

EXTRACTABLE PROTEINS IN LATEX PRODUCTS: EFFECT OF VULCANIZATION METHODS AND LEACHING

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Natural rubber latex is widely used to make various products by casting. Proteins present in latex products are known to cause allergy. In this study, experiments were conducted to find out the combined effect of vulcanization and leaching on extractable protein content in latex products using un-vulcanized and pre-vulcanized latex compounds. Attempt was also made to investigate the particle size variation likely to take place during compounding and pre-vulcanization. The study confirmed that there is considerable reduction of leachable proteins after pre-vulcanization and leaching. Latex compounds with smaller sized particles help in better film formation leading to better physical properties.

Key words: Casting, Extractable proteins, Latex products, Particle size, Pre-vulcanization

INTRODUCTION

Natural rubber (NR) latex refers to the latex obtained from the *Hevea brasiliensis* tree. It is a colloidal dispersion of small polymeric particles (0.02 to 2 μm), in an aqueous serum of NR latex consists of about 25-40 per cent rubber polymer (cis 1, 4 poly-isoprene), 2-2.5 per cent proteins, 1.5-1.8 per cent resins, 1-1.4 per cent sugar, 0.6-0.9 per cent ash and 50-70 per cent water.

The ability of the rubber particles to coalesce and produce a coherent polymer film impermeable to air and water makes latex suitable for an extremely wide variety of products. Vulcanization converts it a significantly versatile raw material for the production of several rubber products by

dipping, moulding, casting or spreading processes. Natural rubber latex products are superior to the synthetic counterparts owing to their high strength properties coupled with relatively low modulus, high wet gel strength (Amir *et al.*, 1999), elasticity, resilience, heat dissipation, abrasion resistance and cold-malleability characteristics which cannot easily be mimicked by synthetic polymers.

Latex undergoes several chemical changes during the different manufacturing steps like compounding, vulcanization and other processing operations like leaching. The proteins present in latex play a major role in deciding the properties of latex products such as elasticity, modulus and barrier functions (Tangpakdee *et al.*, 1997). The proteins are present in latex as adsorbed

on rubber particle surface, as solution in latex serum or associated with the non-rubber ingredients in latex. When processed into latex concentrate, considerable amounts of these soluble proteins are removed. During the conversion of latex to latex products, more of the soluble proteins get removed during leaching and washing steps, so that the remaining levels are very low. Even these small levels of proteins retained on latex products have become a matter of concern to the industry because of the incidence of Type I hypersensitivity associated with the presence of these residual water-soluble proteins retained in latex products.

Hence, there is an urgent need to produce latex products, used in body contact applications like gloves, with minimum extractable proteins (Ng *et al.*, 1994). Although the manufacturing process of latex products like medical gloves, condoms, catheters *etc.*, includes leaching operations for the removal of excess chemicals and proteins, residues of chemicals and/or proteins generally remain in the product in varying levels.

To produce dipped latex products with reduced soluble proteins, the simplest method is to wash in water. Proteins migrate to the surface of the latex film as it dries up (Bahri *et al.*, 1993) and are hence most effectively removed when the film is washed after complete drying. There are many methods to remove extractable protein from latex but a method to remove of them fully is yet to be developed. If this was possible, however, the latex would be less stable.

There are two ways of producing pre-vulcanized latex. One is by heating the stabilized latex containing the vulcanizing ingredients at a suitable temperature for a definite duration so that the rubber molecules

get crosslinked inside the rubber particles thereby producing cured latex films on drying. The other is without the use of crosslinking chemicals like sulphur or peroxides, but by exposure of latex to specific doses of gamma radiation, resulting in radiation pre vulcanized latex, which is processed further in the same way as sulphur pre vulcanized latex.

