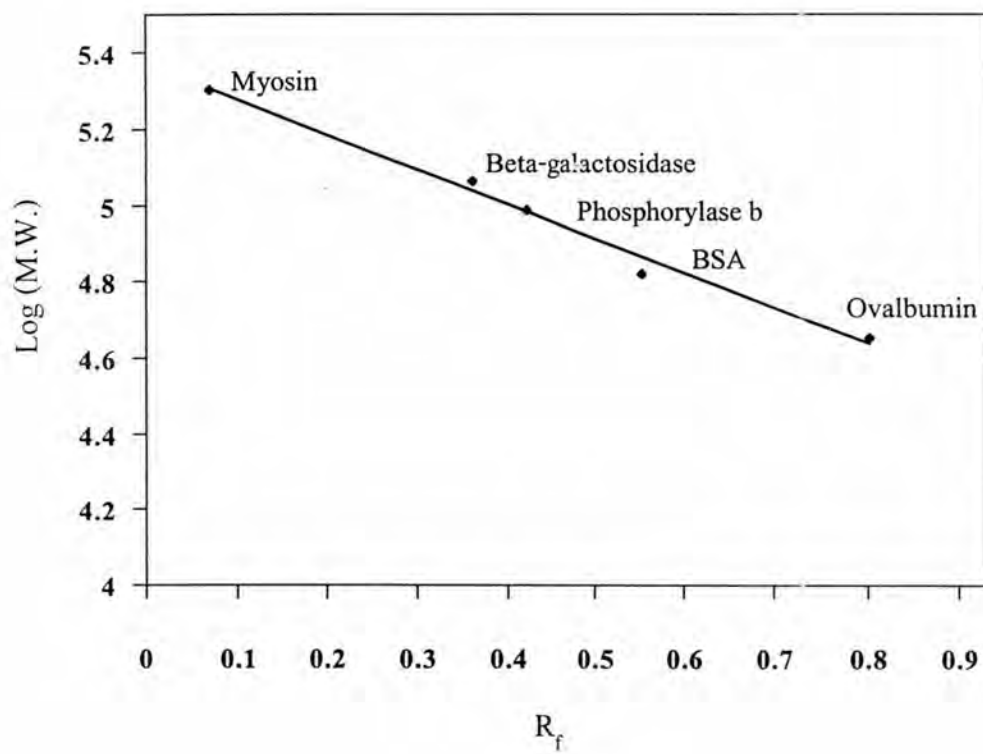
Second destaining solution

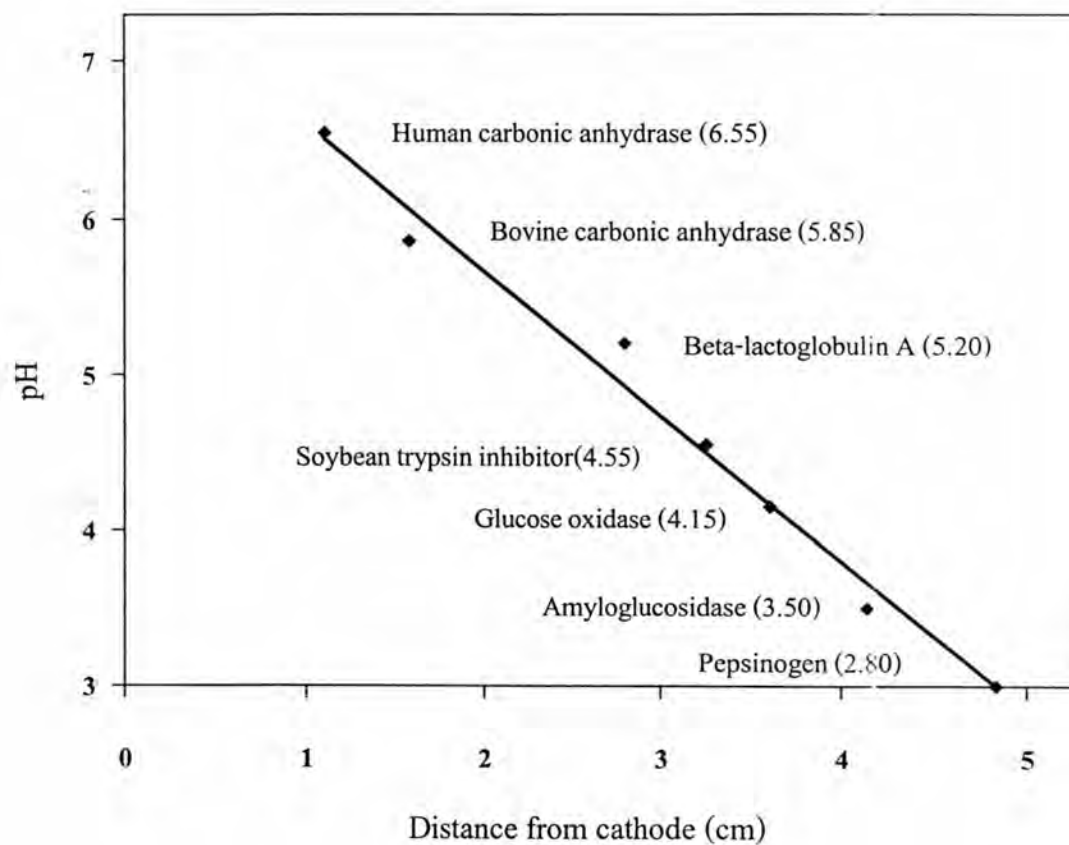
Ethanol	25 ml
Acetic acid	7 ml
