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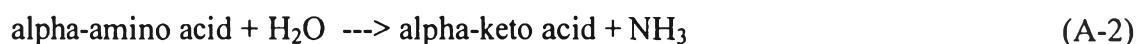
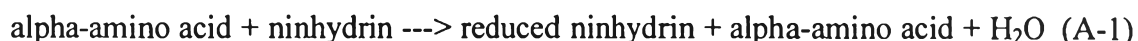
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APPENDICES

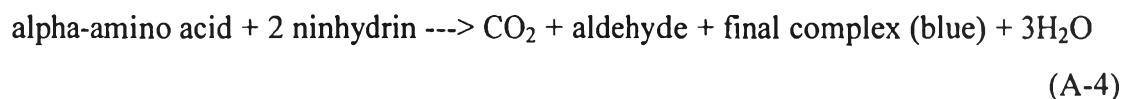
APPENDIX A

Ninhydrin Colorimetric Method

The reaction between alpha-amino acid and ninhydrin involve the development of color which can be described by the following five mechanistic steps:



Step (A-1) is an oxidative deamination reaction that removes two hydrogens from the alpha-amino acid to yield an alpha-imino acid. Simultaneously, the original ninhydrin is reduced and loses an oxygen atom with the formation of a water molecule. In Step (A-2), the NH group in the alpha-amino acid is rapidly hydrolyzed to form an alpha-keto acid with the production of an ammonia molecule. This alpha-keto acid further undergoes decarboxylation reaction in Step (A-3) under heated condition to form an aldehyde that has one less carbon atom than the original amino acid. A carbon dioxide molecule is also produced. These first three steps produce the reduced ninhydrin and ammonia that are required for the production of blue color. The overall reaction for the above reactions is simply expressed in equation (A-4) as follows:



In summary, ninhydrin, which is originally yellow, reacts with amino acid and turns into deep purple. It is this purple color that is detected in this method. Ninhydrin will react with a free alpha-amino group of $\text{NH}_2\text{-CHR-COOH}$. This group is a part of all amino acids, peptides, or proteins. Whereas, the decarboxylation reaction would proceed for a free amino acid and a free amino group at chain end or side chain of protein or peptide, it does not occur for an amino group within peptides and proteins.

Thus, theoretically only amino acids will lead to the color development. However, one should always check out the possible interference from peptides and proteins by performing blank tests especially when such solutions are readily available. For example, one can simply add the ninhydrin reagent to a solution of only proteins and see if there is any color development. There is no excuse for failing to perform such a vital test when the sample mixture contains both proteins and amino acids. There are also reports that chemical compounds other than amino acids also yield positive results.

Although this is a fast and sensitive test for the presence of alpha-amino acids because of the nonselectivity, it cannot be used to analyze the relative individual contents of a mixture of different amino acids. Furthermore, the color intensity developed is dependent on the type of amino acid. Finally, it does not react with tertiary or aromatic amines.

Determination of Amino group on modified PCL film

The quantitative NH_2 amount on aminolyzed PCL film and biomolecule-immobilized PCL was measured by the ninhydrin method. The purple reaction product of ninhydrin with free NH_2 group has the maximum absorbance at 538 nm in the solvent of 1,4-dioxane/isopropanol (IPA) (1/1). Using 1,6-hexamethylenediamine as a standard, a calibration curve was generated as shown in Figure 4.1.

Table A-1 UV absorbance at $\lambda = 538$ nm of standard 1,6-hexamethylenediamine solution for generating a calibration curve

1,6-hexamethylenediamine concentration ($\times 10^{-3}$ M)	Absorbance
0.001	0.012
0.005	0.025
0.010	0.052
0.030	0.164
0.050	0.290
0.100	0.600
0.300	1.489

