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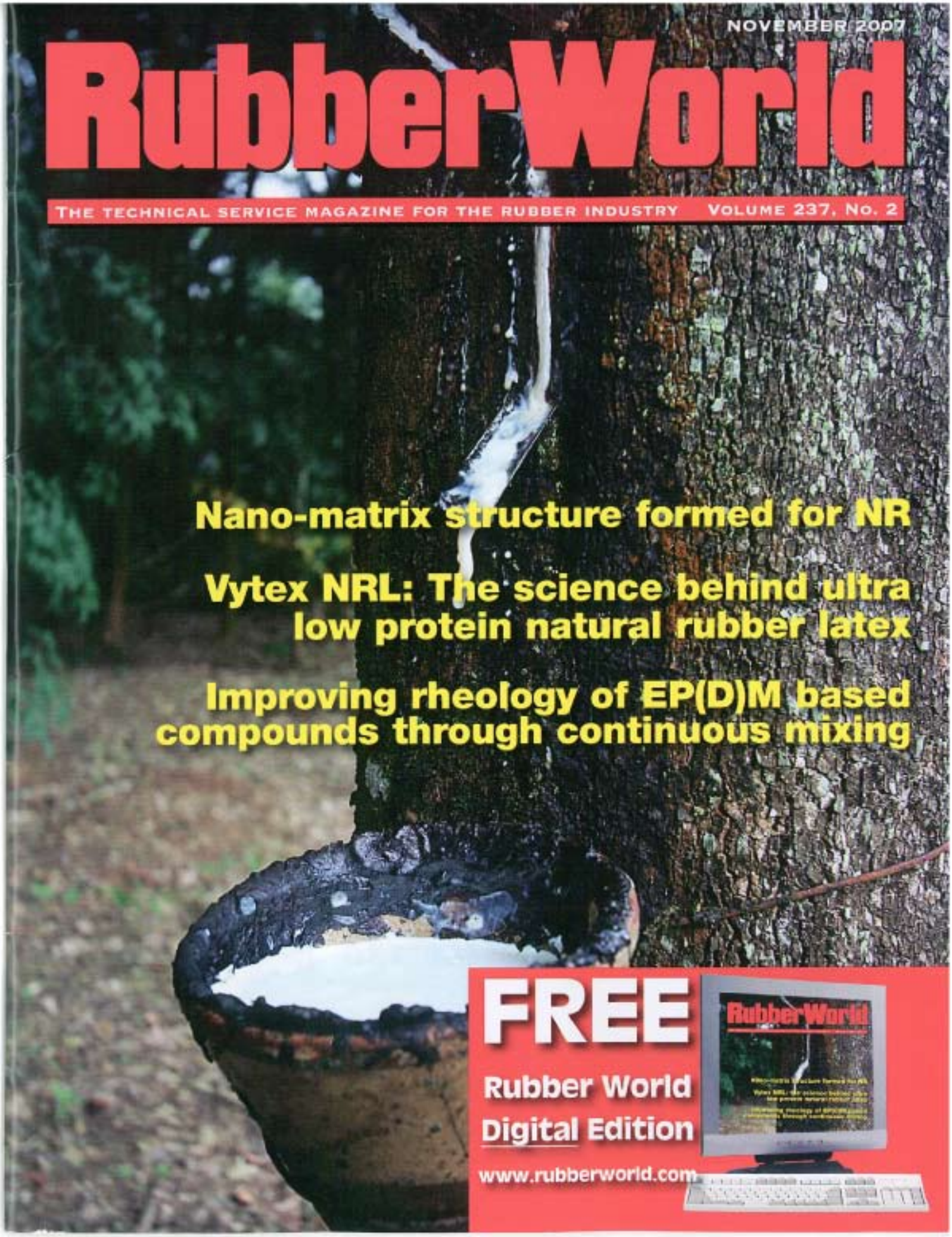

Nano-matrix structure formed for NR

Vytex NRL: The science behind ultra low protein natural rubber latex

Improving rheology of EP(D)M based compounds through continuous mixing

Vytex NRL: The science behind ultra low protein natural rubber latex

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Although latex as a protectant dates back to the 1800s, the use of barrier goods like gloves and condoms increased tremendously in the 1980s. Due to its complex mechanical and chemical properties, latex is considered to have the best broad range of desirable properties. Allergic reactions for human skin in contact with latex have grown tremendously in the past two decades. Naturally occurring antigenic protein in raw latex has been identified as the problem when the finished latex product contacts the skin or mucosa (ref. 1).

