CHAPTER III

RESULTS

3.1 Seasonal variation of dry rubber content (DRC) and water extractable protein (WEP) of fresh field latex (FFL)

Tapping season usually starts in May or June and end in January or February. The FFL was collected and processed to concentrated latex (CL) 60% during various months over the years 2001 - 2003. DRC was shown in Figure 3.1. Specimens from either FFL or CL was casted as thin rubber films, extracted for WEP according to method 2.8.1 and determined for WEP according to methods 2.10.

The results from Figure 3.1-3.2 showed that DRC and WEP of FFL in the beginning of tapping season and also during the rainy season (May-October) 48-73 mg/g rubber , were significantly higher than in the end of tapping season (November-February), 21-27 mg/g rubber. The variation of WEP in FFL is more or less parallel to % DRC which started at about 40-41% DRC in the beginning of tapping season, and decreased to 30% DRC toward the end of tapping season.

3.2 The effect of centrifugation on WEP of concentrated latex (CL) 60%

The same lot of FFL was centrifuged to 60% CL and WEP from dry films was determine as mentioned previously. Figure 3.3 shows the trend of WEP of concentrated latex films which was similar to WEP of FFL, but the amount of protein was only 5-10% of that observed in rubber film (RF) prepared from FFL.

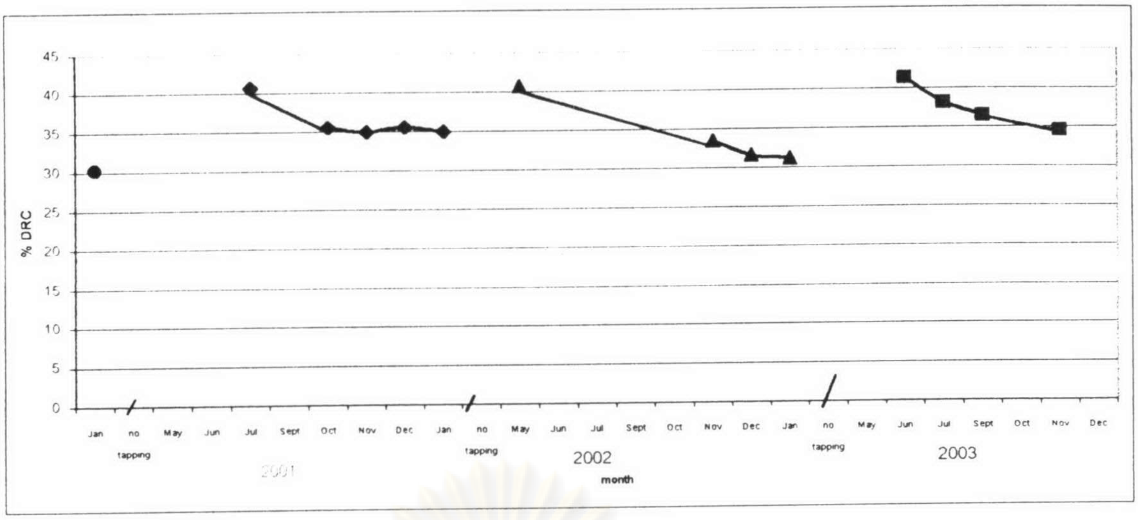


Figure 3.1 Dry rubber content of fresh field latex during 2001-2003

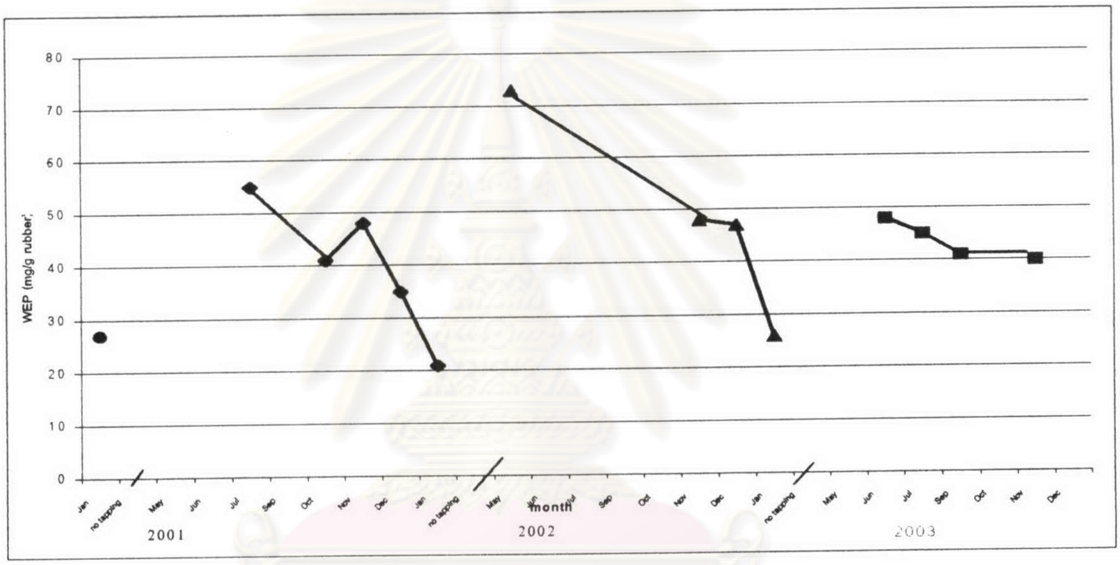


Figure 3.2 Trend of WEP content in rubber film prepared from FFL in 2001-2003

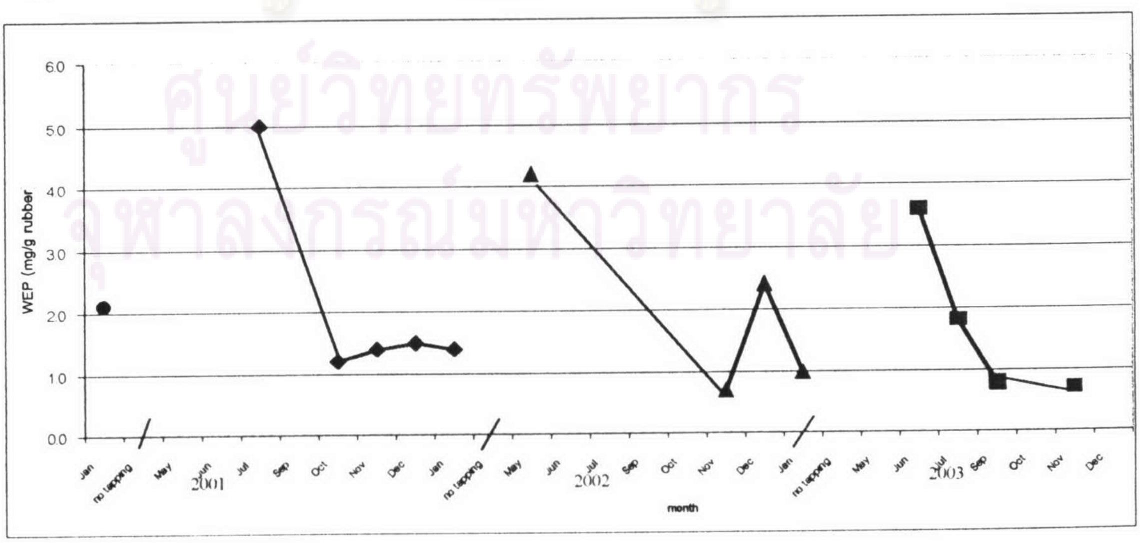


Figure 3.3 Effect of centrifugation on WEP in rubber film from CL 60% in 2001-2003

3.3 Effect of gamma radiation on protein

3.3.1 Effect of dose variation on the quantity of WEP from rubber film

FFL lot No. 9/11/01 was irradiated at dose 1, 2, 3, 20, 40, 60, 80, 100 and 120 kGy. The process was summarized in Figure 3.4

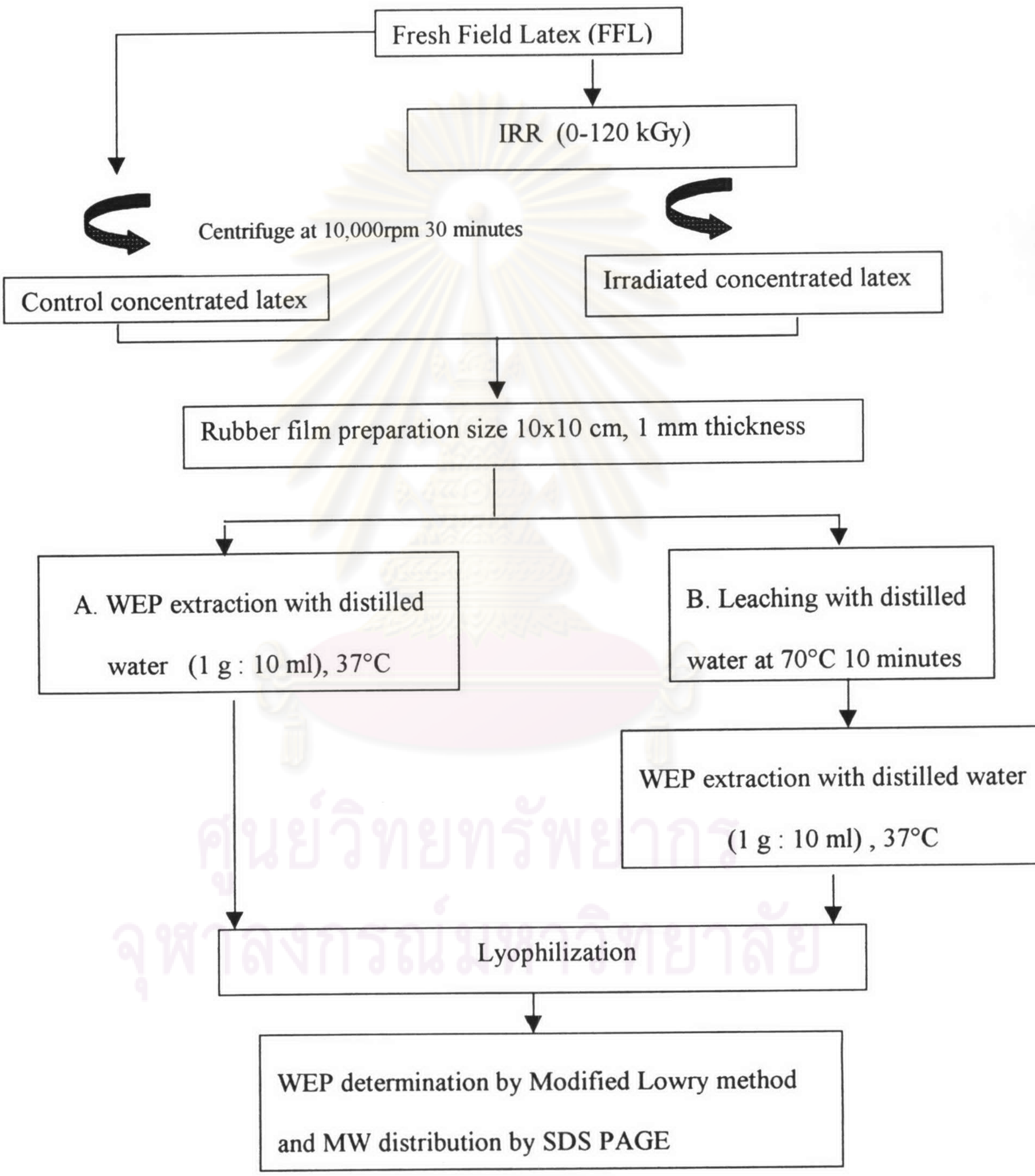


Figure 3.4 The protocol of irradiated film preparation and WEP extraction

The WEP of control non-irradiated CL subjected to centrifugation was significantly lower than FFL (Figure 3.5), which was evident for the effect of centrifugation on reducing WEP. Irradiation of FFL at low dose 1-3 kGy resulting in more or less the same amount of protein comparing to control (1.48 mg/g rubber) probably resulting from centrifugation. At high dose (20-120 kGy), the protein content, which should be less than control (1.48 mg/g rubber) turned out to be increased significantly and even higher than FFL at the dose of 40-120 kGy. This result suggested that water insoluble proteins in latex may be disintegrated and change to WEP at about 20 kGy.

