

Soluble-protein-free Radiation-vulcanised Natural Rubber Latex

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The effect of water-soluble polymers (WSPs), such as polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP), on radiation-vulcanised natural rubber latex (RVNRL) was investigated. Extractable protein measurements showed that mixing of WSP into RVNRL, followed by centrifugation, effectively reduced the extractable protein from RVNRL. However, extractable protein could be further effectively removed from RVNRL by adding low-molecular-weight PVA (LMW-PVA) and PVP (LMW-PVP). It was further observed that, using 3 p.h.r. of either LMW-PVA or LMW-PVP followed by 20 min leaching in 1% ammonia solution, was sufficient to produce RVNRL free of soluble proteins. Based on these results a new process has been developed by the incorporation of LMW-WSP into RVNRL and centrifugation, followed by leaching. Tensile properties such as tensile and tear strength, were comparable to those of conventional RVNL. Fourier Transform Infrared measurements showed that most of the WSP was removed from RVNRL during the centrifugation process. The viscosity of the RVNRL was not much affected by the addition of LMR-WSP.

Key words: NR latex; radiation-vulcanised NR latex; extractable protein; molecular weight; water-soluble polymers; polyvinyl pyrrolidone; centrifugation; FTIR

Radiation vulcanisation confers several advantages such as less nitrosoamine, low cytotoxicity, fewer allergies *etc.*, and has been developed as a viable alternative process to conventional sulphur vulcanisation¹⁻⁴.

Fresh natural rubber latex contains rubber hydrocarbon as well as non-rubber constituents such as proteins, lipids, carbohydrates and inorganic salts. Two kinds of proteins, namely those which are tightly bound to the rubber particles and those which are extractable, are

present in latex products. Some of the extractable proteins that still exist in products could be removed during the leaching process but some proportion will remain in the final products.

There are two types of allergic reactions; namely type I and IV allergic reaction in some sensitised persons by natural rubber products. Some residual water-extractable proteins present in the latex products cause this type I or immediate type allergic reactions.^{5,6}

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Irradiation does have a destructive effect on natural rubber proteins. However, all proteins are not destroyed or removed by irradiation. The remaining proteins are still able to provoke type I allergic reactions⁷. Several methods have been used to reduce the extractable protein content in radiation-vulcanised natural rubber latex (RVNRL)^{8,9}. It has been reported that water-soluble polymers (WSPs) can be used to reduce the concentration of extractable proteins in RVNRL⁸. The soluble protein content in RVNRL is reduced more effectively, by combined treatment with polyvinyl alcohol (PVA) and centrifugation, compared to individual (*i.e.* PVA addition or centrifugation) treatment¹⁰.

In this study two WSPs, PVA and polyvinyl pyrrolidone (PVP) were added to RVNRL and the latex centrifuged, the purpose being to study the effect of WSP and centrifugation on extractable proteins in RVNRL. High-molecular-weight (HMW) and low-molecular-weight (LMW) PVA and PVP were used to investigate the effect of molecular weight of the WSP on extractable protein. This paper discusses the results of the variation of extractable protein content and mechanical properties of RVNRL films with the molecular weight of the added WSP. IR measurements of the WSP incorporated RVNRL films were used to ascertain the percentage of WSP remaining in RVNRL latex films after centrifugation and leaching. This paper also discusses how the stability of the RVNRL is varied when HMW-WSP and LMW-WSP are used to remove soluble protein from RVNRL.

MATERIALS AND METHODS

Materials

Dunlop Malaysia supplied high ammonia centrifuged latex (60% DRC). n-Butyl acrylate and 10% KOH solution were used as radiation

vulcanisation accelerator and stabiliser, respectively. HMW-PVA (MW 90 000), HMW-PVP (MW 360 000), LMW-PVA (MW 22 500) and LMW-PVP (MW 40 000) were used in this work. WSP was prepared as 10% aqueous solution by using an autoclave. RVNRL containing WSP was centrifuged using an SPL – 100 SAITO centrifuging machine.

