

REFERENCES

- Alenius, H., Kalkkinen, N., Lukka, M., Reunala, T., Turjanmaa, K., Makinen-Kiljunen, S., Yip, E. and Palosuo, T. 1995. Prohevein from the rubber tree (*Hevea brasiliensis*) is a major latex allergen. *Clin Exp Allergy*. 24. 659-665.
- Archer, B.L., Barnard, D., Cockbain, E.G., Dickenson, P.B. and McMullen, A.I. 1963. Structure, composition and biochemistry of *Hevea* latex. In L. Bateman (ed). The chemistry and physics of rubber-like substances. London : MacLavenan sons. p 41
- Archer, B.L. 1976. Hevamine : a crystalline basic protein from *Hevea brasiliensis* latex. *Phytochemistry*. 15 : 297-300.
- Aroonvisoot, P. and Makuuchi K. 1990. Selection of Hydroperoxides as Co-Sensitizer for n-butyl acrylate. *Proc. Int. Symp. Radiat. Vulcanisation of natural latex. JAERI-M Japan* : 305.
- Asao, M., Kunisawa, S., Minoura, Y. 1961. Gamma-ray vulcanisation of natural rubber latex. *Kogyo Kahaku Zasshi*. 64: 2066-2071.
- ASTM standard test method for analysis of protein in natural rubber and its products. 1995. ASTM Designation : D 5712-99. American Society for Testing and Materials.
- Blackley, D.C. 1966. High polymer latices. London : Maclarenan sons. p 214-273.
- Bloomfield, G.F. 1973. Super rubbers for engineers. *Rubber Developments*. 26 : 74-77.
- Brehler, R. and Kutting, B. 2001. A problem of interdisciplinary concern in medical. *Arch. Intern. Med*. 161 : 1057-1064.
- Bryan, G.W. and Keith W.S. 1998. Nitrosamine formation in rubber: I. Influence of mixing history. *J.Rub. Chem. Tech*. 71 : 310-322.
- Caruley, M., Shah, S. and Gleeson, R. 1994. Latex hypersensitivity in children with

- myelodysplasia. *J Allergy Clin Immunol.* 93 : 181.
- Dennis, R. and Ownby, M.D. 2002. A history of latex allergy. *J. Allergy Clin Immunol.* 10 : s27-s32.
- Elliotti, D.J., Skinner, T.D. and Smith, J.F. 1970. Compounding of natural rubber for engineering application. *Nat Rub Tech.* 1 : 3.
- Fong, C.S. 1992. Introduction of natural rubber latex. *Rubb. Res. Inst. Malaysia.*
- Fong, C.S. 1992. Protein in natural rubber concentrated latex. *J nat rubb.* 7 : 102-112.
- Ghazaly, H.M., Properties of natural rubber low protein latex. Latex proteins and glove industry. 81- 91. 1993.
- Gehman S.D. and Auerbach I. 1956. Gamma-ray vulcanisation of rubber. *Int. J. App. Radiat. Iso.* 1 : 102-114.
- Habibah, S. and Lim, S.C. 1986. An analysis of current natural rubber production capacity, *Proceedings Rubber Grower's Conference, Res. Inst. Malaysia.* p 19-25.
- Hadijiliadis D., Kan, K., Tarlo, S. 1995. Skin test responses to latex in an allergy and asthma Clinic. *J Allergy Clin Immunol.* 96 : 431-432.
- Haque, M. E., Dafader, N.C., Akhtar, T. and Ahmad, M.U. 1996. Radiation dose required for the vulcanization of natural rubber latex. *Radiat. Phys. Chem.* 48 : 505-510.
- Hasma, H. 1992. Protein of NRL concentrated. *J. Nat. Rubb. Res.* 7 : 102-112.
- Hien, N.Q., Binh, D., and Thien, V.T., Hai, L., Man, N.T. and Thanh V.T. 1990. Development of Surgical gloves from radiation vulcanized natural rubber latex *Proc. Int. Symp. Radiat. Vulcanisation of natural latex, JAERI-M Japan* : 260.
- International standard for determination of water-extractable protein in medical gloves made from natural rubber latex, using the modified Lowry *method Reference number of document* : ISO/DIS 12243. International Standard Organisation.

- Jacob, J. and Vijayakumar, K.C. 1997. Radiation vulcanised latex more safe and better. *Rubber Asia*. 11 : 23-24.
- Jacob, J.L., J., d' Auzac, and Prevot, J.C. 1993. The composition of latex from *Heavea brasiliensis*. *Clin Rev. Al*. 11 : 327-337.
- Jacob, J.L., Prevot, J.C., Roussel, D., Lacrotte., R., Serres, E., d'Auzac, J., Eschbach, J. M., Omont, H. 1989. Yield limiting factors, latex physiological parameters, latex diagnosis and clonal typology. In: d'Auzac, J., Jacob, J-L, Chrestin, H.(Eds.) *Physiology of rubber tree latex. CRC Press. Boca Raton* : 345-382.
- Jager, D., Kleinhans, D., Czuppon, A., Baur, X. 1992. Latex-specific proteins causing immediate-type cutaneous, nasal, bronchial and systemic reactions. *J Allergy Clin Immunol*. 89:759- 768.
- Kartowardoyo S. and Sundardi F. 1977. Studies on the preparation and uses of Co-60 gamma ray irradiated natural latex. *J. Appl. Polym Sci*, 21: 3077.
- Kevin, J.K. 1999. Latex allergy. MD *slide presentation sample* Creative Education Options, LLC.
- Kekwick, R.G.O. 1993. Origin and source of latex protein allergy. Latex protein allergy : the present position. UK : Rubber Consultants. p 21-24.
- Lamm A. and Lamm G. 1964. Some important factors in the radiation treatment of natural rubber latex. *Radiat. Chem., Akade' miai Kiado' Budapest* : 245.
- Linzad, N.J. 1996. Radiation vulcanization improves purity, quality. *Rubber Asia*. 10 : 41.
- McFadden, R.E. 2002. Natural rubber latex sensitivity seminar ; Conference summary, *J. Allergy Clin. Immunol* 110 : s137-s140.
- Mctherell, C. 1980. The effect of protein on the hysterical properties of natural rubber vulcanizaties. *Plast. And Rubb. Mat. Appl.* : 1-15.

