NON-ENZYMATIC DEPROTEINIZATION OF NATURAL RUBBER LATEX

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Natural rubber latex (NRL) is a preferred raw material for latex product manufacturers. However, presence of non-rubbers especially allergenic proteins has inhibited its use in several products and applications. In this study, natural rubber latex was treated with aluminum hydroxide dispersion to bind both extractable (EP) and rubber bound proteins in the latex. The treated latex has superior performance compared to the concentrated latex commercially being used. The advantages include ultra low allergenicity with improved color, absence of rubber odor, improved physicochemical properties, improved gas (air and helium) retentions *etc.* The future potential of the treated latex is also discussed.

Keywords: Aluminium hydroxide, Deprolenization, Natural rubber latex

INTRODUCTION

Natural rubber latex (NRL) contains a small amount of non-rubbers, which include a variety of proteins that have played a role in the biosynthesis and stabilization of rubber latex. The latex of *Hevea brasiliensis* is a complex colloidal dispersion of polyisoprene rubber particles and non-rubber components in an aqueous phase. Some adverse effects of these non-rubbers are well documented. These non-rubber constituents continue to play a role in the processing behavior, long-term stability, and catalyzing the crosslinking reactions of the rubber through free radical and ionic mechanisms resulting in covalent bonds. The protein sheaths, which may be amphoteric in nature, facilitate the movement of curatives into latex particles by providing an intermediate transport mechanism from the water phase to the rubber phase. The removal of the non-rubbers in treated NRL slows down the maturation process, resulting in a longer "pot life."

Aluminum hydroxide-treated natural rubber latex

Aluminum hydroxide treatment found to remove the proteins found in the regular natural rubber latex. For removing the proteins, a dispersion of aluminum hydroxide is introduced in to the latex at the processing stage. The aluminum hydroxide binds with the non-rubber particles, which are subsequently removed during centrifugation.

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The process was originally intended to remove the antigenic proteins associated with latex. However, it was found that the treatment was successful in removing the antigenic proteins along with other less desirable non-rubber contents. It was found that the treated NRL is cleaner and stable and has a number of other benefits which are outlined here.

Natural rubber latex as a physical barrier

The use of gloves and condoms, increased tremendously in the 1980s primarily due to the "universal precaution" policy outlined by US, for Disease Control. The superior physical and barrier properties of natural rubber latex gloves over the synthetic latices have increased its popularity in the healthcare field. NRL is made up of long *cis*-1, 4-polyisoprene chains, and on cross-linking, the films becomes flexible and extendible (Williams, 1999). Flexibility of the NRL films is related to chain lengths, the stereochemistry, rotation of the single C-C chains, while the covalent double bonds remain rigid. The impermeability of the films is related to the film thickness, extension of films, constituents of the polymer, presence of additives, and the size of the gaseous permenent.

Certain chemicals can permeate rapidly through intact NRL surgical gloves (Korniewicz *et al.*, 2002). While there are synthetic alternatives available for NRL gloves, many of their physical properties are not ideal for barrier applications. There is no alternate material that can fully match the characteristics, including transmission of pathogens compared to natural rubber latex. Natural rubber latex is universally agreed to be superior in barrier protection against

Table 1. **Total protein and antigenic protein in TNRL from 4 different locations**

Sample No.	Sample Ref.	Total protein concentration modified Lowry (ASTM D 5712-05)		Antigenic protein concentration inhibition ELISA (ASTM D 6499-07)	
	(Production Location)	(μg g^{-1})	(μg dm^{2-1})	(μg g^{-1})	(μg dm^{2-1})
1	VY KM GL (Guatemala)	55	23	3.0	2.8
2	VY MR 01 (Malaysia)	<42	40	0.4	0.4
3	VY TG GL (Thailand)	<42	<31	<0.2	<0.1
4	VY KA 03 (India)	<42	<34	<0.2	<0.1
Reporting limits		8.3 μg mL^{-1}	0.03 μg mL^{-1}		

Values are determined using the duplicate conducted

< indicates values are below the calculated reporting limit of the assay

Table 2. **Analysis of *Hev* b proteins by the fit kit tests.**

Type	Latex allergen (ng mL^{-1})				
	Hev b 1	Hev b 3	Hev b 5	Hev b 6.02	TOTAL
Enzyme treated	556.15	473.39	15.649	101.74	1146.93
Vytex VY-KM GL 0,1%	494.83	638.70	ND	58.30	1191.83
Control	217.20	187.88	40.24	70.38	515.70

deadly viruses and micro-organisms (Williams, 1999).