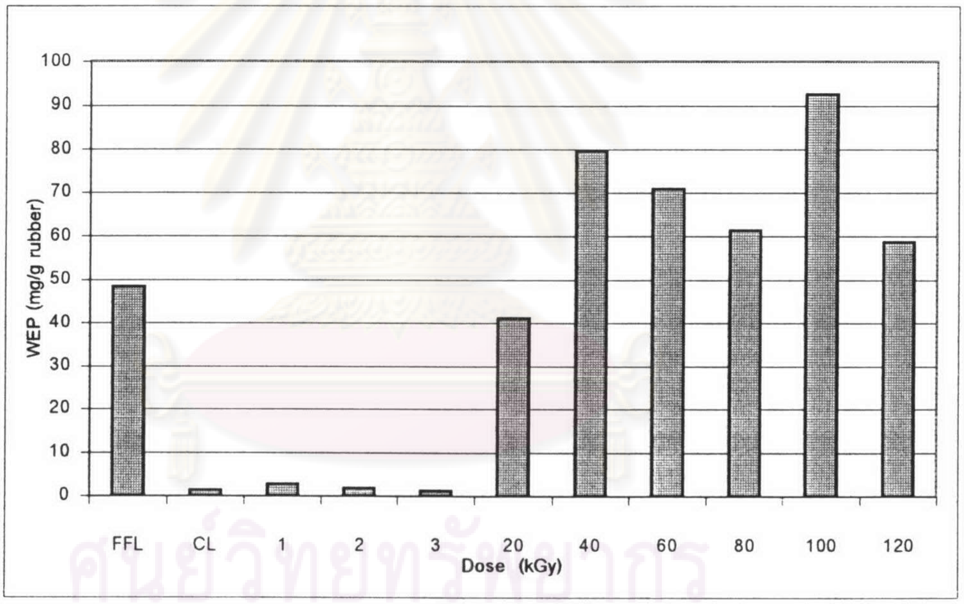


Figure 3.5 Different effects of radiation at low dose (1-3 kGy) and high dose (20-120 kGy) on latex protein

3.3.2 MW distribution of WEP of irradiated latex

MW distribution of WEP of irradiated (low dose, 1-3 kGy) latex was compared with control latex as shown in Figure 3.6. WEP prepared from FFL (lane 2) showed several protein bands in the range of 96-14.4 kDa. CL films (lane 3) display dominant protein band of MW 45, 40, 30 and 14.4 kDa which are the proteins that associate with rubber particles. Irradiation at low dose 1-3 kGy changes the pattern of WEP prepared from CL (lane 4-6) display disintegration of some proteins, resulting in different pattern of MW distribution showing slightly smear bands of proteins at MW lower than 14.4 kDa. Results in lane 7 and 8 also support protein disintegration by irradiation at dose 1 and 2 kGy, because leaching of rubber film at 70°C for 10 min removed most of WEP until not visible in gel.

Figure 3.7 shows the effect of FFL irradiation at dose higher than 20 kGy, resulted in more disintegration of most proteins in the serum and those bound to rubber particles suspended in 0.6% NH_3. Lane 3-8 indicated that after FFL irradiation, 30 kDa and smaller proteins may remain by association with in the cross-linked rubber particles in the CL.

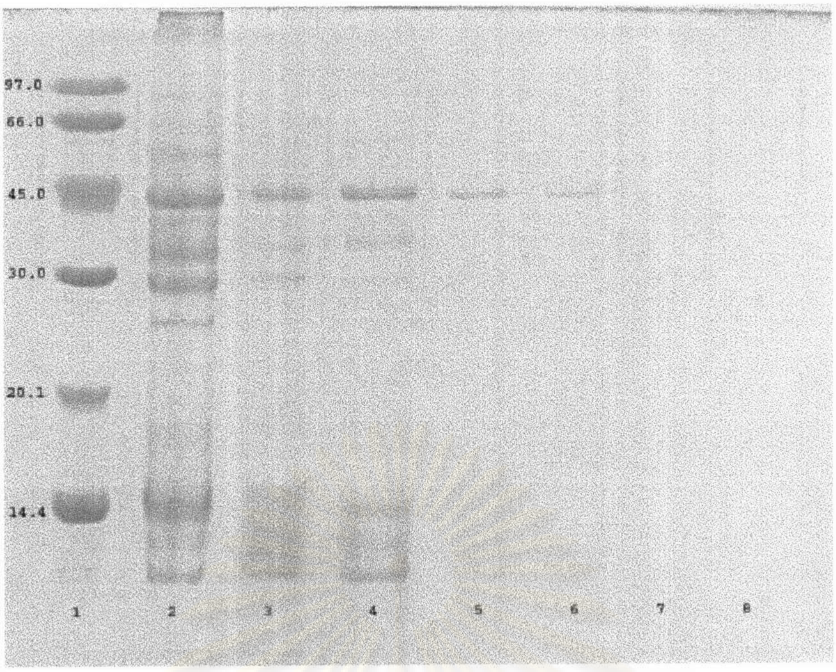


Figure 3.6 SDS PAGE of WEP extracted from dry rubber film prepared from FFL, CL, and FFL IRR with 1-3 kGy

Lane 1	Standard MW markers
Lane 2	WEP of fresh field latex
Lane 3	WEP of non IRR CL (control)
Lane 4	WEP of 1 kGy IRR CL
Lane 5	WEP of 2 kGy IRR CL
Lane 6	WEP of 3 kGy IRR CL
Lane 7	WEP of 1 kGy IRR CL and leaching
Lane 8	WEP of 2 kGy IRR CL and leaching

Well 2-6 was loaded with 20 μg protein and well 7-8 was loaded with 20 μl protein solution containing of non-detectable level protein.

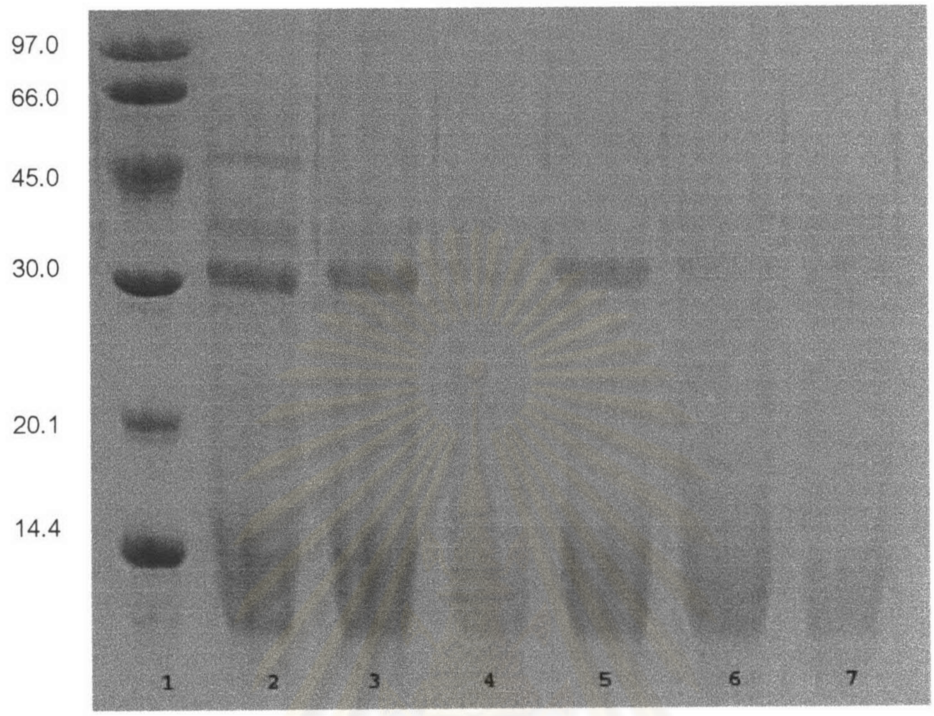


Figure 3.7 SDS PAGE of WEP extracted from dry rubber film prepared from FFL, CL, and FFL IRR with 20-120 kGy

Lane 1	Standard MW markers
Lane 2	WEP of 20 kGy IRR CL
Lane 3	WEP of 40 kGy IRR CL
Lane 4	WEP of 60 kGy IRR CL
Lane 5	WEP of 80 kGy IRR CL
Lane 6	WEP of 100 kGy IRR CL
Lane 7	WEP of 120 kGy IRR CL

Each well was loaded with 40 μg protein.

3.3.3 The effect of gamma radiation on Bovine Serum Albumin (BSA)

To understand the effect of gamma radiation on any single protein, BSA solution 1 mg/ml was irradiated at various doses 1, 3, 5 and 10 kGy and compared for MW distribution by SDS PAGE. Figure 3.8 shows that 66 kDa BSA soluble protein disintegrate slightly at 1 kGy and the band at 66 kDa decreased with increasing dose of irradiation. Smeared bands of protein MW below 66 kDa increased as the intensity of the 66 kDa band decreased.

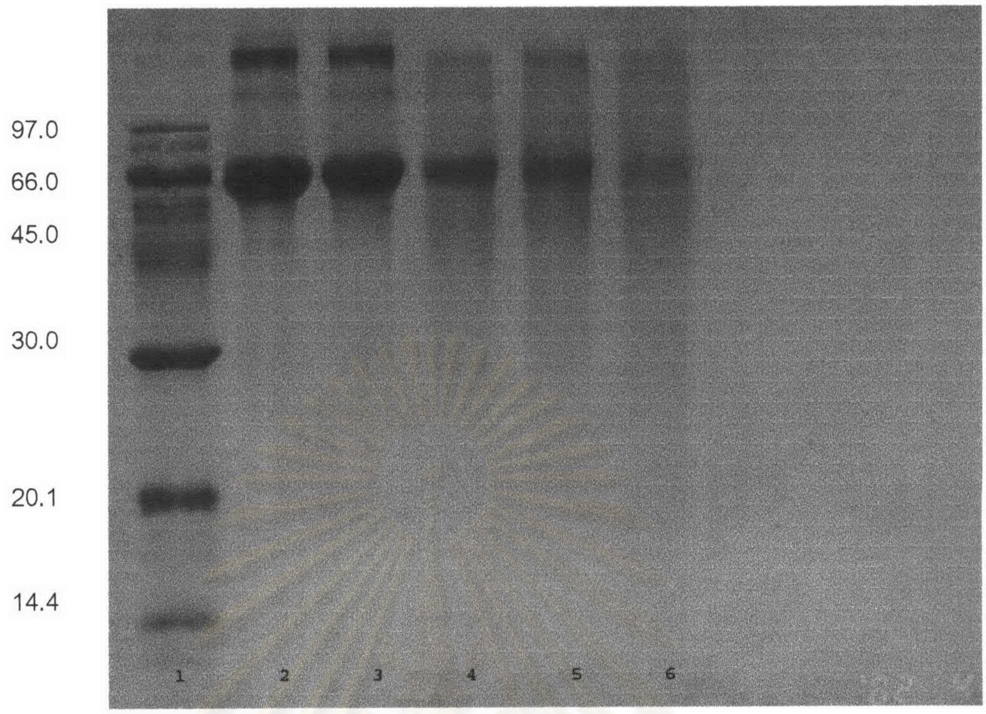


Figure 3.8 SDS PAGE of IRR BSA protein.

Lane 1 Standard MW markers

Lane 2 standard BSA without irradiation

Lane 3 standard BSA IRR at dose 1 kGy

Lane 4 standard BSA IRR at dose 3 kGy

Lane 5 standard BSA IRR at dose 5 kGy

Lane 6 standard BSA IRR at dose 10 kGy

Each well was loaded with 20 μg protein.

3.4 The effect of natural polysaccharide addition on irradiated natural rubber latex

Since it was reported previously that starch powder added in the process of NR – gloves production can act as a carrier for allergen proteins and form aerosol-allergen. Two types of natural polysaccharides: sodium alginate (AG) and carrageenan (CA) were added to FFL after irradiation and before centrifugation as shown in Figure 3.9.

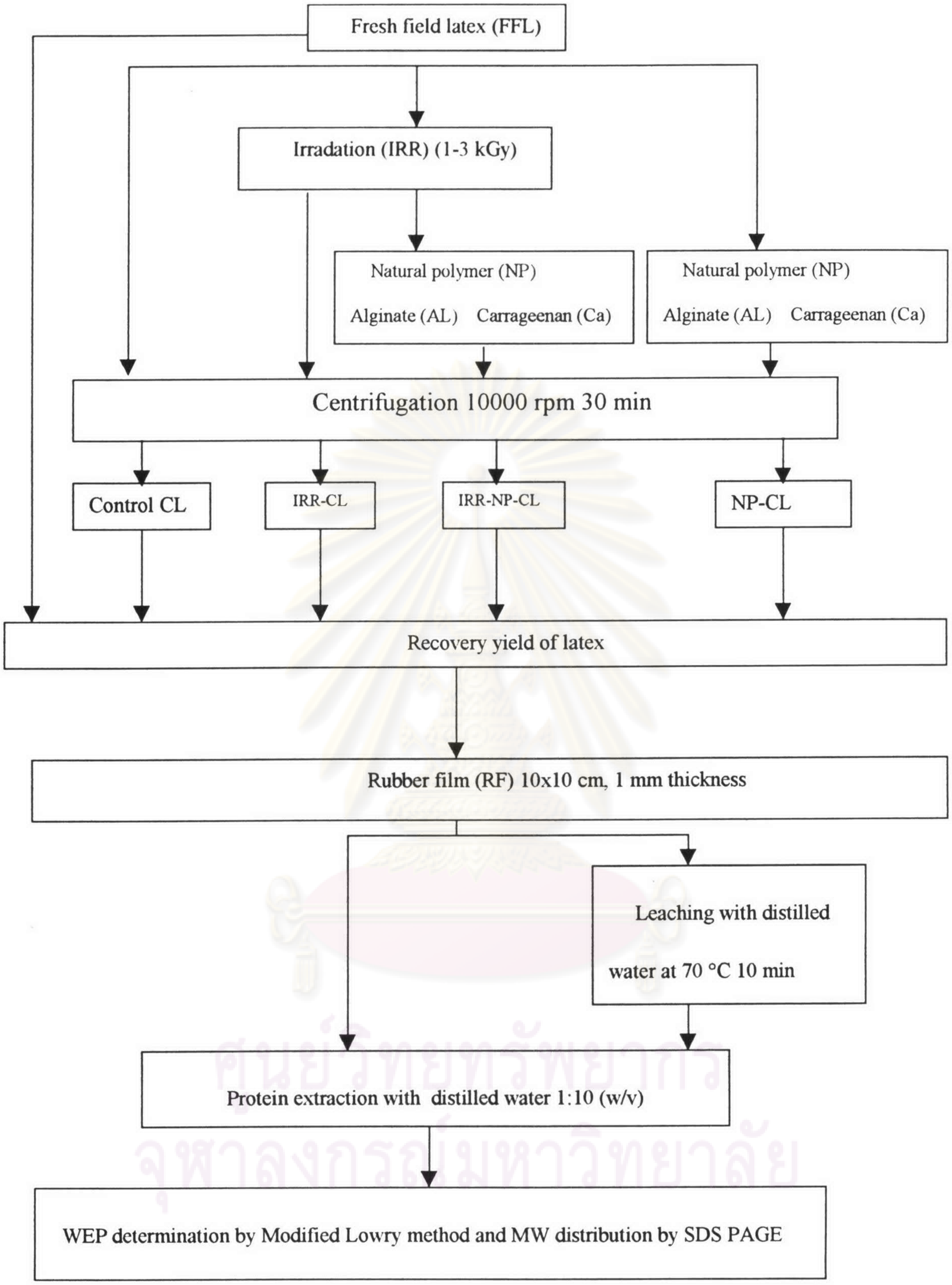


Figure 3.9 The protocol to study effect of IRR, NP addition and leaching on WEP

3.4.1 Recovery yield (%) of irradiated and polymer addition natural rubber latex

According to Figure 3.9, 1-3 phr of sodium alginate and carrageenan were added to 1-3 kGy irradiated natural rubber latex with dose and centrifuged to produce 60% concentrated latex. Recovery yield of these 60% concentrated latice were reduced significantly from 58% in control CL to only 21-32 % in case of adding 1-3 phr carrageenan, and 41-54% when 1-3 phr alginate was added.

