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APPENDICES

APPENDIX A

The average grafting yield

The grafting yield of graft polymerization was calculated by following equation;

$$\text{Grafting yield (\%)} = \frac{(\text{Weight}_{\text{after grafting}} - \text{Weight}_{\text{(before grafting)}})}{\text{Weight}_{\text{(before grafting)}}} \times 100 \qquad \text{A-1}$$

Table A-1. The average grafting yield of vulcanized and unvulcanized rubber as a function of time using 0.5 M PEGMA.

Grafting time (min)	**Average grafting yield (%)**	
	Unvulcanized rubber	**Vulcanized rubber**
30	0.348±0.167	0.146±0.128
60	0.522±0.077	0.237±0.024
90	0.730±0.166	0.528±0.044
120	1.369±0.380	0.791±0.032
150	1.496±0.335	0.846±0.103
180	2.592±0.518	1.096±0.197

Table A-2. The average grafting yield of vulcanized and unvulcanized rubber as a function of PEGMA concentration for 150 min.

Monomer concentration (M)	Average grafting yield (%)	
	Unvulcanized rubber	Vulcanized rubber
0.1	0.505±0.080	0.307±0.043
0.2	0.733±0.150	0.389±0.033
0.3	1.112±0.117	0.571±0.040
0.4	1.030±0.519	0.629±0.052
0.5	1.496±0.335	0.846±0.103

Table A-3. The average grafting yield of vulcanized and unvulcanized rubber as a function of time with 0.5 M VPy.

Grafting time (min)	Average grafting yield (%)	
	Unvulcanized rubber	Vulcanized rubber
30	0.113±0.167	0.000±0.000
60	0.228±0.080	0.101±0.076
90	0.284±0.056	0.153±0.042
120	0.318±0.070	0.155±0.024
150	0.353±0.045	0.189±0.085
180	0.411±0.008	0.242±0.027

Table A-4. The average grafting yield of vulcanized and unvulcanized rubber as a function of VPy concentration for 150 min.

Monomer concentration (M)	Average grafting yield (%)	
	Unvulcanized rubber	**Vulcanized rubber**
0.1	0.209±0.053	0.081±0.017
0.2	0.251±0.074	0.105±0.016
0.3	0.269±0.015	0.136±0.074
0.4	0.292±0.042	0.165±0.073
0.5	0.353±0.045	0.189±0.085

Table A-5. Water contact angle of vulcanized NR and unvulcanized NR after being graft copolymerized by 0.5 M PEGMA and VPy as a function of time.

Grafting time (min)	Water contact angle (degree)			
	U-g-PEGMA	**V-g-PEGMA**	**U-g-VPy**	**V-g-VPy**
0	86.18 ± 1.82	88.45 ± 3.72	86.18 ± 1.82	88.45 ± 3.72
30	79.38 ± 3.29	81.43 ± 1.71	81.08 ± 1.38	87.85 ± 1.13
60	74.23 ± 2.05	79.73 ± 2.80	79.75 ± 0.81	84.70 ± 1.54
90	73.80 ± 1.35	74.10 ± 1.90	78.1 ± 1.48	81.48 ± 3.67
120	72.50 ± 4.54	72.53 ± 1.58	77.70 ± 1.59	79.10 ± 1.42
150	71.28 ± 1.42	70.30 ± 4.60	75.15 ± 1.75	77.85 ± 1.47
180	59.23 ± 3.90	66.95 ± 1.77	66.95 ± 2.25	74.48 ± 3.32

Table A-6. Water contact angle of vulcanized NR and unvulcanized NR after being graft copolymerized by PEGMA and VPy for 150 min as a function of monomer concentration.

