EXTRACTABLE PROTEIN OF RADIATION VULCANIZED NATURAL RUBBER LATEX

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ABSTRACT A new method to reduce the protein level in the latex products by irradiation is reported. Water soluble protein (WSP) solution (10%) was added into radiation vulcanized NR latex (RVNRL) as much as 3 phr in three different processes: added to RVNRL, added to re-centrifuged RVNRL, and added to RVNRL followed by centrifugation. The protein content was determined by enhanced BCA method, and identified by SDS-PAGE analysis. Addition of WSP followed by centrifugation reduces EP up to the minimum protein detection, and shortens the leaching time to 20-30 min. SDS-PAGE analysis confirms the reduction of soluble protein in the serum phase, and disappearance of protein bands in the rubber extract. Protein-WSP interaction produces water soluble complex, and removed by centrifugation. The molecular weight of WSP dictates the efficiency of protein removal.

INTRODUCTION

The addition of WSP into RV NR latex has improved the physical properties of the rubber film such as tear strength and tackiness of the film.[1,2] However, the film transparency depends on the type of WSP. Standard BCA protocol was not able to detect extractable protein (EP) after 15 min leaching of WSP containing rubber films in 1% ammonia solution. Recently it is also reported that dilution followed by centrifugation of RV NRL effectively reduces extractable protein content in RV NR latex.[3]

This paper describes the effect of combining WSP addition and centrifugation on EP. The minimum level of detection is reduced up to 50μg/g by enhanced BCA method. The effect of WSP's molecular weight on the removal of water soluble protein, SDS-PAGE of the serum latex and the rubber extract after washing the films were also studied.

MATERIALS AND METHODS

Preparation of latex films. Centrifuged latex was diluted up to 50% DRC using 1% ammonia solution, stabilized by 0.5phr KOH as 10% solution, 5phr of n-BA was added as the sensitizer to the latex while stirring. Irradiated by γ-rays from a Co-60 at a dose rate of 10 kGy/h for 2hrs. The WSP were high molecular weight (HMW) PVA (90.000), PVP (360.000), and low molecular weight (LMW) PVA (22.500) and PVP (40,000). They were prepared as 10 % solution, and added to RVNRL as much as 3 phr by 3 different processes (Table 1). The treated RVNRL was centrifuged by SPL-100 SAITO centrifuge machine. Rubber films (casting) were dried in air, leached in 1% ammonia solution at r.t for various period of times, post dried in air, and finally heated in the oven at 80℃ for 1hr.

Table 1 Processes of RVNRL treatment

Process	Procedure
STANDARD	Only mixing of WSP with RV NRL (50% DRC)

PRE-centrifugation	(1) dilution of RV NRL to 30% DRC (2) centrifugation (3) addition of WSP to the centrifuged RVNRL
POST-centrifugation	(1) dilution of RV NRL to 30% DRC (2) addition of WSP to the diluted RVNRL (3) centrifugation the WSP added diluted RVNRL

Protein assay. EP was obtained by extraction of 1 g rubber film in 10 ml water for 2 hours. Serum protein was obtained by ultra-centrifugation of the latex at 12.000 rpm for 2hr followed by re-centrifugation of the serum at 12.000rpm for 1hr. The protein was precipitated by centrifugation.[4] The precipitated protein was dissolved directly with BCA working reagent. Enhanced protocol of BCA method (at 60℃ for 30 min.) was used to increase the minimum detection level up to 5μg/ml. The protein concentration was measured at 562nm using Shimadzu 800 UV-visible spectrophotometer.

Polyacrylamide gel electrophoresis. Separation of protein was carried out by glycine SDS-PAGE. It was performed using a discontinuous buffer system according to Laemmli.[5] Protein latex was incubated in the sample buffer solution at 100°C for 5 min. Electrophoresis was performed at 15°C at a constant current of 10 mA for 2 hours. Separated proteins were fixed in the solution containing 50% methanol, 10% acetic acid, and 40% distilled water for 30 min, and stained by silver nitrate (Silver Stain KANTO III).

RESULTS AND DISCUSSION

Protein content, Figure 1 showed the extractable protein (EP) of rubber films achieved by the three processes. The addition of 3 phr WSP (in this case PVA) increased the EP in standard process from 1.00 mg/g to 1.406 mg/g prior to leaching. The same tendency was observed in pre-centrifugation, but the EP was 90% lower due to dilution and centrifugation. Thus, the added PVA has enhanced protein diffusion into the external water phase during dipping.

Irradiation induces protein degradation, and enhances its solubility in the serum phase. Since WSP and protein molecules have polar sites along their chains, interaction of protein-WSP may occur and produces water soluble protein-WSP complexes. In post-centrifugation process, the level of EP achieved prior to leaching was 0.057 mg/g (95% removed). Thus, the WSP-protein complexes were removed with the water phase during centrifugation to obtain concentrated RVNRL.