The first description of allergic reaction to latex gloves appeared in American literature in 1933, yet more than 20 billion gloves from latex are used annually in the United States (refs. 2 and 3). It has been published that in 2003, 16.7% of the cases of intra-operative shock were attributed to latex allergies (ref. 4). Similarly, it is estimated that 17% of American healthcare workers and 73% of frequently exposed patients have become sensitized to latex proteins (refs. 5 and 6).

The amount of protein in natural rubber latex (NRL) has remained fairly constant at 1.6-2.0 wt. % (16,000-20,000 µg/g), but the final concentration varies between 3 to >600 µg/g, depending on the manufacturing technique used (ref. 7). In March 1995, the United States Food and Drug Administration issued interim labeling guidance on permissible levels of protein in medical latex gloves that set the upper limit at 50 µg/g for low protein claims using the Modified Lowry Test. The foremost protein experts from the Guthrie Foundation claim that the ideal glove is one with less than 1 ug/gm by the LEAP assay (ref. 8). Achieving this lower number, plus preserving the superior properties of latex, such as elasticity and liquid barrier, has proven to be elusive.

Since there are 240 proteins in processed latex, and due to the complex nature of the latex extract, accurate protein measurement presented a challenge. Some believe that the optimal estimation of antigenic protein levels is determined by the ELISA Inhibition Assay Method, ASTM D6499-03. At the same time, the Modified Lowry Test, ASTM D5712, quantitatively shows the total protein content, including the potentially allergenic proteins (refs. 9 and 10). For the evaluation of Vytex NRL, we chose the ELISA method performed by LEAP Testing Service Laboratory (Donald Guthrie Foundation Education Research), an independent laboratory located in Sayre, PA, because the Lowry method cannot distinguish between total protein and antigenic protein, or the various sizes of latex proteins.

Many efforts have been made to remove protein from NRL by physical, biological and chemical methods that affect the complex acid-base behavior of proteins. Also, NRL's protein content can be decreased to such levels as 200-300 µg/g through successive centrifugations of NRL, or by enzymatic decomposition; however, appearance and physical and chemical properties usually suffer. AP levels were decreased to 20 ug/g through the use of proteolytic enzymes and surfactants (ref. 11). Ultrasonic leaching or irradiation by cobalt also showed promise to reduce AP levels in the literature (refs. 12 and 13).

Most of the above methods are complex, offer limited effectiveness and result in the inadequate removal of allergenic protein. It was determined that a treatment of NRL in the liquid phase by using selected inorganic and/or organic chemicals prior to processing and manufacturing the end product could be more promising.

The aim of this work is to present a selection of chemicals that were determined to have potential for protein removal as shown in the results discussion of treated NRL and laboratory-produced films. The protein removal occurs without compromising the important physical and chemical properties of latex.

Materials and methods

Two different batches of NRL from Southeast Asia have been used for experimental testing.

- Field latex containing 1,123.7 µg/ml of antigenic protein (AP) and a pH of <10. (Total solids not reported but assumed to be ~30%.)
- Field latex with 26% wt. total solids (TS); 636.3 µg/ml of AP and a pH of 10.92.

(Additional tests are ongoing to determine the effect of antigenic protein removal using centrifuged latex.)

The sample size used for laboratory experiments was 100 grams of NRL. Each sample was poured into a glass flask followed by the introduction of specific additives mixed into latex. When necessary, the pH of the latex was adjusted to 11 by adding NaOH to the solution. Different concentrations of additives (0.01-1.0%) were added to the latex to determine their influence on AP content. Depending on the combination of additives, the insertion methods were different.

Several types of chemicals were used as additives to NRL to remove AP in latex by absorption or binding it chemically. Aldrich, Sigma, Sigma-Aldrich, ALFA AESAR, BASF and Zeolyst supplied all the chemicals as either powders or standard solutions with known concentrations and technical grades.

The first group included inorganic salts: aluminum chloride ($AlCl_3$), aluminum sulfate ($Al_2(SO_4)_3$), magnesium chloride ($MgCl_2$), silver nitrate ($AgNO_3$) and zinc iodide (ZnI_2). These salts were preliminarily dissolved in distilled water, and calculated amounts of solutions were added to NRL.

