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Lipids Associated with Rubber Particles and Their Possible Role in Mechanical Stability of Latex Concentrates

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Lipids, which form one of the major membrane components surrounding the rubber particles (RP) in natural latex, could play a role in the mechanical stability of the latex on storage in ammonia. Results showed that the main lipid components which changed on storage in ammonia were the glycolipids and phospholipids which hydrolysed to free higher fatty acids (HFA). The amount of glycolipids and phospholipids in fresh RP of six Hevea brasiliensis *clones did not vary much. Consequently, the amount of HFA generated in each clone was expected to be equal. The mechanical stability time (MST) of the six clonal latices however differed significantly, some giving MST well above 1000 s while some gave MST below 300 s even after three months' storage. This led to the conclusion that the formation of HFA is not the major factor contributing to the increase in MST of high ammoniated (HA) latex concentrates, contrary to what has been reported previously.*

Mechanical stability time (MST) which assesses the resistance of natural rubber latex to destabilisation by mechanical forces, is an important property of latex concentrate. Freshly prepared HA latex concentrate normally has a MST of less than 100 s but this can increase to more than 1000 s after three months' storage. The amount of increase depends on the clonal origin of the latex[1]. Clones of *H. brasiliensis* stabilised with high ammonia can be broadly divided into two groups: clones with low MST, which register a MST of less than 650 s after three months' storage and clones with high MST, which give higher MST values.

Various factors have been reported to affect the MST of latex concentrate; some are deleterious while some are beneficial. For example, magnesium is the major naturally occurring element associated with the destabilisation of natural rubber latex concentrate[2] while alkalis and sodium pentachlorophenate have been shown to increase the mechanical stability of the latex[3]. However, the much discussed substances among the group of compounds found to increase the MST of latex are the natural free higher fatty acid (HFA) soap formed by hydrolysis of the phospholipids of the latex[3,4]. Chen and Ng[4] found a good correlation between the concentration of HFA and the increase in MST of some clones up to a period of three to six weeks after which the HFA concentration remained constant. They however could not ascertain the factors which affect the further rise in MST after the specified period.

Besides the HFA and phospholipids, there are other lipids associated with the membrane surrounding the rubber particle[5] (RP) which might also have an influence on the MST. This paper aims to demonstrate the role of these lipids on the MST of latex concentrate, focusing on their composition in fresh RP, how it changes on storage in ammonia and their possible contribution to the rise in MST of latices with both high and low MST.

MATERIALS AND METHODS

Materials

Latex was obtained from mature unstimulated trees. The RP were isolated from fresh latex or

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HA latex concentrate by ultracentrifugation of the latex in a Beckman L8-70 ultracentrifuge at 19 500 r.p.m. using rotor 21 for about 1 h. The HA latex concentrates were prepared by centrifuging about 0.5% ammoniated field latex in a De Laval LRH 410-70A centrifugal latex separator. This gave latex concentrates of about 60% dry rubber content, which were further ammoniated to 0.7%.

Extraction, Fractionation and Quantitation of Lipids

Lipids were extracted from the RP by first redispersing the RP in a minimum amount of water and then adding the suspension dropwise to five volumes of a continuously stirred chloroform/methanol (2:1, v/v) mixture. The subsequent procedures for the isolation of lipids, their fractionation and quantitation were as reported[6].

Determination of MST

The MST values of HA latex concentrates and RP redispersed in water ammoniated to 0.7% were determined at 55% total solids content at 35°C using a Klaxon instrument as specified by the International Standards Organisation[7]. The method of assessing the end point of the test was to observe the first signs of flocculation in a thin film of latex spread on the palm of the hand.

RESULTS AND DISCUSSION

MST of Some Clonal Latex Concentrates

Ultracentrifugation of freshly prepared HA latex concentrate at 19 500 r.p.m. for 1 h gave two fractions: an upper rubber phase and a lower aqueous serum phase with some sediments at the bottom of the centrifuge tube. On redispersing the RP in distilled water and ammoniating to 0.7% the resulting latex showed increasing MST with storage time *(Figure 1)*. The RP of both clones with high MST (RRIM 701, RRIM 730 and PB 28/59) and clones with low MST (RRIM 600, RRIM 804 and PR 255) gave MST values of above 1000 s after sixty days of storage. This showed that the increase in the MST of redispersed RP could be governed mainly by changes in the RP on storage in ammonia.

Although all the clonal RP redispersed in ammoniated water gave high MST on storage, the rate of rise in MST differed. The RP of the two clones with high MST, RRIM 730 and PB 28/59, gave the fastest rise in MST, reaching a value of 1000 s before thirty days of storage while the RP of the remaining clone with high MST, RRIM 701, and the RP of all the three clones with low MST gave a more gradual increase in MST to 1000 s after fifty to sixty days' storage. The fast rise in MST was not confined to the RP of clones with high MST. In this study, it was observed that the RP of a clone with low MST, RRIM 729, also gave a fast rise in MST of 1000 s after twenty days of storage. This difference suggested that the components in the membrane might have an influence on the rise in mechanical stability of the RP and they might differ with clones.