It is generally believed that the potentially allergenic protein components are those associated with the aqueous phase of NRL. These are the proteins that are dissolved in the latex serum and tend to concentrate during glove production. The amount of residual extractable proteins present in latex products prepared from the same latex concentrate can vary, depending on the method of pre-vulcanization and other processing conditions during manufacturing. There are several reports to show that proper leaching in water considerably reduces the leachable proteins (Perrella and Gaspari, 2002). There are very few reports on the combined effect of method of vulcanization and leaching process on the amount of leachable proteins retained on rubber films. The work presented here is an attempt in this line. It is also attempted to investigate the particle size variation likely to take place during the different processing operations and its contribution to the development of physical properties of the films.

MATERIALS AND METHODS

High ammonia (HA) centrifuged latex of the same batch conforming to BIS standards, collected from the PLPC Factory of Rubber Board was used to produce un-vulcanized and pre-vulcanized latex compounds. The other chemicals used were commercial grades.

Preparation of radiation pre-vulcanized latex

HA latex was diluted to 50 per cent total solids content with two per cent ammonia solution followed by addition of 0.2 phr of 10 per cent KOH solution. The sensitizer *n*-butyl acrylate (5 phr) was added with constant stirring. The compounded latex was exposed to gamma radiation from a Co⁶⁰ source to a total dose of 10 KGY in a gamma chamber of 5000 cc.

Preparation of sulphur pre-vulcanized latex

Sulphur and other ingredients for curing were added to 60 per cent HA centrifuged latex in the form of dispersions or solutions under slow stirring based on the formulation given in Table 1. The compound was kept in a water bath maintained at 55 ± 1°C for four hours. Loss of ammonia was compensated by adding freshly prepared one per cent ammonia solution. The pre-vulcanized latex was then cooled to room temperature.

Preparation of un-vulcanized latex compound

The compound was prepared in the same way as that used for the preparation of pre-vulcanized latex. The compounded latex was kept at room temperature for 24 h for maturation. Latex films were prepared from radiation vulcanized, sulphur pre-vulcanized and un-vulcanized compounded latices by casting and coagulation methods.

Cast films of approximately 0.2 mm thickness were prepared by casting in side-raised glass plates. Coagulated films were prepared by immersing film deposit in two per cent acetic acid solution. All the films other than the un-vulcanized films were dried at 70°C for two hours. Films from un-vulcanized latex compounds were dried at 70°C for half hour and then vulcanized at

Table1. **Formulation used for preparation of pre-vulcanized and un-vulcanized latex compounds**

Ingredients	Dry wt.	Wet wt.
60 % Natural rubber latex	100	167
10 % Potassium hydroxide	0.25	2.5
10 % Potassium oleate	0.16	1.6
50 % Sulphur dispersion	1.25	2.5
50 % Zinc diethyl dithiocarbamate dispersion	0.8	1.6
50 % Zinc oxide dispersion	0.25	0.5

100°C for one hour. Leaching was done by immersion of films in distilled water for 24h (Distilled water was used to get uniformity and to avoid location wise change in water quality).

The films were analyzed for extractable protein content before and after leaching (ASTM D 5712, 2010). In order to get a deeper insight into the retention of leachable proteins in latex films and changes in particle size during processing that affect film formation, spectroscopic analysis of latex films and particle size determination of un-vulcanized and pre-vulcanized latex samples were conducted. Fourier transform infrared spectroscopic (FTIR) measurements were conducted in the ATR (attenuated total reflection) mode for the films using FTIR Spectrometer model Bruker-Tensor 27 (ASTM D 3677, 2010).

Particle size of latex compounds were measured using a Malvern Mastersizer 3000 based on light scattering technique (ISO 13320-2009). The light scattered from a laser beam, generated by Helium-Neon source, were collected by a detector and transferred to a computer for analysis. Volume based average particle size or volume mean diameter (VMD) D [4, 3]) were measured.

Viscosity was measured using a Brookfield Viscometer as per IS 9316 (part 2) 1987.

Mechanical properties like tensile strength, elongation at break and modulus were determined from stress-strain measurements using a universal testing machine, Instron model 3343.