- Makuuchi, K. 2003. An introduction to radiation vulcanization of natural rubber latex. EB system Corporation, Takasaki Japan.
- Makuuchi, K. and Hagiwara, M. 1984. Radiation vulcanisation of natural rubber latex with polyfunctional monomers. *J. App. Polym. Sci.* 29 : 965.
- Makuuchi, K., Ratnayake, U., Yoshii, F., Soebianto, Y.S. 2000. Protein free radiation vulcanisation NRL. *Proceedings of the IRC 2000*, Helsinki, Finland.
- Makuuchi, K. and Tsushima, K. 1985. Radiation vulcanisation of natural rubber latex with acrylic monomers. *Int. Nat. Rub. Conf.*, Kualalumpur, Malaysia.
- Nagasawa N., Yoshii, F., Motomo, H. and Kume, T. 2000 Radiation degradation of alginate and chitosan. *JAERI-Conf.* 2000-003.
- Ngamlert, P. 2002. Development of test kit for protein allergens in NRL and rubber products. Master's Thesis, Biochemistry, Chulalongkorn University.
- Perera, M.C.S. and Siriwardena, T.A.S. 1985. Effect on moisture on the process ability and physical properties of natural rubber. *J. Rubb. Res. Inst. Sri Lanka.* 64 : 1-6.
- Ratnayake, U., Makuuchi, K. and Yoshii F. 1999. Quality improvement of radiation vulcanized natural rubber latex by addition of polyvinyl alcohol and centrifugation. *J. Rubb. Res. Institute of Sri Lanka*, 82 : 8-21.
- Ratnayake, U., Makuuchi, K. and Yoshii F. 2001. Soluble protein free radiation vulcanised natural rubber latex. *J. Rubb. Res.* 4 : 153-163.
- Robert, O.B. 1978. The vanderbilt rubber handbook. R.T. Vanderbilt company.
- Rogero, S.O., Lugao A.B., Yoshii, F. and Makuuchi, K. 2003. Extractable proteins from irradiated field natural rubber latex. *Radiat Phys chem.* 67 : 501-503.
- RRIM test methods for standard Malaysian Rubbers. SMR bull no. 7 *Rub Res Inst.* Malaysia. 1992.
- Sanjeera, R. P., Sarasriathyamma, C. K. and Sethuraj, M.B. 1998. Studies on the

- relationship between yield and meteorological parameters of para rubber tree (*Hevea brasiliensis*). *Agricultural and Forest Meteorology*. 90 : 235-245.
- Sebastian, M.S. How radiation vulcanised latex is better. 1993. *Rubber Asia* 13 : 76-79.
- Shukri B. HJ., Wagab, AB., Makuuchi. K. Devendra, R. and Pansa, C-P. 1990. Effect of heating and leaching on mechanical properties of radiation vulcanized natural rubber latex film. *Proc. Int. Symp. Radiat. Vulcanisation of natural latex, JAERI-M Japan* : 207.
- Slater, J.E., Trybul, D.E. 1994. Affinity purification of latex antigens. *J Allergy Clin Immunol*. 93: 644- 649.
- Smith, R.H. 1953. Phosphatides of Heavea latex. *Proc. Rubb. Res. Inst. Malaya*. 14 : 169.
- Soebianto, Y. S. and Sundardi, F. 1989. Effect of hydrogen peroxide on radiation vulcanisation of natural rubber latex sensitized with carbon tetrachloride and n-butyl acrylate. *Proc. Int. Symp. Radiat. Vulcanisation of natural latex, JAERI-M Japan* : 329.
- Sumbogo, D.E., Sundardi, F. and Utama, M. 1990. Effect of antioxidant on the aging property of rubber film prepare from radiation natural rubber latex. *Proc. Int. Symp. Radiat. Vulcanisation of natural latex, JAERI-M Japan* : 234.
- Tata, S.J. 1980. Distribution of proteins between the fractions of *Heavea* latex separated by ultracentrifugation. *J Rubb. Res. Inst. Malasia*. 282 : 77-85.
- Tanaka, Y. 1984. NMR and macromolecules. In J.C. Randall (ed.) American Chemical Society. p 233.
- Tomazic, V.J., Champagne, E.L and Lamanna, 1994.A. Cornstarch powder on latex products is an allergen carrier. *J Allergy Clin Immunol*. 93 : 751-758.

- Tsushima, K., Machuuchi, K., Yoshii, F. and Ishigaki, I. 1990 Commercialization of protective rubber gloves by radiation vulcanisation. *Proc. Int. Symp. Radiat. Vulcanisation of natural latex, JAERI-M Japan* : 89-228.
- Turjanmaa, K., Alenius, H., Makinen-Kiljunen, S., Polosuo, T. and Reunala, T. 1996. Natural rubber latex allergy. (review) *Allergy*, 51 : 593-602.
- Turjanmaa, K., Laurila, K., Makinen-Kiljunen, S. and Reunala R. Rubber contact urticaria. 1988. *Allergic properties of 19 brands of latex glove Contact Dermatitis*. 19 : 362-367.
- Wahab, S.B., Sulaiman, S.B., Makuuchi, K., Devendra, R. and Pansa, C.P. 1990. Stabilization of radiation vulcanised natural rubber latex. *Proc. Int. Symp. Radiat. Vulcanisation of natural latex, JAERI-M Japan* : 245.
- Vaghese, S., Katsumura, Y., Makuuchi, K. and Yosii, F. 2000. Production of soluble protein free latex by radiation process. *Rubb. Chem. Tech.* 73 : 80-88.
- Varghese, S. Makuuchi, K., Yoshii, F. and Katsumura, Y. 1999. Effect of water soluble polymer on radiation vulcanization natural rubber latex. *Rubb. Chem. Technol.* 72 : 308-312.
- Vongbupnimit, K. 1992. A study of gamma radiation vulcanisation of natural rubber latex. Master's Thesis, Mahidol University.
- Zhonghai, C. and Makuuchi, K. 1989. N-butyl acrylate as a sensitizer of radiation vulcanisation of natural rubber latex. *Proc. Int. Symp. Radiat. Vulcanisation of natural latex, JAERI-M Japan* : 326.



