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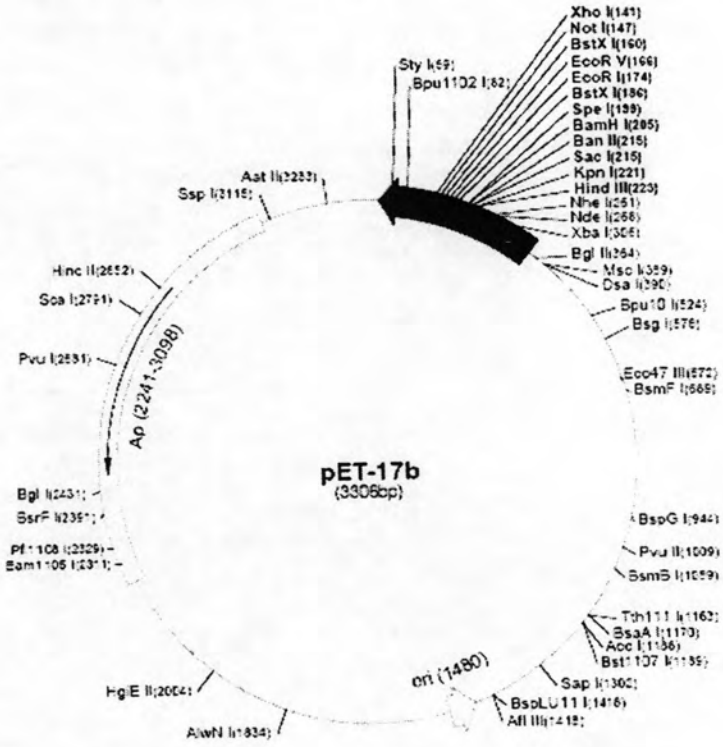
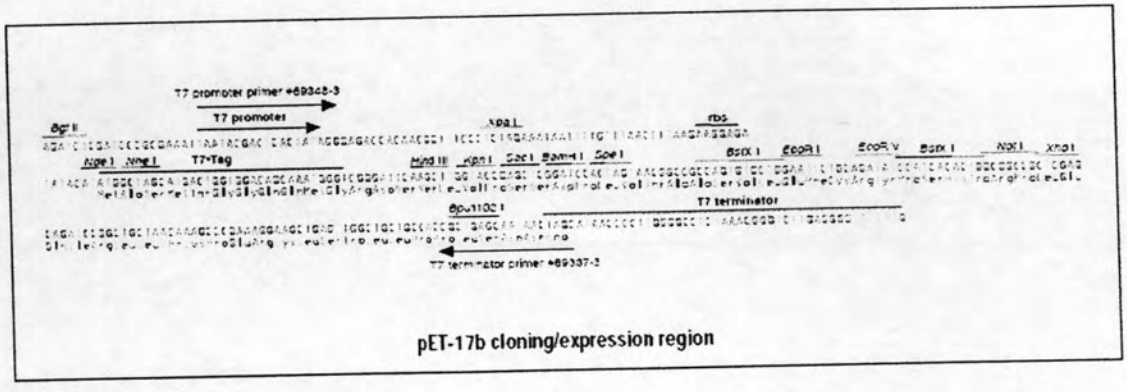
APPENDICES

APPENDIX A

Restriction map of pET-17b

pET-17b sequence landmarks	
T7 promoter	333-349
T7 transcription start	332
T7•Tag coding sequence	237-269
Multiple cloning sites (*Hind* III - *Xho* I)	141-228
T7 terminator	28-74
pBR322 origin	1480
bla coding sequence	2241-3098





APPENDIX B

QIAquick® gel extraction kit protocol

1. The DNA fragment from the agarose gel was excised with a clean and sharp scalpel.
2. The gel slice was weighed in a colorless tube. Then, 3 volumes of buffer QG was added to 1 volume of gel (100 mg ~100 µl).
3. The gel was incubated at 50°C for 10 min (or until the gel slice has completely dissolved) and mixed by vortexing the tube every 2-3 minutes during the incubation.
4. After the gel slice has dissolved completely, 1 gel volume of isopropanol was added to the sample and mixed.
5. QIAquick spin column was placed in a provided 2-ml collection tube.
6. To bind DNA, the sample was applied to the QIAquick column and centrifuged at 10,000x*g* for 1 minute.
7. The flow-through was discarded and QIAquick column was placed back in the same collection tube.
8. Then, 0.5 ml of buffer QG was added to QIAquick column and centrifuged at 10,000x*g* for1 minute.
9. Buffer PE 0.75 ml was added to QIAquick column to wash and further centrifuged at 10,000x*g* for 1 minute.
10. The flow-through was discarded and QIAquick column was centrifuged at 12,000x*g* for an additional 1 minute.
11. To elute DNA, 50 µl of buffer EB (10 mM Tris-Cl, pH 8.5) or H_2O was added to the center of QIAquick membrane and centrifuged at 10,000x*g* for 1 minute.

APPENDIX C

Preparation for protein determination

Reagent for determination of protein concentration (modified from Lowry *et al.*, 1951)

Solution A (0.5% copper sulfate and 1% potassium tartate, pH 7.0)

Potassium tartate	1	g
Copper sulfate	0.5	g

Adjusted pH to 7.0 and adjust the solution volume to 100 ml.

Solution B (2% sodium carbonate and 1 N sodium hydroxide)