Table 3.1 Effect of NP addition after irradiation of FFL on recovery yield.

Source of RF	Treatment		Recovery yield (%) of rubber in 60%CL under treatment with	
	IRR (kGy)	NP (phr)	Alginate	Carrageenan
Control CL	0	0	58	58
IRR-CL	1	0	42	42
	2	0	41	41
	3	0	42	42
NP-CL	0	1	45	26
	0	2	45	28
	0	3	45	29
IRR-NP-CL	1	1	45	21
	1	2	48	23
	1	3	43	25
	2	1	47	25
	2	2	54	32
	2	3	52	30
	3	1	51	25
	3	2	46	28
	3	3	47	27
	1-3	1-3	41-54	21-32

CL= Concentrated latex IRR = Irradiation

NP = Natural polysaccharide RF = Rubber film

Because addition of carrageenan resulting in about half recovery yield of concentrated latex comparing to control and alginate added concentrated latex, the result suggest that not only protein but rubber molecules may be associated with carrageenan.

3.4.2 WEP of irradiated FFL plus NP before centrifugation concentrated latex films

The fresh latex was irradiated at various dose (1-3 kGy) before adding natural polysaccharide, which was either 1-3 phr alginate or carrageenan . After centrifugation and film preparation, the WEP was determined by modified Lowry method. The results are shown in Table 3.2 and 3.3.

Table 3.2 shows that irradiation or alginate addition alone did not reduce WEP but irradiation followed the addition of alginate before centrifugation showed synergistic effect at all irradiation dose 1-3 kGy and alginate 1-2 phr. Table 3.3 shows that carrageenan addition alone resulted in increased WEP. When irradiation was followed by carrageenan addition, 1-3 phr, synergistic effect at all irradiation dose 1-3 kGy was observed. Although irradiation plus carrageenan have lower WEP comparing to irradiation plus alginate, the percent recovery yield of rubber in CL of the carrageenan added latex was very low and it was very difficult to add carrageenan in the irradiated latex because it was very viscous after dissolve. So carrageenan is not used in any further experiment.

Table 3.2 Effect of FFL irradiation and alginate addition on WEP of rubber film.

Source of RF	Treatment			WEP content(mg/g rubber)		% WEP remained
	IRR (kGy)	Alginate(phr)	Centrifugation	Lot No 9/11/01	Lot No 25/5/02	
FFL	0	0	-	48.3	73.1	100
CL	0	0	+	1.4	4.2	2.90 - 5.75
IRR-CL	1	0	+	2.7	3.6	
	2	0	+	1.8	2.3	
	3	0	+	1.3	2.4	
Average				1.9	2.8	3.84 - 3.96
AG-CL	0	1	+	5.8	3.7	
	0	2	+	3.5	1.6	
	0	3	+	6.9	4.2	
Average				5.4	3.2	4.38 – 11.25
IRR-AG-CL	1	1	+	2.4	2.2	
	1	2	+	1.5	2.0	
	1	3	+	1.7	0.7	
Average				1.9	1.6	2.19 – 3.87
IRR-AG-CL	2	1	+	1.1	0.4	
	2	2	+	1.4	0.5	
	2	3	+	1.8	0.9	
Average				1.2	0.6	0.82 – 2.50
IRR-AG-CL	3	1	+	1.4	2.4	
	3	2	+	1.1	1.1	
	3	3	+	8.7	5.3	
Average				3.7	2.9	3.97 – 7.77

Table 3.3 Effect of FFL irradiation and carrageenan addition on WEP of rubber film.

Source of RF	Treatment			WEP content(mg/g rubber)	% WEP
	IRR (kGy)	Carrageenan (phr)	Centrifugation	Lot no 9/11/01	remained
FFL	0	0	-	48.3	100
CL	0	0	+	1.4	2.90
IRR-CL	1	0	+	2.7	
	2	0	+	1.8	
	3	0	+	1.3	
Average				1.9	3.96
CA-CL	0	1	+	1.8	
	0	2	+	1.0	
	0	3	+	2.2	
Average				1.7	3.54
IRR-CA-CL	1	1	+	0.9	
	1	2	+	2.5	
	1	3	+	0.9	
Average				1.4	2.91
IRR-CA-CL	2	1	+	0.2	
	2	2	+	2.6	
	2	3	+	0.9	
Average				1.2	2.50
IRR-CA-CL	3	1	+	0.9	
	3	2	+	1.7	
	3	3	+	0.7	
Average				1.1	2.29

3.4.3 Effect of leaching on WEP of irradiated latex film

Leaching rubber film with distilled (1 g rubber: 10 ml distilled water at 70 °C for 10 minutes) reduced WEP significantly to 2.1 mg/g rubber – non-detectable level for alginate added latex (Table 3.4) and 1.1 mg/g rubber – non-detectable level for carrageenan added latex (Table 3.5).

Table 3.4 Effect of leaching on WEP of IRR-AG-CL films

Source of RF	Treatment			WEP content (mg/g rubber) Lot no 9/11/01	
	IRR (kGy)	Alginate (phr)	Centrifugation	- Leaching	+ Leaching
FFL	0	0	-	48.3	1.1
CL	0	0	+	1.4	ND
IRR-CL	1	0	+	2.7	ND
	2	0	+	1.8	ND
	3	0	+	1.3	ND
AG-CL	0	1	+	5.8	1.5
	0	2	+	3.5	0.6
	0	3	+	6.9	2.1
IRR-AG-CL	1	1	+	2.4	0.06
	1	2	+	1.5	ND
	1	3	+	1.7	ND
IRR-AG-CL	2	1	+	1.1	ND
	2	2	+	1.4	1.0
	2	3	+	1.8	0.6
IRR-AG-CL	3	1	+	1.4	ND
	3	2	+	1.1	ND
	3	3	+	8.7	0.02
Range of WEP				1.1-8.7	ND-2.1

ND = Non detectable level

Table 3.5 Effect of leaching on WEP of IRR-CA-CL films

Source of RF	Treatment			WEP content (mg/g rubber) Lot no 9/11/01	
	IRR (kGy)	carrageenan (phr)	Centrifugation	- Leaching	+ Leaching
FFL	0	0	-	48.3	1.1
CL	0	0	+	1.4	ND
IRR-CL	1	0	+	2.7	ND
	2	0	+	1.8	ND
	3	0	+	1.3	ND
CA-CL	0	1	+	1.8	ND
	0	2	+	1.0	ND
	0	3	+	2.2	ND
IRR-CA-CL	1	1	+	0.9	ND
	1	2	+	2.5	ND
	1	3	+	0.9	ND
IRR-CA-CL	2	1	+	0.2	ND
	2	2	+	2.6	ND
	2	3	+	0.9	ND
IRR-CA-CL	3	1	+	0.9	ND
	3	2	+	1.7	ND
	3	3	+	0.7	ND
Range of WEP				0.9-2.7	ND-1.1

ND = Non detectable level

3.4.4 Effect of leaching on MW distribution of WEP from IRR , alginate and centrifugation

Figure 3.10 shows separation of WEP by SDS-PAGE from 1-2 kGy irradiated added 1-2 phr alginate CL film non-leaching and leaching in comparison with standard MW marker (lane 1). 1 kGy irradiated with 1 phr alginate added CL film and 2 kGy irradiated with 1 phr alginate added CL film (lane 3,5) show several protein band in the range 66.0- lower than14.4 kDa . 2 kGy irradiated with 2 phr alginate added CL film (lane 7) displays bands of proteins MW in the range higher than 97.7 kDa – lower then 14.4 kDa. The latex films leaching with 70°C water for 30 min (lane 2, 4, 6 and 8) that was loaded with 20 μl / well because the amount of proteins was non-detectable, show no band of protein.

Figure 3.11 display separation of WEP by SDS-PAGE from 3 kGy films leaching with 70°c water for 10 min, faint band of protein at 30 kDa was observed (lane 2). The other leaching film (lane4, 6 and 8) that was loaded with 20 μl / well because the amount of proteins, was non-detectable display no band of protein. 3 kGy irradiated and added 1 and 2 phr alginate CL film (lane 5, 7) display the same pattern of protein band. It also shows several protein bands in the range 66.0-14.4 kDa and slightly smear band of protein MW lower than 14.4 kDa. 1 kGy irradiated and added 3 phr alginate CL films (lane 3) show the high intensity band of protein MW 14.4 and 45 kDa. It also shows smear band of protein MW higher than 45 kDa and lower than 14.4 kDa.

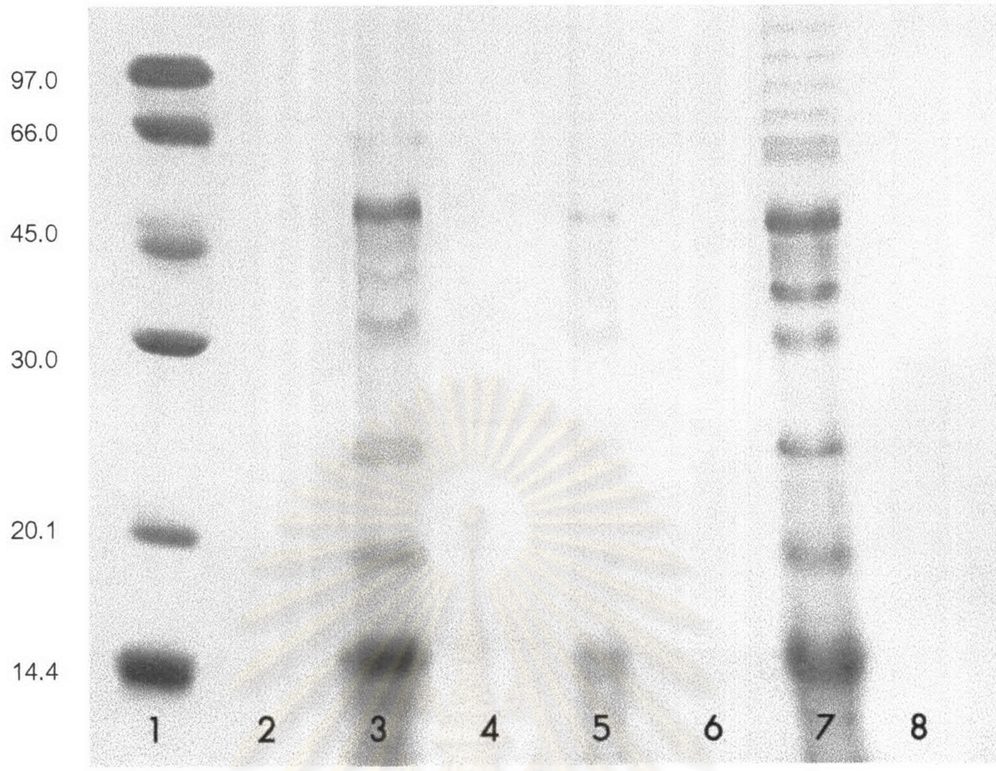


Figure 3.10 Effect of leaching on MW distribution of WEP from IRR, AG and centrifugation.

Lane 1	Standard MW markers
Lane 2	WEP of rubber film prepared from fresh field latex after leaching
Lane 3	WEP of 1 kGy IRR with 1 phr AG added CL
Lane 4	WEP of 1 kGy IRR with 1 phr AG added CL, then leaching
Lane 5	WEP of 2 kGy IRR with 1 phr AG added CL
Lane 6	WEP of 2 kGy IRR with 1 phr AG added CL, then leaching
Lane 7	WEP of 2 kGy IRR with 2 phr AG added CL
Lane 8	WEP of 2 kGy IRR with 2 phr AG added CL, then leaching

Well 3, 5 and 7 was loaded with 60 μg protein and well 2, 4, 6 and 8 was loaded with 20 μl protein solution containing protein at non-detectable level.