APPENDICES

ศูนย์วิทยทรัพยากร
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Appendix 1

Determination of Dry Rubber Content (%)

Lot No.	DRC (%)
8/01/01	30.25
27/07/01	40.76
11/10/01	35.57
9/11/01	34.99
12/12/01	35.58
19/01/02	34.99
25/5/02	40.78
12/11/02	33.54
14/12/02	31.66
19/01/03	31.25
26/06/03	41.39
21/07/03	35.91
12/09/03	36.50
4/11/03	34.56

Calculation : FFL lot No. 27/07/01 5.21 g was pipetted in to a petrifidish and coagulated with 5 % acetic acid in ethyl alcohol. After complete coagulation, the coalum was then removed, washed with water, creped and dried in a oven at 60°C. Dried coagulum was 5.2134 g and calculated DRC content by the equation below.

$$\% \text{ DRC} = W_1/W_0 \times 100$$

Where W_1 = weight of the dry rubber (g)

W_0 = weight of the latex taken (g)

$$\% \text{ DRC} = 2.12/5.21 \times 100 = 40.76 \%$$

Appendix 2

Protein determination by modified Lowry method

1. Solution for modified Lowry method
 - 1.1 With presence of CuSO_4

Solution C : 6 % w/v of sodium carbonate

Solution D : 1.5% w/v of copper sulfate in 3%w/v of sodium citrate

Reagent A: Alkali copper sulfate (10 parts of C: 0.2 part of D)

Reagent B : Diluted Folin Reagent
 - 1.2 With absence of CuSO_4

Solution DC : 6 % w/v of sodium carbonate

Solution DD : 3%w/v of sodium citrate

Reagent A : (10 parts of DC: 0.2 part of DD)

Reagent B : Diluted Folin Reagent
2. Measurement water extractable protein with CuSO_4 by modified Lowry method
(ASTM D 5712-99 and ISO DIS 12243)

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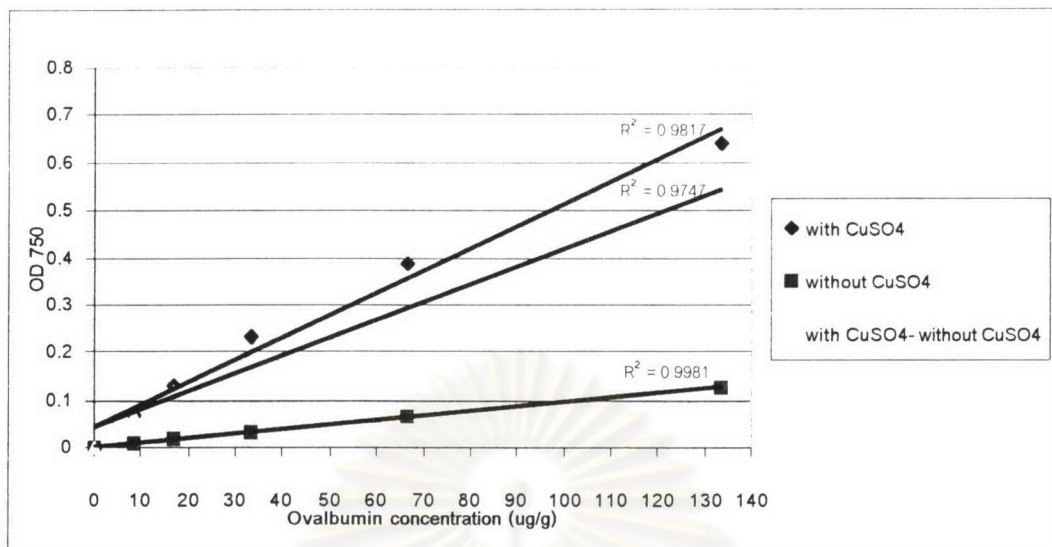


Figure A1 standard curve of ovalbumin measured by modified Lowry method (ASTM D5712-99)

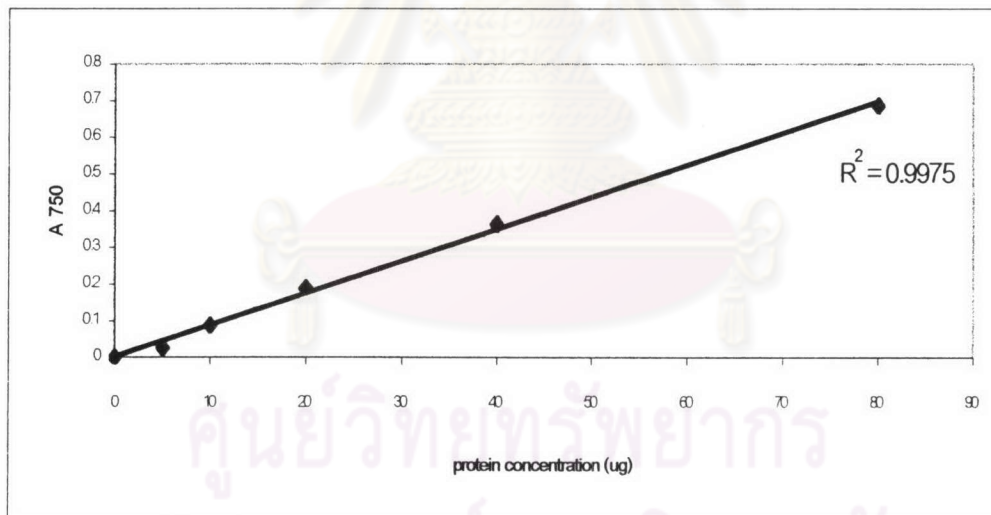


Figure A2 standard curve of ovalbumin measured by modified Lowry method (ISO DIS 12243)

1. WEP of fresh field latex

Lot No.	WEP content (mg/g rubber)
8/01/01	27
27/07/01	55
11/10/01	41
9/11/01	48
12/12/01	35
19/01/02	21
25/5/02	73
12/11/02	48
14/12/02	47
19/01/03	27
26/06/03	48
21/07/03	45
12/09/03	42
4/11/03	40

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2.WEP of 60% concentrated latex

Lot No.	WEP content (mg/g rubber)
8/01/01	2.1
27/07/01	5.0
11/10/01	1.2
9/11/01	1.4
19/01/02	1.4
25/5/02	4.2
12/11/02	0.7
14/12/02	2.4
19/01/03	1.1
26/06/03	3.6
21/07/03	1.8
12/09/03	0.8
4/11/03	0.7