Further EP reduction was achieved by leaching the rubber films in the ammonia solution (1%). After 10 min leaching, the remained EP following the standard process was 0.171 mg/g (83% removed), but it still remained 0.07 mg/g even after 30 min leaching time (93% removed). The effect of leaching on films obtained from pre- and post-centrifugation processes were not obvious, because the two processes have induced low protein RVNRL before leaching. However, minimum EP could be achieved after a short leaching time. After 20-30 min leaching, EP of the rubber films were hardly detected by the enhanced BCA method (less than 50μg/g.)

Table 2 listed the EP and the serum protein of post-centrifuged RVNRL. Higher concentration of WSP (3 phr) lead to low level of EP, and high level serum protein regardless the chemical structure and molecular weight of the WSP. These showed that protein were bound to WSP in the serum phase, and would be removed by re-centrifugation of RVNRL.

Effect of WSP's molecular weight on the water soluble protein removal. Table 2 showed lowest EP (0.019 and 0.025 mg/g) were achieved by the addition of 3 phr LMW- WSP (PVA, 22,500 and PVP, 40,000, respectively). The efficiency of protein removal depends on the water solubility of WSP which is dictated by the molecular weight, degree of saponification (PVA), and association between and within the chains.[6] High molecular weight (HMW) PVP (360,000) showed better efficiency than HMW-PVA (90,000), because PVP has higher polarity than PVA (carbonyl group in the cyclic ring).

Table 2 Protein content in the post-centrifuged RVNRL

Water Soluble Polymer	Molecular Weight ($\times 10^3$)	Concentration (phr)	Protein Content	
			Rubber *) (mg/g)	Serum (mg/g)
-	-	-	0.107	3.06
PVA	22.5	1.0	0.033	3.32
		3.0	0.019	3.68
	90	1.0	0.077	3.18
		3.0	0.062	3.34
PVP	40	1.0	0.039	3.22
		3.0	0.025	3.56
	360	1.0	0.049	3.12
		3.0	0.043	3.44

*) EP prior to leaching

Gel electrophoresis. Electrophoresis of the serum latex obtained from ultra-centrifugation of the latex are shown in Fig. 2. Non irradiated latex (lane 2) showed protein components mainly about 14 kDa, and some amount about 30 kDa. In the present experiment protein band at 67 kDa as reported by Jaeger et. al did not appear in the results.[7] Jaeger et.al used latex extract by precipitation of rubber phase by acetic acid. The protein bands became more intense after irradiation (lane 3 and 4) which mean the increase of these proteins in the serum phase. Lane 5 showed the remained protein in the serum after addition of PVA (22,500) followed by re-centrifugation. The protein-WSP complex molecules have very high molecular weight which are not possible to pass through the pores of the gel. Therefore, the protein bands intensity significantly reduced in comparison with the irradiated ones. No protein bands appeared from the rubber extract (24 hours) even after concentration prior to sample incubation. It showed that the remained proteins in the serum latex (17%) after centrifugation were leached out during leaching in the ammonia solution.

CONCLUSION

Combining dilution, WSP addition, and centrifugation of RVNRL has beeb able to reduce the EP of the rubber films to a level less than 50μg/g, and shorten the leaching time to 20-30 min. SDS-PAGE analysis confirmed the reduction of water soluble protein in the serum latex after post-centrifugation, and disappearance of the protein bands in the rubber extract after leaching . Protein- WSP interaction produces water soluble complexes which will be removed during re-centrifugation of the treated RVNRL. Low molecular weight WSP is more effective than high molecular one due to its higher solubility in the serum phase.

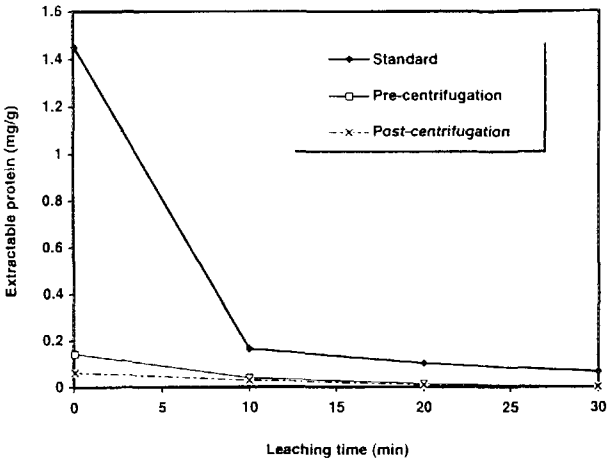

Fig.1 Comparison of EP from 3 processes (3phr of PVA)

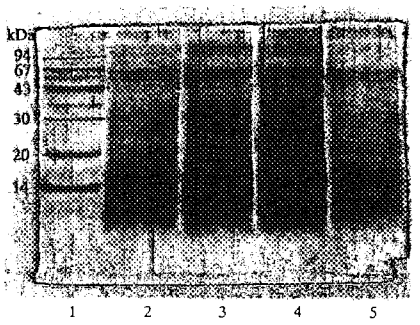

Fig. 2. SDS-PAGE (15%gel) of serum protein: (1)marker, (2)non-irrad, (3)irrad 20 kGy, (4)40 kGy, (5)post-centrifuged (20 kGy,PVA 22500)

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