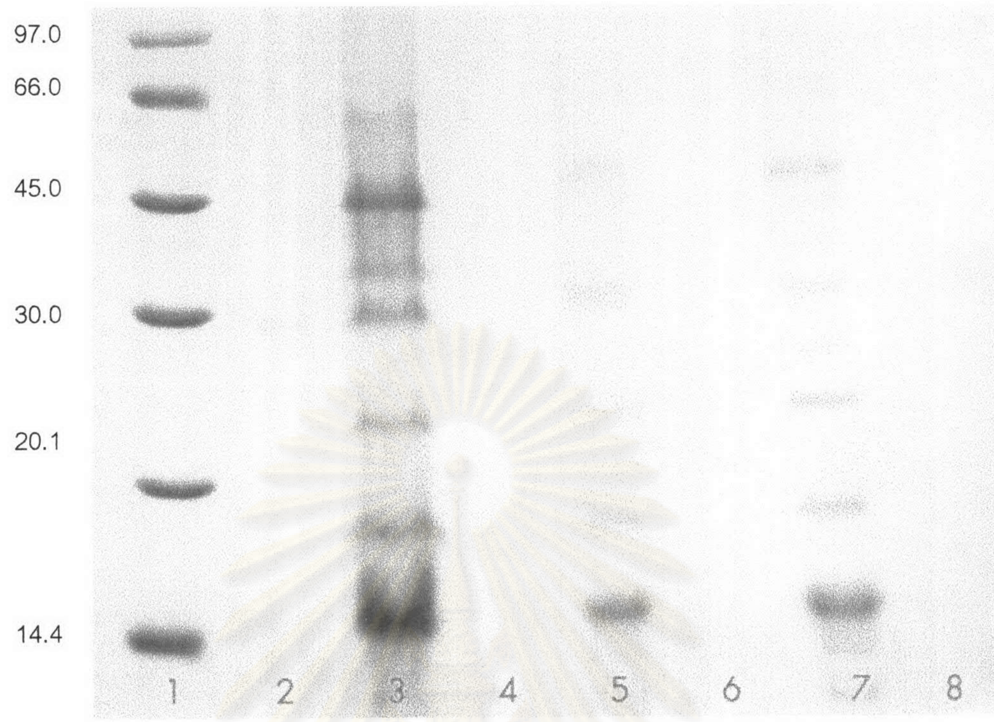


Figure 3.11 Effect of leaching on MW distribution of WEP from IRR, AG and centrifugation.

Lane 1 Standard MW markers

Lane 2 WEP of 3 kGy IRR, then leaching

Lane 3 WEP of 1 kGy IRR with 3 phr AG added CL

Lane 4 WEP of 1 kGy IRR with 3 phr AG added CL, then leaching

Lane 5 WEP of 3 kGy IRR with 1 phr AG added CL

Lane 6 WEP of 3 kGy IRR with 1 phr AG added CL, then leaching

Lane 7 WEP of 3 kGy IRR with 2 phr AG added CL

Lane 8 WEP of 3 kGy IRR with 2 phr AG added CL, then leaching

Well 3, 5 and 7 was loaded with 60 μg protein and well 2, 4, 6 and 8 was loaded with 20 μl protein solution containing protein at non-detectable level.

3.4.5 MW distribution of WEP of carrageenan added after irradiated latex

Figure 3.13 demonstrated distribution of WEP from irradiated latex and carrageenan added film in comparison of MW marker. A clear band of protein MW 45 kDa appears when irradiated latex at 1 kGy and added 1 phr carrageenan and irradiated latex at 1 kGy and added 3 phr carrageenan (lane 2, 6). Irradiated 1 kGy and added 2 phr carrageenan latex (lane 4) shows a clear band of 45 kDa and slightly smear bands below. Irradiated at 3 kGy and added 2 phr carrageenan latex films (lane 8) shows 3 clear bands of MW 45, 40.1 and 35 kDa. In lane 3, 5 and 7, no band of protein was observed because of leaching effect.

From Figure 3.14, irradiated 2 kGy latex and added 1 phr carrageenan (lane 2) shows no band of protein. The irradiated 2 kGy and added 2 phr carrageenan latex (lane 4) has a slightly band MW 45 kDa. Irradiated 2 kGy added and 3 phr carrageenan latex (lane 6) shows 3 clear bands of MW 30, 35 and 45 kDa and a smear band of 14.4 kDa. There have no band of protein lane 3, 5, 7 and 8 when leaching rubber films.

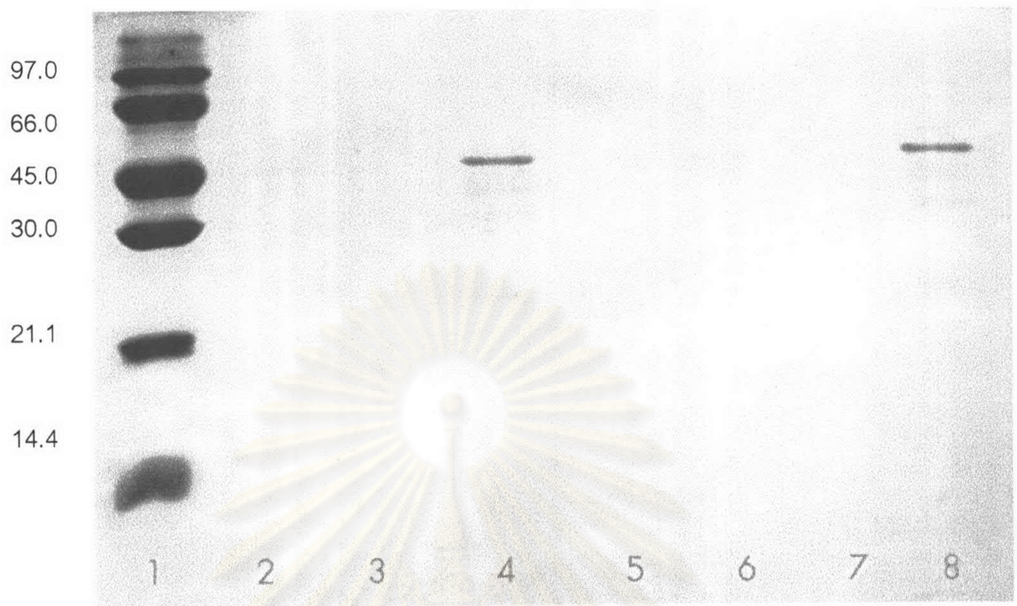


Figure 3.12 Effect of leaching on MW distribution of WEP from IRR CA addition and centrifugation.

Lane 1	Standard MW markers
Lane 2	WEP of 1 kGy IRR with 1 phr CA added CL
Lane 3	WEP of 1 kGy IRR with 1 phr CA added CL, then leaching
Lane 4	WEP of 1 kGy IRR with 2 phr CA added CL
Lane 5	WEP of 1 kGy IRR with 2 phr CA added CL, then leaching
Lane 6	WEP of 1 kGy IRR with 3 phr added CA CL
Lane 7	WEP of 1 kGy IRR with 3 phr CA added CL, then leaching
Lane 8	WEP of 3 kGy IRR with 2 phr CA added CL

Well 2, 4, 6 and 8 was loaded with 15 μg protein and well 3, 5 and 7 was loaded with 20 μl protein solution containing protein at non-detectable level.

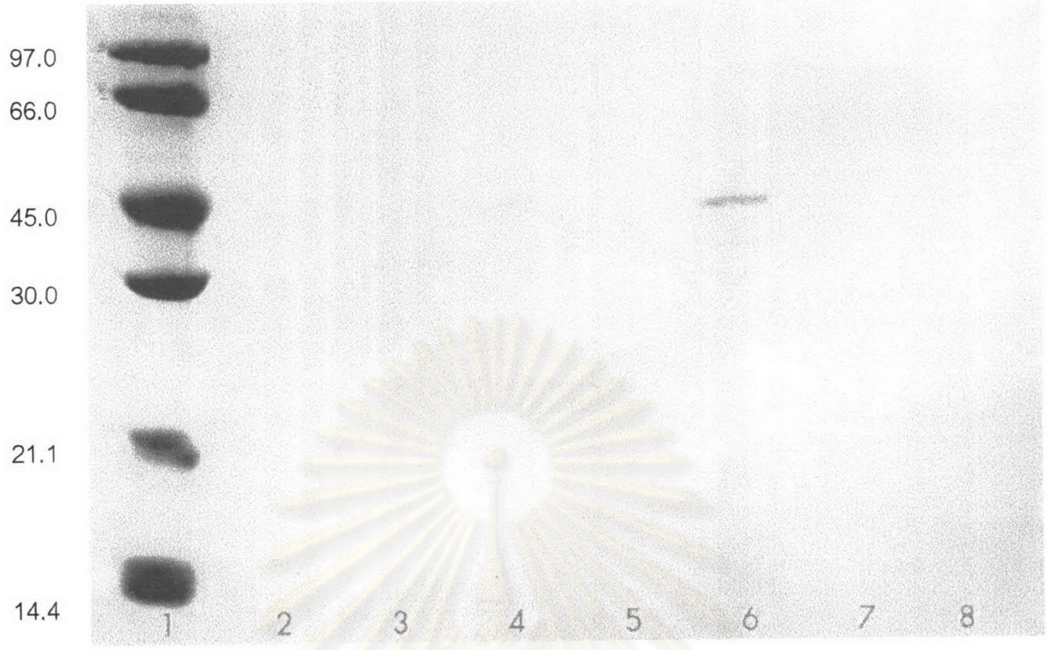


Figure 3.13 Effect of leaching on MW distribution of WEP from IRR, CA addition and centrifugation.

Lane 1 Standard MW markers

Lane 2 WEP of 2 kGy IRR with 1 phr CA added CL

Lane 3 WEP of 2 kGy IRR with 1 phr CA added CL,then leaching

Lane 4 WEP of 2 kGy IRR with 2 phr CA added CL

Lane 5 WEP of 2 kGy IRR with 2 phr CA added CL, then leaching

Lane 6 WEP of 2 kGy IRR with 3 phr CA added CL

Lane 7 WEP of 2 kGy IRR with 3 phr CA added CL,then leaching

Lane 8 WEP of 3 kGy IRR with 1 phr CA added CL,then leaching

Well 2, 4 and 6 was loaded with 15 μg protein and well 3, 5, 7 and 8 was loaded with 20 μl protein solution containing protein at non-detectable level.

3.5 The effect of radiation on WEP of alginate addition latex

Since alginate addition after irradiation shows synergistic effect in reduction of WEP of rubber film. And It has been reported that alginate can be degraded due to scission of glycosidic bond by radiation. So in this experiment alginate was added before irradiation to improve efficiency of removal WEP by centrifugation. The protocol was followed by Figure 3.12.