Sodium carbonate	20	g
Sodium hydroxide	4	g

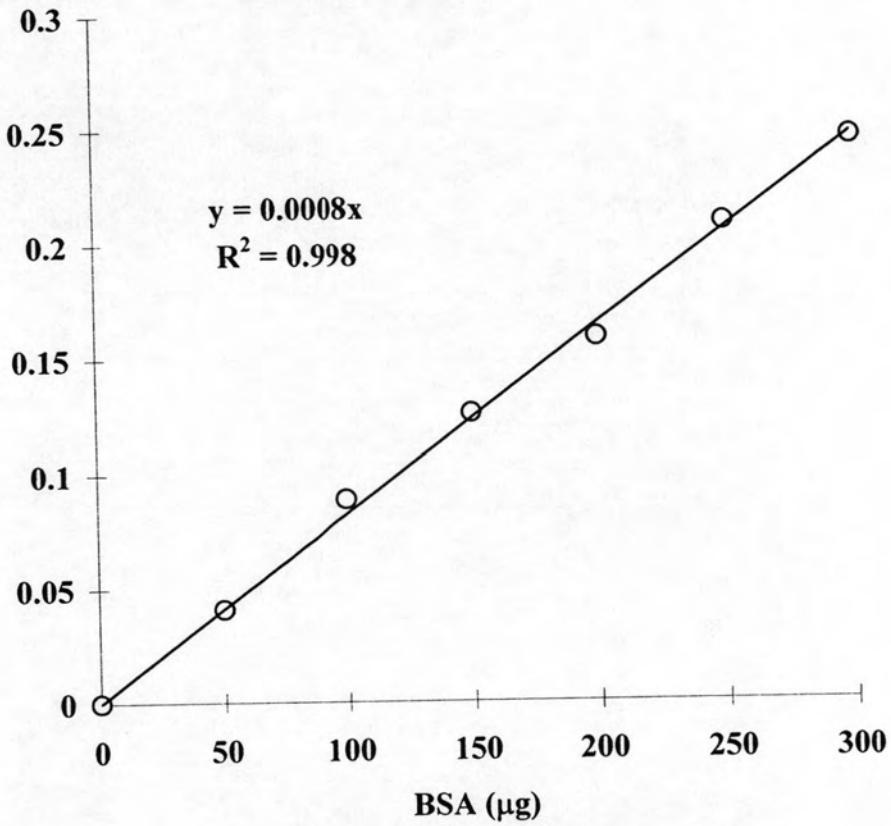
Dissolved in distilled water to 1 liter.

Solution C (phenol reagent)

Folin-Ciocalteu phenol reagent used in this work was reagent grade from Carlo Erba, Italy.

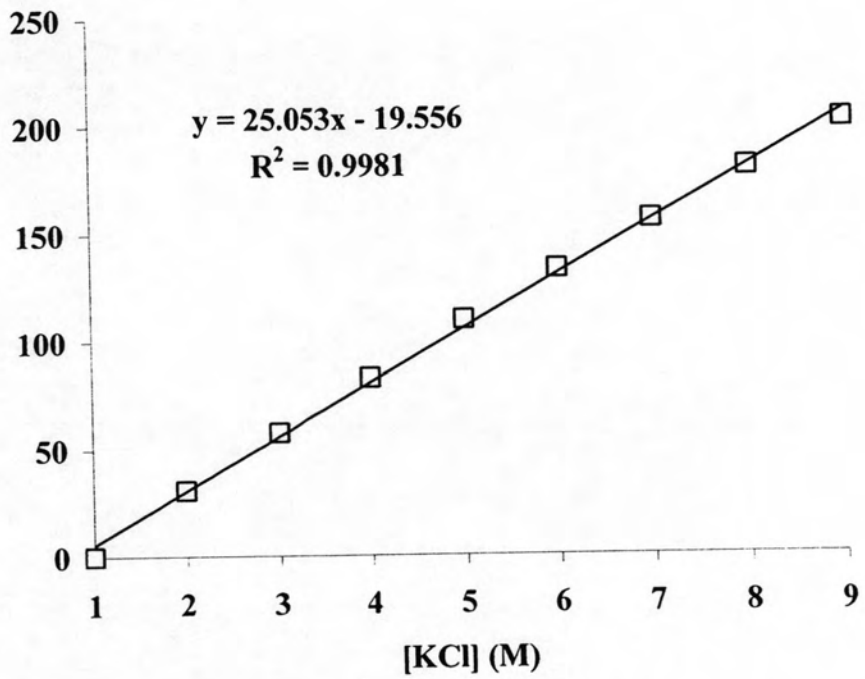
APPENDIX D

Standard curve for protein determination by Lowry's method



APPENDIX E

Calibration curve for conductivity of potassium chloride



APPENDIX F

Preparation for non-denaturing polyacrylamide gel electrophoresis (Native-PAGE)

1. Stock solutions

2 M Tris-HCl (pH 8.8)

Tris (hydroxymethyl)-aminomethane	24.2	g

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

1% (w/v) Bromophenol blue

Bromophenol blue	100	mg

Brought to 10 ml with distilled water and stirred until dissolved.

The aggregated dye was removed by filtration.

2. Working solutions

Solution A (30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide)

Acrylamide	29.2	g
N, N'-methylene-bis-acrylamide	0.8	g

Adjusted volume to 100 ml with distilled water.

Solution B (1.5 M Tris-HCl, pH 8.8)

2 M Tris-HCl (pH 8.8)	75	ml
Distilled water	25	ml

Solution C (0.5 M Tris-HCl, pH 6.8)

1 M Tris-HCl (pH 6.8)	50	ml
Distilled water	50	ml

APPENDIX F (continued)

10% (w/v) Ammonium persulfate

Ammonium persulfate	0.5	g
Distilled water	5.0	ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3.0	g
Glycine	14.4	ml

Dissolved and adjusted to total volunm 1 liter with distilled water

(final pH should be approximately 8.3)

5x Sample buffer (312.5 mM Tris-HCl pH 6.8, 50% (v/v) glycerol, 1% (v/v) bromophenol blue)

1 M Tris-HCl (pH 6.8)	0.6	ml
Glycerol	5.0	ml
1% Bromophenol blue	0.5	ml
Distilled water	1.4	ml

3. Native-PAGE

7.7% Separating gel

Solution A	2.6	ml
Solution B	2.5	ml
Distilled water	4.9	ml
10% (w/v) Ammonium persulfate	50	µl
TEMED	5.0	µl

5.0% Stacking gel

Solution A	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10% (w/v) Ammonium persulfate	30	µl
TEMED	5.0	µl

APPENDIX F (continued)

4. Protein staining solution

Staining solution, 1 liter

Coomassie brilliant blue R-250	1.0	g
Glacial acetic acid	100	ml
Methanol	450	ml
Distilled water	450	ml

Destaining solution, 1 liter

Methanol	100	ml
Glacial acetic acid	100	ml
Distilled water	800	ml

5. Enzyme activity staining solution

1 M Tris-HCl, pH 8.5

Tris (hydroxymethyl)-aminomethane 6.06 g

Adjusted to pH 8.5 with 1 N HCl and made up volume to 100 ml with distilled water