Preparation of latex films

Concentrated latex was diluted to 50% dry rubber content (DRC) using 1% ammonia solution. 0.5 p.h.r. of 10% KOH was added, followed by 5 p.h.r. of n-butyl acrylate to the latex while stirring. Irradiation was carried out by gamma rays from a Co-60 source at a rate of 10 kGy/h for 2 h. The irradiated latex was diluted to 30% DRC and was mixed with a 10% solution of WSP at different proportions. The viscosity of the RVNRL was increased when 10% WSP was added to irradiated latex. The viscosity of the WSP added RVNRL, was measured at 25°C using a viscometer made by Tokyo Keki Co. Ltd., Japan. RVNRL containing WSP was centrifuged and the rubber phase was separated from serum. Latex films were cast on glass plates and were dried in air until the films became transparent. Dried latex films were leached in 1% ammonia solution at room temperature for various time intervals, then post dried in air and in an oven at 80°C for 1 h.

Protein Assay

After leaching the RVNRL films, the content of residual soluble protein in the latex films was measured using bicinchonic acid (BCA) method. One gram of latex film sample was extracted with 10 mL of distilled water at 37°C for 2 h. Interfering substances

were precipitated by centrifugation¹¹. The precipitated protein was dissolved directly in the BCA working reagent. Enhanced protocol of BCA method (30 min at 60°C) was used to increase the minimum detection level up to 5 µg/mL. The protein concentration was measured at 562 nm using Shimadzu 800 uv-visible spectrophotometer.

Infrared (IR) Measurements

The latex films for IR measurements were prepared by dipping small glass slides into the RVNRL. Latex films were dried in an air oven at 100°C until their weights were constant. IR measurements were carried out on an FTIR-800 Shimadzu spectrophotometer. Due to the difficulty of preparing films of the same thickness, the height ratio of the two peaks corresponding to C-H bonding of rubber hydrocarbon at 1449 cm⁻¹ and stretch vibrations of N-H and O-H, respectively of rubber protein and PVA at 3300 – 3330 cm⁻¹ were calculated in order to determine the PVA remaining in the RVNRL after leaching and centrifugation.

Mechanical Properties

After leaching for 20 min in 1% ammonia solution and drying in an oven at 80°C for 1 h, the tensile properties of the latex films were determined using a Stograph RI (Toyoseki Co. Ltd.) tensile machine.

RESULTS AND DISCUSSION

In a previous study it was observed that PVA which has 90 000 molecular weight could be used to reduce the soluble protein content in RVNRL¹⁰. It was further found that combined treatment of PVA addition and centrifugation was more effective than individual treatment.

Figure 1 shows the effect of leaching time on extractable protein content when HMW (90 000) and LMW (22 500) PVA were added to RVNRL followed by centrifugation. The figure clearly shows the significant effect of the addition of PVA in reducing soluble protein in RVNRL. This was due to removal of soluble protein with PVA, when PVA treated latex was centrifuged. When 3 p.h.r. of HMW-PVA were used, the soluble protein content was reduced from 0.110 mg/g to 0.062 mg/g (*i.e.* by 44%); whereas when the same concentration of LMW-PVA was used, the soluble protein content was reduced from 0.110 mg/g to 0.019 mg/g (*i.e.* by 83%). Comparing the effect of the addition of high- and low- molecular weight PVA, about 40% more reduction of soluble protein was achieved by using 3 p.h.r. LMW-PVA. This could be attributed to the fact that the more water-soluble LMW-PVA had a greater affinity to soluble proteins than had the less soluble HMW-PVA and, therefore soluble protein was removed with LMW-PVA more effectively during centrifugation process. Proper leaching of RVNRL films is known to be an important process for reducing the soluble protein content to a lower level^{12,13}. Leaching of RVNRL films containing PVA seems to play an important role in removing the remaining extractable proteins. Figure 1 also shows that 20 min leaching time was sufficient to produce RVNRL films free of soluble protein when the films were prepared by addition of 3 p.h.r. of LMW-PVA to RVNRL and centrifuged.