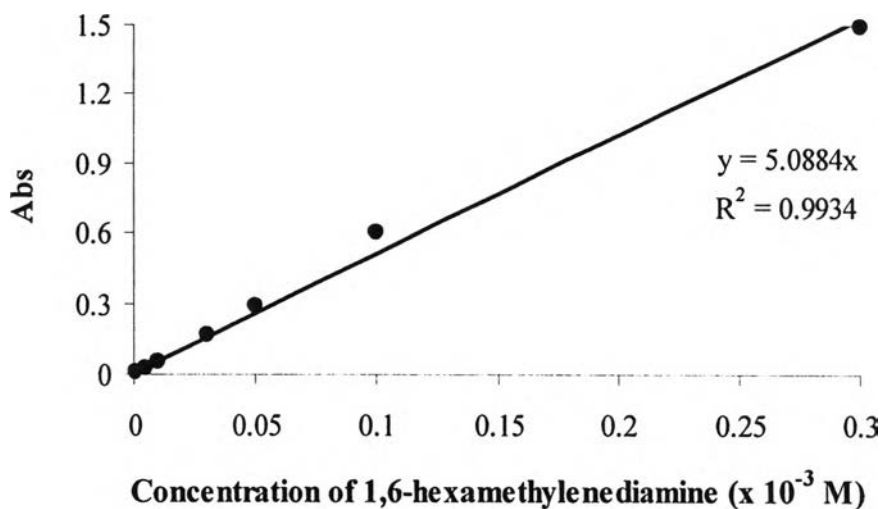


Figure A-1 Calibration curve of UV absorbance as a function of 1,6-hexamethylenediamine concentration analyzed by ninhydrin method

The amount of amino group (C) obtained from the calibration curve was then calculated by these following equations:

$$\text{NH}_2 \text{ concentration} = \left[\frac{C \text{ (mmol/L)}}{1000 \text{ (mmol/mol)}} \right] \times \left[\frac{1}{1000} \text{ (L/mL)} \right] \times 2 \text{ (mL)}$$

$$\text{NH}_2 \text{ concentration per surface area} = \frac{\text{NH}_2 \text{ concentration (mol)}}{\text{Sample area (cm}^2\text{)} \times 2}$$

Toluidine Blue O Method

Toluidine blue O method is a method used for determination of the amount of carboxyl groups. The complex formed by the carboxyl group and toluidine blue o has the λ_{max} at 633 nm.

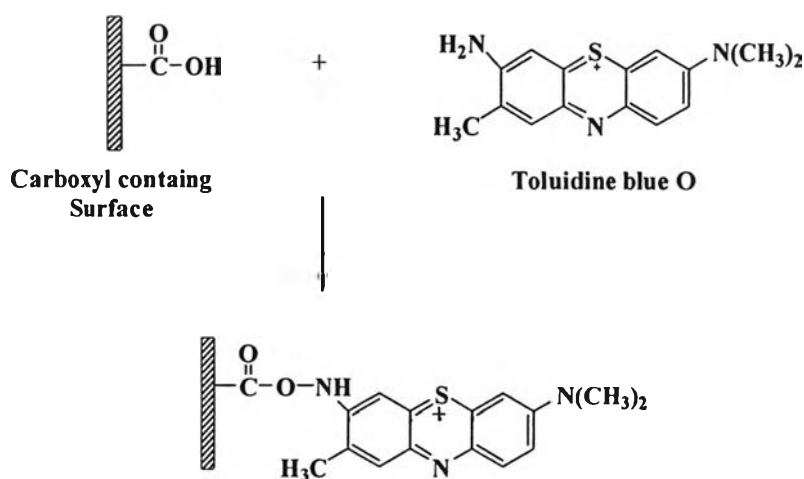


Figure A-2 Formation of the complex between toluidine blue O and carboxyl group

Determination of Carboxyl group on PCL-g-PAA film

The quantitative density of COOH groups grafted on PCL membrane was determined by the reaction with toluidine blue o, generating the absorbance at 633 nm. The COOH content was obtained from a calibration plot of the optical density versus dye concentration assuming a 1:1 stoichiometric ratio (Figure A-3) between the dye and the COOH group.