THE PROPERTIES OF TREATED LATEX

The protein content values for the treated NRL are given in Table 1.

In a separate test with different samples the amount of the *Hev* b proteins was reported as below.

The *Hev* b1 is 14 k Da in size and is described as the rubber elongation protein.

The *Hev* b2 is a 35 kDa protein of -1,3 glucanase enzyme and is a defence related protein in the plant. The results of subsequent work on the reduction of these two proteins will be described in a future paper. Current levels are below that reported in Table 2 for materials from all locations.

Table 3 illustrates the property difference between the treated (TNRL) and standard NRL. Treated NRL withstands mechanical shear and is more stable than standard NRL.

Table 3. **Comparison between treated NRL and standard NRL**

Property	Treated TNRL	Standard NRL
Dry rubber content, % (m/m)	60.03	60.08
Total solids content % (m/m)	60.94	61.21
Non rubber solids, % (m/m)	0.91*	1.13
pH	10.05	10.55
Alkalinity (as NH_3), % (m/m)	0.25	0.62
Volatile fatty acid number (VFA)	0.02	0.03
KOH number	0.56	0.4
Mechanical stability time (MST), S	960	586
Viscosity (sp2/60), cP	70	79.5
Coagulum content, % (m/m)	0.002	

*This is adjusted between 0.7-1.0 depending on specific requirements

Treated NRL performed within the acceptable limits of ISO 4074 standards, exhibiting parity with standard NRL (Table 4).

The reduction of the non-rubber content in treated NRL results in improved dynamic properties (resilience and rebound), which is ideal for products for dynamic applications (Sakdapipanich *et al.*, 2012).

The modified NRL though more expensive than standard NRL, is nevertheless less expensive than nitrile, chloroprene, and other alternate synthetic materials. The treated NRL provides a significant reduction in cost when compared to other synthetic latices. Previous study has shown that the increased price for the modified NRL can be offset by reducing the number of manufacturing steps required to

BENEFITS OF TREATED LATEX IN APPLICATIONS

1) Condoms/gloves

Condoms manufactured from treated NRL show improved burst pressure (Table 4). Additionally, reduced pinholes are suggestive of improved quality. These results have been confirmed by several manufacturers currently using treated NRL.

Table 4. **Burst analysis of treated NRL performed by company A**

ISO 4074	Burst pressure (kPa)	Burst volume (L)	Pinhole
Treated NRL	2.19 ± 0.28	35.4 ± 4.7	0
(Average of 315 pieces)	pass	pass	pass

Table 5. **Physical properties of examination gloves made from treated NRL and standard NRL**

	Formulation	Tensile strength MPa					
		Before (ASTM D 3578: Min. 14MPa)			After (ASTM D 3578: Min. 14MPa)		
		Highest	Lowest	Average	Highest	Lowest	Average
A	Treated NRL	33.3	30.1	31.5	23.2	19.4	20.9
Control	L9D	31.3	27.9	29.6	25.6	17.8	20.8
	Formulation	Elongation %					
		Before (ASTM D 3578: Min. 650 %)			After (ASTM D 3578: Min. 500 %)		
		Highest	Lowest	Average	Highest	Lowest	Average
A	Treated NRL	966.9	884.0	927.8	999.3	867.7	932.5
Control	L9D	845.8	770.2	808.7	893.3	782.8	831.6
	Formulation	300 % MPa modulus					
		Before (ASTM D 3578: Not stated)			After (ASTM D 3578: Not stated)		
		Highest	Lowest	Average	Highest	Lowest	Average
A	Treated NRL	3.85	3.43	3.63	2.71	1.95	2.34
Control	L9D	7.05	5.83	6.37	5.50	3.20	4.20

achieve acceptable protein levels. The improved stability of the modified NRL enables greater flexibility to the processor, in terms of longer shelf-life and a greater filler loading.