Figure 2 shows the variation of extractable protein content of RVNRL with increasing leaching time after addition of PVPs of different molecular weight followed by centrifuging. The effect of PVP on extractable protein was similar to that of PVA. However comparing low- and high-molecular weight PVP, 3 p.h.r. of LMW-PVP was able to reduce extractable protein by a further 20%.

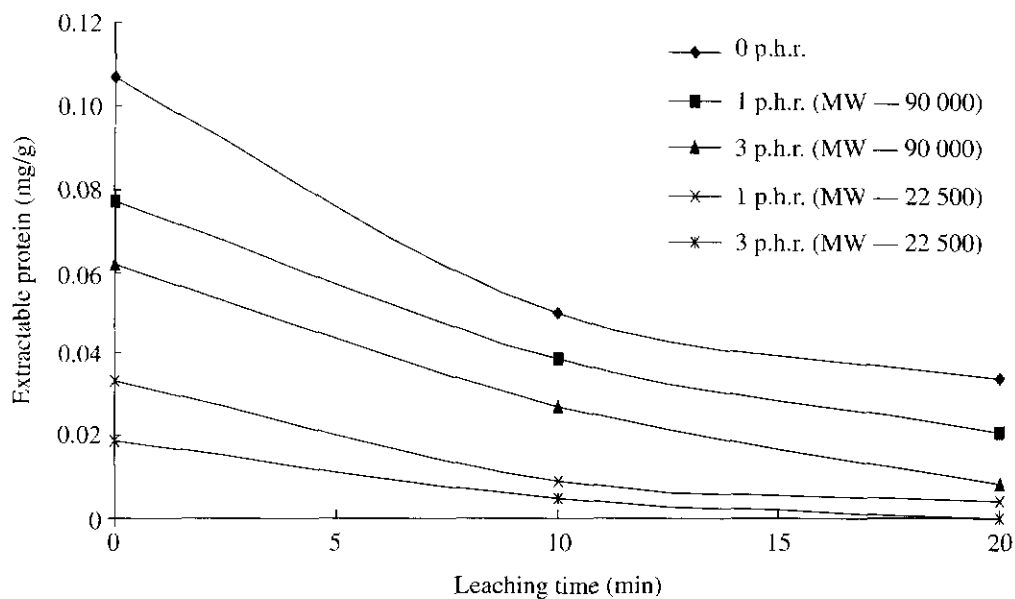


Figure 1. Effect of PVA and centrifugation on extractable protein content.

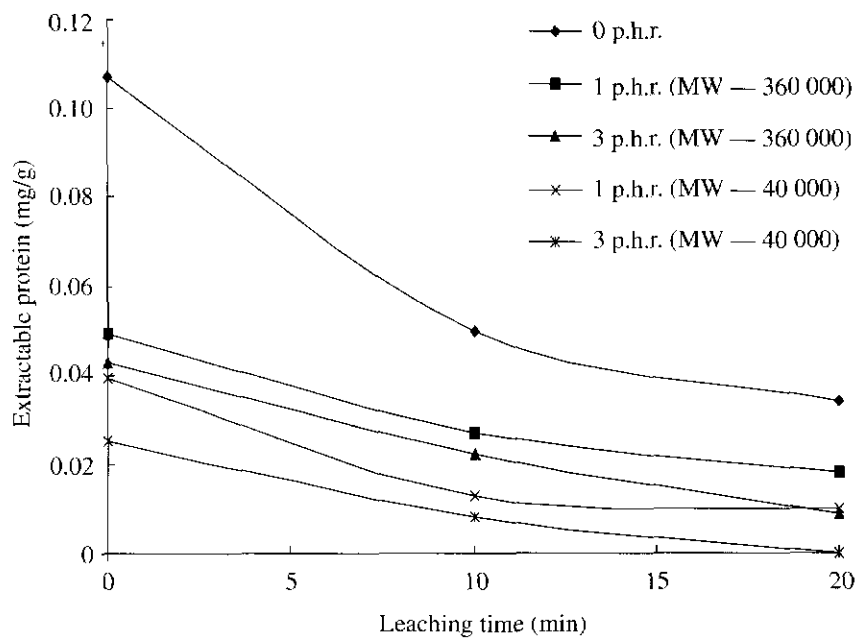


Figure 2. Effect of PVP and centrifugation on extractable protein content.