Monomer Concentration (M)	Water contact angle (degree)			
	U-g-PEGMA	V-g-PEGMA	U-g-VPy	V-g-VPy
0	86.18 ± 1.82	88.45 ± 3.72	86.18 ± 1.82	88.45 ± 3.72
0.1	76.50 ± 2.64	76.68 ± 1.13	80.00 ± 2.03	82.28 ± 2.87
0.2	76.33 ± 1.61	74.90 ± 2.22	79.03 ± 1.74	81.83 ± 2.95
0.3	74.63 ± 2.79	73.4 ± 0.94	78.25 ± 1.05	79.43 ± 1.77
0.4	73.78 ± 3.89	72.35 ± 2.09	76.90 ± 3.04	78.40 ± 0.88
0.5	71.28 ± 1.42	70.3 ± 4.60	75.15 ± 1.75	77.85 ± 1.47

Determination of Benzophenone residue

Table A-7 Standard benzophenone solution, for the calibration curve, was prepared according to the following table.

Standard	Solution (mL)	Ethanol (mL)	Benzophenone conc (μg/mL)
S_1	10.0 of Benzophenone solution (100 (μg/mL)[a]	30	25
S_2	12 of S_1	3.0	20
S_3	9.0 of S_2	3.0	15
S_4	6.0 of S_3	3.0	10
S_5	4.0 of S_4	4.0	5
S_6	4.0 of S_5	4.0	2.5
S_7	4.0 of S_6	6.0	1.0
S_8	4.0 of S_7	4.0	0.5
S_9	4.0 of S_7	4.0	0.25

After reading the UV absorbance of the samples and standard benzophenone solution at λ = 251 nm. the calibration curve was plotted as shown in Figure A-1. The benzophenone concentration was determined from the calibration curve (C; μg/mL). The amount of benzophenone (B) in the original solution (5 mL) from the sampling sample (1 mL)

Total amount of benzophenone (B) = C (μg/mL) x 5 (mL) A-2

After that the determined of the amount of adsorbed protein/surface area was calculated as follow;

Adsorbed protein/surface area B_{ads} = B/surface area (2 sides) (μg/cm^2) A-3

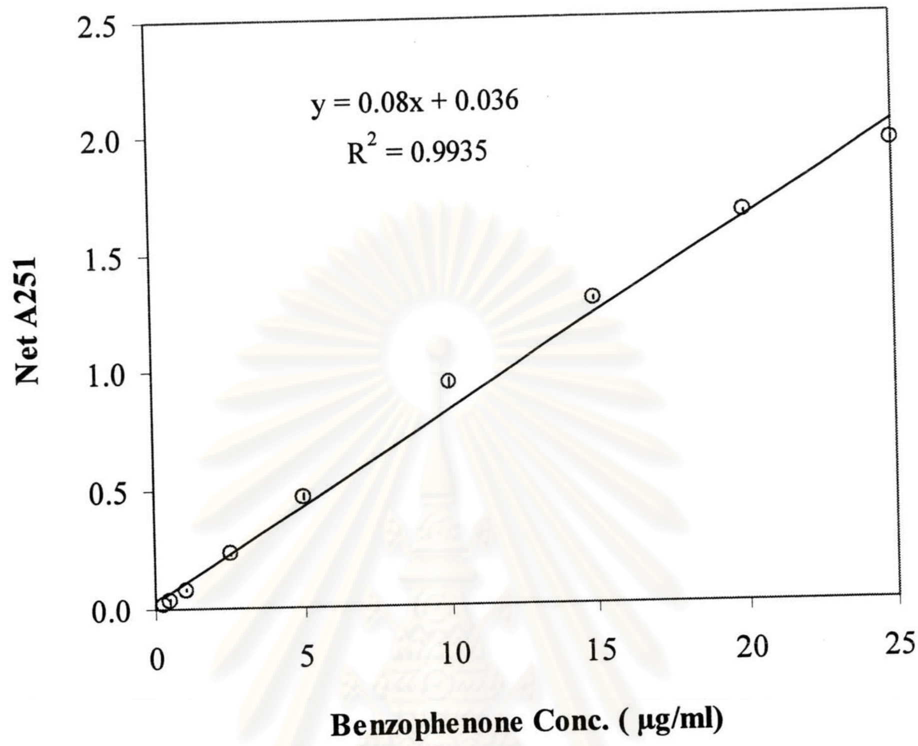

Figure A-1 A calibration curve of the amount of benzophenone as a function of UV absorbance at 251 nm.