The slower maturation rate of modified NRL is beneficial to glove manufacturers as it imparts prolonged shelf-life and longer span of dipping hours. The softer feel of the modified gloves and the comparable

Table 6. **Compounding of treated and standard NRL**

Ingredients	Level of addition (phr)		
	Treated/G45	Treated/ G40	Standard/G40
Treated NRL	100	100	-
60% Concentrated latex	-	-	100
20% Potassium hydroxide	0.1	0.1	0.1
20% Potassium laurate	0.15	0.15	0.15
60% Sulphur	1.2	1.2	1.2
50% Zinc oxide	0.6	0.6	0.6
50% ZDEC	0.4	0.4	0.4
50% BZ	0.2	0.2	0.2
50% Wingstay L	0.5	0.5	0.5
70% Calcium carbonate	45	40	40

Table 7. **Toluene swell index analysis**

Period, h	Toluene swell results (%)		
	Treated G/45	Treated G/40	Common G/40
6	136	134	132
12	120	118	116
18	116	112	112
24	108	106	102
30	100	98	94
36	96	94	88
42	92	90	82
48	88	88	TSI LOWER LIMIT
			78
54	84	82	78
60	80	80	-
	TSI LOWER LIMIT		
66	78	78 (overcured)	-
72	78	78	-

physical properties even at the increased filler level (45 phr), makes the modified NRL a better choice for cost saving. The high loading of filler in the modified NRL gloves can be done without any quality deterioration as seen in Table 5. Overall, lower price for modified NRL is due to the higher accommodation of filler as compared to standard NRL.

Modified NRL and standard NRL were compounded for production of gloves (Table 6).

Based on the toluene swell index of both compounded and modified latex, their suitability for the production of gloves were assessed. Once the toluene swell reached the acceptable limit (90 per cent), dipped films of 0.1 mm thickness, were prepared. Significantly, films prepared from both treated NRL compounds had similar toluene swell indices (Table 7). However, the treated NRL had a slower maturation level than to standard NRL. Treated NRL needed to be dipped within 42 h after compounding while

Table 8. **Physical properties of different compounded treated NRL and standard NRL**

Physical Properties	Treated G/45			Treated G/40			Standard G/40		
	Before	After	% retention	Before	After	% retention	Before	After	% retention
Tensile strength, Mpa	22.9	16.3	71.2	24.1	17.2	71.4	24.9	15.7	63.1
Elongation, %	712	705	99.0	751	720	104.3	640	697	108.9
300% modulus, MPa	3.26	3.07	94.2	3.72	3.1	83.3	3.93	3.02	76.8
500% modulus, MPa	10.0	7.96	79.6	11.3	7.99	70.7	13.3	7.99	70.7
700% modulus, MPa	19.7	14.5	73.6	20.3	14.7	72.4	-	-	-

Table 9. **Quantitative color determination**

	Treated NRL			Standard NRL			Difference		
	L	a	b	L	a	b	ΔL	Δa	Δb
Orange	39.31	61.17	49.73	35.96	57.37	48.22	3.35	3.8	1.51
Red	-21.07	85.13	0.09	-37.11	80.17	0.03	16.04	4.96	0.06
Green	-31.76	-79.89	-0.15	-39.45	-72.13	-0.08	7.69	-7.76	0.13
Yellow	79.02	0.11	85.13	71.77	0.21	76.19	7.25	-0.1	8.94
Plain	69.86	0.04	3.54	45.87	0.08	10.43	23.99	-0.04	-6.89

standard NRL required to be dipped within 30h. This gives better time flexibility in the production line when using treated NRL as source material.

The physical properties of the dipped films were assessed. The modified and standard NRL with 40 phr filler had comparable tensile strength (Table 8). Significantly, the modified NRL gloves had a softer feel than the standard NRL glove due to the higher elongation at break. This translated into more room for filler in the compounding formula. The modified NRL with the extra 5 phr filler had a higher elongation break than the standard NRL at 40 phr filler. The 700 per cent elongation values of the modified NRL compounds with 40 and 45 phr filler were still softer than the standard *Hevea* NRL having 60 per cent rubber content.

The results presented in Table 8 illustrate that treated NRL had significantly lower modulus than standard NRL. Hence treated NRL is particularly attractive for the manufacture of surgical and examination gloves.