40 mM L-lysine

L-lysine 0.064 g

Dissolved with 10 ml distilled water

50 mM NAD^+

NAD^+ 0.359 g

Dissolved with 10 ml distilled water

0.25 mg/ml phenazine methosulfate

Phenazine methosulfate 0.0025 g

Dissolved with 10 ml distilled water

2.5 mg/ml nitroblue tetrazolium

Nitroblue tetrazolium 0.025 g

Dissolved with 10 ml distilled water

APPENDIX F (continued)

Activity staining solution (4.25 mM Tris-HCl, pH 8.5, 40 μM L-lysine, 50 μM NAD^+, 250 μg phenazine methosulfate and 2.5 mg nitroblue tetrazolium)

1 M Tris-HCl, pH 8.5	4.25	ml
40 mM L-lysine	1.0	ml
50 mM NAD^+	1.0	ml
0.25 mg/ml phenazine methosulfate	1.0	ml
2.5 mg/ml nitroblue tetrazolium	1.0	ml
Distilled water	1.75	ml

APPENDIX G

Preparation for denaturing polyacrylamide gel electrophoresis

1. Stock solution

2 M Tris-HCl (pH 8.8)

Tris (hydroxymethyl)-aminomethane 24.2 g

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

10% (w/v) SDS

Sodium dodecyl sulfate (SDS) 10 g

Added distilled water to a total volume of 100 ml.

50% (w/v) Glycerol

100% Glycerol 50 ml

Added distilled water to a total volume of 100 ml.

1% (w/v) Bromophenol blue

Bromophenol blue 100 mg

Brought to 10 ml with distilled water and stirred until dissolved.

The aggregated dye was removed by filtration.

2. Working solutions

Solution A (30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide)

Acrylamide 29.2 g

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

Adjusted volume to 100 ml with distilled water.

Filtered and stored in dark (brown bottle) at 4°C

APPENDIX G (continued)

Solution B (1.5 M Tris-HCl, pH 8.8 and 0.4% SDS)

2 M Tris-HCl (pH 8.8)	75	ml
10% (w/v) SDS	4.0	ml
Distilled water	21	ml

Solution C (0.5 M Tris-HCl, pH 6.8, 0.4% SDS)

1 M Tris-HCl (pH 6.8)	50	ml
10% (w/v) SDS	4.0	ml
Distilled water	46	ml

10% (w/v) Ammonium persulfate

Ammonium persulfate	0.5	g
Distilled water	5.0	ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine and 0.1% (w/v) SDS)

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

Dissolved and adjusted to total volume to 1 liter with distilled water

(final pH should be approximately 8.3)

5x Sample buffer (312.5 mM Tris-HCl pH 6.8, 50% (v/v) glycerol, 1% (w/v) bromophenol blue)

1 M Tris-HCl (pH 6.8)	0.6	ml
50% (v/v) Glycerol	5.0	ml
10% (w/v) SDS	2.0	ml
1% (w/v) Bromophenol blue	1.0	ml
β-Mercaptoethanol	0.5	ml
Distilled water	1.4	ml

APPENDIX G (continued)

3. SDS-PAGE

Separating gel	**10%**	**12.5%**	
Solution A	3.3	3.45	ml
Solution B	2.5	2.5	ml
Distilled water	4.2	2.5	ml
10% (w/v) Ammonium persulfate	50	50	µl
TEMED	5.0	5.0	µl

5.0% Stacking gel		
Solution A	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10% (w/v) Ammonium persulfate	30	µl
TEMED	5.0	µl

4. Protein staining solution

Staining solution, 1 liter		
Coomassie brilliant blue R-250	1.0	ml
Methanol	450	ml
Distilled water	450	ml

Destaining solution, 1 liter		
Methanol	100	ml
Glacial acetic acid	100	ml
Distilled water	800	ml

APPENDIX I

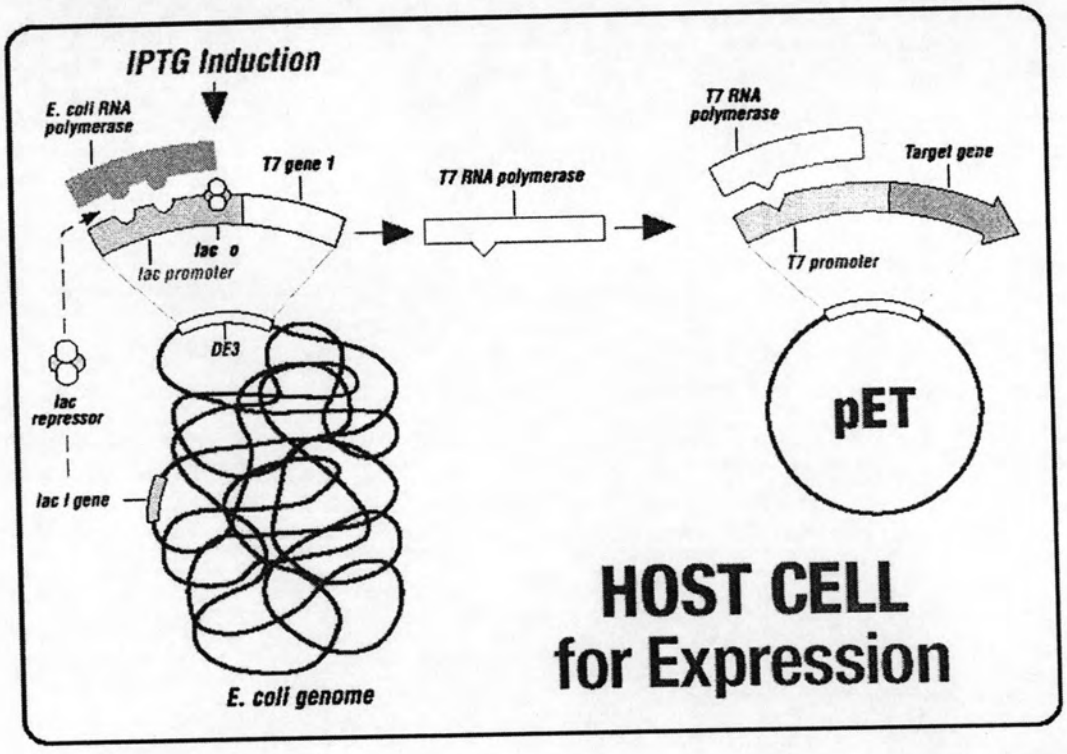
Abbreviation for amino acid residues

Amino acid	3 Letters-Abbreviation	1-Letter-Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Source: Voet, 2004