The second group of additives included inorganic oxides and hydroxides: aluminum hydroxide ($Al(OH)_3$), titanium dioxide (TiO_2), fumed silica (SiO_2) and zeolytes. Because these compounds are insoluble in water, $Al(OH)_3$ was added as a solution in 45-50% NaOH or KOH. However, TiO_2, which is practically insoluble in alkali, was added as a suspension of TiO_2 in 50% NaOH. In some tests, TiO_2 and $Al(OH)_3$ were added to latex in their powder form, as was done with zeolytes and silica.

The third group of additives included metal powders aluminum (Al) and silver (Ag), and aluminum wire (Al-wire). The powders were added directly to NRL; however, the Al-wire was immersed into the latex and exposed for a set period of time to get the aluminum ions into the latex as the wire corroded.

The fourth group included organic compounds: formaldehyde, sodium salts of benzenesulfonic and benzenesulfinic acids, sodium decylsulfate, acrylamide, zinc gluconate, zinc acetate and copper acetate. They were added to latex as liquids, solutions or solids.

The fifth group included polymeric absorbents: Lupamin 9010, Lupamin 1595 (BASF), Lupasol FG, Lupasol G20 (BASF) and lignins.

After the additives were added and thoroughly mixed with a magnetic stirrer, the pH was evaluated using a digital pH meter. Next, the latex with additives was agitated for 72 hours. Ten-gram samples of treated latex were taken to determine the AP content. The balance of the latex was centrifuged for one hour at 3,000 rpm using a Dimon/IEC HN-SII centrifuge. Ten-gram samples of treated latex were taken after the centrifuge process for evaluation.

The mix was then compounded by using standard additives commonly used to produce latex goods. Added compounds and concentrations used were:

Additive	*Phr*
Potassium hydroxide	0.05
2-mercaptobenzothiazole, zinc salt	0.25
Sodium polynaphthalenesulfonate	1.00
Zinc oxide	0.70
Sulfur	0.70
Butylated hydroxytoluene	0.50
Zinc dimethyldithiocarbamate	0.45

The compounded latex was agitated for 48 hours in a covered glass flask. The mix was then used to produce films. About 40 g of liquid latex were poured on a leveled glass plate, drawn by an adhesive spreader and then air dried for 8 to10 hours. Next, the glass plate and film were placed in an oven at 70ºC for 10 minutes, and then placed into a bath with distilled water. The film was detached from glass and then leached in distilled water at 100º C for two minutes. The rubber film was then mounted on metal frame and placed into an oven for vulcanization at 130ºC for 25 minutes. Next, samples were cut from the treated latex film to analyze AP.

Certain selected films were tested to determine elongation, and samples with a 20 mm width and a 70 mm length were cut from the film. One end of the sample was attached to a metal crossbeam frame, and the sample was examined by loading weights at the lower end. The measurement for each sample (20 mm) was recorded after loading and release.

Results and discussion

Influence of pH and method of pH adjustment on antigenic protein content

Maintaining pH in latex is important for latex processing. The pH can be adjusted by using potassium hydroxide (KOH) and/or potassium hydroxide + ammonium hydroxide (NH_4OH) or sodium hydroxide. The reactive functional groups of protein are acids or bases, thus their reactivity must vary with the pH of latex (ref. 13).

Table 1 illustrates the results of AP value in latex after a pH adjustment. Because an initial pH of latex used for these tests was <10, the pH had to be increased to 11 before adding additional chemicals. The

Table 1 - influence of pH at different methods of adjustment on the AP content

#	*Chemical*	*Additives Formula*	*Wt. %*	*Test conditions pH*	*Exposition after introduction of additives, hours*	*Centrifuging*	*AP, µg/ml*	*Notes*
1	Sodium hydroxide	NaOH	-	11	72	No	14,700.0	
	Sodium hydroxide	NaOH	-	11	72	Yes	30,365.4	
2	Sodium hydroxide + ammonium hydroxide	NaOH	-	11	72	No		
		NH_4OH	1.0	11+	-	Yes	31,487.4	
		NaOH	-	11	72	No		Double treatment
		NH_4OH	1.0	11+	-	Yes		
		NH_4OH	1.0	11+	-	Yes	60,782.5	
3	Sodium hydroxide + formaldehyde	NaOH	-	11	72	No		
		HCHO	0.01	11	24	Yes	3,758.6	Treatment by NaOH then HCHO
		NaOH	-	11	72	No		
		HCHO	0.10	11	24	Yes	5,203.4	
		NaOH	-	11	72	No		
		HCHO	0.50	11	24	Yes	7,328.6	
4	Formaldehyde	HCHO	0.01	9	24	Yes	314.9	
		HCHO	0.10	9	24	Yes	379.2	
		HCHO	0.50	9	24	Yes	555.2	
5	Sodium hydroxide + aluminum hydroxide	NaOH		11	72	No		
		$Al(OH)_3$	0.01	11+	24	Yes	243.1	Treatment by NaOH then $Al(OH)_3$
		NaOH		11	72	No		
		$Al(OH)_3$	0.05	12	24	Yes	22.8	
		NaOH		11	72	No		
		$Al(OH)_3$	0.10	13	24	Yes	14.5	