Table A-8 The amount of benzophenone per surface area (µg/cm^2) dissolving out from vulcanized and unvulcanized NR before and after graft copolymerization.

Grafting condition	Amount of Benzophenone residue (µg/ml)	
	Unvulcanized rubber	Vulcanized rubber
Before grafting	32.14 ± 1.77	42.91 ± 1.78
After grafting for 30 min	5.09 ± 0.45	3.58 ± 0.84
After grafting for 180 min	4.80 ± 1.20	4.30 ± 0.73

APPENDIX B

Bicinchoninic Acid Assay

Bicinchoninic acid assay is a method used for determination of the amount of proteins. The standard reagents used in this method are reagent A, reagent B and reagent C. Reagent A consists of an aqueous solution of Na_2tartrate, Na_2CO_3, $NaHCO_3$ in 0.2 M NaOH, pH 11.25. Reagent B is 4% (W/V) bicinchoninic acid solution, pH 8.5. Reagent C is 4% $CuSO_4 \cdot 5H_2O$ in deionized water.

The principle of the bicinchoninic assay (BCA) relies on the formation of a Cu^{2+}-protein complex under alkaline conditions, followed by reduction of the Cu^{2+} to Cu^{1+}. The amount of reduction is proportional to protein present. It has been shown that the peptide bond is able to reduce Cu^{2+} to Cu^{1+}. BCA forms a purple-blue complex with Cu^{1+} in alkaline environments, thus providing a basis to monitor the reduction of alkaline Cu^{2+} by proteins.[30] Figure B-1 shows complexation between bicinchoninic acid and Cu^{1+}.

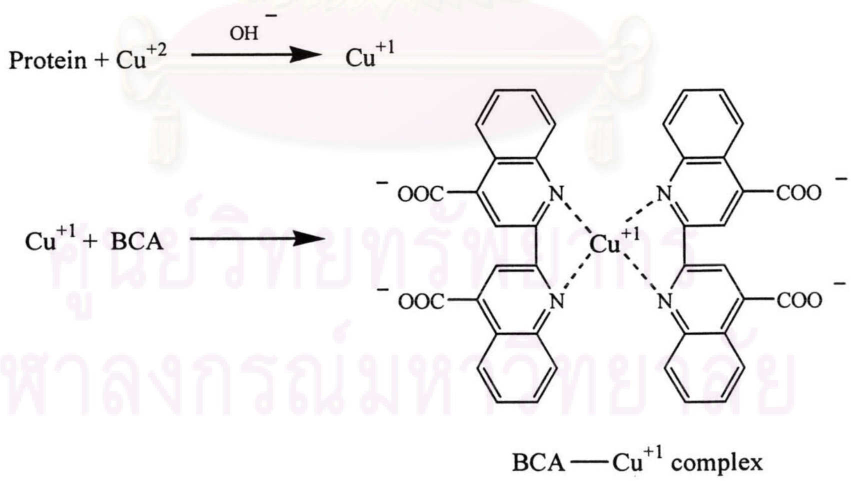

Figure B-1. Formation of purple complex between BCA and cuprous ion generated from the biuret reaction.

Calculation of Protein Adsorption

Table B-1 Standard BSA solution, for the calibration curve.

Standard	Solution (mL)	SDS (mL)	BSA conc (μg/mL)
S_1	0.5 of BSA (1000 (μg/mL)[a]	4.5	100
S_2	4.0 of S_1	4.0	5.0
S_3	4.0 of S_2	4.0	25
S_4	4.0 of S_3	6.0	10
S_5	4.0 of S_4	4.0	5
S_6	4.0 of S_5	4.0	2.5
S_7	4.0 of S_6	6.0	1.0
S_8	4.0 of S_7	4.0	0.5

a : standard BSA was pipette from 1 mg/mL ampule

After reading the UV absorbance of the samples and standard BSA solution at λ = 562 nm. , the result was then calculated for the net absorbance by substracting the absorbance of the blank (SDS).