As shown in *Figures 1 and 2*, addition of 3 p h r of either LMW-PVA or LMW-PVP, and centrifuging followed by 20 min leaching of the films could produce RVNRL films free of soluble proteins. Further, the treatment with LMW-WSP is more effective compared to the treatment with HMW WSP in removing soluble proteins from RVNRL.

Figure 3 shows that the variations of extractable protein content with the increase of molecular weight of WSP. It clearly shows that up to a molecular weight of 10^7 , the extractable protein content decreased with decrease in the molecular weight of WSP. However, when the molecular weight of the WSP was increased above 10^5 , there was no significant variation in the extractable protein content. These results indicate that the molecular weight of the WSP up to 10^5 is a significant factor for removing extractable protein in RVNRL.

Mechanical Properties

Irrespective of the nature of WSP, incorporation of WSP decreases the tensile strength of RVNRL films⁸. *Figure 4* shows the effect of WSP on tensile strength. Tensile strength was significantly decreased with increase of PVA concentration when PVA was added to RVNRL and the films were leached for 20 min. However, only a slight decrease in tensile strength was observed when the films were prepared, by addition of high concentration (3 p h r or 4 p h r) of either HMW PVA or HMW PVP to RVNRL was centrifuged. No significant change in tensile strength was observed when RVNRL containing LMW PVA or LMW PVP was centrifuged. This could be due to the removal of LMW PVA and LMW-PVP, almost completely with serum phase during the centrifugation process, since LMW-PVA and LMW-PVP are more soluble in water compared to HMW-PVA and HMW-PVP.

The removal of LMW WSP almost completely during centrifugation was further confirmed by analysing the FTIR spectra of RVNRL films containing WSP. *Figures 5, 6 and 7* show IR spectra for RVNRL PVA mixed and centrifuged RVNRL and PVA mixed and leached RVNRL, respectively. In these spectra, peak A corresponds to PVA and rubber protein whereas peak B corresponds to rubber hydrocarbon. *Figure 8* shows the variation of A/B peak height ratio of IR spectrum against PVA concentration. A/B peak height ratio gives a measure of the concentration of PVA in films. Higher peak height ratio implies that higher level of PVA remained in the RVNRL films. According to these results, relatively high percentage of PVA remained in the RVNRL films when only a leaching was involved, but low percentage of PVA remained in films when the PVA treated RVNRL was centrifuged.

From the results of tensile strength and IR spectra, it could be confirmed that leaching of PVA added RVNRL films did not remove PVA completely. Hence the tensile strength was adversely affected by the residual PVA. On the other hand, centrifugation of PVA added RVNRL does remove PVA almost completely, hence tensile strength was not affected by PVA addition.

As shown in *Figure 9*, tear strength increased with PVA concentration if PVA added RVNRL was not centrifuged. Any increase of tear strength was probably due to hydrogen bonding with remaining WSP in RVNRL. However, after centrifugation of HMW-WSP or LMW-WSP, mixed RVNRL did not show any significant change in tear strength since most of the WSP was removed during the centrifugation process.