adjustment was made by adding NaOH to the latex, followed by the introduction of other chemicals. The addition of chemicals also changed the pH because of the alkalinity of the added chemicals (solutions).

Table 1 also indicates that increasing the pH to 11 by adding NaOH and NH_4OH resulted in a significant increase of AP in latex (from 1,123.7 µg/ml initially up to more than 60,000 µg/ml). This AP increase suggests that strong alkalis provide an activation of proteins releasing AP. Formaldehyde with NaOH partially neutralizes this action and even reduces AP content 2-3 times, when used alone. This decrease in AP may not be enough to obtain the status "protein-free" latex. Better results were observed by the addition of aluminum hydroxide as a 2% solution in saturated NaOH. Table 1 demonstrates that this additive drastically reduces the AP in latex compared with the initial AP value, even at a slightly higher pH of the final product. The required pH of 11 can be achieved by introducing the appropriate amount of alkali in added solutions.

Influence of soluble inorganic and organic compounds

The AP content in liquid latex after treatment with soluble inorganic and organic compounds reveals that inorganic compounds are more effective for AP reduction than organic compounds. The inorganic compounds evaluated were magnesium chloride, aluminum hydroxide, aluminum chloride, silver nitrate, aluminum sulfate and zinc iodide. The organic compounds evaluated were copper acetate, zinc gluconate, zinc acetate, sodium dodecyl sulfate, sodium salt of benzenesulfinic acid and sodium salt of benzenesulfonic acid. Of particular interest was the effectiveness of inorganic additive aluminum hydroxide becoming higher with increased concentration.

Influence of metallic additives and absorbents

Several experiments were conducted using various concentrations of metallic additives and organic and inorganic absorbents. The metal additives included silver and aluminum. The results of treating latex with metallic additives show conclusively that protein reduction is possible by this procedure (approximately half the AP value of the control sample); however, these results were not enough to achieve the targeted levels.

Inorganic absorbents used included titanium dioxide, fumed silica and zeolyte, all at various concentrations. The titanium dioxide and fumed silica samples yielded the lowest AP values (from 116 ug/ml to 384 ug/ml). Zeolyte demonstrated limited effectiveness in reducing the AP value of latex.

Organic absorbents used included Merrifield's peptide resin, acrylamide, polyacrylamide, vinylamine-vinylformamide and polyethylenimine. These absorbents demonstrated limited effectiveness in reducing the AP value in NRL. It became apparent that absorption alone is not enough to achieve low AP status.

Influence of mixed additives on AP content in liquid latex

Assuming that a combination of the most effective additives can improve AP removal, several combinations of $Al(OH)_3$ with other chemicals were tested. Table 3 results indicate that this assumption was only partially confirmed by tests. Latex, which was treated with $Al(OH)_3$ dissolved in NaOH in combination with absorbents (fumed silica and Lignin Curan 2711P), provided the best results. The AP levels in latex treated by these combined additives are similar to the results obtained from treating latex with $Al(OH)_3$ alone dissolved in alkali.

The additives on the base of $Al(OH)_3$ in various combinations with alkali and other chemicals were chosen for further research to determine their influence on AP content in vulcanized NRL and on other physical properties of NRL.

Influence of additives on AP content in NRL film

NRL films were created from the treated liquid latex as previously described. A control film sample was made from the field latex that contains 636.3 µg/ml of AP.

Potassium hydroxide (KOH) was substituted in some tests for NaOH because it is a typical component for compounding latex products and is considered less harsh compared to NaOH. Table 4 illustrates that the AP value is significantly reduced in vulcanized rubber film compared to the AP value in liquid latex. This observation is also consistent with the control sample.