RESULTS AND DISCUSSION

Particle size and particle size distribution

From the particle size data of compounded latex shown in Table 2 and Figure 1, it is observed that pre-vulcanization using sulphur results in increased average size of rubber particles and also in particles with higher size due to the large distribution range. Similar occurrence was reported by Suteewong and Tangboriboonrat (2007) where the broad particles size distribution of another type of natural rubber pre vulcanized latex caused larger average size. After radiation vulcanization the average size of particles showed slight increase but the particle size range do not increase. Consequently the average specific surface area of the particles is the lowest for latex pre vulcanized using sulphur and accelerator. Average particle size of the pre-vulcanized latices are almost triple the size of un-vulcanized high ammonia latex compound (Ruslimie *et al.*, 2015). After analyzing the distribution curves (Fig. 1), it was noted that the distribution range is up to 1.65 μm and 2.13 μm sizes in RVNRL and UVLC, respectively.

When comparing with un-vulcanized and radiation vulcanized compounds, pre-vulcanized latex, contains only 74.2 per cent volume of submicron particles; the rest 25.8 per cent is in the higher side (Fig. 2). This clearly shows the presence of large particles, possibly on account of particle aggregation, in the sulphur pre-vulcanized latex.

It is well known that during the process of pre-vulcanization there is formation of sulphur accelerator intermediates in the serum which get adsorbed on rubber and migrate into the hydrophobic interior of rubber particles to initiate the formation of crosslinks between rubber molecules inside the rubber particles (Blackley, 1997).

Hence, it is expected that there is no change in rubber particle size, shape and particle size distribution (Blackley, 1997) during pre-vulcanization. However, there is change in the colloidal stabilizers present in latex. The presence of zinc oxide could reduce the colloidal stability by inactivating the soluble fatty acid soaps and this would lead to some sort of aggregation in sulphur pre vulcanized latex. This type of zinc oxide thickening is absent in RVNRL as zinc oxide was absent in radiation processing. It is well known that smaller sized particle in latex helps in better film formation than the particles of comparatively higher size (Steward *et al.*, 2000). Hence, it is expected that the film formation ability will be better

Table 2. Data on particle size analysis

Properties	UVLC	PVNRL	RVNRL
Particle size (μm)	0.494	1.23	0.643
Particle size range (μm)	0.01-2.13	0.0114-40.1	0.01-1.65
Specific surface area ($\text{m}^2 \text{kg}^{-1}$)	54050	19840	31070

UVLC-Un-vulcanized latex compound PVNRL-Pre-vulcanized latex RVNRL-Radiation vulcanized latex