Figure 10 shows the effect of WSP on viscosity of RVNRL. HMW-PVA can

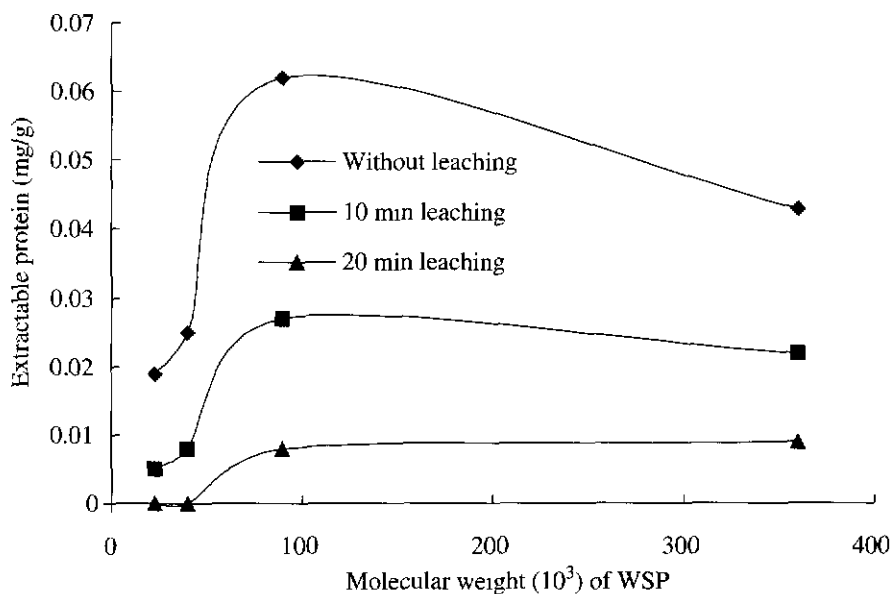


Figure 3. Effect of molecular weight of WSP (PVA and PVP) on extractable protein content.

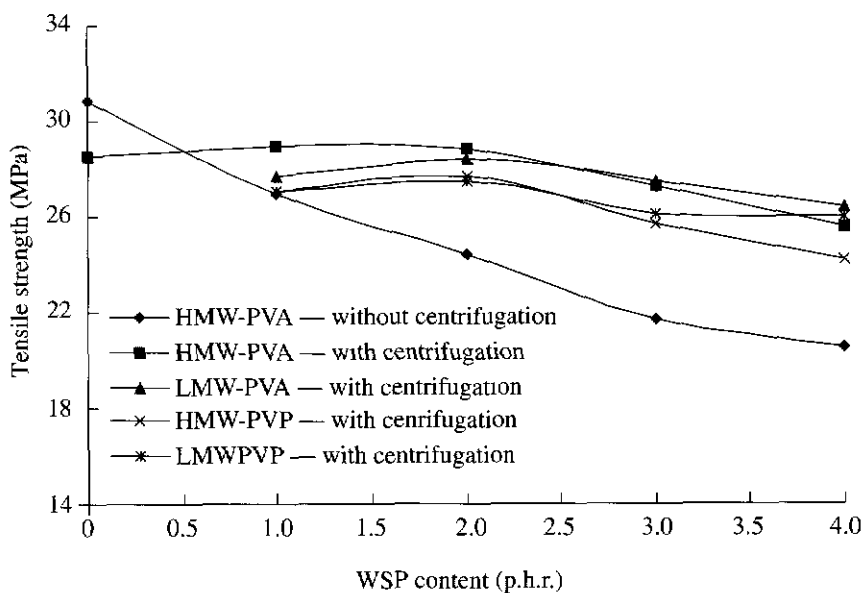


Figure 4. Effect of WSP on tensile strength of 20 min-leached RVNRL films.

