7. Extractable Protein of Radiation Vulcanized Natural Rubber Latex

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Abstract

Protein remained in the latex products are reported to cause serious allergy. 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) in three different processes. The amount of WSP was 3 phr. It was only added to RVNRL (standard), added to re-centrifuged RVNRL (pre-centrifugation), and added to RVNRL followed by centrifugation (post-centrifugation). The protein content was determined by enhanced BCA method, and identified by SDS-PAGE. Extractable protein (EP) from the rubber has been reduced up to the minimum protein detection by combining WSP addition and centrifugation. Short leaching time (20-30 min.) can be achieved after the combine treatment, and SDS-PAGE 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 efficiency of protein removal by WSP depends on its molecular weight of WSP which relates to its water solubility.

Keywords: Extractable protein, Water soluble protein, Post-centrifugation, Gel electrophoresis

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. It was also reported that addition of WSP lead to low residual protein content after leaching of the rubber films. After 15 min. leaching in 1% ammonia solution no extractable protein can be detected by standard BCA protocol, which shows that the protein level is less than 200μg/g. More recently we have also found out that dilution of RVNRL followed by centrifugation effectively reduces EP. The minimum protein detection was reduced up to 0.50μg/g by using enhanced BCA method.[3]

In this paper we report the effect of combining WSP addition and centrifugation on EP of RVNRL films. The protein level in the latex (serum and rubber) was determined 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% dry rubber content (DRC) using 1% ammonia solution, stabilized by 0.5phr KOH as 10% solution, 5phr of n-butyl acrylate (n-BA) was added as the sensitizer to the latex while stirring. Gamma-rays irradiation from a Co-60 source was carried out at a dose rate of 10 kGy/h for 2hrs.

The WSP in the present experiment 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 were prepared by casting on the glass plates, dried in air until it became transparent. The dried films were then leached in 1% ammonia solution at room temperature for various period of times. Post drying of the films in air until they became transparent , 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.

Extractable protein (EP) content is the remaining extractable protein after a rubber film has been leached and properly dried. It was obtained by extraction of 1 g rubber film in 10 ml water for 2 hrs. Serum protein was obtained by ultra-centrifugation of the latex at 12.000 rpm for 2hrs to collect the serum, and the collected serum was again centrifuged at 12.000rpm for 1hr. Interference substances were separated by centrifugation.[4] The precipitated protein was dissolved directly with BCA working reagent. Enhanced protocol of BCA method (at 60°C 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 SDS-PAGE. It was performed using a discontinuous buffer system according to Laemmli.[5] Vertical slab of gel (stacking gel and separation gels were 7.5%, and 15% acrylamide, respectively). The gel dimension was 80×80×1 mm. The sample and stacking gel contain Tris.Cl (pH 6.8), the separation gel contains Tris.Cl (pH 8.8), and the running buffer contains Tris-glycine (pH 8.3). All components contain 0.1% SDS. Protein latex was incubated in the sample buffer solution (pH 6.8) 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 EP obtained from untreated RVNRL without leaching was 1.00 mg/g, and the presence of 3 phr PVA in the standard process increased the EP to 1.406 mg/g (about 40%). In pre-centrifugation process, the water soluble proteins were first removed during re-concentration of the diluted RVNRL (EP = 0.1005 mg/g, 90% removal), and it became slightly higher (0.1428 mg/g) if PVA was added. However, EP prior to leaching reached the lowest level (0.057 mg/g, 95% removal) in post-centrifugation process where PVA was added prior to centrifugation. Thus, there was a tendency of EP increase if PVA was added, and EP reduction if the PVA added RVNRL was re-centrifuged. The results suggested that the added PVA has enhanced protein diffusion into the external water phase during dipping.

Since WSP and protein molecules have polar sites along their chains, interaction of protein-WSP may occur and produces water soluble protein-WSP complexes. From the results we suggested the mechanism of enhancement as follows: the protein-WSP complexes move to the film surface during drying, and they will be leached out during dipping in the water such as leaching water or dipping water for protein extraction. The removal become easier since the protein macromolecules have been degraded by irradiation. In post-centrifugation process, the protein-WSP complexes were removed during centrifugation, therefore, the level of EP achieved the lowest value prior to leaching (0.057 mg/g).

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). The drastic reduction of EP has been explained in the previous paragraph. However, after 30 min leaching the value of EP was still 0.07 mg/g which means only 93% removal. 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.)

Effect of WSP's molecular weight on the water soluble protein removal.

Figure 2 shows the effect of 3 phr WSP on the EP of rubber films obtained by post-centrifugation process. The results showed that WSP used in the present experiment induce EP reduction regardless their molecular structure. However, lowest EP (prior to leaching) were achieved by the addition of LMW- WSP (PVA, 22,500 and PVP, 40,000): 0.019 and 0.025 mg/g, respectively. Thus, efficiency of protein removal depends on the molecular weight of the WSP. This molecular weight dependence associates with the water solubility of WSP. Moreover, water solubility of PVA is also determined by the degree of saponification, and in general water solubility is also determined by some other factors.[6] It can be seen in the results that HMW-PVP (360,000) has better efficiency than HMW-PVA (90,000). The reason might be the difference of polarity of WSP caused by conformational changes in the solution.