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3. 3. WEP of irradiated latex film (Determination by modified Lowry method
(ASTM D 5712-99)

sample	OD750, CuSO ₄	OD750, NO CuSO ₄	OD750, CuSO ₄ -NO CuSO ₄	Protein, μg/well of titer plate	Protein, mg/g rubber
FFL	0.57	0.11	0.46	112.15	48.3
CL	0.15	0.03	0.12	21.10	1.40
1	0.95	0.18	0.77	119.97	2.70
2	0.42	0.14	0.28	57.50	1.80
3	0.30	0.07	0.23	52.10	1.30
20k	0.39	0.07	0.32	72.77	41.10
40k	0.35	0.07	0.28	62.77	79.70
60k	0.28	0.05	0.23	52.74	70.90
80k	0.19	0.03	0.16	31.19	61.50
100k	0.34	0.06	0.28	61.76	92.60
120k	0.26	0.04	0.22	46.86	58.70

Calculation:

CL : OD 750 = 0.12, Protein evaluated from standard protein ovalbumin = 21.1

Extraction : 3.556 g of CL film/ 35 ml of water then the solution was lyophilized and re-dissolved of 500 μl water

Therefore WEP = $21.10 \times 500 / 2 = 5,275$

Total WEP = $5275 / 3.55 = 1,483 \mu\text{g/g rubber}$

= 1.40 mg/g rubber

4. WEP of irradiated and added alginate Lot No.9/11/01 (Determination by modified Lowry method (ASTM D 5712-99)

Source of RF	Treatment		OD750, CuSO ₄	OD750, NO CuSO ₄	OD750, CuSO ₄ -NO CuSO ₄	Protein, ug/well of titer plate	Protein, mg/g rubber
	kGy	polymer					
FFL	0	0	0.72	0.14	0.58	112.15	48.30
CI	0	0	0.23	0.05	0.18	21.10	1.40
IRR-CL	1	0	0.95	0.18	0.77	119.97	2.70
	2	0	0.42	0.14	0.28	57.50	1.80
	3	0	0.30	0.07	0.23	52.10	1.30
AG-CL	0	1	0.71	0.15	0.56	134.96	5.80
	0	2	0.37	0.07	0.30	65.46	3.50
	0	3	0.72	0.15	0.57	136.52	6.90
IRR-AG-CL	1	1	0.66	0.13	0.53	124.62	2.40
	1	2	0.43	0.08	0.35	73.25	1.50
	1	3	0.67	0.13	0.54	124.80	1.70
	2	1	0.50	0.09	0.41	89.20	1.10
	2	2	0.15	0.04	0.11	14.59	1.40
	2	3	0.20	0.03	0.17	26.00	1.80
	3	1	0.50	0.11	0.39	112.00	1.40
	3	2	0.47	0.08	0.39	87.00	1.10
	3	3	0.67	0.13	0.54	125.00	8.70

5. WEP of irradiated and added alginate Lot No.25/5/01 (Determination by modified Lowry method (ASTM D 5712-99))

Source of RF	Treatment		OD750, CuSO ₄	OD750, NO CuSO ₄	OD750, CuSO ₄ -NO CuSO ₄	Protein, ug/well of titer plate	Protein, mg/g rubber
	kGy	polymer					
FFL	0	0	0.66	0.13	0.53	125.00	73.10
CI	0	0	0.40	0.08	0.32	71.53	4.20
IRR-CL	1	0	0.59	0.12	0.47	110.00	3.60
	2	0	0.42	0.08	0.34	73.82	2.30
	3	0	0.49	0.09	0.40	91.65	2.40
AG-CL	0	1	0.39	0.06	0.33	68.82	3.70
	0	2	0.22	0.03	0.19	35.80	1.60
	0	3	0.39	0.07	0.32	69.10	4.20
IRR-AG-CL	1	1	0.23	0.03	0.20	40.20	2.20
	1	2	0.19	0.03	0.16	31.37	2.00
	1	3	0.09	0.01	0.08	8.84	0.70
	2	1	0.04	0.00	0.04	5.99	0.40
	2	2	0.09	0.01	0.08	9.90	0.50
	2	3	0.13	0.03	0.10	16.73	0.90
	3	1	0.21	0.03	0.18	36.96	2.40
	3	2	0.12	0.02	0.10	14.17	1.10
	3	3	0.37	0.07	0.30	69.83	5.30

6. WEP of irradiated and added carrageenan Lot No.9/11/01 (Determination by modified Lowry method (ASTM D 5712-99))

Source of RF	Treatment		OD750,	OD750,	OD750,	Protein,	Protein,
	kGy	polymer	CuSO ₄	NO CuSO ₄	CuSO ₄ -NO CuSO ₄	ug/well of titer plate	mg/g rubber
FFL	0	0	0.58	0.12	0.46	112.15	48.30
CI	0	0	0.10	0.01	0.09	10.55	1.40
IRR-CL	1	0	0.65	0.13	0.52	130.00	2.70
	2	0	0.35	0.07	0.28	57.50	1.80
	3	0	0.29	0.05	0.24	52.12	1.30
CA-CL	0	1	0.25	0.04	0.21	41.00	1.80
	0	2	0.14	0.02	0.12	20.00	1.00
	0	3	0.24	0.04	0.20	42.00	2.20
IRR-CA-CL	1	1	0.60	0.12	0.48	114.30	0.90
	1	2	0.56	0.11	0.45	107.34	2.50
	1	3	0.36	0.07	0.29	66.47	0.90
	2	1	0.08	0.00	0.08	7.54	0.20
	2	2	0.31	0.06	0.25	56.51	2.60
	2	3	0.18	0.03	0.15	25.40	0.90
	3	1	0.24	0.03	0.21	44.58	0.90
	3	2	0.42	0.08	0.34	79.94	1.70
	3	3	0.26	0.08	0.18	32.56	0.70

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7. WEP of irradiated, added alginate and leaching 10 min Lot No.9/11/01

(Determination by modified Lowry method (ASTM D 5712-99)