Table 2 - effectiveness of aluminum hydroxide (initial field latex 636.3 g/ml of AP)

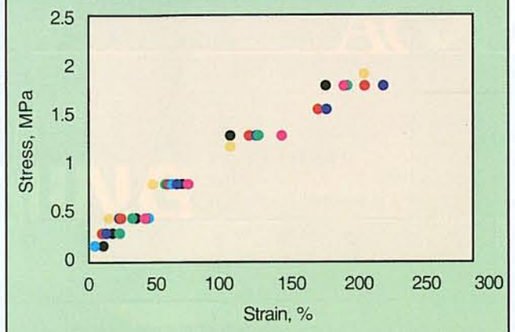
Additives			Test conditions		AP,
Chemical	*Formula*	*Wt. %*	*pH*	*Centrifuged*	*µg/ml*
		0.05	12	No	12.1
Aluminum hydroxide	$Al(OH)_3$	0.04	11	No	39.5
		0.04	11	Yes	41.6
		0.02	11	Yes	147.3
		0.01	11	Yes	403.9

Table 3 - effectiveness of combined additives on AP content

#	Title	Additives *Wt. %*	*pH*	*AP, µg/ml*	*Notes*
28	Fumed silica	0.50		22.2	$Al(OH)_3$ dissolved in
	+ aluminum	0.04	11		NaOH solution,
	hydroxide	0.50		15.5	
		0.05	12	215.2	Powder of SiO_2
29	Acrylamide	0.5	11		$Al(OH)_3$ disolved in
	aluminum	0.04			NaOH solution
	hydroxide				Powder of acrylamide
30	Lignin +	0.5		48.0	*Type of lignin:*
	aluminum	0.04	11	12.3	Curan 2711P
	hydroxide	0.5	11		
		0.05		71.6	Curan 2711P
		0.5	11		
		0.04		76.9	Norlig
		0.5	11		Lignosite 100
		0.04		68.0	
		0.5	11		Lignin alkali
		0.04			

It is important to note that an increase in viscosity and a coagulum formation was observed during extended agitation of the latex. This was primarily observed with samples using sodium hydroxide. Under these circumstances, water was added as needed (~10 to 15%) to the latex to reduce the viscosity in order to process the latex into the film. The mixture was stirred for one hour by mixing with a magnetic stirrer. The coagulated particles were removed before the addition of compounding additives and before film creation. The formation of the large conglomerates occurred in some cases. It is believed that this could have occurred due to some coagulation of latex prior to compounding. We can also assume that this process can facilitate the binding and deactivation of antigenic protein. It should be noted that we observed a reverse correlation with the lowest levels of AP occurring when the highest coagulation took place.

Figure 1 - elasticity of latex



Elasticity of NRL films

The elasticity of NRL films made from various types of latex are plotted together in figure 1. It demonstrates that NRL films produced from latex treated with "anti-protein" additives have elasticity similar to industrial rubber films. Latex films made with anti-protein additives do not significantly alter the elasticity of NRL within the limits of measurement error, which was close to data available in the literature (ref. 14). The length of the samples after testing returned to their original length. (Note: The figure 1 legend above has two symbols with the same identification. Vystar confirmed that this is correct – apparently a repeat test. – ed.)

The possible mechanism of influence of $Al(OH)_3$ on removal of AP

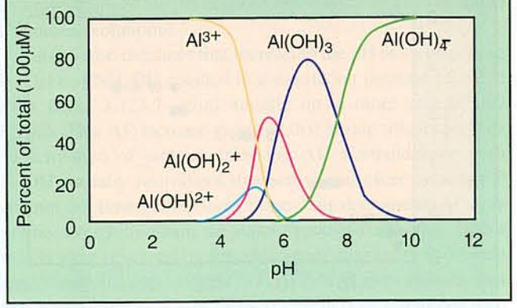
Fresh precipitated aluminum hydroxide is used in the purification of water because it can form a jelly-like structure suspending any unwanted materials in water, including most bacteria (ref. 15). Furthermore, because of a high ionic strength (low ionic radius and high positive charge), aluminum can form a different kind of oxide-hydroxide complex ion that can have a positive, neutral or negative total charge, depending on the pH of a solution. Figure 2 shows the dependency of aluminum ionic composition on pH (ref. 16).