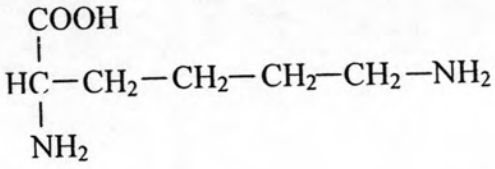
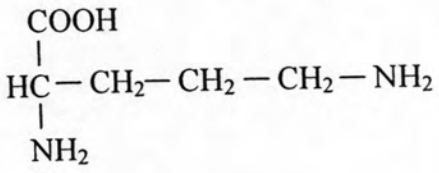
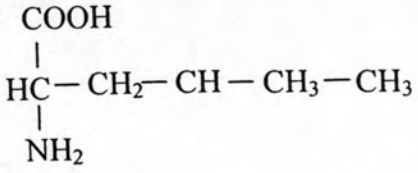
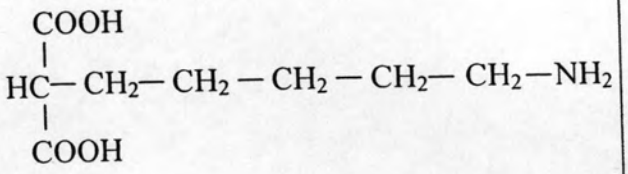
APPENDIX J

Control element of the pET system



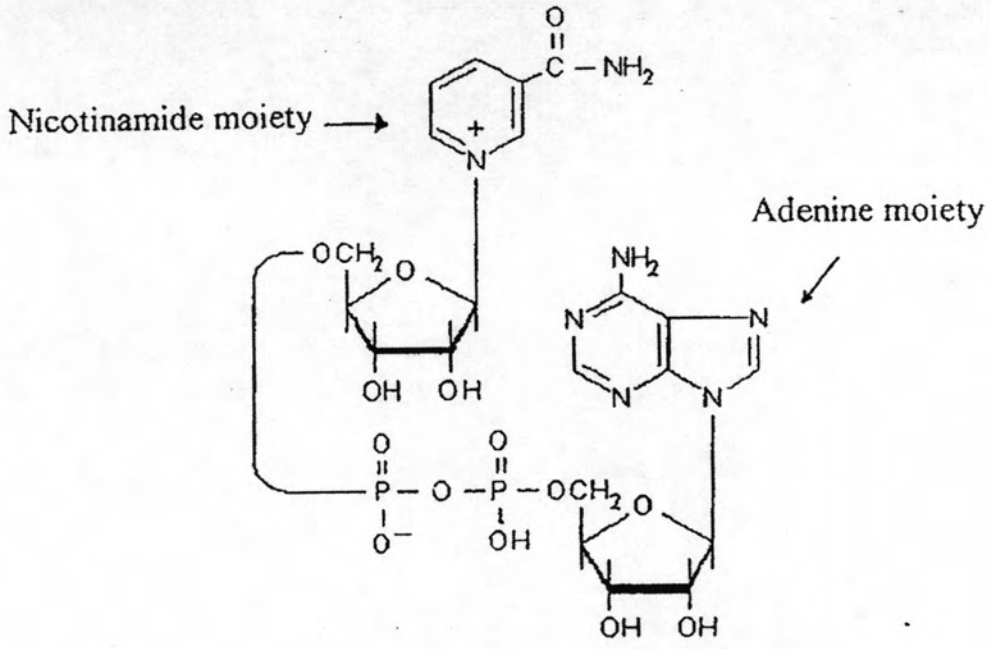
APPENDIX K

Structure of amino acids and their analogs

Amino acids and analogs	Structure
L-lysine	
L-ornithine	
L-norleucine	
6-amino-*n*-carpoic acid	

APPENDIX L

NAD^+ structure



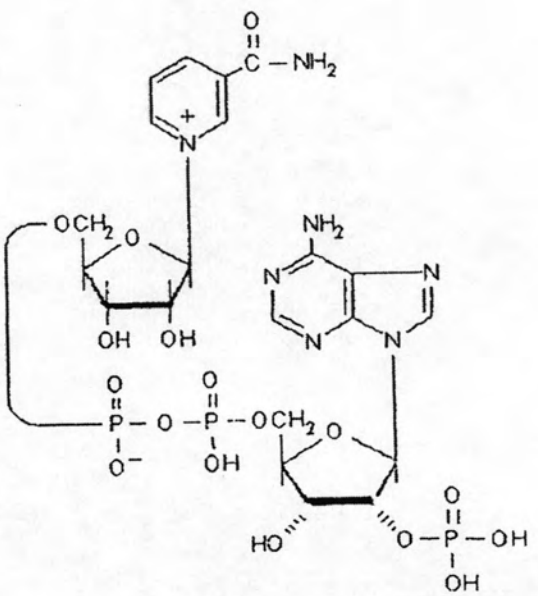
Nicotinamide adenine dinucleotide (NAD^+)

APPENDIX L (continued)

NAD^+ analogs

The NAD^+ analogs used in this work can be divided into 3 groups based on their modified structure.

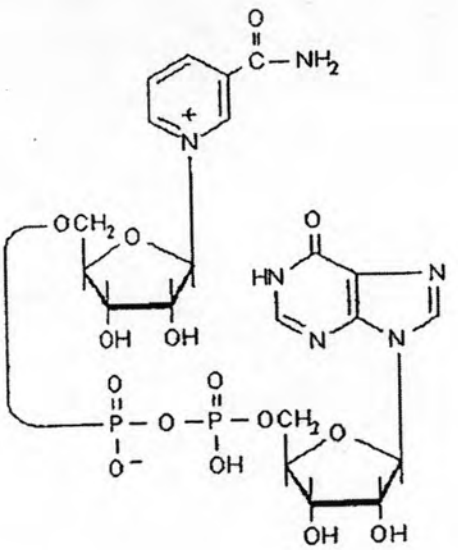
1. Coenzyme analog modified at C-2 position of the adenosylribose