Source of RF	Treatment		OD750, CuSO ₄	OD750, NO CuSO ₄	OD750, CuSO ₄ -NO CuSO ₄	Protein, μg/well of titer plate	Protein, mg/g rubber
	kGy	polymer					
FFL	0	0	0.27	0.04	0.23	42.97	1.10
CI	0	0	0.04	0.02	0.02	ND	ND
IRR-CL	1	0	0.02	0.01	0.01	ND	ND
	2	0	0.03	0.02	0.01	ND	ND
	3	0	0.01	0.01	0.00	ND	ND
AG-CL	0	1	0.21	0.02	0.19	34.50	1.50
	0	2	0.09	0.01	0.08	9.80	0.50
	0	3	0.22	0.20	0.20	39.10	2.10
IRR-AG-CL	1	1	0.05	0.03	0.02	21.00	0.06
	1	2	0.03	0.02	0.01	ND	ND
	1	3	0.07	0.01	0.06	ND	ND
	2	1	0.03	0.02	0.01	ND	ND
	2	2	0.20	0.01	0.19	25.42	1.00
	2	3	0.07	0.03	0.04	16.00	0.60
	3	1	0.05	0.03	0.02	ND	ND
	3	2	0.05	0.03	0.02	ND	ND
	3	3	0.05	0.03	0.02	12	0.02

8. WEP of added alginate and irradiated Lot No.19/1/03 (Determination by modified Lowry method (ISO DIS 12243))

Source of RF	Treatment		rubber (g)	v	F	OD 750	Protein, ug/tube	* protein (ug/g rubber) = (c*v*5)/(w*F)	Protein, mg/g rubt
	kGy	polymer							
FFL	0	0	1.61	17	1	0.51	51.40	2710.30	27.10
CI	0	0	2.13	22	1	0.24	20.74	1069.57	1.10
IRR-CL	1	0	1.90	20	1	0.26	25.45	1339.47	1.30
	2	0	1.87	19	1	0.32	31.91	1619.36	1.60
	3	0	2.56	26	1	0.42	41.62	2113.52	2.10
CA-CL	0	1	2.08	21	1	0.42	42.08	2125.25	2.10
	0	2	2.74	28	1	0.26	26.36	1345.39	1.30
	0	3	2.35	24	1	0.37	37.25	1901.32	1.90
IRR-CA-CL	1	1	1.26	13	1	0.08	8.20	424.36	0.40
	1	2	1.69	17	1	0.41	40.98	2063.57	1.20
	1	3	2.45	25	1	0.27	26.87	1373.72	1.40
	2	1	2.84	29	1	0.04	3.55	181.19	0.20
	2	2	2.59	26	1	0.03	3.26	163.76	0.20
	2	3	2.42	25	1	0.58	58.21	3005.47	3.00
	3	1	2.06	21	1	0.08	7.71	392.22	0.40
	3	2	1.89	19	1	0.16	15.63	785.22	0.80
	3	3	2.16	22	1	0.36	36.20	1846.94	1.80

9. WEP of added alginate irradiated and leaching 10 min Lot No.19/1/03

(Determination by modified Lowry method (ISO DIS 12243))

Source of RF	Treatment		rubber (g)	v	F	OD 750	Protein, ug/tube	Protein, mg/tube
	kGy	polymer						
FFL	0	0	2.51	26	1.00	0.06	6.44	0.30
CI	0	0	1.75	18	2.50	0.08	7.71	0.20
IRR-CL	1	0	1.76	18	2.50	0.08	8.12	0.20
	2	0	1.55	16	2.50	0.37	36.50	0.20
	3	0	2.24	23	2.50	0.07	7.42	0.20
AG-CL	0	1	1.72	18	2.50	0.10	9.98	0.20
	0	2	2.62	27	2.50	0.15	14.67	0.30
	0	3	3.98	40	2.50	0.06	5.80	0.10
IRR-AG-CL	1	1	1.61	17	2.50	0.12	11.50	0.20
	1	2	1.73	18	2.50	0.18	17.66	0.40
	1	3	1.87	19	2.50	0.30	29.99	0.60
	2	1	1.41	15	2.50	0.01	1.10	ND
	2	2	1.46	15	2.50	0.01	1.20	ND
	2	3	1.79	18	2.50	0.09	24.00	0.50
	3	1	1.54	16	2.50	0.10	9.88	0.20
	3	2	1.95	20	2.50	0.12	12.00	0.20
	3	3	1.96	20	2.50	0.41	15.00	0.30

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10. WEP of CCL, DP-CL, Ag-CL, IRR CL and leaching film in various time.

(Determination by modified Lowry method ISO DIS 12243)

Lot No.	Source of sample	rubber (g)	v	F	OD 750	Protein, Evaluated from std protein	protein (ug/g)
24/6/03	CCL	3.63	37	1.00	0.68	71.26	3563.00
	DPCL	3.48	35	5.00	0.08	8.35	84.00
	C CL-IRR	3.15	32	1.00	0.71	74.17	3708.00
	DPCL-IRR	3.25	33	2.50	0.21	22.29	446.00
11/7/03	CCL	3.23	33	1.00	0.33	36.60	1830.00
	DPCL	3.15	32	5.00	0.05	5.45	54.00
	CCL-IRR	1.25	13	1.00	0.39	42.12	2105.00
	DPCL-IRR	1.22	13	1.67	0.11	10.11	303.00
21/7/03	CCL	2.59	26	1.00	0.40	36.81	1840.00
	DPCL	3.27	33	5.00	0.12	10.92	109.00
	CCL-IRR	0.93	10	1.25	0.55	53.80	2152.00
	DPCL-IRR	0.29	3	2.50	0.19	18.54	371.00
12/9/03	CCL	1.55	16	2.50	0.40	38.78	776.00
	AGCL	1.49	15	2.50	0.14	13.65	273.00
	CCL-IRR	0.97	10	1.67	0.33	31.63	947.00
	AGCL-IRR	0.96	10	2.50	0.20	19.40	388.00
4/11/03	CCL	3.63	37	1.00	0.13	13.93	697.00
	AGCL	3.64	37	1.00	0.05	5.07	253.00
	CCL-IRR	1.42	15	1.25	0.21	23.49	940.00
	AGCL-IRR	1.59	16	1.25	0.11	12.74	510.00