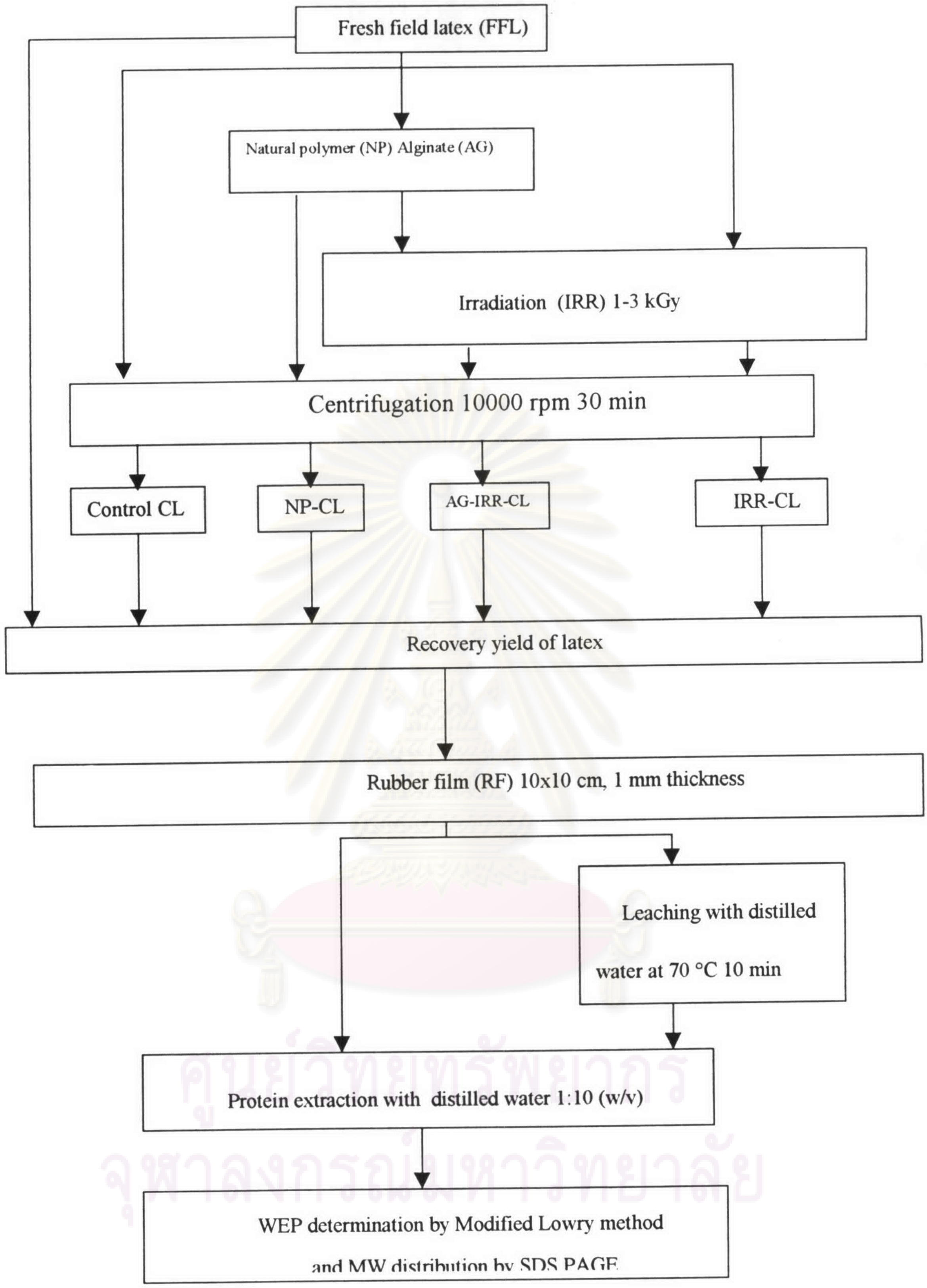


Figure 3.15 The protocol to study effect of AG addition, IRR and leaching on WEP

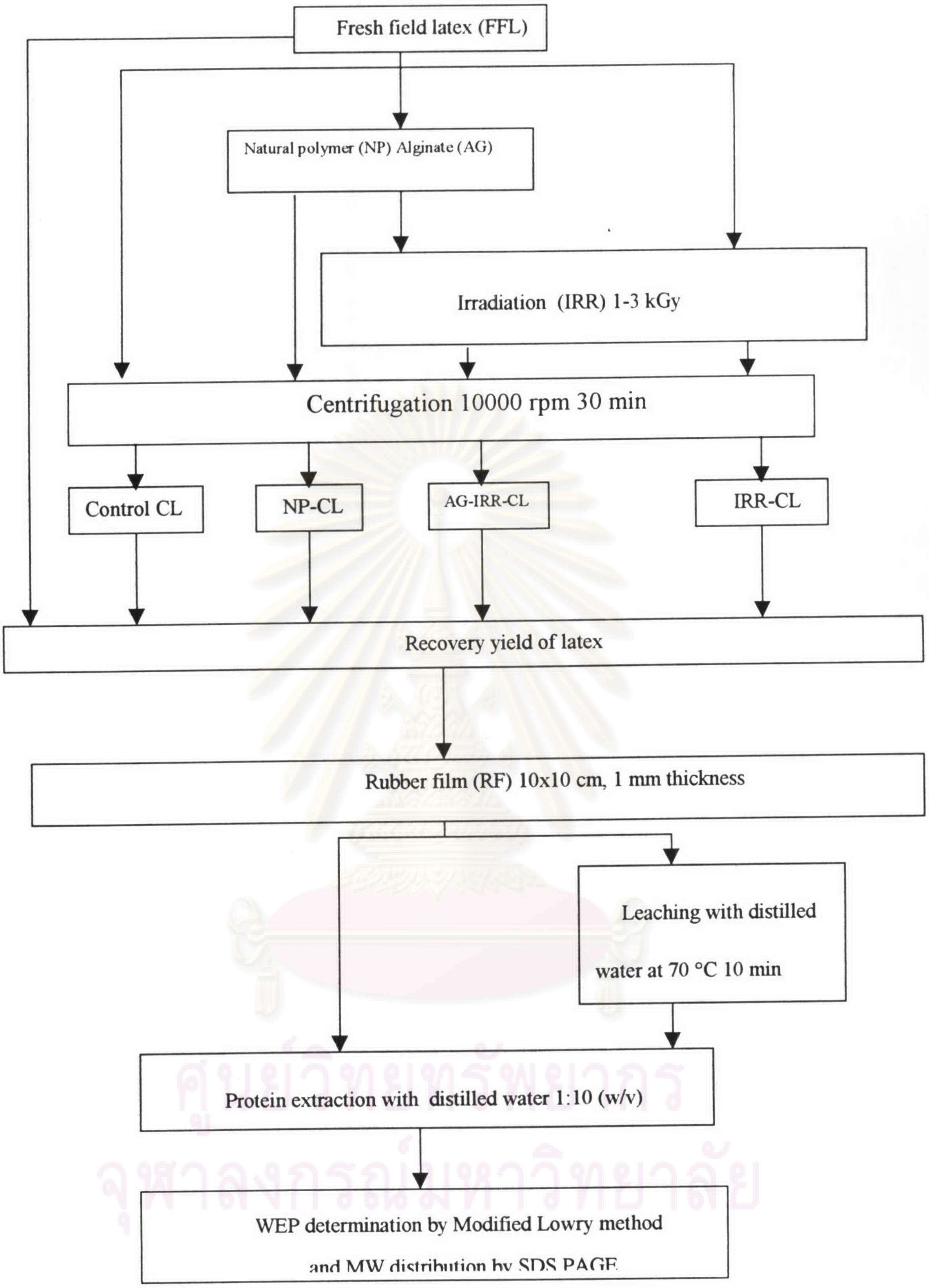


Figure 3.15 The protocol to study effect of AG addition, IRand leaching on WEP

3.5.1 Recovery yield (%) of alginate addition and irradiated latex

Table 3.6 shows that added alginate in to latex before irradiation had recovery yield better than added alginate after irradiated.

Table 3.6 Effect of Alginate addition before irradiation of FFL on recovery yield.

Source of RF	Treatment		Recovery yield (%) of rubber in
	AG (phr)	IRR (kGy)	60%CL
Control CL	0	0	58
IRR-CL	0	1	51
	0	2	55
	0	3	55
AG-CL	1	0	56
	2	0	57
	3	0	57
IRR-AG-CL	1	1	51
	2	1	52
	3	1	58
	1	2	52
	2	2	53
	3	2	53
	1	3	51
	2	3	51
	3	3	53
	1-3	1-3	51-58

CL= Concentrated latex , IRR = Irradiation, AG=Alginate, RF= Rubber film

3.5.2 Leaching effect with WEP of rubber AG-IRR-CL films

From Table 3.7 it was shown that only AG addition WEP increased from CL. It may be cause of alginate interaction with rubber protein in rubber particle and improve efficiency of solubility of protein so it can be extract more easily. WEP extracted form rubber film prepared from AG addition plus IR and centrifugation increased when increasing dose and amount of alginate. Leaching rubber films with 70°C for 10 min could reduce WEP significantly (0.6 μg/g rubber – ND). Especially condition added alginate 1-2 phr and irradiated at 2 kGy, the WEP reduced to non-detectable level.

Table 3.7 WEP of FFL plus AG before irradiated and centrifugation and leaching effect with WEP of rubber films

Source of RF	Treatment			WEP content (mg/g rubber) Lot no 19/1/03	
	Alginate (phr)	IRR (kGy)	Centrifugation	-leaching	+ leaching
FFL	0	0	-	27.1	0.3
CL	0	0	+	1.1	0.2
IRR-CL	0	1	+	1.3	0.2
	0	2	+	1.6	0.2
	0	3	+	2.1	0.2
AG-CL	1	0	+	2.1	0.2
	2	0	+	1.3	0.3
	3	0	+	1.9	0.1
AG-IRR-CL	1	1	+	0.4	0.2
	2	1	+	1.2	0.4
	3	1	+	1.4	0.6
AG-IRR-CL	1	2	+	0.2	ND
	2	2	+	0.2	ND
	3	2	+	3.0	0.5
AG-IRR-CL	1	3	+	0.4	0.2
	2	3	+	0.8	0.2
	3	3	+	1.8	0.3
Range of WEP				0.2-27.1	ND-0.6

ND = Non detectable level

3.6 Effect of irradiation after centrifugation

Since previous results (Figure 3.2-3.3) indicated that centrifugation could remove about 90% of WEP from FFL, and irradiation at various dose, 1-5 kGy disintegrates proteins into smaller fragments which may become more soluble and resulted in increasing number of small molecular weigh polypeptides, which can be easily removed by leaching. It is also known that irradiation of concentrated latex at 10-15 kGy, in the presence of nBA, induces cross-linking between rubber molecules, which can replace sulfur vulcanization process. To remove WEP from FFL the scale-up lot of approximately 400 L. According to Figure 3.14, FFL was treated with DAP to precipitate Mg^{2+}, and separated into two tanks of 200 L each. The control tank was concentrated using the factory centrifuge, whereas the other tank was treated with alkali protease before centrifugation. The concentrated latice obtained were adjusted to 60% DRC, and determined for their physical properties according to ISO 2004 specifications. Irradiation was performed at 10 kGy according to Method 2.6 at various time after centrifugation (different MST).

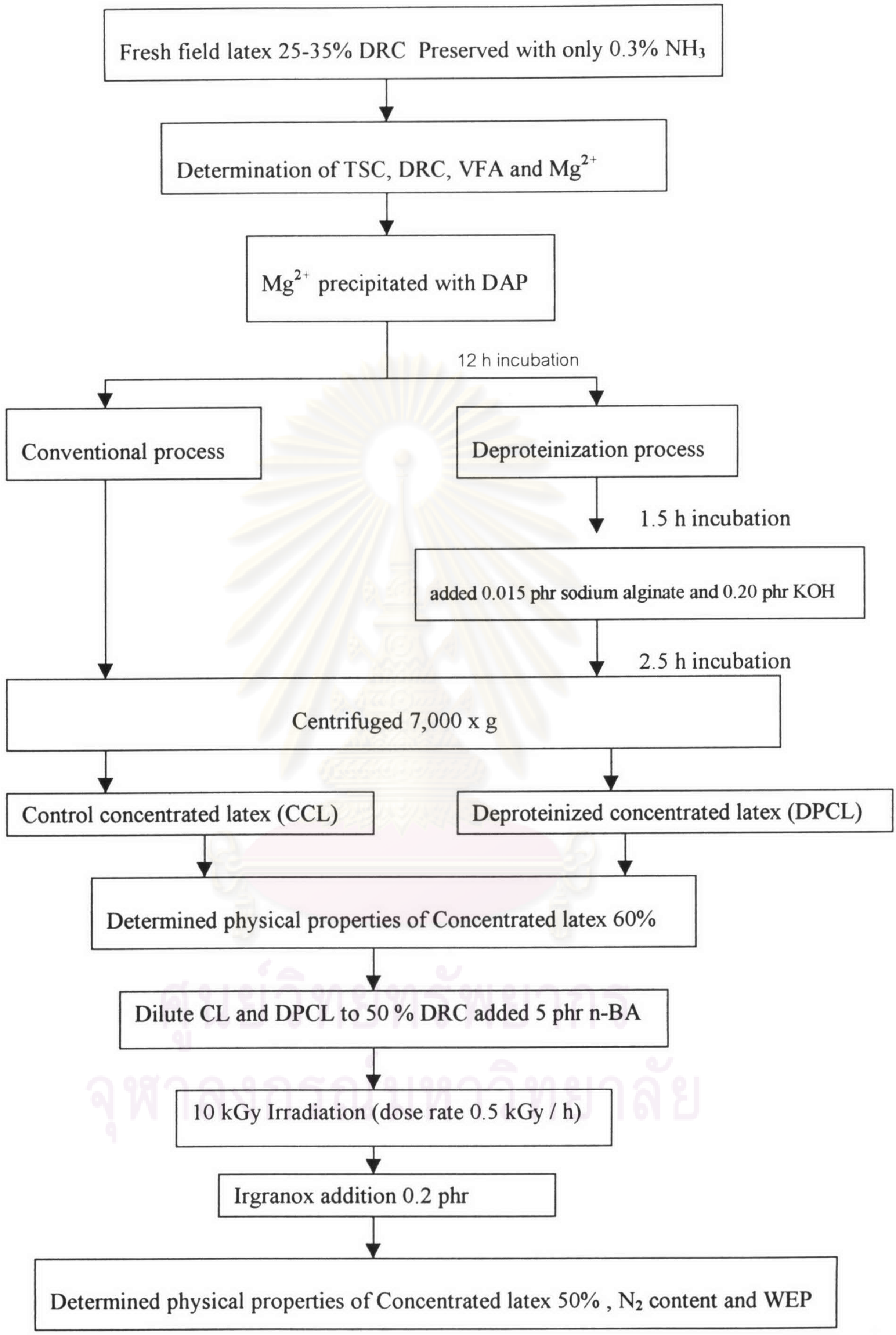


Figure 3.15 Production of concentrated latex followed by irradiation

3.6.1 Recovery yield of control and deproteinized concentrated latex

The recovery yield of 3 lots of control and deproteinized latex were shown in table 3.8.

Table 3.8 Recovery yield of DPCL compared with control concentrated latex

Sample lot no.	Recovery yield (%)	
	Control CL	DPCL
24/6/03	57.46	67.17
11/7/03	62.33	55.56
21/7/03	58.69	58.50
Average	59.49	60.41

DPCL = Deproteinized concentrated latex

Table 3.8 shows that deproteinization had no significant effect on recovery yield of rubber in the concentrated latex 60%.

3.6.2 Physical properties of control and deproteinized concentrated latex

The comparison of properties of control and deproteinzed concentrated latex latex was shown in Table 3.9.