The effect of protein removal from natural latex can be contributed to the absorption of protein by a jelly-like freshly precipitated alumi-

Table 4 - antigenic protein in latex films

#	Additives *Chemical or formula*	*Wt. %*	Technological features *Aging before compounding, hours*	*Premixing before compounding additives, hours*	*Increase of + - viscosity C - conglomeration*	*AP, µg/ml*
1	Control sample	-	0	0	Not observed	33.0
2	$Al(OH)_3$ in NaOH	0.04	432	0	+	23
	$Al(OH)_3$ in NaOH	0.05	456	0	++	6.7
3	Fumed silica	0.5	312	0	Not observed	46.5
	$Al(OH)_3$ in NaOH	0.04				
	Fumed silica	0.5	360	0	++	10.8
	$Al(OH)_3$ in NaOH	0.05				
	Fumed silica	0.25	504	0	+++	1.8
	$Al(OH)_3$ in NaOH	0.05			C	
	Fumed silica	0.5	648	0	+++	1.1
	$Al(OH)_3$ in NaOH	0.06			C	
	Fumed silica	0.5	0	0	Not observed	37.3
	$Al(OH)_3$ in NaOH	0.1				
4	Lignin	0.5	528	0	++	4.5
	$Al(OH)_3$ in NaOH	0.05			C	
	Lignin	0.5	624	0	+++	0.4
	$Al(OH)_3$ in NaOH	0.06			C	
	Lignin	0.5	72	0	Not observed	22.4
	$Al(OH)_3$ in NaOH	0.06				
	Lignin	0.5	72	0	Not observed	112.0
	$Al(OH)_3$ in KOH	0.06				
	Lignin	0.5	0	0	Not observed	53.8
	$Al(OH)_3$ in KOH	0.06				
	Lignin	0.5	72	0	Not observed	31.0
	$Al(OH)_3$ in KOH	0.1				
5	Lignin	0.5	0	0	Not observed	7.2
	$Al(OH)_3$ in KOH	0.1				
	Lignin	0.5	0	72	Not observed	1.9
	$Al(OH)_3$ in KOH	0.1				
	Lignin	0.5	0	72	Not observed	<0.2
	$Al(OH)_3$ in KOH	0.15			C	
	Lignin	0.5	0	72	Not observed	0.8
	$Al(OH)_3$ in KOH	0.20			C	

Figure 2 - aluminum ionic composition vs. pH



num hydroxide. The precipitation of aluminum hydroxide occurs when an alkaline solution with a rather high concentration of aluminum ions is added to latex. The alkalinity of natural latex emulsion allows the $Al(OH)_3$ to precipitate, thus forming thin particles which have very strong absorbing ability. Furthermore, aluminum ions can bind protein chemically. The ability to form complexes with carboxylic groups (which are present in proteins) is well known from literature (ref. 17). These complexes can be rather stable in a solution with a high alkalinity. Therefore, the proteins are capable of replacing the hydroxide group in the complex aluminum-hydroxide ion partially or completely. The formation of pure protein-aluminum complex could form a soluble compound, but the partially replaced complex can be insoluble in the solution that would remove proteins from latex.

A possible scenario of the reactions with AP could be presented as:

$H_3N^+CHRCOO^- + (OH)Al(OH)^-_3 \rightarrow$
$H_2NCHRCOOAl(OH)^-_3 + H_2O$
(antigenic protein)

$H_3N^+CHRCOO^- + (OH)Al(OH)^-_3 \rightarrow$
$H_2NCHRCOOAl(OH)_2 + H_2O + OH^-$
(antigenic protein)

$2H_3N^+CHRCOO^- + (OH)Al(OH)^-_3 \rightarrow$
$(H_2NCHRCOO)_2Al(OH) + 2H_2O + OH^-$
(antigenic protein)

$3H_3N^+CHRCOO^- + (OH)Al(OH)^-_3 \rightarrow$
$(H_2NCHRCOO)_3Al + 3H_2O + OH^-$
(antigenic protein)

The presence of an insoluble coagulum has been observed in the latex emulsion after treatment in all of our successful tests. There is evidence of a formation of insoluble compounds, which can provoke coagulation of the latex containing protein.

Conclusion

It is quite apparent from the test data that a dramatic reduction in protein levels is achieved by the relatively simple processes with aluminum hydroxide alone or with lignin and fumed silica. All of these processes are employed prior to vulcanization (ref. 18) of the natural rubber latex. In doing so, products can be produced while reducing risks imposed upon users of natural rubber latex products, including healthcare professionals, as a result of type I hypersensitivity. Most importantly, this can be accomplished without diminishing the physical properties of natural rubber latex that makes commercial products made from this material so desirable.

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