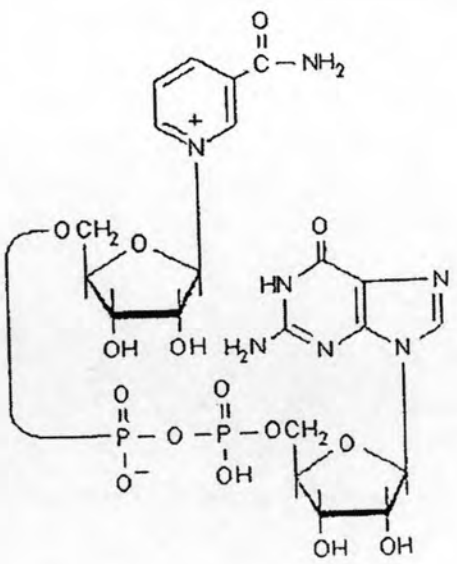
APPENDIX L (continued)

NAD^+ analogs

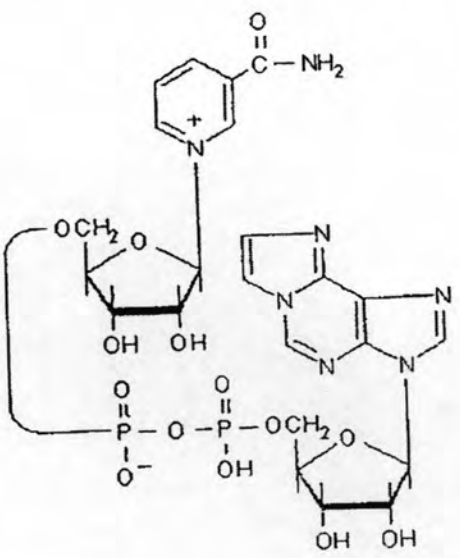
2. Coenzyme analog modified at the amino group in the adenine moiety



Nicotinamide hypoxanthine dinucleotide (Deamino-NAD^+)



Nicotinamide guanine dinucleotide

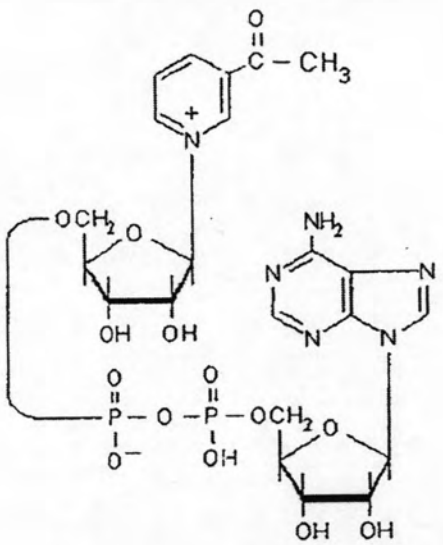


Nicotinamide 1, N^6-ethenoadenine dinucleotide

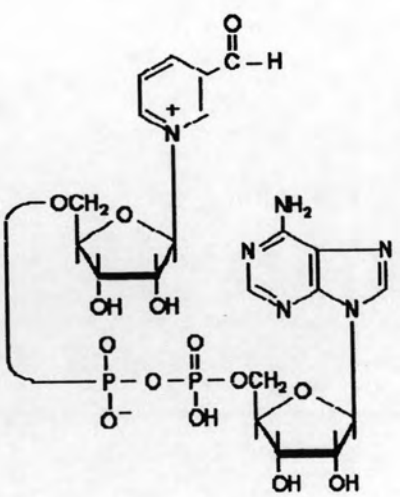
APPENDIX L (continued)

NAD^+ analogs

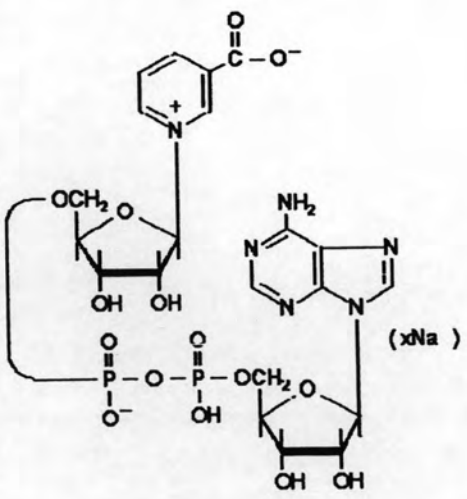
3. Coenzyme analog modified at the nicotinamide moiety



3-Acetylpyridine adenine dinucleotide



3-Pyridinealdehyde adenine dinucleotide



Nicotinic acid adenine dinucleotide (deamido-NAD^+)

APPENDIX M

Dielectric constants and hydrophobicity characteristics of solvents (log *P*) values of some organic solvents