Source of sample	Source of sample	rubber (g)	v	F	A 750	Protein, Evaluated from std protein	protein (ug/g)
11/7/03	CCL- L10	1.32	14	5	0.53	57.36	608.00
	DPCL- L10	0.89	9	5	0.02	2.47	25.00
	CCL-IRR- L10	1.25	13	5	0.55	59.83	620.00
	DPCL-IRR- L10	1.27	13	5	0.26	28.47	293.00
	CCL- L30	1.236	13	5	0.24	26.49	279.00
	DPCL- L30	1.541	16	5	0.01	0.95	10.00
	CCL-IRR- L30	1.145	12	5	0.22	23.84	250.00
	DPCL-IRR -L30	1.256	13	5	0.11	12.23	127.00
21/7/03	CCL- L10	1.295	13	5	0.70	76.20	765.00
	DPCL- L10	1.271	13	5	0.06	6.63	68.00
	CCL-IRR- L10	1.253	13	5	0.22	23.74	246.00
	DPCL-IRR- L10	1.295	13	5	0.04	4.10	41.00
	Cont CL- L30	1.145	12	5	0.26	27.96	293.00
	DPCL- L30	1.365	14	5	0.04	3.91	40.00
	CCL-IRR- L30	1.236	13	5	0.09	9.88	104.00
	DPCL-IRR -L30	1.325	14	5	0.03	3.07	32.00
12/9/03	CCL -L10	1.541	16	5	0.22	24.03	250.00
	AGCL- L10	1.352	14	5	0.08	8.57	89.00
	CCL-IRR- L10	1.256	13	5	0.23	24.77	256.00
	AGCL-IRR- L10	1.958	20	5	0.04	3.90	40.00
	CCL- L30	1.325	14	5	0.11	11.83	125.00
	AGCL- L30	1.231	13	5	0.06	6.76	71.00
	CCL-IRR- L30	1.255	13	5	0.12	12.80	133.00

	Source of sample	rubber (g)	v	F	A 750	Protein, Evaluated from std protein	protein (ug/g)
4/11/03	AGCL-IRR- L30	1.35	14	5	0.02	1.924	20.00
	CCL -L10	1.37	14	5	0.39	42.50	436.00
	AGCL- L10	1.37	14	5	0.16	17.41	179.00
	CCL-IRR- L10	1.66	17	5	0.30	33.00	339.00
	AGCL-IRR- L10	1.26	13	5	0.06	6.28	65.00
	C CL- L30	1.59	16	5	0.18	19.95	201.00
	AGCL- L30	1.24	13	5	0.04	3.84	40.00
	CCL-IRR- L30	1.24	13	5	0.14	14.67	154.00
	AGCL-IRR- L30	1.37	14	5	0.03	2.78	29.00

L10 : Leaching 10 min, L30 : Leaching 30 min

Calculation :

Ag 4/11/03L10 : OD₇₅₀ = 0.08, Protein evaluated from standard protein ovalbumin = 17.40

µg

Extraction: 1.37 g of DP / 14 ml of water then the solution was precipitated and re-dissolved of 0.8 ml

$$\begin{aligned} \text{Extractable protein in } \mu\text{g/g rubber} &= (V \times C \times 5) / (F \times M) \\ &= (14 \times 17.40 \times 5) / (5 \times 1.37) \\ &= 178.50 \mu\text{g/g rubber} \end{aligned}$$

where, V= volume of extraction medium used (ml)

C = protein concentration of the redissolved protein solution (µg)

F = concentration factor

M = mass in grams of rubber film

Appendix 3

Recovery yield (%) of concentrated latex

1. Recovery yield of irradiated and polymer added concentrated latex

sample	treatment		alginate			carrageenan		
	IRR (kGy)	NP (phr)	DRC (%)	Latex recovery (g)	% recovery	DRC (%)	Latex recovery (g)	% recovery
CL lot 9/11/01	0	0	59.57	82	58	59.57	82	58
IRR-CL	1	0	51.56	70	42	51.56	70	42
IRR-CL	2	0	51.24	68	41	51.24	68	41
IRR-CL	3	0	53.06	67	42	53.06	67	42
NP-CL	0	1	54.4	70	45	42.51	52	26
NP-CL	0	2	55.1	69	45	43.22	55	28
NP-CL	0	3	53.83	70	45	42.25	57	29
IRR-NP-CL	1	1	53.83	70	45	43.83	40	21
IRR-NP-CL	1	2	54.15	75	48	44.15	43	23
IRR-NP-CL	1	3	55.97	65	43	35.97	59	25
IRR-NP-CL	2	1	53.01	75	47	43.01	49	25
IRR-NP-CL	2	2	51.14	89	54	41.14	65	32
IRR-NP-CL	2	3	54.17	80	52	34.17	73	30
IRR-NP-CL	3	1	57.08	75	51	47.08	43	25
IRR-NP-CL	3	2	50.37	76	46	40.37	59	28
IRR-NP-CL	3	3	49.08	80	47	37.08	62	27

DRC of Fresh field latex = 34.99% (lot no 9/11/01), each treatment started with 240 g rubber.

2. Recovery yield of polymer added and irradiated concentrated latex

sample	treatment		Recovery yield of alginate added CL-IRR		
	IRR (kGy)	NP (phr)	DRC (%)	Latex recovery (g)	% Recovery
CL lot 19/1/03	0	0	61.02	82	58
IRR-CL	1	0	54.24	71	51
IRR-CL	2	0	54.98	75	55
IRR-CL	3	0	55.24	75	55
NP-CL	0	1	56.55	74	56
NP-CL	0	2	56.9	75	57
NP-CL	0	3	57.24	75	57
IRR-NP-CL	1	1	54.36	70	51
IRR-NP-CL	1	2	54.21	72	52
IRR-NP-CL	1	3	55.97	78	58
IRR-NP-CL	2	1	53.01	74	52
IRR-NP-CL	2	2	53.21	75	53
IRR-NP-CL	2	3	54.17	74	53
IRR-NP-CL	3	1	54.98	69	51
IRR-NP-CL	3	2	55.21	69	51
IRR-NP-CL	3	3	55.44	80	53

DRC of Fresh field latex = 31.25% (lot no. 9/1/03), each treatment started with 240 g rubber.