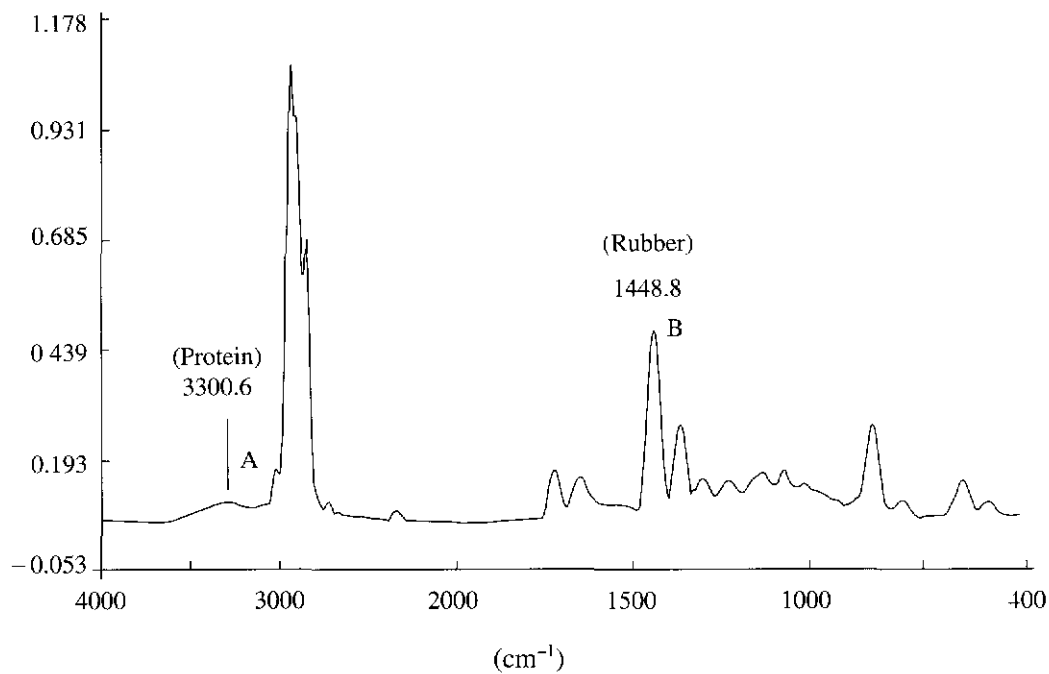


Figure 5. FTIR spectrum of RVNRL.

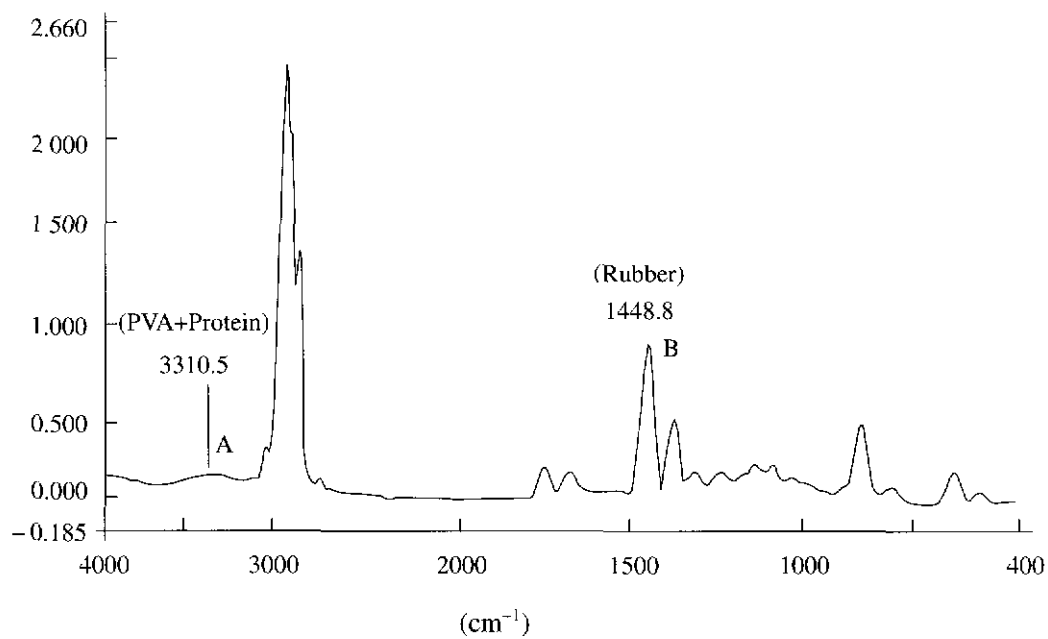


Figure 6. FTIR spectrum of PVA mixed and centrifuged RVNRL.