Table A-2 UV absorbance at $\lambda = 633$ nm as a function of toluidine blue o concentration

Toluidine blue o concentration ($\times 10^{-3}$ M)	Absorbance
0.001	0.013
0.005	0.112
0.007	0.177
0.010	0.216
0.030	0.939
0.050	1.490

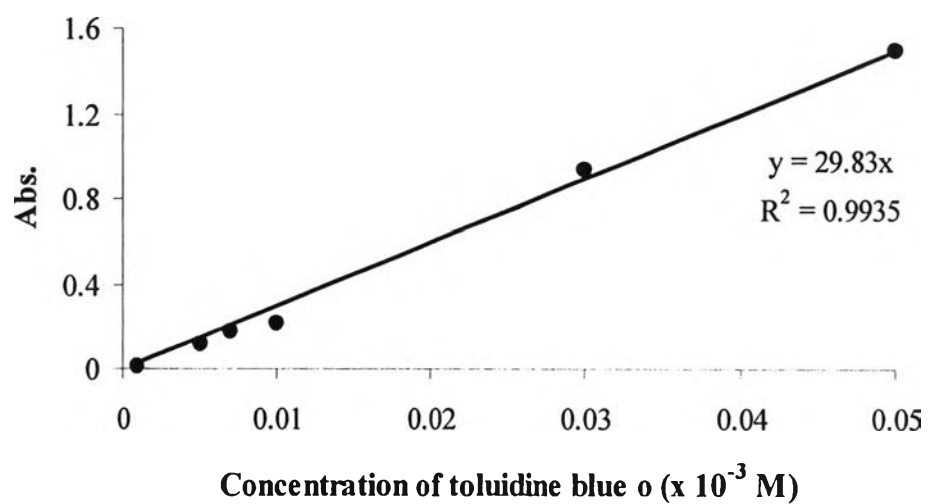


Figure A-3 Calibration curve of UV absorbance as a function of toluidine blue o concentration

APPENDIX B

Table B-1 Water contact angle and amount of amino group of PCL films after reaction with 1 M 1,6-hexamethylenediamine/IPA as a function of reaction time

Aminolyzing time (h)	Water contact angle (degree)		NH ₂ concentration/surface area (x10 ⁻⁸ mol/cm ²)
	θ_A	θ_R	
0	85 ± 1.3	50 ± 1.3	0 ± 0.0
1	79 ± 2.9	47 ± 2.3	1.8 ± 0.1
2	77 ± 2.1	46 ± 1.9	2.8 ± 0.3
4	75 ± 2.7	44 ± 2.1	3.8 ± 0.4
8	75 ± 2.3	43 ± 2.4	4.4 ± 0.3
12	76 ± 2.4	44 ± 1.6	5.0 ± 0.5
24	78 ± 2.4	45 ± 2.1	5.8 ± 0.3

Table B-2 Water contact angle and amount of amino group of PCL films after reaction with 1,6-hexamethylenediamine/IPA for 8 h as a function of amine concentration

Amine concentration (M)	Water contact angle (degree)		NH ₂ concentration/surface area (x10 ⁻⁸ mol/cm ²)
	θ_A	θ_R	
0.00	85 ± 1.3	50 ± 1.3	0 ± 0.0
0.25	82 ± 1.6	47 ± 3.2	0.6 ± 0.1
0.50	78 ± 2.1	45 ± 2.4	0.8 ± 0.1
1.00	75 ± 2.3	43 ± 2.4	4.4 ± 0.3
1.50	73 ± 1.9	42 ± 1.5	6.3 ± 0.2
2.00	73 ± 2.3	43 ± 1.8	12.2 ± 0.9
3.00	74 ± 3.2	45 ± 3.3	14.7 ± 0.7

Table B-3 Water contact angle of aminolyzed PCL films after the reaction with a solution of 0.1 M DSC/DMSO as a function of activation time

Activation time (h)	Water contact angle (degree)	
	θ_A	θ_R
0.00	73 ± 1.9	42 ± 1.5
0.50	78 ± 2.4	43 ± 1.5
0.75	87 ± 2.4	43 ± 1.7
1.00	89 ± 2.1	45 ± 1.1
2.00	89 ± 1.9	43 ± 1.9
3.00	91 ± 2.6	44 ± 1.1

Table B-4 Advancing water contact angle (θ_A) and amount of amino group of activated aminolyzed PCL films after the reaction with 10 mg/mL collagen solution as a function of immobilization time