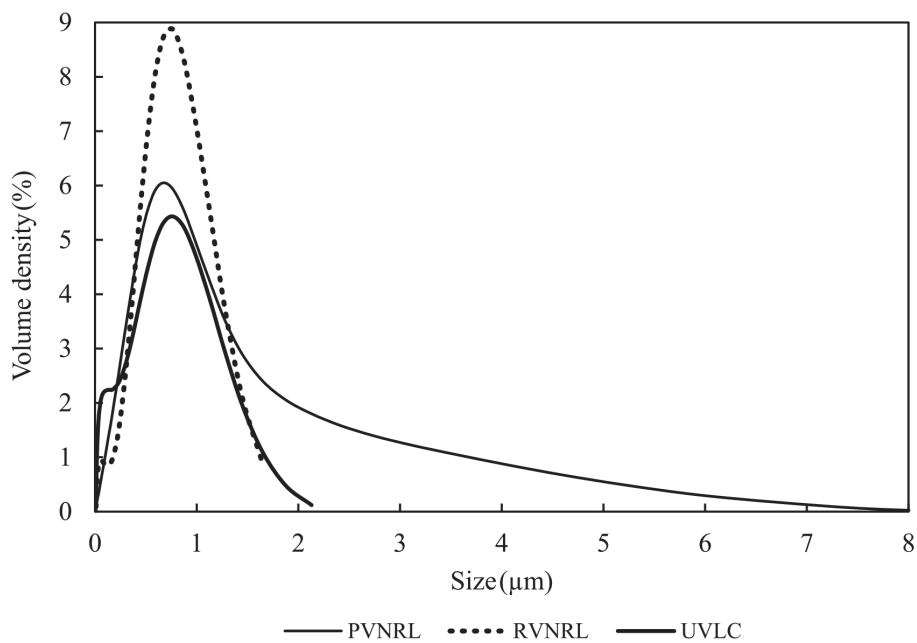


Fig. 1. Distribution curves of particles present in different latices

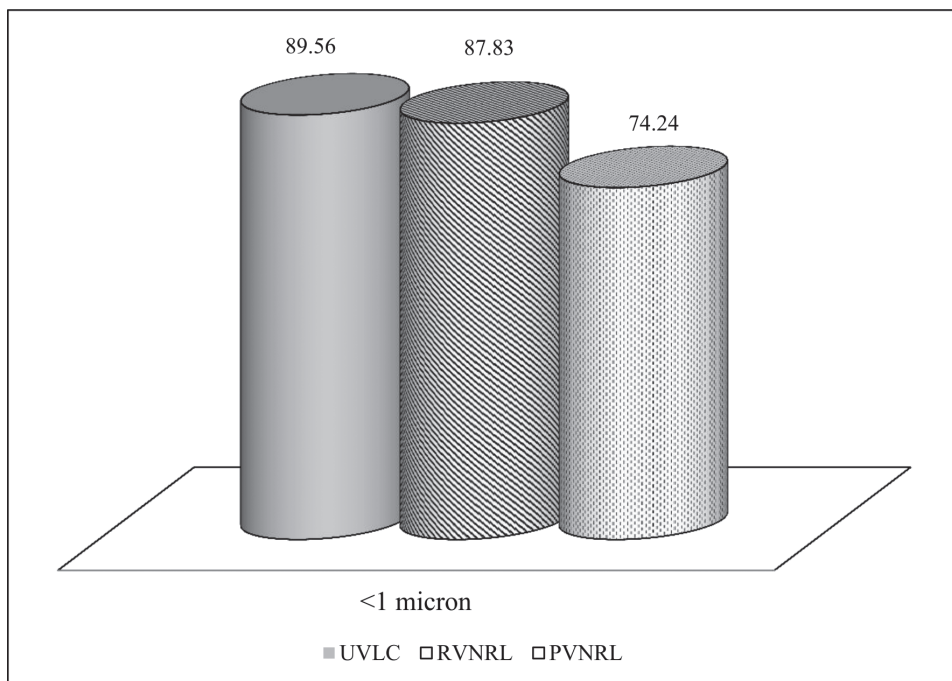


Fig. 2. Percentage volume of submicron particles in different latex compounds

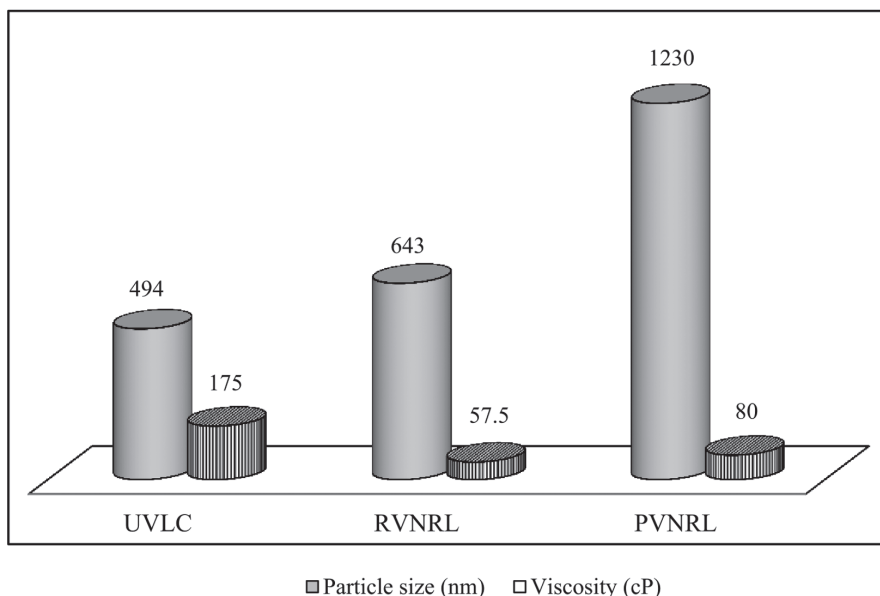


Fig. 3. Brookfield viscosity and particle size of latex samples

for RVNRL and post-vulcanized latex film than for the sulphur pre-vulcanized one.

Brookfield viscosity

Viscosity of the un-vulcanized and pre-vulcanized latex samples is given in Figure 3. It is observed that viscosity of pre-vulcanized latex is the highest among the three latex samples while that of the pre-vulcanized and radiation post-vulcanized latex samples are slightly closer. It is known that the particle size as such does not contribute to viscosity. The changes in viscosity could be associated