$$\textbf{Net } A_{562} = \textbf{recorded } A_{562} - A_{562}\textbf{ (blank)} \qquad \text{B-1}$$

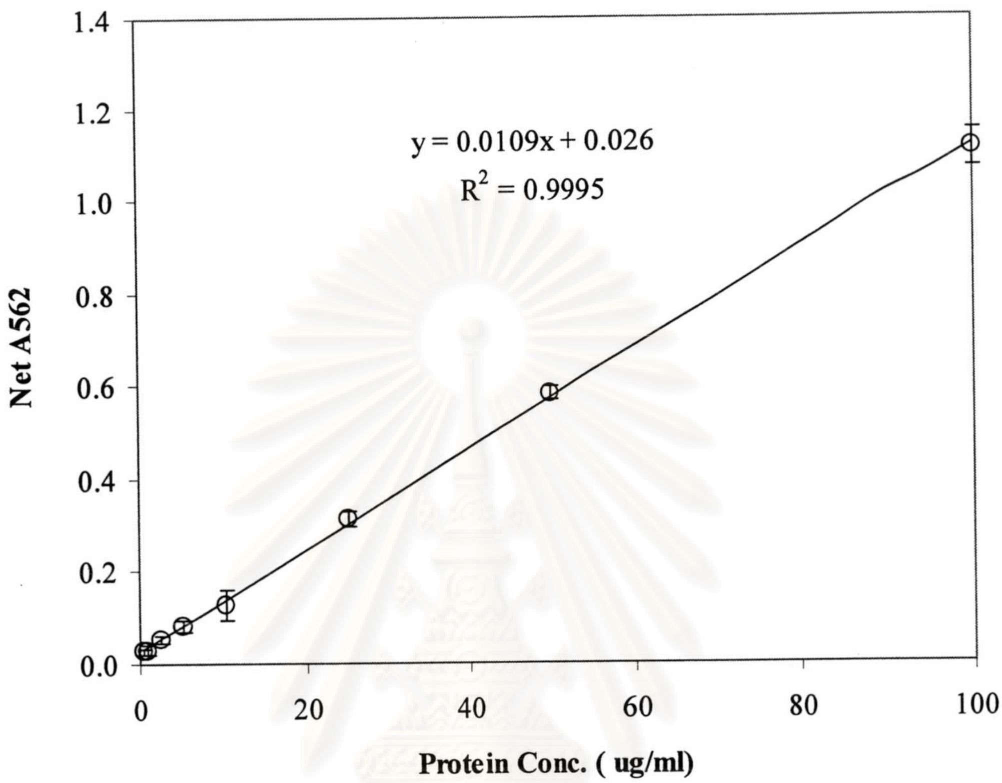

Figure B-2 A calibration curve of the amount of albumin adsorbed and the absorbance obtained from BCA microassay.

The protein concentration (C; µg/mL) in each well was determined from the calibration curve. The total amount of protein (P) in the original solution (1 mL) was calculated from the sampling sample (100 µL) + BCA working solution (100 µL)

$$\textbf{Total amount of protein (P)} = \frac{C\ (\mu g/mL) \times 200\ (\mu L)}{1000\ (\mu L/mL)} \times \frac{1000\ (\mu L)}{100\ (\mu L)} \qquad \text{B-2}$$

$$\textbf{Adsorbed protein/surface area } P_{ads} = \text{P/surface area (2 sides) } (\mu g/cm^2) \qquad \text{B-3}$$

Table B-2 The amount of plasma protein adsorption per surface area (μg/cm^2) of Vulcanized and unvulcanized rubber before and after graft copolymerization with PEGMA and VPy as function of time.