Immobilization time (h)	θ_A (degree)	NH ₂ concentration/surface area ($\times 10^{-8}$ mol/cm ²)
0	89 ± 2.1	-
4	67 ± 2.4	0.71 ± 0.1
8	62 ± 1.9	1.03 ± 0.2
24	59 ± 1.3	1.31 ± 0.1
48	60 ± 2.4	1.38 ± 0.2

Table B-5 Advancing water contact angle (θ_A) and amount of amino group of activated aminolyzed PCL films after the immobilization with biomolecules for 24 h as a function of biomolecule concentration

Biomolecule concentration (mg/mL)	θ_A (degree)		
	Collagen marine	Chitosan (MW = 15,000)	Chitosan (MW = 83,000)
1	69 ± 1.9	74 ± 1.3	76 ± 1.7
3	65 ± 1.7	71 ± 1.1	70 ± 1.5
5	61 ± 2.1	72 ± 2.2	71 ± 2.3
7	62 ± 1.6	73 ± 2.3	71 ± 2.6
10	59 ± 1.3	72 ± 2.5	73 ± 1.8

Table B-6 Amount of amino group on activated aminolyzed PCL films after the immobilization with biomolecules for 24 h as a function of biomolecule concentration

Biomolecule concentration (mg/mL)	NH ₂ concentration/surface area ($\times 10^{-8}$ mol/cm ²)		
	Collagen marine	Chitosan (MW = 15,000)	Chitosan (MW = 83,000)
1	0.49 ± 0.01	0.11 ± 0.01	0.21 ± 0.02
3	0.63 ± 0.01	0.23 ± 0.02	0.27 ± 0.01
5	1.05 ± 0.02	0.22 ± 0.01	0.30 ± 0.02
7	1.26 ± 0.02	0.23 ± 0.03	0.28 ± 0.03
10	1.31 ± 0.01	0.25 ± 0.01	0.31 ± 0.03

Table B-7 Water contact angle and amount of carboxyl group of PCL-g-PAA films as a function of photo-oxidation time. The graft copolymerization was conducted in 10% AA solution at 30°C for 1 h

Photo-oxidation time (h)	Water contact angle (degree)		COOH concentration/surface area ($\times 10^{-8}$ mol/cm ²)
	θ_A	θ_R	
0.00	85 ± 1.3	50 ± 1.3	0 ± 0.0
0.25	67 ± 1.8	30 ± 3.2	6.3 ± 0.5
0.50	45 ± 3.3	0 ± 0.0	9.1 ± 0.4
1.00	62 ± 1.1	32 ± 2.3	8.8 ± 0.5
2.00	70 ± 1.5	34 ± 3.1	6.2 ± 0.3

Table B-8 Water contact angle and amount of carboxyl group of PCL-g-PAA films as a function of grafting time. The graft copolymerization was conducted in 10% AA solution at 30°C after photo-oxidation for 30 min

Grafting time (h)	Water contact angle (degree)		COOH concentration/surface area ($\times 10^{-8}$ mol/cm ²)
	θ_A	θ_R	
0.00	85 ± 1.3	50 ± 1.3	0 ± 0.0
0.50	61 ± 2.6	34 ± 2.3	4.1 ± 0.4
0.75	55 ± 2.7	25 ± 2.7	5.9 ± 0.2
1.00	45 ± 3.3	0 ± 0.0	9.1 ± 0.4
2.00	58 ± 3.1	31 ± 1.4	7.8 ± 0.4
3.00	68 ± 3.7	32 ± 2.5	7.1 ± 0.3

Table B-9 Water contact angle and amount of carboxyl group of PCL-g-PAA films as a function of acrylic acid concentration. The graft copolymerization was conducted at 30°C for 1 h after photo-oxidation for 30 min

Acrylic acid concentration (%)	Water contact angle (degree)		COOH concentration/surface area ($\times 10^{-8}$ mol/cm ²)
	θ_A	θ_R	
0	85 ± 1.3	50 ± 1.3	0 ± 0.0
1	66 ± 2.6	30 ± 2.3	3.2 ± 0.3
5	61 ± 2.7	28 ± 2.7	6.6 ± 0.5
10	45 ± 2.6	0 ± 0.0	9.1 ± 0.4
15	54 ± 3.1	27 ± 1.4	6.3 ± 0.3