with the stabilizers present in latex. In the presence of ZnO the soluble soaps were converted into insoluble ones during pre-vulcanization process and would result as increased viscosity (Lai, 1991). If proteins get hydrolyzed to low molecular weight fractions that are acidic in nature, the same also result in an increase in viscosity.

Mechanical properties of vulcanized films

Modulus and tensile strength of the cast latex films before and after leaching are given in Table 3. In all the cases leached film has

Table 3. Physical properties of vulcanized cast films

Parameters	UVLC		RVNRL		PVNRL	
	(UL)	(L)	(UL)	(L)	(UL)	(L)
Tensile strength (MPa)	26.1	27.2	14.5	19.4	19.2	21.0
Elongation at break (percentage)	790	802	817	758	1094	959

UL-Un-leached film, L- Leached film, UVLC film - High ammonia compounded latex (Films after pre-vulcanization - RVNRL- Radiation Vulcanized Latex - PVNRL- Sulphur pre-vulcanized latex

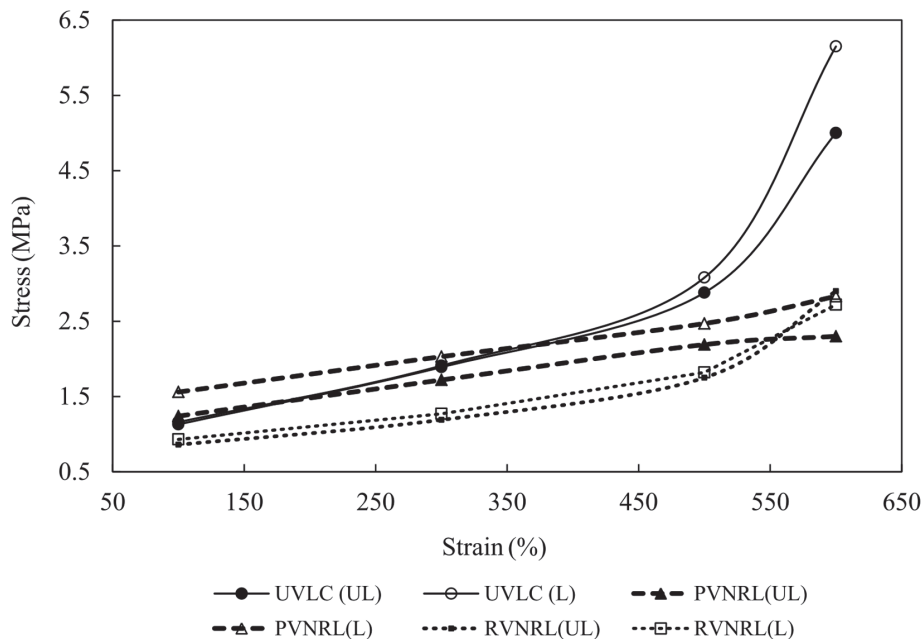


Fig. 4. Stress-strain properties of leached (L) and un-leached (UL) films of different type of latex compounds