Distilled water	68 ml

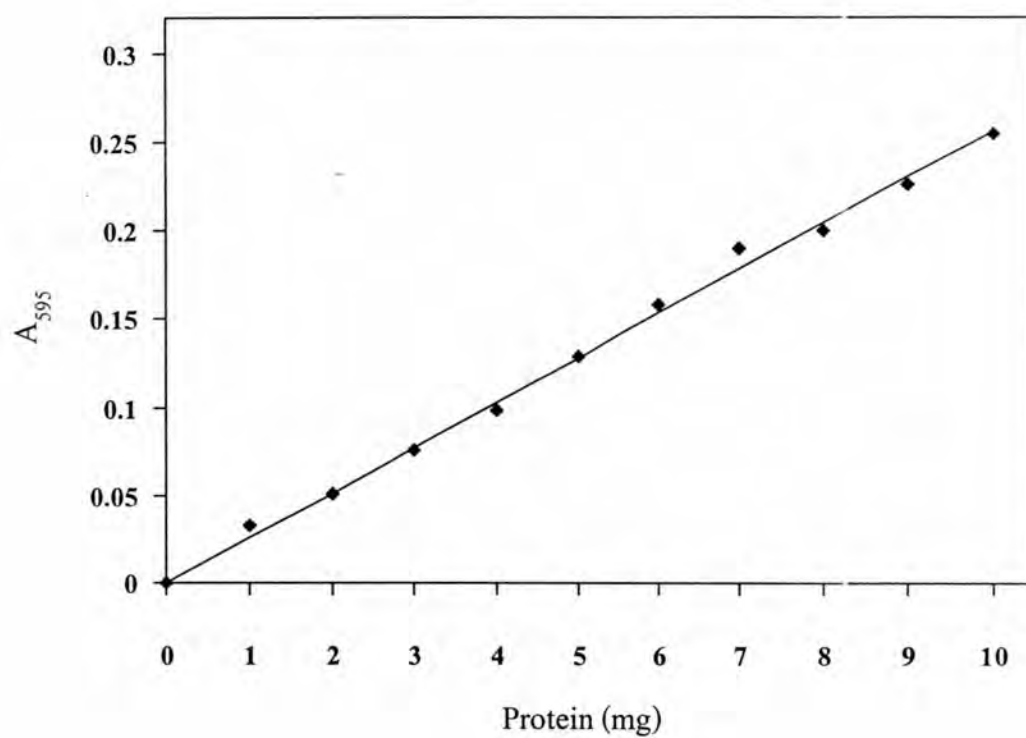
Immerse the gel in this solution to remove the last traces of stain and CuSO₄.