Grafting time (min)	The amount of plasma protein adsorption (μg/cm^2)			
	U-g-PEGMA	V-g-PEGMA	U-g-VPy	V-g-VPy
0	26.396±3.980	12.834±1.570	26.396±3.980	12.834±1.570
30	8.380±4.100	11.184±4.100	19.419±0.711	12.437±3.115
60	3.286±0.999	10.648±0.999	13.673±3.799	11.069±1.375
90	0.596±0.531	6.424±0.531	7.807±3.174	8.016±3.497
120	-0.304±1.153	2.275±1.153	5.536±0.887	7.671±2.923
150	-1.027±0.844	0.551±0.844	3.046±0.431	6.449±1.053
180	-0.767±2.839	-0.174±2.839	2.612±0.862	3.743±0.526

Table B-3 The amount of plasma protein adsorption per surface area (μg/cm^2) of Vulcanized and unvulcanized rubber before and after graft copolymerization with PEGMA and VPy as function of monomer concentration.

Monomer Concentration (M)	The amount of plasma protein adsorption (μg/cm^2)			
	U-g-PEGMA	V-g-PEGMA	U-g-VPy	V-g-VPy
0	26.396±3.980	12.834±1.570	26.396±3.980	12.834±1.570
0.1	4.939±0.612	10.596±2.358	8.102±4.347	11.220±1.620
0.2	4.536±1.669	7.064±3.177	7.013±3.085	10.447±1.966
0.3	0.099±0.642	3.261±0.714	5.723±1.156	9.134±1.083
0.4	-0.347±0.311	1.122±1.059	3.269±1.077	8.443±0.547
0.5	-1.027±1.794	0.551±0.774	3.046±0.431	6.449±1.053

APPENDIX C

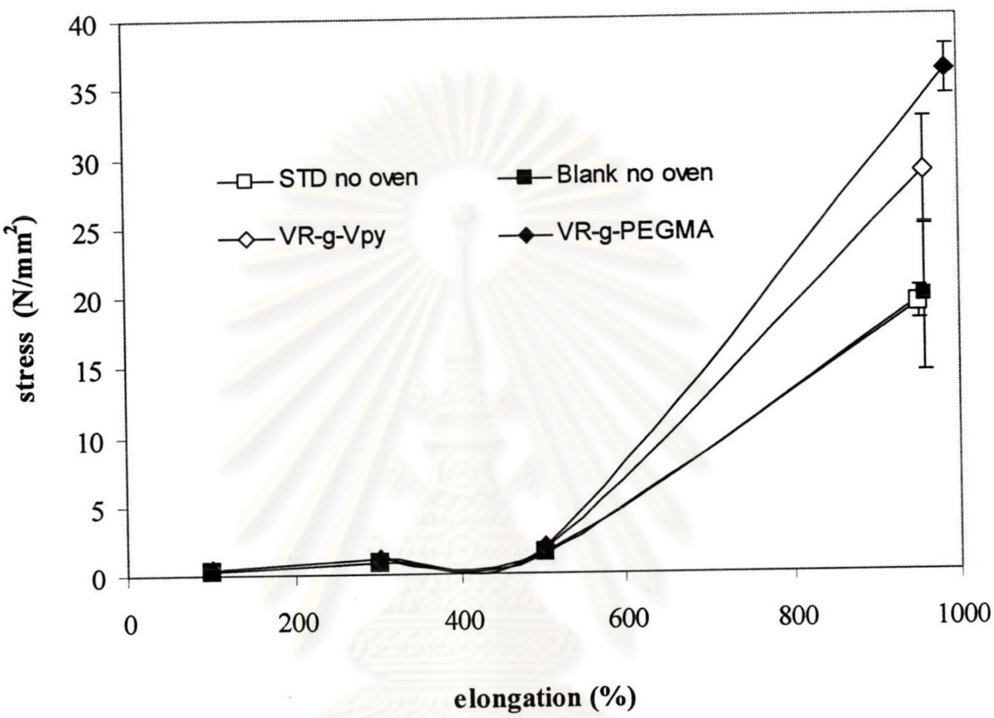

Figure C-1 Stress-strain curves of NR latex films before and after graft copolymerization using 0.5 M PEGMA and VPy for 150 min

VITAE

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