The protein content of serum latex obtained by post-centrifugation process were listed in Table 2 together with the EP values prior to leaching. It was clear that LMW-WSP induced higher protein content in the serum at low (0.1 phr) and high (0.3 phr) concentration. These results showed that protein preferred to combine with WSP rather than being adsorbed on the rubber phase, and existed in the serum phase. Due to this preference, the remaining protein in the rubber phase became less and the EP decreased.

Upon casting and drying of the rubber films, these WSP-protein complexes will migrate to the film surface. They would be leached out during leaching or dipping in the water for EP measurement as described in the earlier paragraph. .

Table 2 Protein content in the post-centrifuged RVNRL

WSP	Mw ($\times 10^3$)	Conc. (phr)	Protein Content (mg/g)	
			Rubber *)	Serum
-	-	-	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. 3. 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.

Physical properties.

Since physical and mechanical properties are not the main concern in this report, they will be not discussed here. However, it can be reported that the presence of LMW-WSP up to 3 phr hardly changed the viscosity of post-centrifuged RVNRL, although the tensile strength of the rubber films decreased as reported before. Tackiness of the rubber films (prior to leaching) drastically improved by post-centrifugation process, but after leaching the tackiness increased again although it was not as high as the non treated one (conventional RVNRL). It showed that WSP together with the protein were leached out by water during leaching. Therefore, short leaching time is recommended.

Conclusion

Combining dilution, WSP addition, and centrifugation of RVNRL has been 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.

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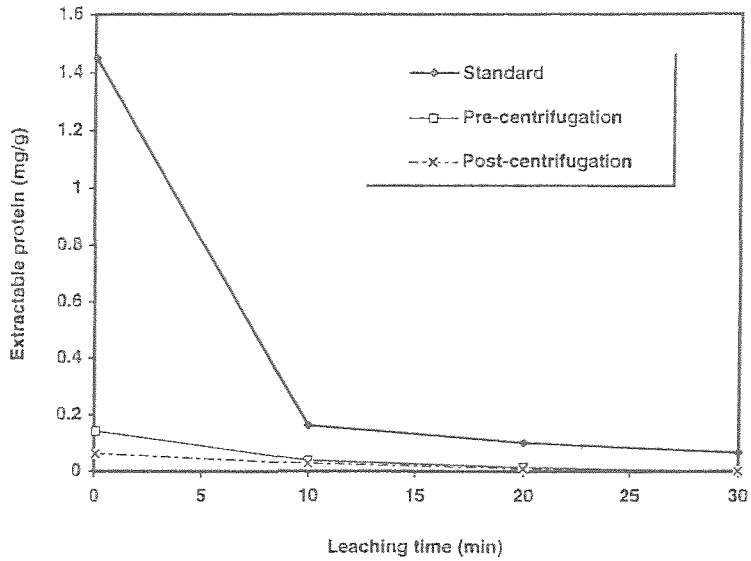

Fig.1 Comparison of EP from 3 processes (3phr of PVA)

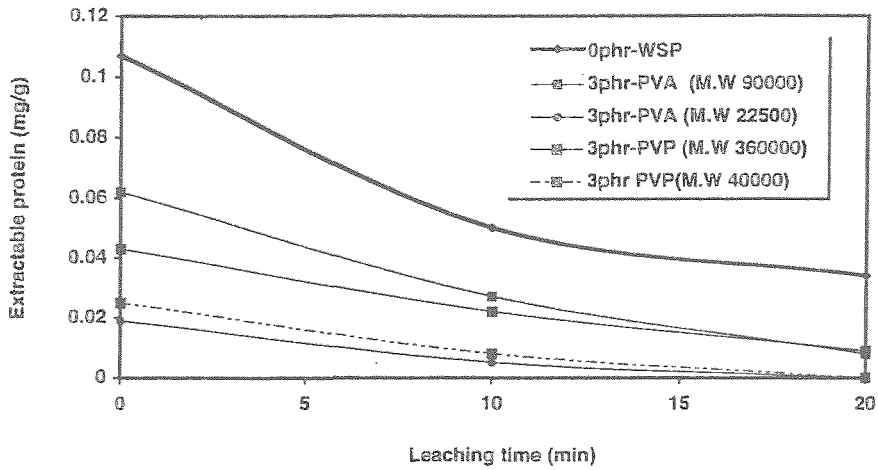

Fig.2. Effect of different WSP on EP (post-centrifugation)

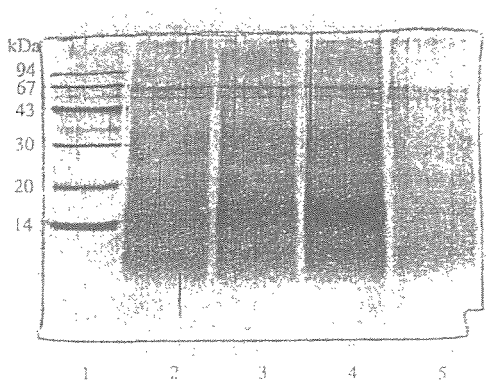

Fig. 3. SDS-PAGE (15%gel) of serum protein: (1)marker, (2)non-irrad, (3)irrad 20 kGy, (4)40 kGy, (5)post-centrifuged (20 kGy,PVA 22500)