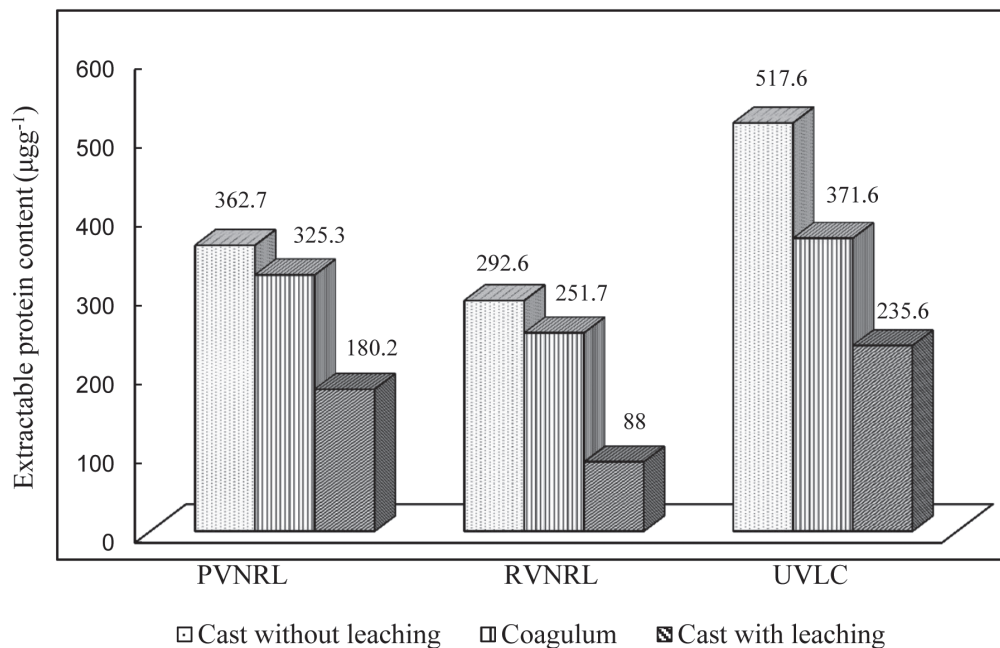


Fig. 5. Extractable protein content in cast films made from different latices

higher mechanical properties than un-leached films. Tensile strength is higher for UVLC films than the prevulcanized latices after vulcanization. If post vulcanization technique such as drying at 70°C for half an hour is given to the pre-vulcanized latex films, its physical properties may be improved (McGlothlin, 2001).

Earlier studies have shown that intra-particle crosslinks were found to be higher in a sulphur post-vulcanized film as compared with a sulphur pre-vulcanized film (Cook *et al.*, 1997; Che *et al.*, 2012). For sulphur vulcanization, the additional heat treatment (post-vulcanization) increased the interactions between rubber particles and unreacted vulcanizing agents. It is also expected that smaller particles can lead to better film formation (Blackley, 1997) as shown earlier and this also could contribute to better mechanical properties.

As seen from Table 3, leaching improves the tensile strength and modulus. It is believed that, upon drying, the non-rubber

components, principally proteins which are adsorbed on the surface of rubber particles tend to prevent effective coalescence of the rubber particles (Porter and Wong, 1989). Hence, removal of the proteins by leaching caused better interaction between rubber molecules from the adjacent particles, resulting in a more coherent film (Ramli *et al.*, 2014). The removal of excess calcium nitrate and water-soluble non-rubbers such as proteins and added compounding ingredients also results in improvement of physical properties.