Table 3.9 Comparison of physical properties in control concentrated latex and DPCL

Physical properties according to ISO 2004 Specification	Control CL (n=3)	DPCL (n=3)	Difference from control (%)
Concentrated latex 60%			
Total solid content, (TSC; 61.5% min)	59.18 ± 2.35	60.95 ± 3.16	3
Dry rubber content, (DRC; 60.0% min)	58.46 ± 2.22	60.13 ± 3.37	3
Non rubber content, (NRC; 2.0% max)	0.72 ± 0.19	0.83 ± 0.36	15
Ammonia (HA-L; 0.06% min)	0.60 ± 0.01	0.66 ± 0.05	10
KOH (1 % max)	0.36 ± 0.06	0.43 ± 0.11	20
Volatile fatty acid (VFA; 0.2% max)	0.02 ± 0.01[b]	0.04 ± 0.03[a]	100
Magnesium content (50 ppm max)	65.01 ± 13.90	60.05 ± 17.89	-8
* Mechanical stability time, MST (650 sec min)	1482 ± 272.09[a]	283 ± 119.65[b]	-80
**Deproteinized CL 60%			
Nitrogen content (0.09% max)	0.16 ± 0.005[a]	0.09 ± 0.01[b]	-44
WEP (μg /g rubber)	2411± 998[a]	82± 28[b]	-97

* MST – determined 25 days after centrifugation

** Addition physical properties of DPCL

Significant difference of physical properties between control concentrated latex and deproteinized concentrated latex are marked by different letter (a, b) analyzed by t-test at 95% confident.

Table 3.9 shows that control concentrated latex did not meet the 60% DRC of ISO specification because of the minimum volume 200 lits used for centrifugation.. MST, nitrogen content and WEP of DPCL was decreased from control CL significantly, indicating that Alcalase enzyme had digested proteins that stabilized rubber particles. Volatile Fatty acid (VFA) also increased 100% in DPCL suggesting that there should be lipoproteins in latex, so that when proteins were removed, free fatty acids increased.

3.6.3 The effect of irradiation on control and deproteinized concentrated latex

The comparison of properties of control and deproteinzed irradiated concentrated latex was shown in Table 3.10.

Table 3.10 Effect of irradiation on physical properties of 50% control and deproteinized concentrated latice.

Physical properties according to ISO 2004 Specification	Control CL-IRR (n=3)	DPCL-IRR (n=3)	Difference from control (%)
Concentrated latex 50%			
Total solid content, (TSC; %)	52.15 ± 1.14	52.94 ± 0.84	1.5
Dry rubber content, (DRC; %)	51.42 ± 1.24	51.54 ± 1.24	0.2
Non rubber content, (NRC, %)	0.78 ± 0.40	1.4 ± 0.41	79
Ammonia (HA-L, %)	0.68 ± 0.15	0.68 ± 0.09	0
Volatile fatty acid (VFA, %)	0.06 ± 0.01	0.04 ± 0.02	-33
Magnesium content (ppm, %)	60.71 ± 8.91	69.90 ± 18.96	15
*Mechanical stability time, MST (sec)	1057 ± 105.91[b]	204 ± 172.70[a]	-80
**Deproteinized CL			
Nitrogen content (%)	0.16 ± 0.005[a]	0.09 ± 0.01[b]	-44
WEP (μg /g rubber)	2655± 912[b]	373± 72[a]	-86

* MST – determined 25 days after centrifugation

** Addition physical properties of DPCL

Significant difference of physical properties between control irradiated concentrated latex and deproteinized irradiated concentrated latex are marked by different letter (a, b) analyzed by t-test at 95% confidence.

Table 3.10 shows MST, nitrogen content and WEP of control and DPCL irradiated latex were significantly different. There were no significant difference in other physical properties in other %TSC, % DRC, %NH_3, contaminants (non-rubber content, KOH, Mg). WEP of control CL and DPCL increased after irradiated.

3.7 Effect of irradiation on NP addition and centrifugation

The alginate addition concentrated latex was prepared 2 lots (12/9/03 and 4/11/03) at batch scale 200 lits. The process was as followed in Figure 3.16.

3.7.1 Recovery yield of alginate addition concentrated latex

The recovery yield of alginate added concentrated latex was higher than control in both lots of sample very good recovery yield was obtained when added alginate to fresh latex before centrifuge (Table 3.11).

Table 3.11 Recovery yield (%) of control and alginate added concentrated latex

Sample lot no.	Recovery yield (%)	
	Control CL	Ag CL
12/9/03	59.81	69.65
4/11/03	58.14	68.02
Average	58.98	68.84

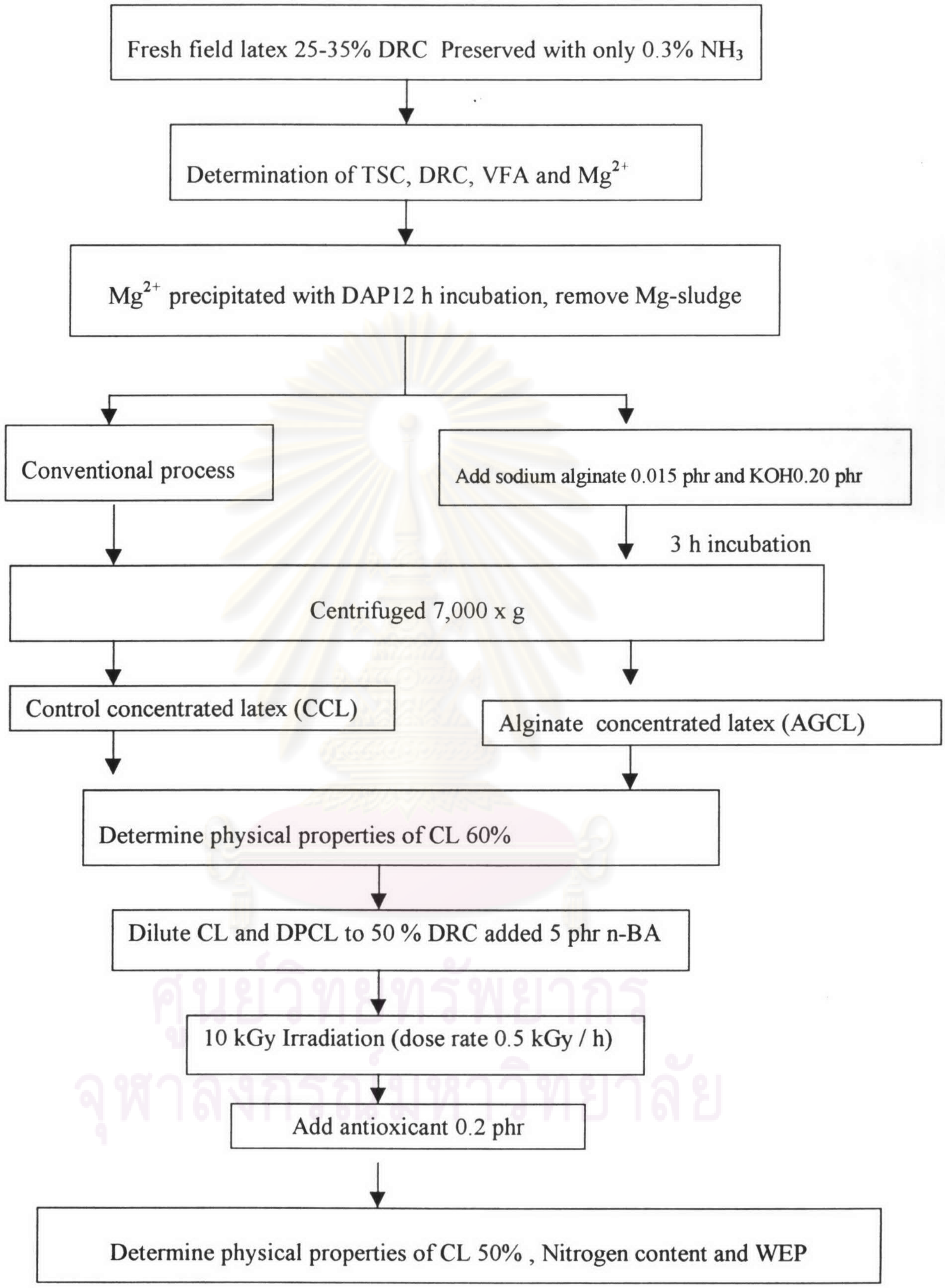


Figure 3.16 Production of control and AG CL followed by irradiation

3.7.2 Physical properties of control and alginate addition concentrated latex 60%

The physical properties of 2 lots of control and alginate addition concentrated latex were shown in Table 3.12.

Table 3.12 Physical properties of control and alginate added concentrated latex

Physical properties according to ISO 2004 Specification	CCL		AGCL	
	12/9/03	4/11/03	12/9/03	4/11/03
Concentrated latex 60%				
Total solid content, (TSC; 61.5% min)	62.14	60.74	64.52	64.66
Dry rubber content, (DRC; 60.0% min)	61.85	59.98	64.02	63.53
Non rubber content, (NRC; 2.0% max)	0.29	0.76	0.50	1.13
Ammonia (HA-L; 0.06% min)	0.66	0.60	0.68	0.60
KOH (1 % max)	0.35	0.54	0.37	0.42
Volatile fatty acid (VFA; 0.2% max)	0.01	0.03	0.02	0.01
Magnesium content (50 ppm max)	42.40	49.32	70.18	90.12
* Mechanical stability time, MST (650 sec min)	1396	1192	1447	1101
Nitrogen content (%)	0.18	0.20	0.18	0.20
WEP (μg /g rubber)	776	697	273	253

* MST – determined 27 days after centrifugation

From 2 lots of CCL and AGCL, the % DRC of AGCL (63 – 64%) was slightly higher than control (60-61%). The results indicated that addition of alginate at 0.015 phr may increase recovery yield of CL, but Mg content was also higher than control.

It seems that in the presence of sodium alginate, centrifugation could not reduce Mg content. However both control and AGCL had low amount of VFA which indicated for low contamination of microorganisms and MST also meet ISO 2004 specification. Although nitrogen content or total proteins of control and AGCL were not different but WEP of control was higher than AGCL.

3.7.3. The effect of 10 kGy irradiation on CCL and AGCL

The specification of CCL-IRR and AGCL-IRR were determined in the term of impurity, stability and protein content. It was shown in Table 3.13.

After 10 kGy irradiation, VFA and MST increased in both irradiated lots. Mg content of control still higher than AGCl. Nitrogen content of CL and CL-IR was not different, but WEP of non-irradiated latex increased after irradiation.

Table 3.13 Effect of Irradiation on WEP and 50% concentrated latex

Physical properties according to ISO 2004 Specification	CCL-IRR		AGCL-IRR	
	12/9/03	4/11/03	12/9/03	4/11/03
Concentrated latex 50%				
Total solid content, (TSC; %)	52.78	53.93	53.10	54.90
Dry rubber content, (DRC; %)	51.38	52.31	51.39	53.54
Non rubber content, (NRC, %)	1.46	1.62	1.71	1.36
Ammonia (HA-L, %)	0.49	0.45	0.45	0.43
Volatile fatty acid (VFA, %)	0.02	0.08	0.03	0.06
Magnesium content (ppm, %)	39.75	48.32	69.69	85.32
Mechanical stability time, MST (sec)	1186*	1239**	1195***	931****
Nitrogen content (%)	0.18	0.20	0.17	0.19
WEP (μg /g rubber)	947	940	388	510

Lot 12/9/03 and lot 4/11/03 irradiated days 143 and days 80 after centrifugation.

* MST – determined days 172 after centrifugation, **MST – determined days 86 after centrifugation

***MST – determined days 165 after centrifugation, ****MST – determined days 93 after centrifugation

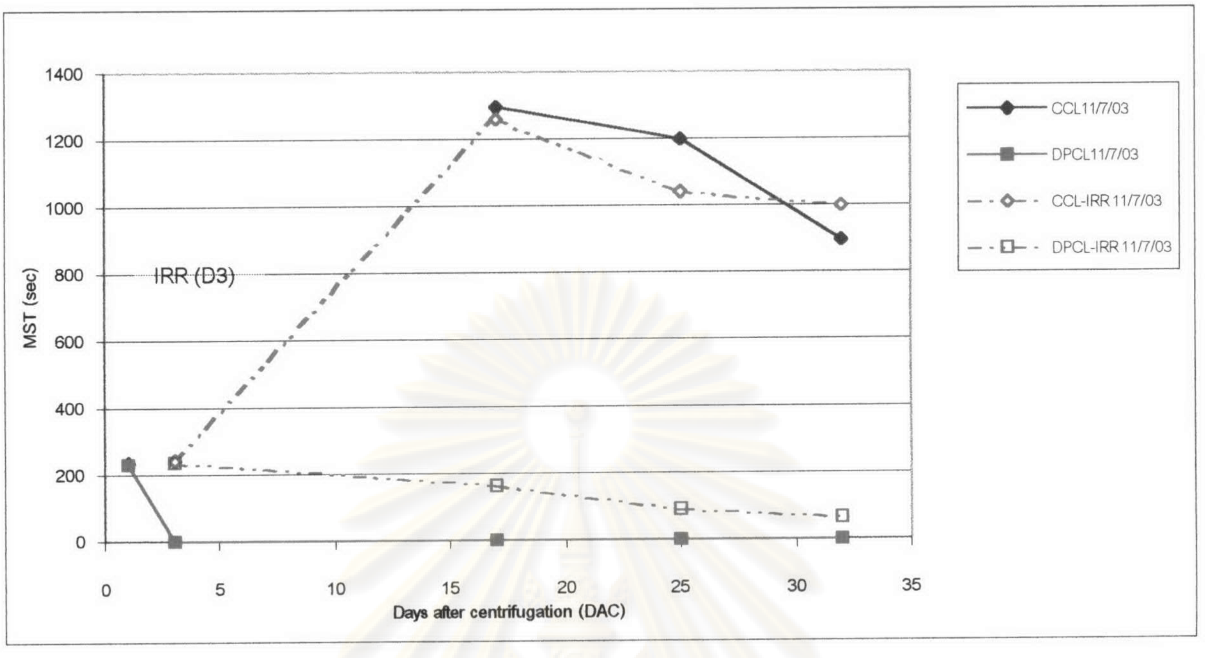
3.8 The effect of gamma radiation on MST of CCL, DPCL and AGCL

The mechanical stability of latex is usually defined as the resistance to those mechanical influences, which increase the number and violence of the collisions between particles and as a consequence tend to coagulate the latex. In this research, MST of enzyme-deproteinized latex was obviously low (200-300 sec) comparing to CCL. Figure 3.17 A and B show that 10 kGy irradiation on day 3 after centrifugation had no significant effect on MST of both CCL and DPCL 50%.