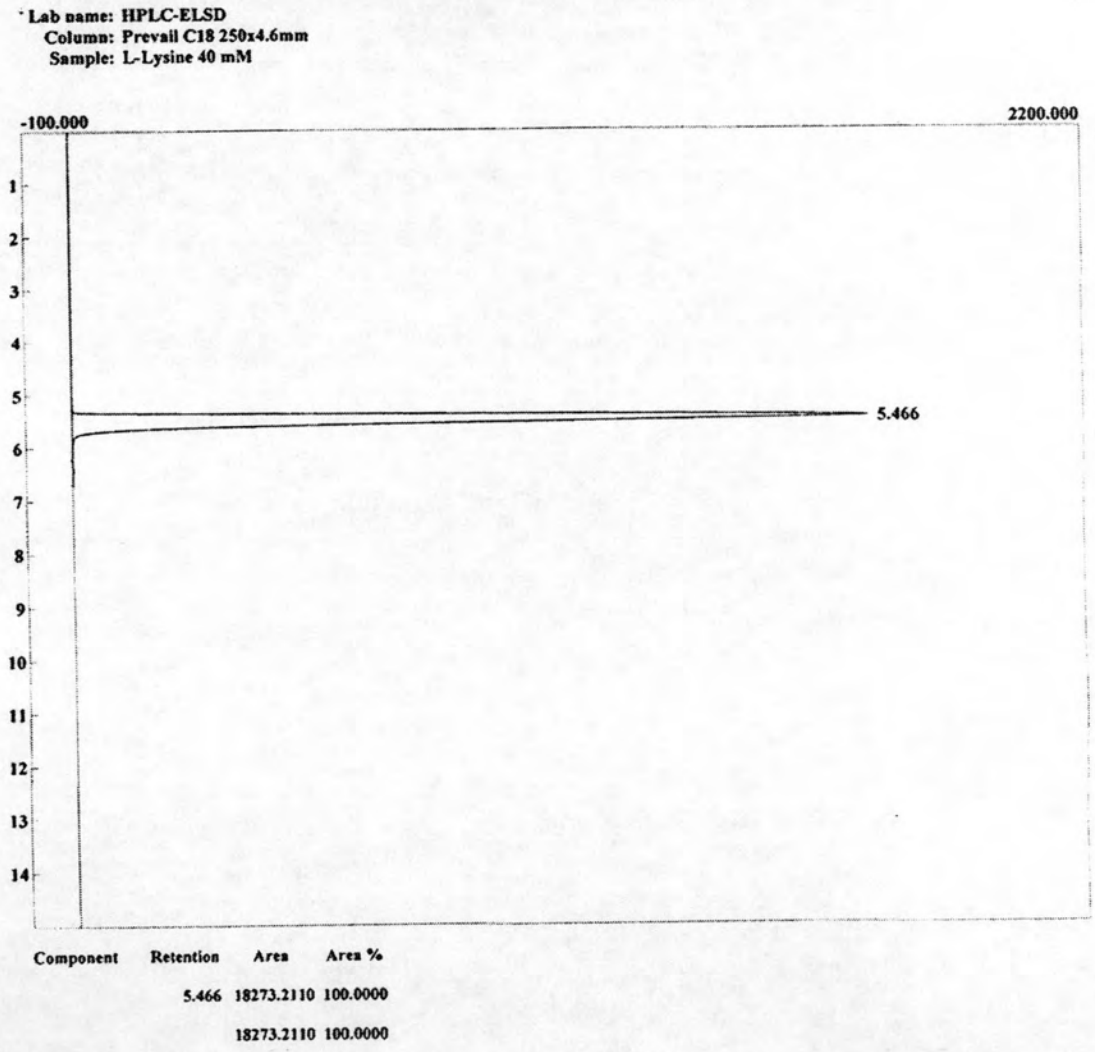
Solvent	Dielectric constant (C^2/Nm^2)	Log *P* (-)
Hexane	1.89	3.50
Benzene	2.28	2.00
Toluene	2.38	2.50
Xylene	2.40	3.10
Chloroform	4.80	2.00
Pyridine	12.5	0.71
Isopropyl alcohol	18 .3	0.05
Acetone	20.7	-0.23
Ethanol	24.3	-0.24
Methanol	32.6	-0.76
Water	78.5	-

Source: Dielectric constants and log *P*, where *P* is the partition coefficient of the solvent between 1-octanol and water, were taken from (http://www.asiinstr.com/technical/Dielectric%20Constants.htm and Laane *et al.*,. 1987, respectively).

APPENDIX N

ELSD-HPLC profile of L-lysine and L-pipecolic acid

A) L-lysine standard (40 mM)



Component	Retention	Area	Area %
	5.466	18273.2110	100.0000
		18273.2110	100.0000

Condition: Prevail C18 (250x4.6cm) column

Temperature : 90 °C

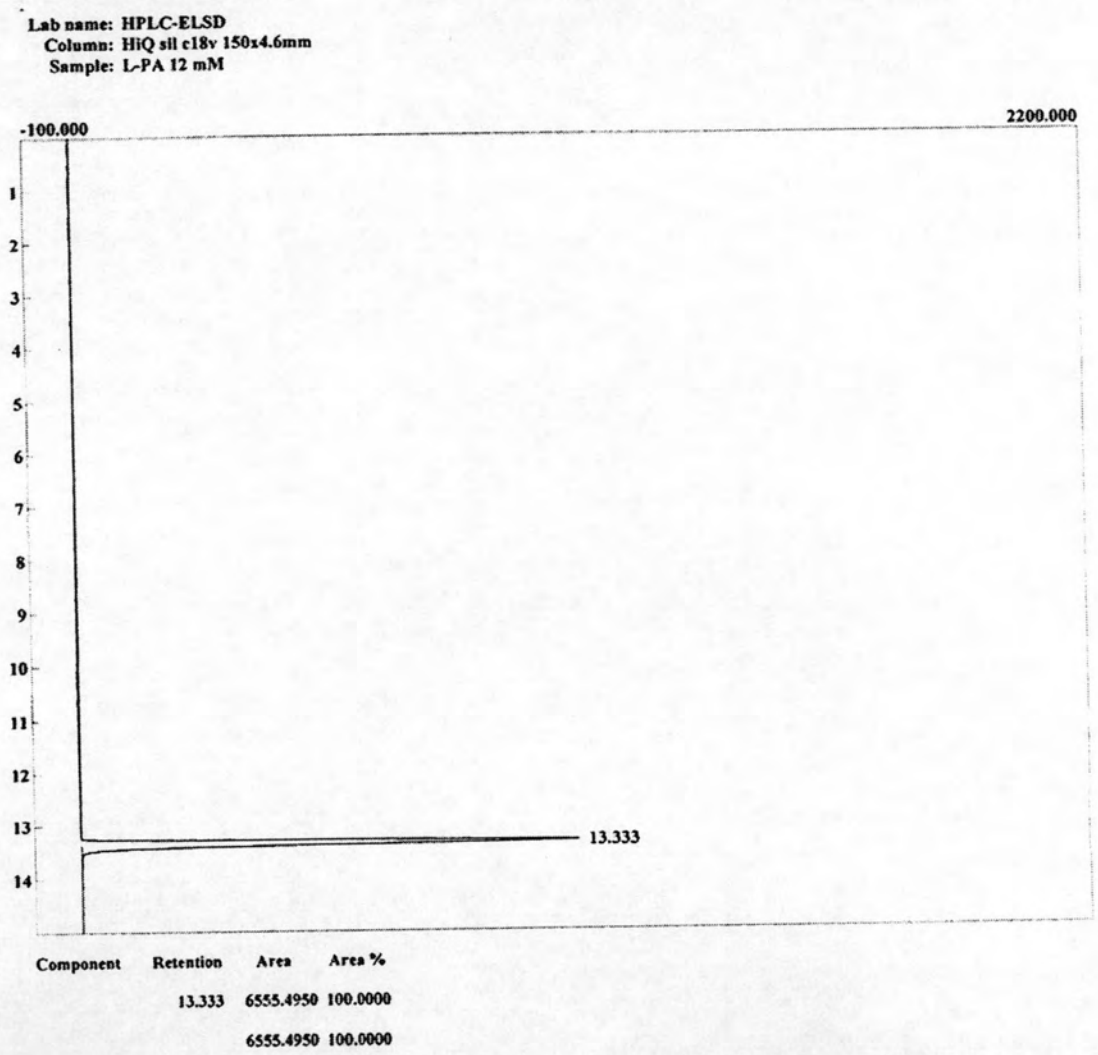
Flow rate : 0.6 ml/min.