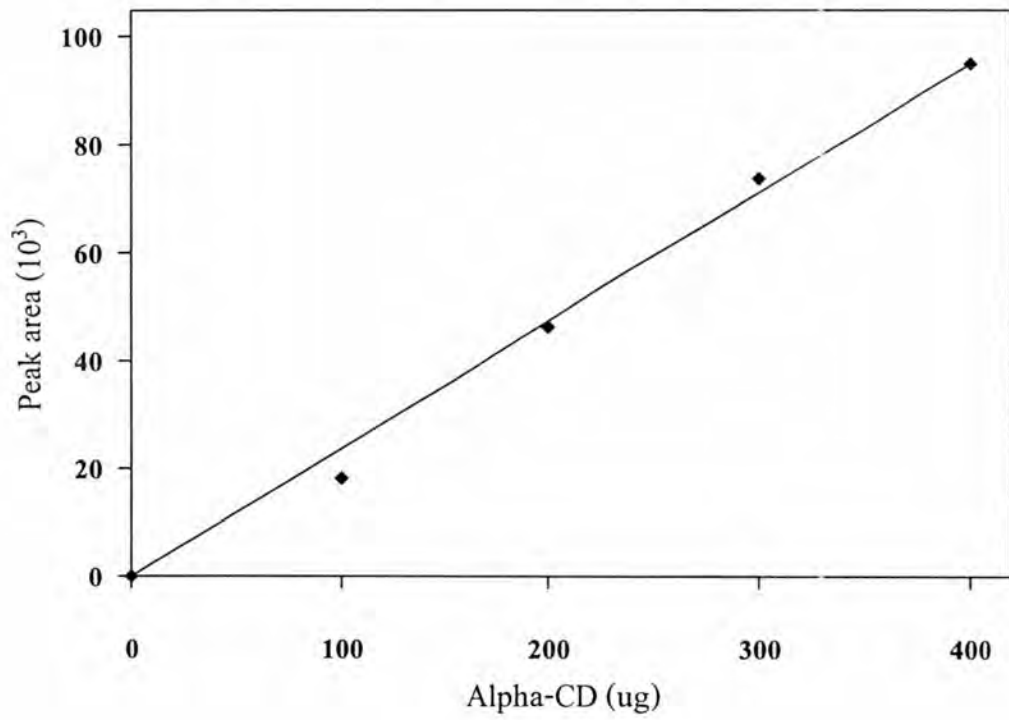
APPENDIX D : Standard curve of glucose by Phenol-sulfuric acid method

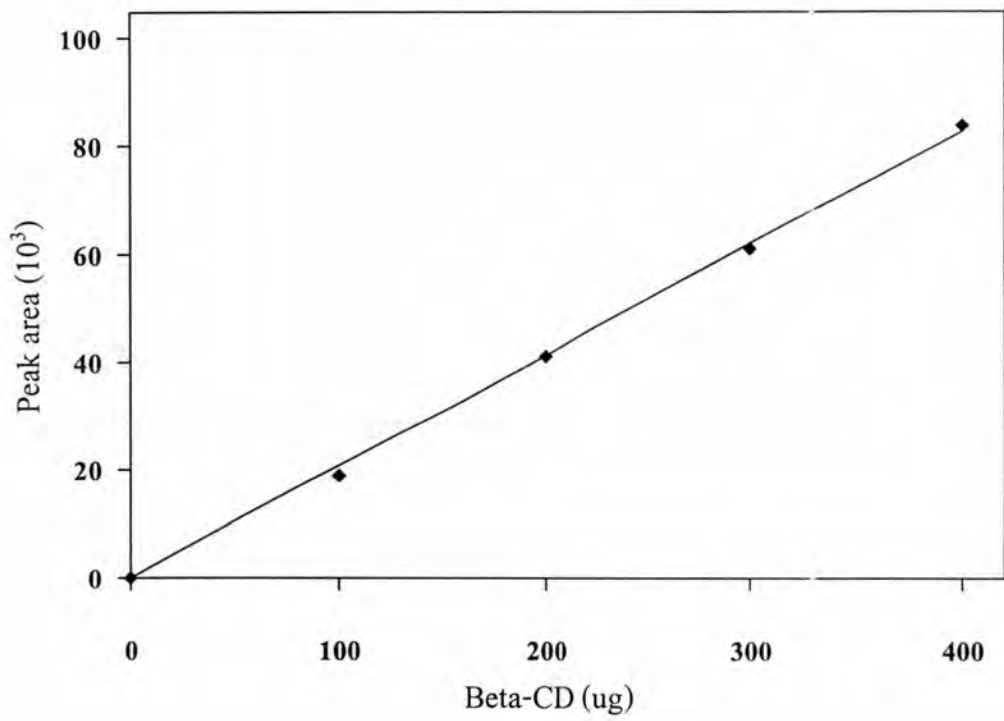
APPENDIX E : Molecular weight calibration curve of standard protein on 7.5% SDS-PAGE