The MST profile of AGCL is very similar to CCL, that is the maximum MST about 1200-1400 sec was reached in about 20-30 days after centrifugation. Storage of control and AGCL for 90 days without stirring resulted in decreasing MST to about 900 sec, and both lots of AGCL showed slightly low MST comparing to CCL (Figure 3.18 A and B).

Irradiation on day 3 after centrifugation, where the MST were about 200 sec did not change the MST profile. On the other hand irradiation on day 80, 143 and 150 after centrifugation, where MST were about 800-900 sec increase the MST profile of IRRCL. The result indicated that the amount of proteins asssociated with rubber particles should affect MST of CL. Enzymatic deproteinization can attack the rubber protein, both soluble and protein associated with rubber particle. So the latex could not stabilize and had low MST. Although the IRR could not stabilize DPCL.

A. Lot No. 11/7/03



B. Lot No. 21/7/03

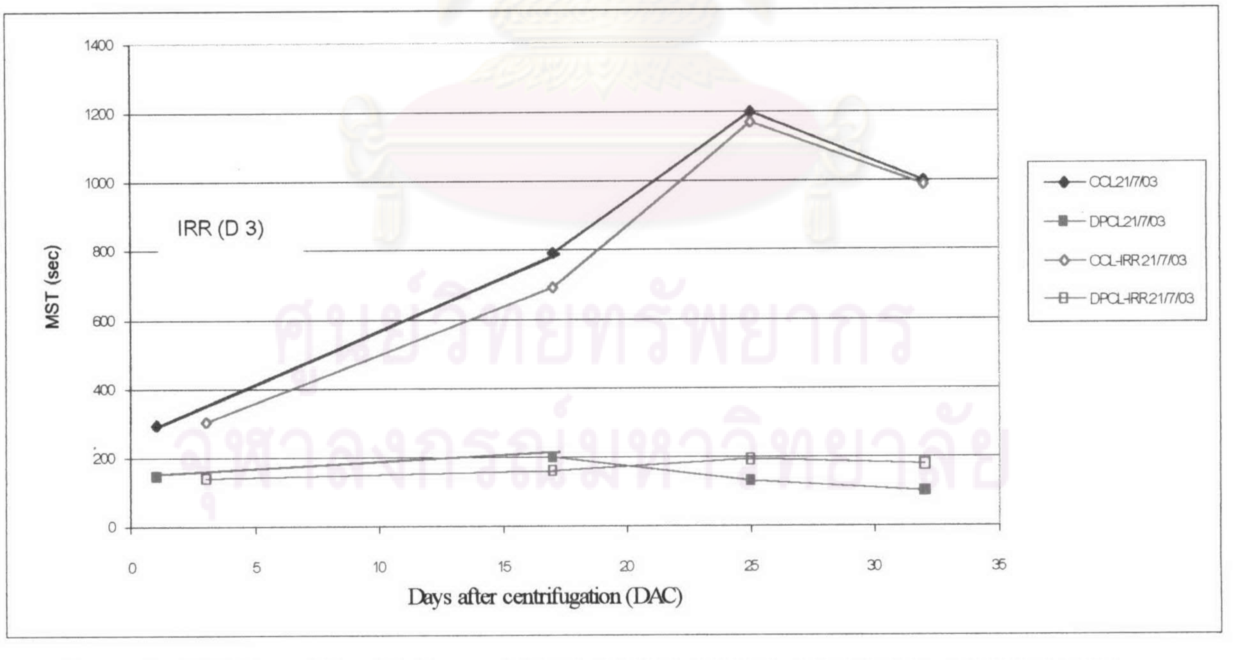
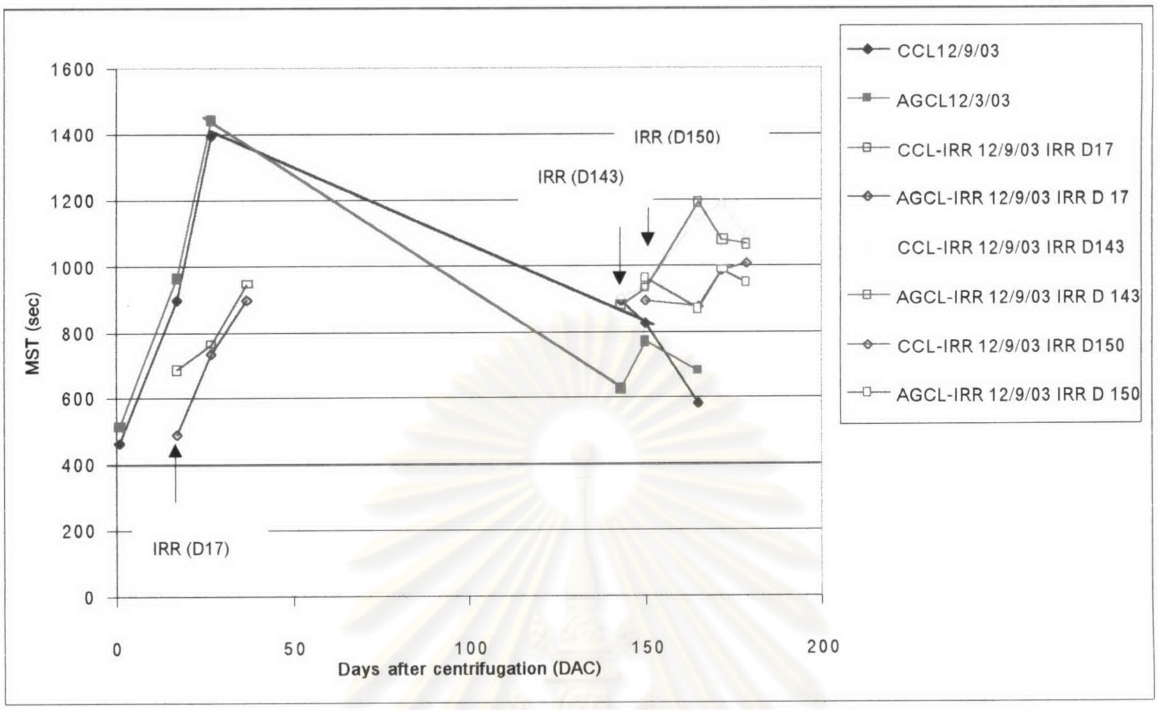


Figure 3.17 Effect of irradiation on MST of CCL, DPCL, CCL IRRand DPCL IRR

Irradiation 10 kGy on day 3 after centrifugation

A. Lot No. 12/9/03 Irradiation 10 kGy on 143 and 150 days after centrifugation



B Lot No. 4/11/03 : Irradiation 10 kGy on 80 days after centrifugation

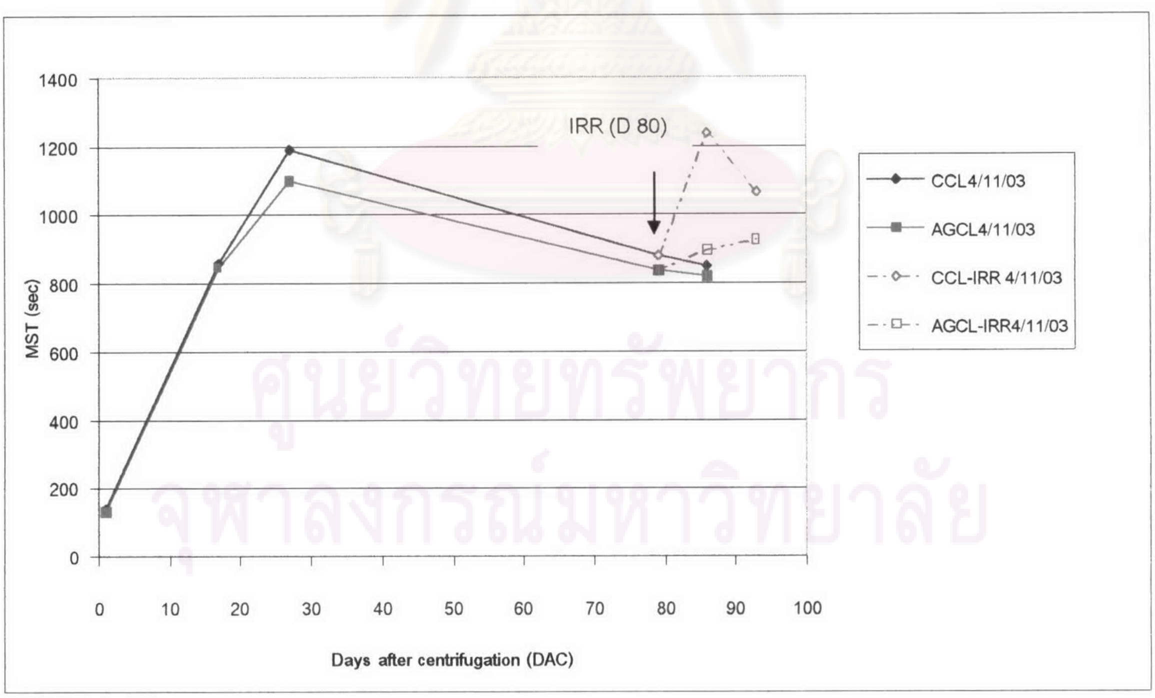


Figure 3.18 Effect of irradiation on MST of CCL, AGCL, CCL IRR and AGCL IRR irradiation 10 kGy .

3.9 The effect of leaching on WEP of latex films

Results of the effect of leaching latex films on WEP of irradiated latex films are given in Table 3.14.

Leaching latex film with 70°C distilled water could reduce WEP significantly. Increasing leaching time could increase WEP reduction efficiency. After irradiation at 10 kGy, rubber film had high crosslinking and it could trap protein into the crosslinking molecule so it can not leaching WEP better than non-irradiation.

Table 3.14 Effect of radiation vulcanization and leaching on WEP

Source of rubber film		WEP (μg/g rubber)				
		No Leaching	Leaching (RF : water = 1.0 g : 10 ml)			
Lot No.	Treatment		10 minutes	% reduction from no leaching	30 minutes	% reduction from no leaching
11/7/03	CCL	1830	608	66.78	279	84.75
	DPCL	54	25	53.70	10	81.48
	CCL-IR	2105	620	70.55	250	88.12
	DPCL-IR	303	293	3.30	127	58.09
21/7/03	CCL	1840	765	58.42	293	84.08
	DPCL	109	68	37.61	40	63.30
	CCL-IR	2152	246	88.57	104	95.18
	DPCL-IR	371	41	88.95	32	91.37
12/9/03	CCL	776	250	67.78	125	83.89
	AGCL	273	89	67.40	71	73.99
	CCL-IR	947	256	72.97	133	85.93
	AGCL-IR	388	40	89.69	20	94.85
4/11/03	CCL	697	436	37.45	201	71.16
	AGCL	253	179	29.25	40	84.19
	CCL-IR	940	339	63.94	154	83.62
	AGCL-IR	510	65	87.25	29	94.31
Reduction range				3.30 - 89.69		58.09 - 95.18

3.10 Effect of deprotenization, alginate addition and irradiation on MW distribution of WEP

From Figure 3.19, according to standard molecular weight markers (lane 1), FFL film (lane 2) shows several protein bands in the range of 97.0 to lower than 14.4 kDa. CCL film (lane 3) shows 4 clear bands of protein at 14.4, 27, 30 and 45 kDa where protein MW 14.4 kDa shows the highest intensity. It also displays smear band of protein MW lower 14.4 kDa. DPCL-film (lane 4) shows only 2 weak protein bands at 17.5 and 28.5 kDa. The control 10 kGy irradiated (lane 5) displays smear band of protein molecular weight lower than 30 kDa and 3 faint band of protein with molecular weight 30, 38 and 51. These 3 faint bands also appear in irradiated DPCL films (lane 6) but they had very low intensity. Leaching of latex films with 70°c water for 30 min (lane 7 and 8) show no band of protein although it was loaded at the maximum volume per well (20 μl protein solution / well).