Solvent A : 0.1% TFA in water

Solvent B : 0.1% TFA in acetonitride

APPENDIX N (continued)

B) L-pipecolic acid standard (12 mM)



Component	Retention	Area	Area %
	13.333	6555.4950	100.0000
		6555.4950	100.0000

Condition: Prevail C18 (250x4.6cm) column

Temperature : 90 °C

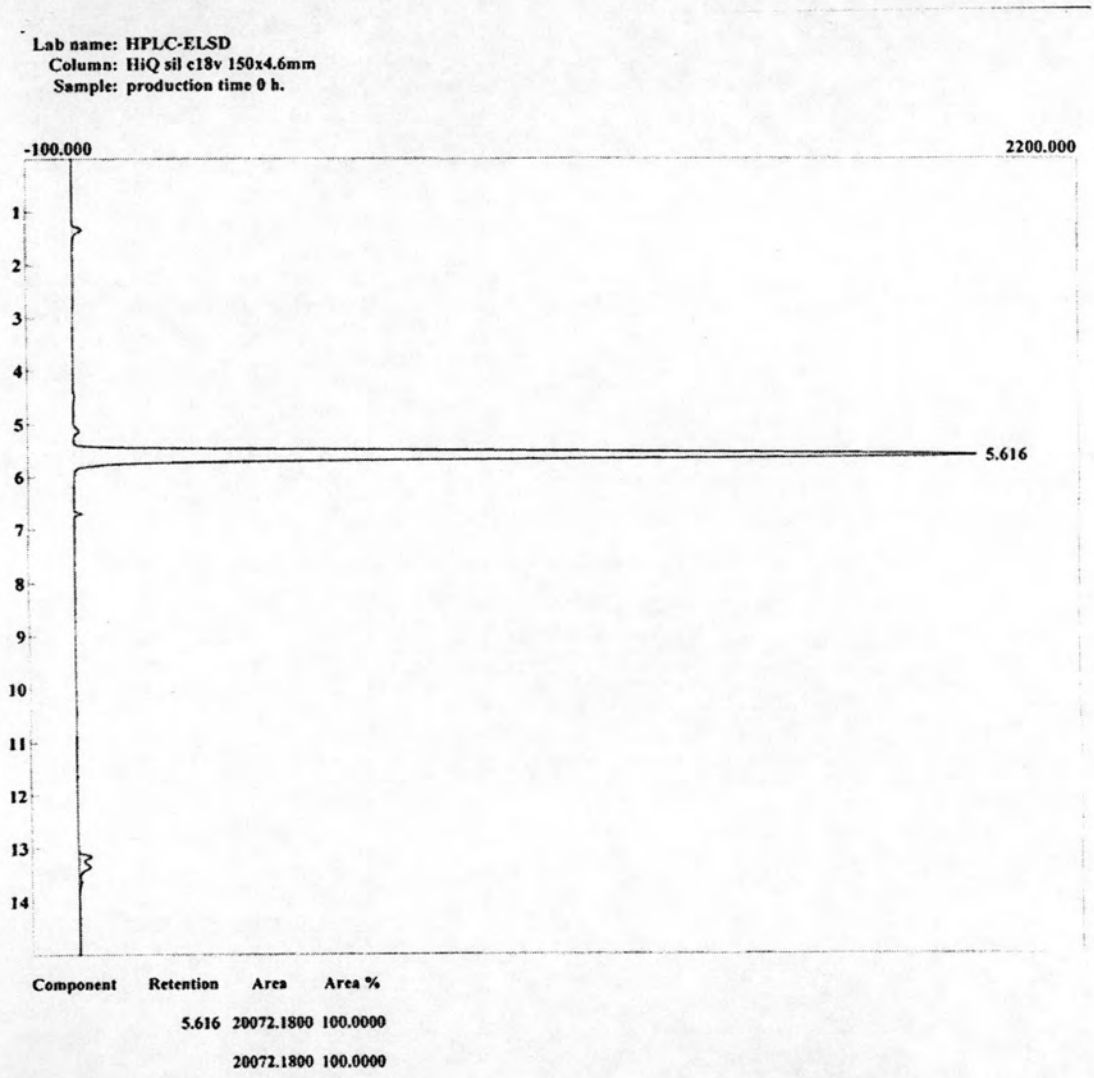
Flow rate : 0.6 ml/min.

Solvent A : 0.1% TFA in water

Solvent B : 0.1% TFA in acetonitride

APPENDIX N (continued)