The same trend in tensile strength is seen in stress-strain properties also. As the strain increases stress is also increasing. This may be due to strain induced crystallisation of natural rubber (Che *et al.*, 2012). Here also leached sample (L) has higher stress values than its corresponding un-leached sample (UL). Figure 4 shows the trend discussed above. In all cases leached film has higher modulus than the un-leached ones at all elongations. Moreover, radiation vulcanized

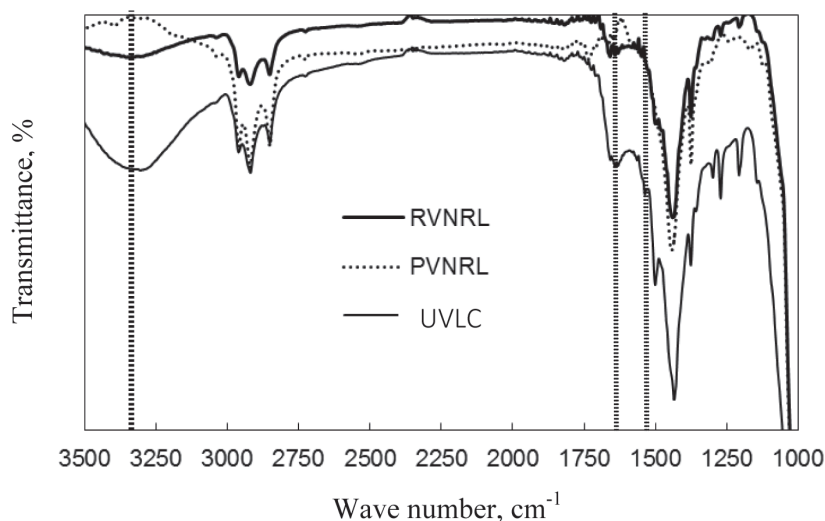


Fig. 6. FTIR spectra of cast films after leaching from different types of latex compounds

film shows lower modulus than the pre- and post-sulphur vulcanized films at all elongations (Hossain and Chowdhury, 2010)

Extractable protein content of different types of vulcanized latex compounds

The extractable protein content in cast films/coagulum made from different latex compounds are shown in Figure 5. It is observed that pre-vulcanization results in the reduction of leachable proteins. Within the two pre-vulcanized latex samples, radiation pre vulcanized sample showed a lower leachable protein content. For cast/coagulated films without leaching extractable protein content is higher in the film prepared from un-vulcanized latex compound compared to all other latex films. It can be seen that in all the cases *i.e.*, cast (leached, un-leached) and coagulated films the trend of leachable proteins is UVLC > PVNRL > RVNRL. Earlier reports show that pre-vulcanization results in a lower extractable protein content than its un-vulcanized counterpart (Eng *et al.*, 1999).

In all cases irrespective of the method of vulcanization, leaching reduces the extractable protein content. The lowest extractable proteins are obtained for samples prepared from radiation processed latex after leaching. Earlier reports show that leachable proteins get reduced after radiation processing and leaching (Eng *et al.*, 1999). It is expected that chemical changes take place for the proteins present in latex during preservation and other processing operations like pre-vulcanization (hydrolysis of proteins to low molecular

weight fractions). During leaching the low molecular weight proteins formed during the various processing operations are removed by water.

Characterization using FTIR Spectroscopy

FTIR technique was also used in the study. FTIR spectra of leached films are given in Figure 6. The extractable protein values of un-leached and coagulated films showed the same trend like leached films as described above. It justifies the same trend as shown in Figure 5.

In FTIR spectra (Fig. 6), the principal peaks of proteins in NR were observed at 3281, 1656 and 1539 cm^{-1} , corresponding to N-H stretching, C=O stretching (amide I) and N-H bending (amide II), respectively (Kalapat *et al.*, 2009) in all the samples. However, the absorption at 1539 cm^{-1} is more prominent in the film that is post-vulcanized (UVLC) that contains a higher amount of proteins.

CONCLUSION

There is considerable reduction of leachable proteins after pre-vulcanization and leaching. Within the two pre-vulcanization methods, radiation vulcanization results in remarkably low amount of leachable proteins. The strength related properties are better for post-vulcanized latex films. Since smaller sized particle in latex concentrate helps in better film formation, it is expected that film formation ability will be better for RVNRL and post-vulcanized latex film than the sulphur pre-vulcanized one.

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