2) Balloons

The treated NRL has high potential in the manufacture of balloons. Treated NRL

Table 10. **Gas retention of treated NRL (TNRL) vs standard NRL**

Days	Hours	Circumference measurement of balloons at intervals (cm)							
		Helium retention				Normal air retention			
		TNRL 1	TNRL 2	NRL 1	NRL 2	TNRL 1	TNRL 2	NRL 1	NRL 2
1	12	69.0	69.0	69.0	69.0	69.0	69.0	69.0	69.0
	24	34.9	35.2	22.4	26.9	59.5	58.3	48.0	49.7
2	360	25.4	26.0	20.1	20.4	53.0	58.0	35.5	32.8
	48	21.0	20.5	19.5	19.6	52.5	57.3	29.4	28.1
3	60	20.2	19.4	18.9	18.5	49.4	52.6	20.4	22.0
	72	20.1	19.4	18.9	18.5	48.9	50.2	19.5	19.9
4	84	20.1	19.4	18.6	18.5	45.2	47.4	19.1	18.2
	96	20.1	19.4	18.6	18.5	44.0	44.2	19.0	18.2
5	108	20.1	19.4	18.1	18.4	42.5	41.4	19.0	18.2
	120	20.0	19.1	18.0	18.4	40.8	39.4	18.9	18.2

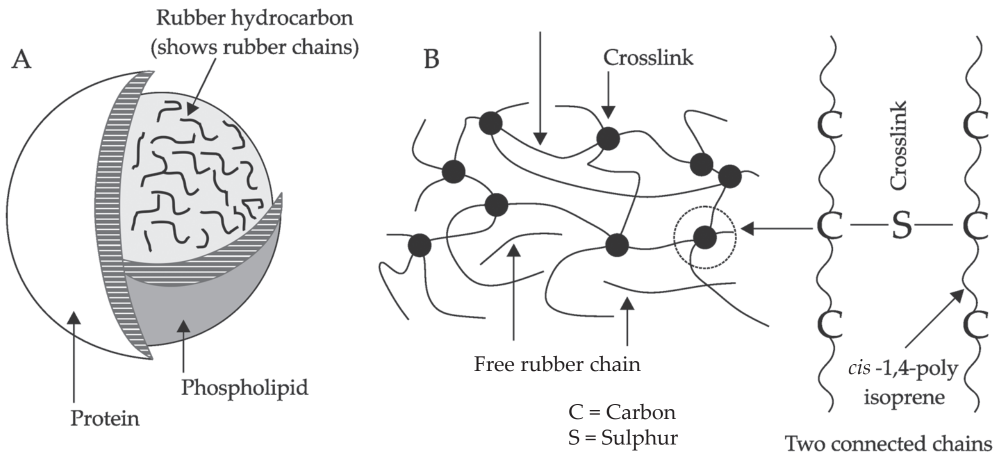

Fig. 1. A) Model of rubber latex particle, B) Depiction of rubber polyisoprene chains cross-linked by sulfur (Blackley, 1997; Bilgili, *et al*., 2001).

produces a very high quality, more translucent balloon that has better barrier properties than ordinary NRL. This is due to the removal of the lutoids and Frey-Wyssling particles (Fig. 1). Accordingly, rubber to non-rubber ratio can be reduced and also odorous low molecular weight acids can be minimized.

A comparison study was performed between colored balloons made with treated and standard NRL (Table 9). The data showed that the treated NRL was brighter in color (Fig. 2). More significantly, for each color of balloon tested, the treated NRL retains the original colour than that of the standard NRL.

L (lightness) axis - positive values are whiter; negative values are blacker and 0 is transparent

a (red-green) axis - positive values are red; negative values are green and 0 is neutral

b (blue-yellow) axis - positive values are yellow; negative values are blue and 0 is neutral

Gas retention has been assessed (Fig. 3) as a function of tightly packed rubber particle in the matrix. Balloons made of modified NRL and standard NRL were inflated to a specified circumference using helium gas or normal air. The circumferences were measured at intervals of 12 h. The modified NRL retained about 30 per cent higher helium as compared to the standard NRL. Additionally, modified NRL retained 58 per cent more air on the fifth day as compared to standard NRL which completely deflated by 60 h (Table 10). The removal of non-rubbers causes more rubber particle integration, preventing the loss of gas from the balloons. Helium molecules being lighter and smaller are able to permeate faster than the heavier molecules of nitrogen and oxygen.