C) Sample at production time 0 hour



Component	Retention	Area	Area %
	5.616	20072.1800	100.0000
		20072.1800	100.0000

Condition: Prevail C18 (250x4.6cm) column

Temperature : 90 °C

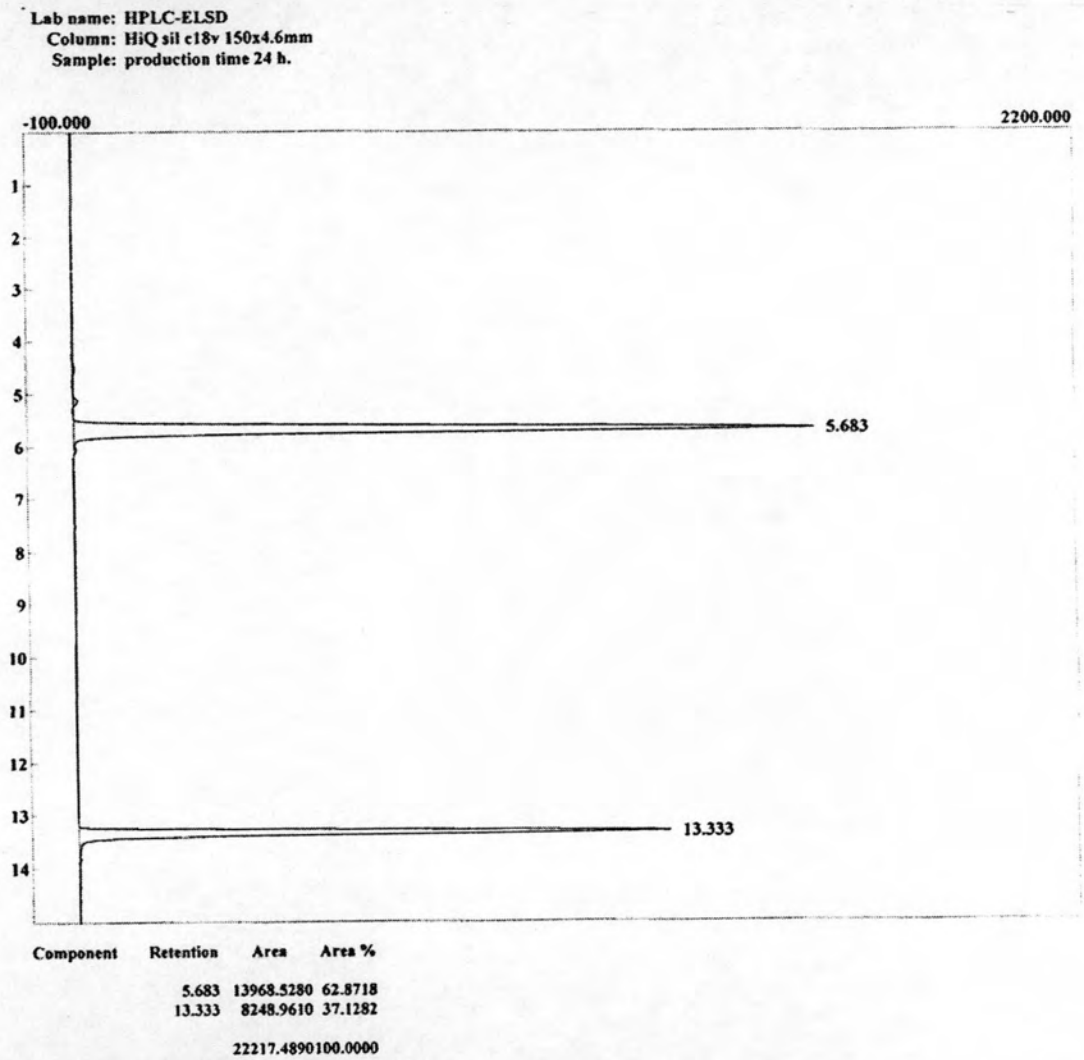
Flow rate : 0.6 ml/min.

Solvent A : 0.1% TFA in water

Solvent B : 0.1% TFA in acetonitride

APPENDIX N (continued)

D) Sample at production time 24 hours



Component	Retention	Area	Area %
	5.683	13968.5280	62.8718
	13.333	8248.9610	37.1282
		22217.4890	100.0000

Condition: Prevail C18 (250x4.6cm) column

Temperature : 90 °C

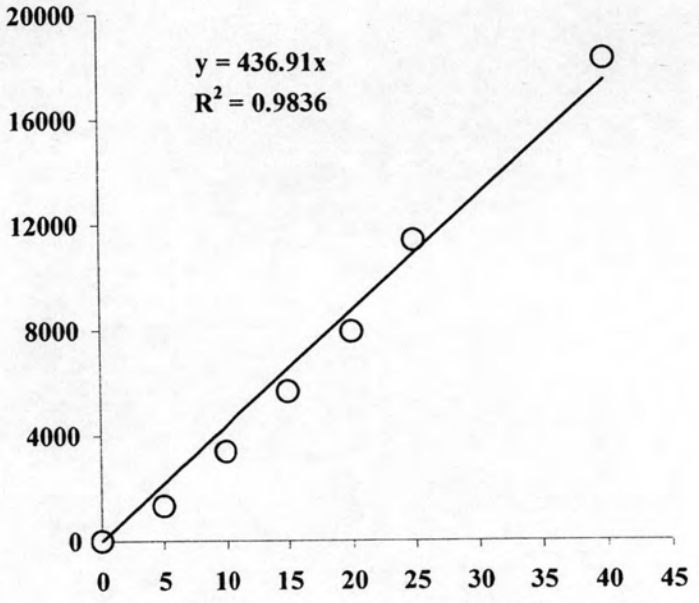
Flow rate : 0.6 ml/min.

Solvent A : 0.1% TFA in water

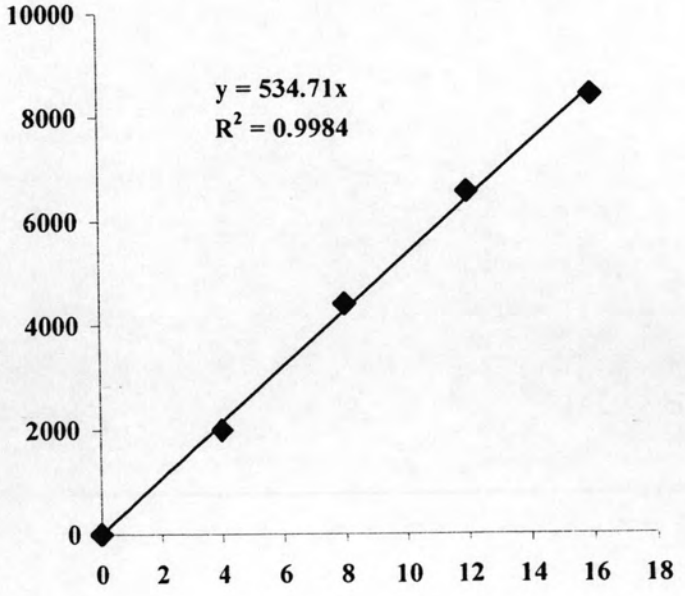
Solvent B : 0.1% TFA in acetonitride

APPENDIX O

Standard curve for L-lysine concentration by ELSD-HPLC



Standard curve for L-pipecolic acid concentration by ELSD-HPLC



BIOGRAPHY

Mr. Prakarn Ruldeekulthamrong was born on December 25th, 1975 in Bangkok, Thailand. He graduated with a Bachelor of Science (Health Science), Faculty of Science, Thammasat University in 1996. After graduating with degree of Bachelor degree, He enrolled in the Master of Science in Biotechnology Program, Faculty of Science, Chulalongkorn University during 1997-2001. Then, He continued studying for the Degree of Philosophy of Science in Biological Science Program in the field of Cell and Molecular Biology, Faculty of Science, Chulalongkorn University in that year.