Table B-10 Water contact angle of PCL-g-PAA films after the reaction with aqueous solution of 0.1 M NHS/0.4 M EDCI as a function of activation time

Activation time (h)	Water contact angle (degree)	
	θ_A	θ_R
0.00	45 ± 1.9	0 ± 0.0
0.50	54 ± 2.4	32 ± 2.3
0.75	58 ± 2.9	35 ± 2.4
1.00	70 ± 2.1	38 ± 2.7
2.00	68 ± 2.4	37 ± 2.1
3.00	67 ± 1.9	38 ± 2.1

Table B-11 Advancing water contact angle (θ_A) and amount of amino group of activated PCL-g-PAA films after the reaction with 10 mg/mL collagen solution as a function of immobilization time

Immobilization time (h)	θ_A (degree)	NH ₂ concentration/surface area ($\times 10^{-8}$ mol/cm ²)
0	70 \pm 2.1	-
4	54 \pm 2.2	1.12 \pm 0.1
8	48 \pm 2.1	1.43 \pm 0.1
24	34 \pm 2.3	1.77 \pm 0.2
48	34 \pm 1.9	1.78 \pm 0.2

Table B-12 Advancing water contact angle (θ_A) of activated PCL-g-PAA films after the immobilization with biomolecules for 24 h as a function of biomolecule concentration

Biomolecule concentration (mg/mL)	θ_A (degree)		
	Collagen marine	Chitosan (MW = 15,000)	Chitosan (MW = 83,000)
1	59 \pm 2.6	72 \pm 1.5	67 \pm 1.1
3	46 \pm 2.3	69 \pm 2.4	62 \pm 2.1
5	35 \pm 2.4	70 \pm 2.4	63 \pm 2.1
7	36 \pm 1.3	69 \pm 1.6	64 \pm 1.3
10	34 \pm 2.3	67 \pm 1.7	63 \pm 1.6

Table B-13 Amount of amino group on activated PCL-g-PAA films after the immobilization with biomolecules for 24 h as a function of biomolecule concentration

Biomolecule concentration (mg/mL)	NH ₂ concentration/surface area ($\times 10^{-8}$ mol/cm ²)		
	Collagen marine	Chitosan (MW = 15,000)	Chitosan (MW = 83,000)
1	0.95 ± 0.1	0.51 ± 0.08	0.58 ± 0.09
3	1.24 ± 0.1	0.78 ± 0.03	0.87 ± 0.04
5	1.66 ± 0.2	0.77 ± 0.05	0.87 ± 0.06
7	1.75 ± 0.1	0.76 ± 0.05	0.89 ± 0.03
10	1.77 ± 0.2	0.78 ± 0.05	0.88 ± 0.05

APPENDIX C

Table C-1 Keratinocyte (HEK001) cell adhesion and proliferation on modified and unmodified PCL films

Materials	12 h Adhesion		2 day Proliferation		4 day Proliferation	
	Abs	% relative to TCPS	Abs	% relative to TCPS	Abs	% relative to TCPS
TCPS	0.437 ± 0.05	100 ± 11	0.470 ± 0.02	100 ± 5	0.549 ± 0.05	100 ± 9
PCL	0.268 ± 0.01	61 ± 3	0.294 ± 0.01	62 ± 2	0.310 ± 0.04	56 ± 7
Aminolyzed PCL	0.388 ± 0.03	77 ± 7	0.342 ± 0.01	73 ± 3	0.352 ± 0.06	64 ± 10
Aminolyzed PCL-collagen marine	0.339 ± 0.03	78 ± 7	0.345 ± 0.02	73 ± 4	0.357 ± 0.03	65 ± 5
Aminolyzed PCL-collagen type I	0.385 ± 0.03	88 ± 7	0.392 ± 0.03	83 ± 7	0.403 ± 0.03	73 ± 6
Aminolyzed PCL-collagen type IV	0.356 ± 0.04	82 ± 9	0.383 ± 0.03	81 ± 6	0.399 ± 0.05	73 ± 9
Aminolyzed PCL-chitosan 15000	0.339 ± 0.03	78 ± 6	0.352 ± 0.04	75 ± 8	0.375 ± 0.04	68 ± 8
Aminolyzed PCL-chitosan 83000	0.354 ± 0.04	81 ± 9	0.360 ± 0.03	77 ± 6	0.391 ± 0.02	71 ± 3
PCL-g-PAA	0.396 ± 0.02	91 ± 5	0.405 ± 0.03	86 ± 6	0.411 ± 0.03	75 ± 5
PCL-g-PAA-collagen marine	0.384 ± 0.04	88 ± 10	0.394 ± 0.02	84 ± 4	0.412 ± 0.04	75 ± 8
PCL-g-PAA-collagen type I	0.450 ± 0.02	103 ± 4	0.461 ± 0.02	98 ± 4	0.470 ± 0.01	86 ± 2
PCL-g-PAA-collagen type IV	0.410 ± 0.02	94 ± 5	0.451 ± 0.04	96 ± 9	0.459 ± 0.03	84 ± 5
PCL-g-PAA-chitosan 15000	0.335 ± 0.04	77 ± 9	0.404 ± 0.01	86 ± 2	0.418 ± 0.03	76 ± 6
PCL-g-PAA-chitosan 83000	0.380 ± 0.04	87 ± 9	0.414 ± 0.01	88 ± 3	0.419 ± 0.03	76 ± 5