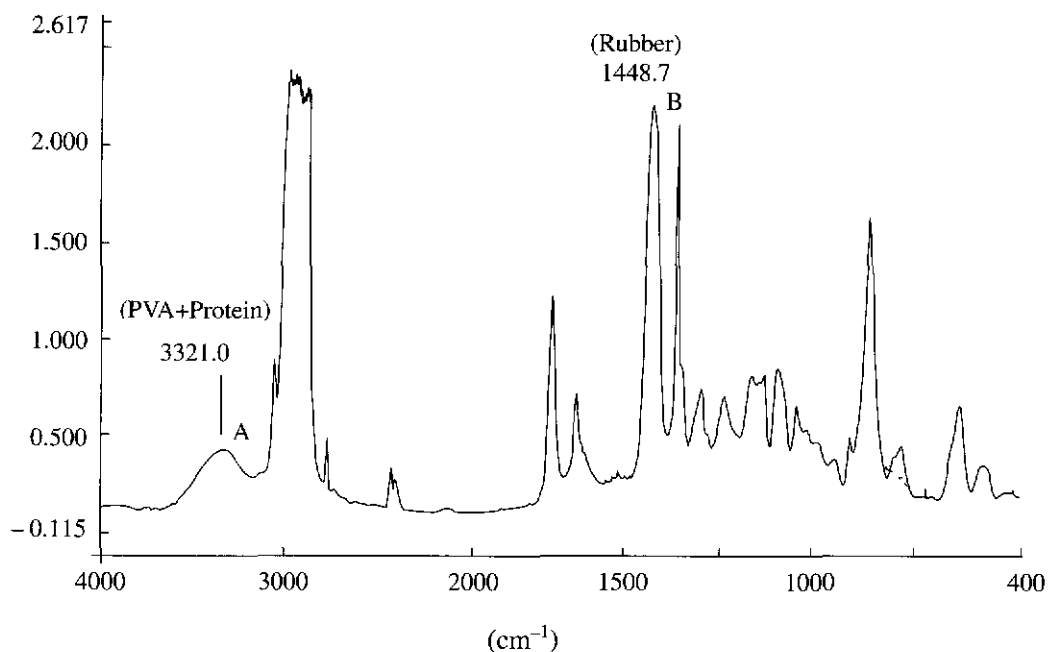


Figure 7. FTIR spectrum of PVA mixed and leached RVNRL.

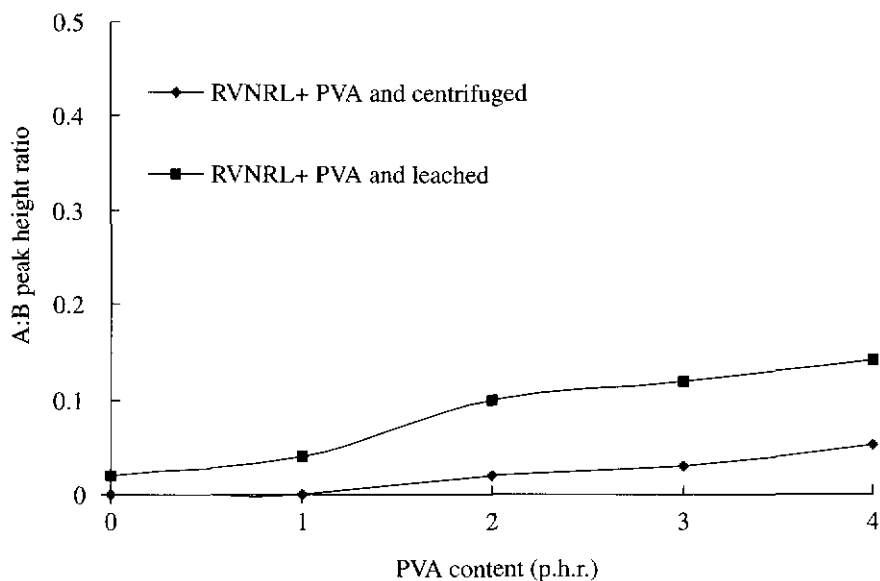


Figure 8. Remaining PVA content in RVNRL films.