CONCLUSIONS

Aluminum hydroxide treated NRL is an ideal material of choice due to the good colour retension of products made out of it. Glove manufacturers prefer to use low

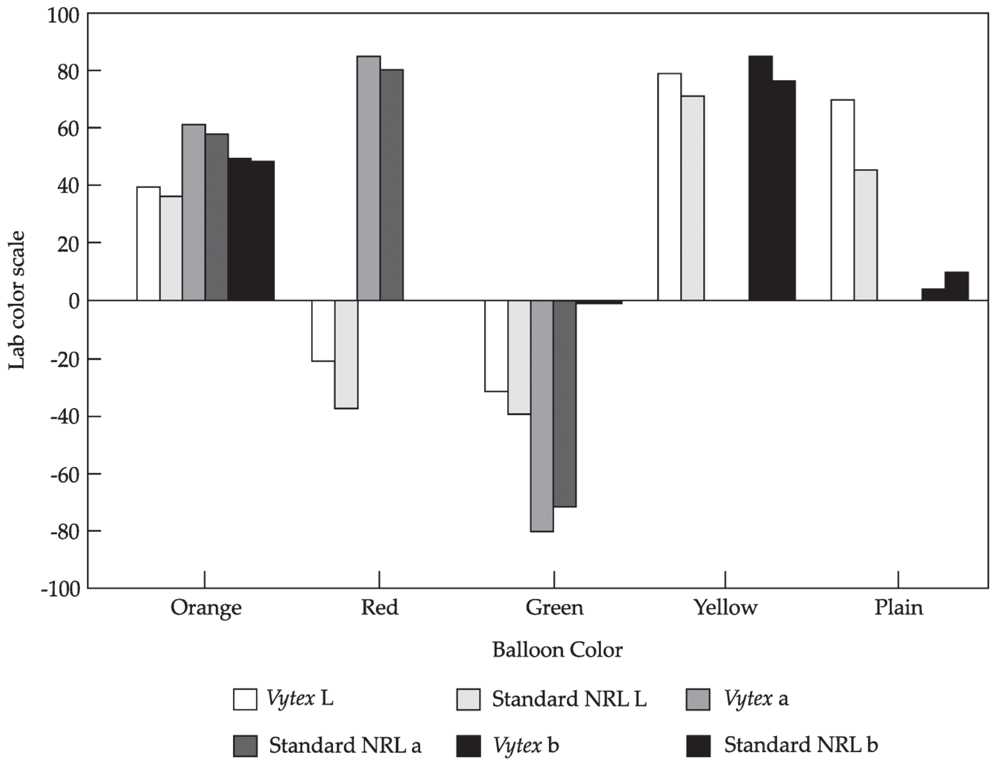

Fig. 2. Lab color analysis of balloons

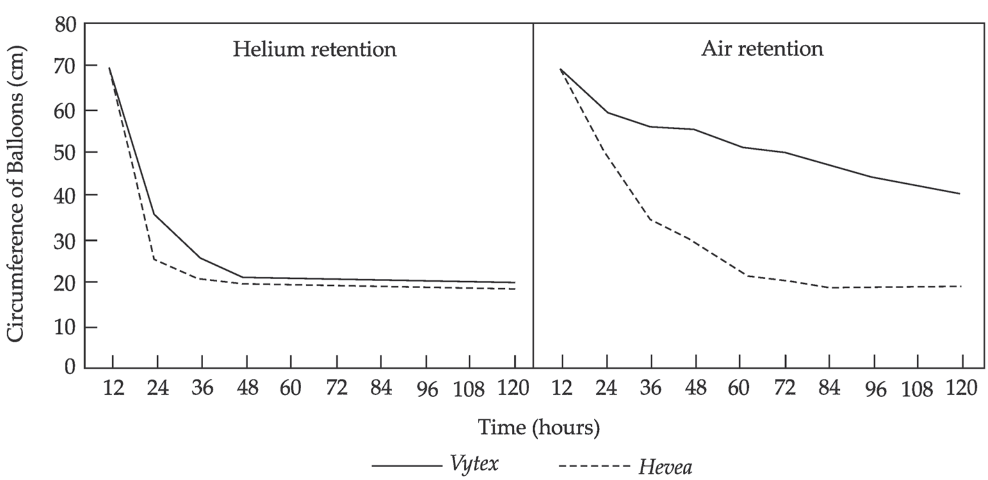

Fig. 3. Helium and air retention

modulus TNRL as it can accommodate more filler due to the absence of non-rubber materials. Products made out of TNRL require less leaching in water and hence processing cost is low. The aluminum hydroxide-treated NRL has a longer "pot life" than regular NRL which allows longer shelf life and more dipping flexibility to the compound. These attributes are attractive in other applications, such as foam, where less rubber odour and a whiter colour are highly desirable. Aluminum hydroxide treated-NRL has a low non-rubber content compared to regular NRL and is virtually free of the 14kD and 30kD polypeptide proteins which are reported to cause Type 1 latex allergies. These characteristics make TNRL, the material of choice for applications seeking high-quality and safer end products.

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