Table C-2 Fibroblast (L929) cell adhesion and proliferation on modified and unmodified PCL films

Materials	12 h Adhesion		2 day Proliferation		4 day Proliferation	
	Abs	% relative to TCPS	Abs	% relative to TCPS	Abs	% relative to TCPS
TCPS	0.275 ± 0.04	100 ± 14	0.336 ± 0.01	100 ± 4	0.767 ± 0.04	100 ± 5
PCL	0.191 ± 0.01	69 ± 2	0.234 ± 0.01	70 ± 3	0.503 ± 0.03	66 ± 4
Aminolyzed PCL	0.221 ± 0.03	80 ± 10	0.283 ± 0.03	84 ± 8	0.630 ± 0.07	82 ± 9
Aminolyzed PCL-collagen marine	0.227 ± 0.02	83 ± 8	0.297 ± 0.02	88 ± 6	0.596 ± 0.03	78 ± 3
Aminolyzed PCL-collagen type I	0.273 ± 0.04	99 ± 13	0.288 ± 0.02	86 ± 5	0.607 ± 0.04	79 ± 5
Aminolyzed PCL-collagen type IV	0.253 ± 0.03	92 ± 10	0.265 ± 0.03	79 ± 8	0.638 ± 0.04	83 ± 5
Aminolyzed PCL-chitosan 15000	0.259 ± 0.03	94 ± 11	0.278 ± 0.03	83 ± 8	0.654 ± 0.08	85 ± 10
Aminolyzed PCL-chitosan 83000	0.271 ± 0.02	98 ± 6	0.295 ± 0.04	88 ± 11	0.631 ± 0.02	82 ± 2
PCL-g-PAA	0.261 ± 0.01	95 ± 4	0.278 ± 0.04	83 ± 12	0.557 ± 0.05	73 ± 7
PCL-g-PAA-collagen marine	0.241 ± 0.03	88 ± 10	0.303 ± 0.03	90 ± 10	0.586 ± 0.10	76 ± 13
PCL-g-PAA-collagen type I	0.285 ± 0.01	104 ± 4	0.323 ± 0.04	96 ± 12	0.625 ± 0.04	82 ± 5
PCL-g-PAA-collagen type IV	0.279 ± 0.03	102 ± 9	0.299 ± 0.02	89 ± 5	0.614 ± 0.04	80 ± 5
PCL-g-PAA-chitosan 15000	0.253 ± 0.03	92 ± 10	0.284 ± 0.04	85 ± 12	0.579 ± 0.03	76 ± 4
PCL-g-PAA-chitosan 83000	0.297 ± 0.03	108 ± 11	0.311 ± 0.01	93 ± 4	0.602 ± 0.04	79 ± 6



VITAE

Miss Waradda Mattanavee was born in Chonburi, Thailand, on September 11th, 1980. She received Bachelor Degree of Science (Industrial Chemistry) from Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang in 2003. In the same year, she started as a Master Degree student with a major in Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University and completed the program in 2006.