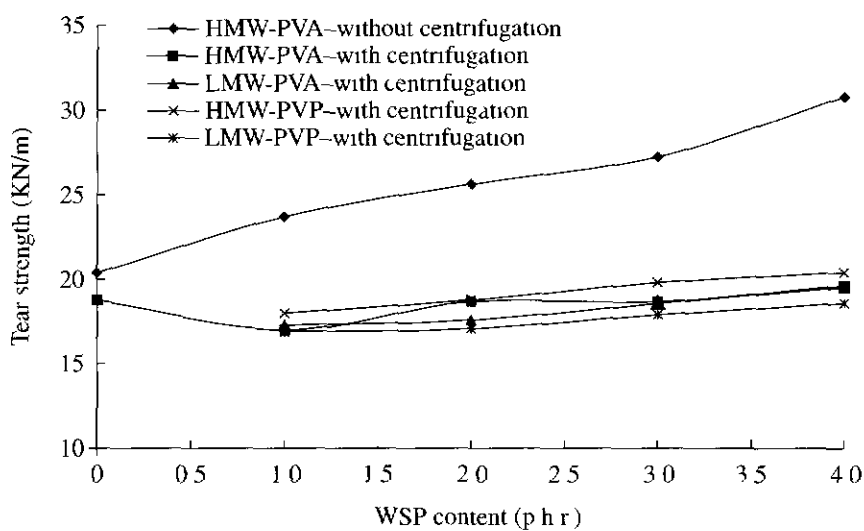


Figure 9 Effect of WSP on tear strength of 20 min-leached RVNRL films

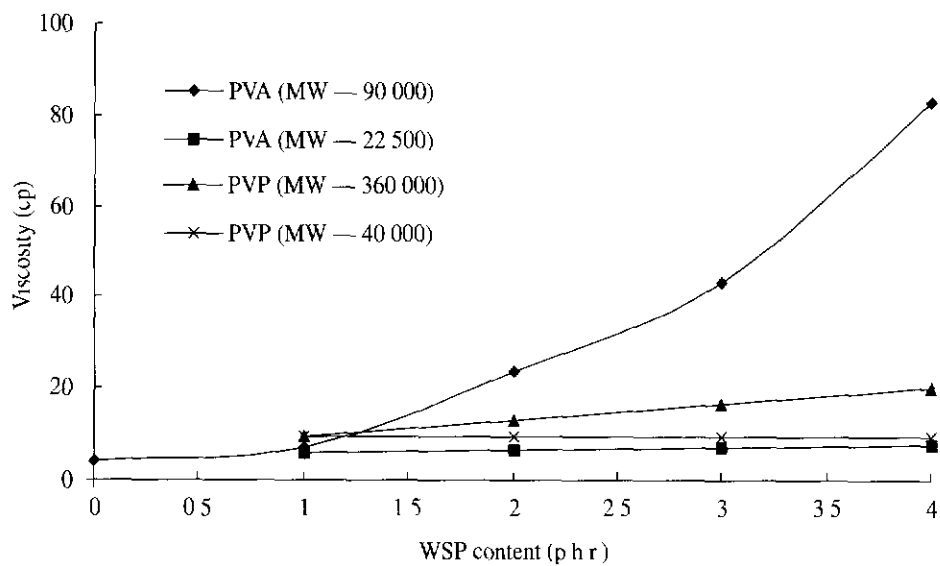


Figure 10 Effect of WSP on viscosity

drastically increase the viscosity of RVNRL. The stability of the RVNRL was affected by this high viscosity increase. However, HMW-PVP appeared to have little effect on the viscosity of RVNRL. Viscosity remained unchanged when LMW-PVA or PVP was added to the RVNRL. These results indicate that LMW-WSP did not affect the stability of the RVNRL.

CONCLUSION

Extractable proteins could be effectively removed from RVNRL by the addition of WSPs such as PVA and PVP to the RVNRL followed by centrifugation. Addition of LMW-PVA and LMW-PVP could further enhance the reduction of extractable proteins in RVNRL. Molecular weight of WSP, up to 10^5 was a significant factor in removing extractable protein in RVNRL. RVNRL films, free from extractable proteins could be produced by mixing either LMW-PVA or LMW-PVP and centrifuging followed by 20 min leaching. Tensile properties, such as tensile strength and tear strength, were not significantly affected when RVNRL containing LMW-WSP was centrifuged, since most of the LMW-WSP was removed during the centrifugation process.

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