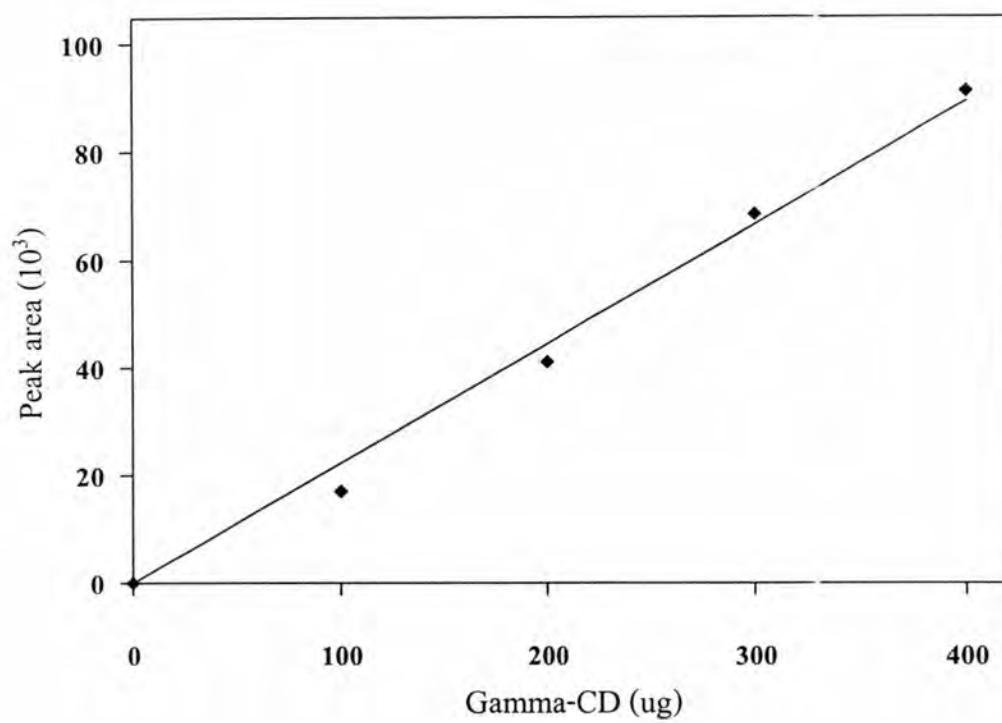


APPENDIX F : Standard pI calibration curve (pI 2.5-6.5)

APPENDIX G : Standard curve for protein determination by Bradford's method

APPENDIX H : Standard curve of Alpha-CD by HPLC method

APPENDIX I : Standard curve of Beta-CD by HPLC method

APPENDIX J : Standard curve of Gamma-CD by HPLC method

BIOGRAPHY

Miss Kannika Kaskangam was born on Nov. 4, 1974. She graduated with the Bachelor Degree of Science in Radiation Technology with the first class honor from Mahidol University in 1995 and continued studying for Master in Biotechnology Program.