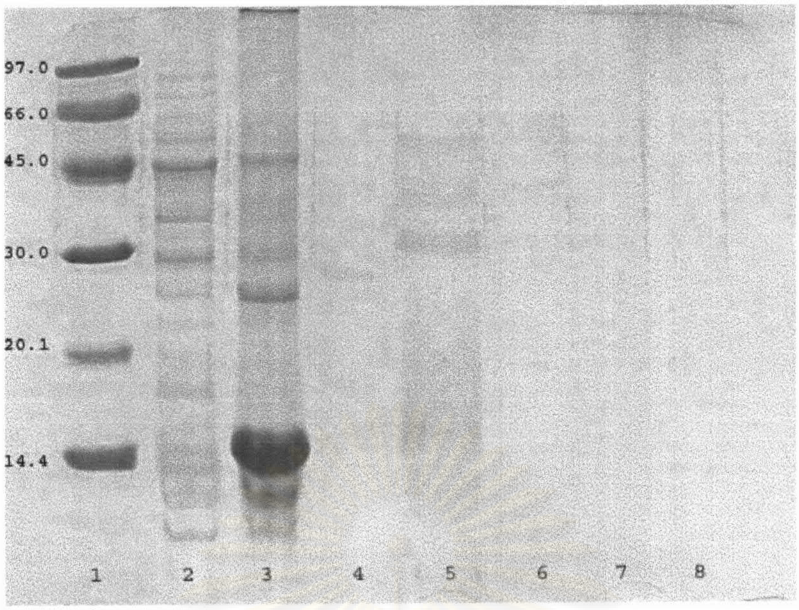


Figure 3.19 Effect of centrifugation, deproteinization and irradiation on MW distribution of WEP lot No 11/7/03

Lane 1 Standard molecular weight markers

Lane 2 WEP of FFL lot No.11/7/03

Lane 3 WEP of CCL lot No. 11/7/03

Lane 4 WEP of DPCL lot No. 11/7/03

Lane 5 WEP of CCL-IRR 10 kGy lot No. 11/7/03

Lane 6 WEP of DPCL-IRR 10 kGy lot No.11/7/03

Lane 7 WEP of CCL-IRR 10 kGy lot No. 11/7/03, then leaching 30 min

Lane 8 WEP of DPCL-IRR 10 kGy lot No 11/7/03, then leaching 30 min

Well 2-6 was loaded with 20 μg protein and well 7-8 was loaded with 20 μl protein solution containing protein lower 1 μg protein.

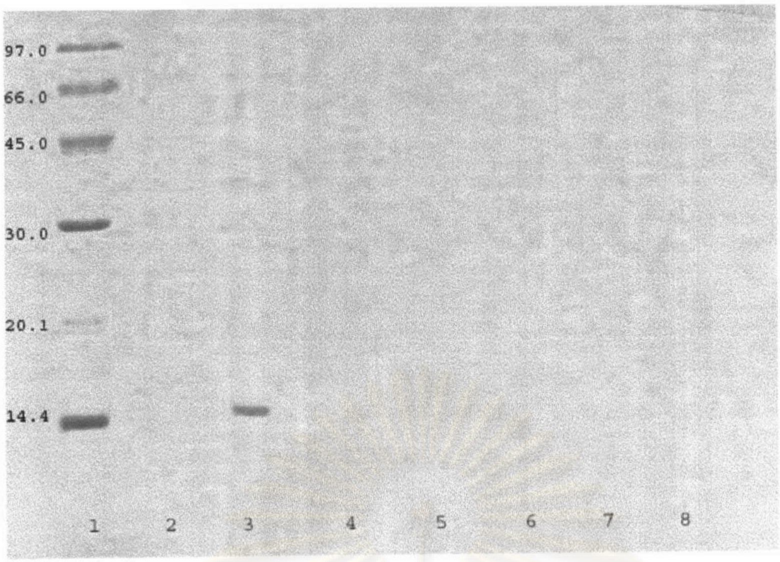


Figure 3.20 Effect of centrifugation, deproteinization and irradiation on MW distribution of WEP lot No.21/7/03

Lane 1 Standard molecular weight markers

Lane 3 WEP of CCL lot No. 21/7/03

Lane 4 WEP of DPCL lot No. 21/7/03

Lane 5 WEP of CCL-IRR 10 kGy lot No. 21/7/03

Lane 6 WEP of DPCL-IRR 10 kGy lot No.21/7/03

Lane 7 WEP of CCL-IRR 10 kGy lot No. 21/7/03, then leaching 30 min

Lane 8 WEP of DPCL-IRR 10 kGy lot No 21/7/03, then leaching 30 min

Well 2-6 was loaded with 20 μg protein and well 7-8 was loaded with 20 μl protein solution containing protein lower 1 μg protein.

Figure 3.20 ,according to standard molecular weight markers (lane 1) FFL film (lane 2) shows several protein bands in the range 97.0 – 14.4 kDa. CCL (lane 3) show major band of 14.4 kDa. DPCL film (lane 4) show a very slightly band of protein with molecular weight of 28.5 kDa. CCL-IRR (lane 5) show the 3 major band of 30, 35 and 45 kDa. It also displays smear band of protein MW lower 14.4 kDa too. But all of these proteins disappear in DPCL-IRR. Leaching film can remove the proteins confirm by no band of protein although it was loaded at the maximum volume per well (20 μl protein solution / well).

Figure 3.21 and 3.22 demonstrated distribution patterns of WEP from non-irradiated and irradiated alginate addition latex lot No.12/9/03 and 4/11/03. There are several proteins band of FFL and CCL (lane 2, 3). They are the same pattern of protein band of CCL and AGCL (lane 3,4). The major bands are 14.4 , 26, 30, 32, 37 and 45 kDa. After irradiated and leaching (lane5, 6,7 and 8) there are no protein band appear although it was loaded at the maximum volume per well (20 μl protein solution / well).

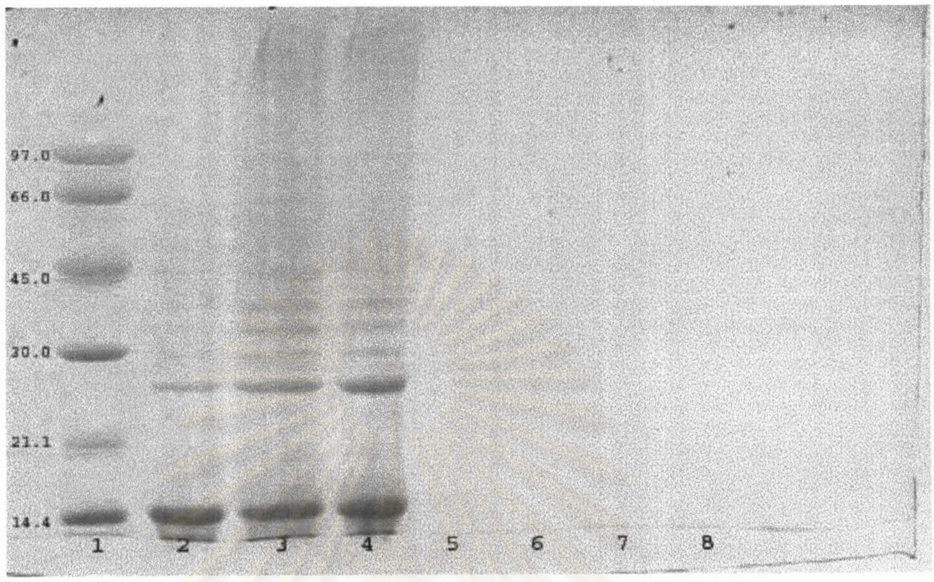


Figure 3.21 Effect of centrifugation, alginate addition and irradiation on MW distribution of WEP lot No.12/9/03

Lane 1 Standard molecular weight markers

Lane 2 WEP of FFL lot No.12/9/03

Lane 3 WEP of CCL lot No. 12/9/03

Lane 4 WEP of AGCL lot No. 12/9/03

Lane 5 WEP of CCL-IRR 10 kGy lot No. 12/9/03

Lane 6 WEP of AGCL-IRR 10 kGy lot No.12/9/03

Lane 7 WEP of CCL-IRR 10 kGy lot No. 12/9/03, then leaching 30 min

Lane 8 WEP of AGCL-IRR 10 kGy lot No 12/9/03, then leaching 30 min

Well 2-4 was loaded with 40 μg protein and well 5-8 was loaded with 20 μl protein solution containing protein lower 5 μg protein.

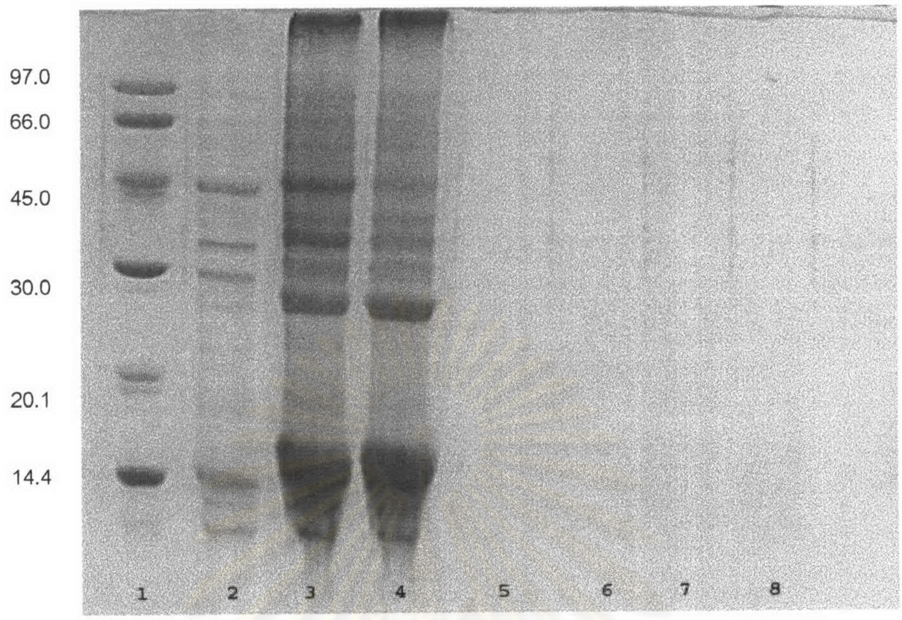


Figure 3.22 Effect of centrifugation, alginate addition and irradiation on MW distribution of WEP lot No 4/11/03

Lane 1	Standard molecular weight markers
Lane 2	WEP of FFL lot No.4/11/03
Lane 3	WEP of CCL lot No. 4/11/03
Lane 4	WEP of AGCL lot No. 4/11/03
Lane 5	WEP of CCL-IRR 10 kGy lot No. 4/11/03
Lane 6	WEP of AGCL-IRR 10 kGy lot No.4/11/03
Lane 7	WEP of CCL-IRR 10 kGy lot No. 4/11/03, then leaching 30 min
Lane 8	WEP of AGCL-IRR 10 kGy lot No 4/11/03, then leaching 30 min

Well 2 was loaded 20 μg protein, Well 3-4 was loaded with 40 μg protein and well 5-8 was loaded with 20 μl protein solution containing protein lower 5 μg protein.

3.11 Allergen detection by skin prick test (SPT)

This study was approved by the Human Right and Ethics Committee of the Division of Dermatology, Department of Medicine, Faculty of Medicine, Chulalongkorn Hospital. There were 2 persons who had volunteered for SPT. The volunteered had been sensitized with latex glove. The SPT was kindly conducted by Assoc. Prof., Dr. Porntip Puvabanditsin. Table 3.15 shows that 2 subjects gave positive SPT results when using latex proteins prepared from commercial standard latex allergen. In contrast both of latex proteins prepared from control and AG-IR-CL Leaching showed negative results. These results indicated that the latex proteins though visible by SDS PAGE are not allergen and could not response in subjects.

Table 3.15 Allergen detection by SPT

Test solution	Protein (μg/ml)	Skin prick testing
Normal saline (negative control)	-	NEG
Histamine phosphate 1000 μg/ml (Positive control)	-	POS
Commercial standard latex allergens (Stallergene, Extraints Allergeniques, France)	40,000	POS
CL-IRR Leaching	13	NEG
AG-IRR-CL Leaching	0.2	NEG

CL : concentrated latex

AG-IRR-CL : Alginate addition plus irradiation and centrifugation.

POS : Wheal ≥ 50% of Histamine wheal.

NEG : Wheal < 50% of Histamine wheal.

3.12 Physical properties on RVNRL

The tensile strengths and other physical properties of the irradiated rubber films (0.2-0.32 and 2 mm thickness) was prepared from the irradiated rubber latex are shown in Table 3.16 and 3.17.

Table 3.16 Physical properties on RVNRL at thickness 0.2-0.3 mm

Source of rubber film		Physical properties				
Lot No.	Treatment	Tensile strength MPa	% 300 modulus MPa	Elongation at break (%)	Tear strength (N/cm)	Thickness (mm)
21/7/03	CCL-IRR	7.16	0.59	845	186	0.20-0.30
	DPCL-IRR	8.73	0.59	1015	167	0.2-0.30
12/9/03	CCl-IRR	7.84	0.78	925	235	0.25-0.32
	AGCL-IRR	8.04	0.78	988	255	0.23-0.31
Dental rubber dam	Heavy	9.90	1.27	800	519	0.30
	Soft	9.21	0.98	750	402	0.20

Table 3.17 Physical properties on RVNRL at thickness 2.0 mm

Source of rubber film	Physical properties	
	% 500 modulus (MPa)	Shore A
CCl-IR	0.33	15
AGCL-IR	0.37	17
SVNR	0.57	20

From Table 3.16 shows that the tensile strength, 300% modulus, elongation at break and tear strength were no different between control CL-IR and DPCL-IR or AGCL-IR. This indicated that the treatment of enzymatic deproteinization and alginate addition didn't affect with physical properties after irradiation. Comparison of dental dam and RVNRL found that tear strength of dental dam higher than RVNRL. This indicate that rubber dam or SVNRL have higher resistance to tear forces than RVNRL.

From Table 3.17 shows that both of 500 % modulus and Shore A of SVNR higher than control and AGCL-IR. This indicate that RVNRL had lower strength and softer than SVNRL.