3.Recovery yield of concentrated latex

sample	DRC of FFL	Latex start (kg)	DRC of 60%CL	Latex recovery (kg)	% Recovery
control 24/6/03	41.39	0.8	55.97	0.34	57.46
DPCL 24/6/03	41.39	0.8	63.55	0.35	67.17
control 11/7/03	33.23	200	59.18	70	62.33
DPCL 11/7/03	33.23	200	56.81	65	55.56
control 21/7/03	35.91	200	60.22	70	58.69
DPCL21/7/03	35.91	200	60.02	70	58.50
control 12/9/03	36.5	170	61.85	60	59.81
AI 12/9/03	36.5	170	54.02	80	69.65
control 4/11/03	34.56	200	59.98	67	58.14
AI 4/11/03	34.56	200	63.53	74	68.02

Calculation

Fresh field latex lot 24/6/03 0.8 kg, DRC = 41.39 %

Total dry rubber content is $(41.39 \times 0.8) / 100 = 0.33$ kg rubber/ 0.8 kg latex

CL prepared from FFL 0.34 kg, DRC = 55.97 %

Total dry rubber content is $(55.97 \times 0.34) / 100 = 0.19$ kg rubber / 0.34 kg latex

% recovery = $(0.19 \times 100) / 0.33 = 57.47\%$

Appendix 4

Solution for SDS-PAGE

1. Tris glycine electrode buffer (25 mM Tris, 192 mM glycine)

Tris	3.0	g
Glycine	14.4	g
SDS	1.0	g
H ₂ O	1	L

2. Tris-HCl stock solution pH 8.8

(2 M Tris)

Tris	24.2	g
H ₂ O	100	ml

(adjust pH to 8.8 with HCl_{conc.} Or 0.1 M NaOH)

3. Tris-HCl stock solution pH 6.8

(1 M Tris)

Tris	12.2	g
H ₂ O	100	ml

(adjust pH to 8.8 with HCl_{conc.} Or 0.1 M NaOH)

4. Sample buffer

Tris-HCl stock solution pH 6.8	0.6	ml
10 % SDS	2	ml
2-mercaptoethanol	0.5	ml
1% bromophenol blue	1	ml
H ₂ O	0.9	ml

5. Acrylamide stock (30%)

	Acrylamide	29.2	g
	Bis	0.8	g
	H ₂ O	100	ml
6.	Ammonium persulfate	0.1	g/ml
7.	15% Separating gel		
	Stock gel (30%)	10	ml
	Stock buffer pH8.8	5	ml
	H ₂ O	5	ml
	Ammonium persulfate	100	μl
	TEMED	10	μl
8.	Stacking gel		
	Stock gel (30%)	1.34	ml
	Stock buffer pH 6.8	2.0	ml
	H ₂ O	4.6	ml
	Ammonium persulfate	60	μl
	TEMED	10	μl
1.	Staining solution		
	Commassie Blue R-250	1.0	g
	Methanol	450	ml
	Glacial acetic acid	100	ml
	H ₂ O	450	ml
2.	Destain solution		
	Glacial acetic acid	100	ml
	Methanol	100	ml
	H ₂ O	800	ml

Appendix 5

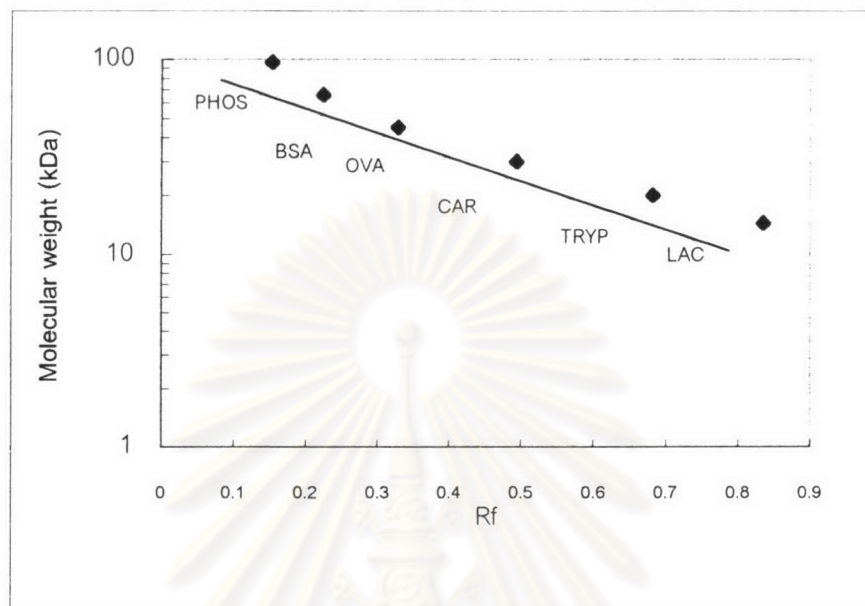


Figure: A3 Molecular weight markers calibration curve of SDS-PAGE

PHOS: Phosphorylase B 97 kDa,

BSA: Bovine serum albumin 66 kDa,

OVA: Ovabumin 66 kDa,

CAR: Carbonic anhydrase 30 kDa,

TRYP: Trypsin inhibitor 20.1 kDa

LAC: α - Lactalbumin 14.4 kDa

Appendix 6

Statistical calculation:

Statistical analysis of variance of TSC from DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	59.27	2.20	0.92
DPCL	3	60.95	3.16	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of DRC from DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	58.46	2.21	0.87
DPCL	3	60.27	3.60	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of NR from DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	0.72	0.19	0.51
DPCL	3	0.83	0.36	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of NH_3 from DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	0.60	0.006	2.44
DPCL	3	0.66	0.047	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of KOH from DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	0.36	0.06	1.14
DPCL	3	0.43	0.11	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of VFA DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	0.02	0.005	1.66
DPCL	3	0.04	0.021	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of Mg^{++} from DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	65.01	13.9	-0.47
DPCL	3	60.05	17.9	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of MST from DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	1482	272	-17.36
DPCL	3	283	119	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of nitrogen content from DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	0.16	0.005	-12.7
DPCL	3	0.09	0.01	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of WEP from DPCL

Sample	DF	\bar{X}	S.D	t
CL	3	2411	998	-146.5
DPCL	3	82	28	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical calculation:

Statistical analysis of variance of TSC from DPCL IRR

Sample	DF	\bar{X}	S.D	t
CL	3	52.15	1.14	1.65
DPCL	3	52.94	0.84	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of DRC from DPCL IRR

Sample	DF	\bar{X}	S.D	T
CL	3	51.42	1.24	0.17
DPCL	3	51.54	1.24	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of NR from DPCL IRR

Sample	DF	\bar{X}	S.D	T
CL	3	0.75	0.40	2.76
DPCL	3	1.40	0.41	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of NH_3 from DPCL IRR

Sample	DF	\bar{X}	S.D	T
CL	3	0.68	0.15	0.13
DPCL	3	0.68	0.09	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of VFA DPCL IRR

Sample	DF	\bar{X}	S.D	t
CL	3	0.05	0.006	-1.11
DPCL	3	0.04	0.020	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of Mg^{++} from DPCL IRR

Sample	DF	\bar{X}	S.D	T
CL	3	60.71	8.91	0.84
DPCL	3	69.90	18.96	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of MST from DPCL IRR

Sample	DF	\bar{X}	S.D	T
CL	3	1057	105	-8.55
DPCL	3	204	172	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of nitrogen content from DPCL -IRR

Sample	DF	\bar{X}	S.D	T
CL	3	0.16	0.005	-9.8
DPCL	3	0.10	0.01	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$

Statistical analysis of variance of WEP from DPCL-IRR

Sample	DF	\bar{X}	S.D	T
CL	3	2655	912	-55.2
DPCL	3	373	72	

$t_{0.05}$ – value from table at df. 2 is 4.3027

Accept $H_0 : \mu_1 = \mu_2$



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Appendix 7

Physical properties of 60% control and DPCL

Physical properties	Production date					
	24/6/03		11/7/03		21/7/03	
	Control	DPCL	Control	DPCL	Control	DPCL
Total solid content, (TSC; %)	56.58	63.99	59.81	57.69	61.16	61.18
Dry rubber content, (DRC; %)	55.97	63.55	59.18	56.81	60.22	60.02
Non rubber content, (NRC; %)	0.61	0.44	0.61	0.88	0.94	1.16
Ammonia (HA-L; %)	0.59	0.61	0.60	0.68	0.60	0.70
KOH (%)	0.36	0.45	0.30	0.53	0.41	0.31
Volatile fatty acid (VFA; %)	0.03	0.02	0.02	0.07	0.02	0.02
Magnesium content (ppm)	48.98	46.24	72.18	80.26	73.86	53.66
*Mechanical stability time, (MST; sec)	1358	420	1294	230	1794	199
**Deproteinized CL 60%						
Nitrogen content (%)	0.17	0.09	0.16	0.08	0.16	0.10
WEP ($\mu\text{g/g}$ rubber)	3563	84	1830	54	1840	109

* MST – determined 25 days after centrifugation

** Addition physical properties of DPCL

Effect of irradiation on physical properties of control and deproteinized irradiated concentrated latex

Physical properties	Production date					
	24/6/03		11/7/03		21/7/03	
	Control	DPCL	Control	DPCL	Control	DPCL
Total solid content, (TSC; %)	53.39	53.87	51.91	52.72	51.14	52.24
Dry rubber content, (DRC; %)	52.51	52.94	51.67	51.09	50.07	50.59
Non rubber content, (NRC; %)	0.88	0.93	0.3	1.63	1.07	1.65
Ammonia (HA-L; %)	0.51	0.59	0.79	0.69	0.73	0.77
Volatile fatty acid (VFA; %)	0.06	0.02	0.06	0.06	0.05	0.05
Magnesium content (ppm)	52.01	51.25	69.82	69.30	60.29	89.16
*Mechanical stability time, (MST; sec)	960	120	1041	90	1170	403
**Deproteinized CL 60%						
Nitrogen content (%)	0.16	0.10	0.16	0.09	0.15	0.11
WEP ($\mu\text{g/g}$ rubber)	3708	446	2105	303	2152	371

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Appendix 8

Trend of MST of concentrated latex

1. MST of non irradiated and irradiated CCL and DPCL lot No.11/7/03 and 21/7/03.

Source of sample	Days after centrifugation			
	3	17	25	32
CCL 11/7/03	230	1294	1200	900
DPCL 11/7/03	230	0	0	0
CCL-IRR 11/7/03	235	1261	1041	1003
DPCL-IRR 11/7/03	230	161	91	66
CCL 21/7/03	292	794	1200	1000
DPCL 21/7/03	143	199	129	100
CCL-IRR 21/7/03	292	694	1170	992
DPCL-IRR 21/7/03	143	161	193	180

Irradiated at day 3 after centrifugation

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2. MST of CCL and AGCL lot No.12/9/03 irradiated at various times

Source of sample	Days after centrifugation								
	1	17	27	37	143	150	165	172	179
CCL12/9/03	465	896	1396		894	823	585		
ACCL12/9/03	517	965	1447		630	769	679		
CCL-IRR 12/9/03 IRR D 17*		690	760	945					
AGCL-IRR 12/9/03 IRR D 17*		492	736	897					
CCL-IRR 12/9/03 IRR D143**					902	939	1146	1186	1094
AGCL-IRR 12/9/03 IRR D 143**					881	931	1195	1081	1068
CCL-IRR 12/9/03 IRR D150***						892	874	984	1008
AGCL-IRR 12/9/03 IRR D 150***						963	872	985	949

*Irradiated at day 17 after centrifugation

**Irradiated at day 143 after centrifugation

***Irradiated at day 150 after centrifugation

3. MST of non irradiated and irradiated control and added alginate latex lot No.4/11/03

Source of sample	Days after centrifugation					
	1	17	27	79	86	93
CCL4/11/03	143	859	1192	882	854	
AGCL4/11/03	131	874	1101	840	823	
CCL-IRR 4/11/03				882	1239	1067
AGCL-IRR4/11/03				840	900	931

Irradiated at day 79 after centrifugation

BIOGRAPHY

Miss Oranoot Haowuttikul was born on April 25, 1978. She graduated with the degree of Bachelor of Science in Biology from Burapha University in 2000. She continued her study in the Master Program of Biochemistry, Faculty of Science at Chulalongkorn University



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