When the RP were dispersed in their own serum, as in the normal preparation of HA latex concentrate, only the RP of clones with high MST gave a MST of over 1000 s while the RP of clones with low MST gave a MST of less than 300 s even though the latex concentrate was stored for more than two months *(Figure 2)*. The apparent inhibition of the rise in MST of RP of clones with low MST was not only observed when the RP were dispersed in their own serum but also when they were redispersed in the sera of clones with high MST *(Table 1)*. On the contrary, the RP of clones with high MST consistently gave high MST values even when redispersed in the sera of clones with low MST *(Table 1)*. The serum constituents thus seemed to preferentially inhibit the rise in MST of RP of clones with low MST but seemed to exhibit no significant effect on the rise in MST of RP of clones with high MST. The results indicate that there is an interaction between the RP of clones with low MST and serum (of both types) and this interaction does not occur with clones with high MST. This also means that there is a factor on the surface of RP from clones with low MST, which is absent on the surface of RP of clones with high MST. The latter difference may be reflected in their

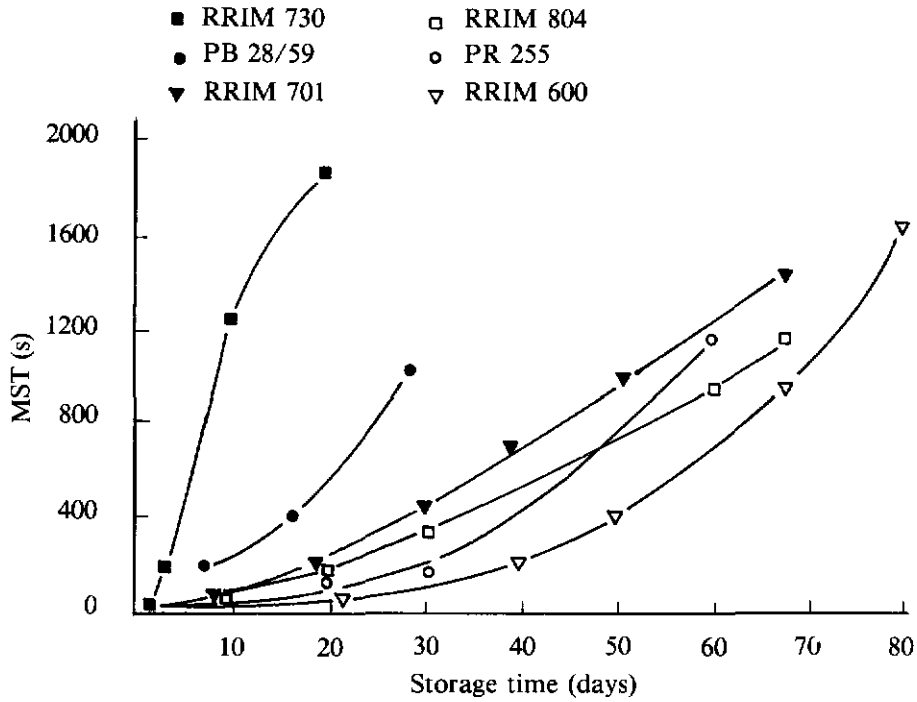

Figure 1. MST of 0.7% ammoniated redispersed RP from six H. brasiliensis *clones.*

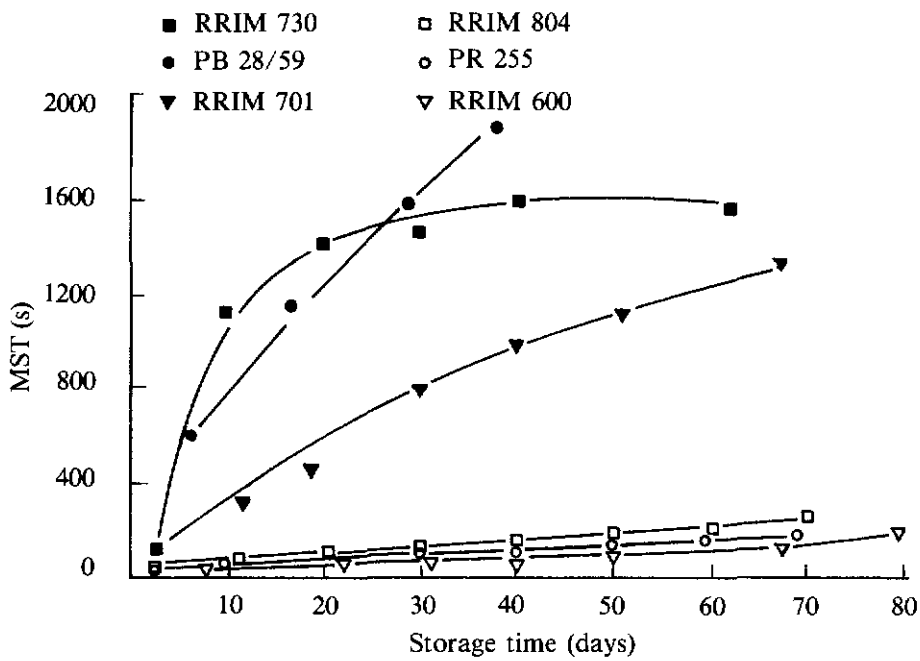

Figure 2. MST of HA latex concentrates from six H. brasiliensis *clones.*

TABLE 1 EFFECTS OF SERUM ON THE MST OF REDISPERSED RUBBER PARTICLES AFTER TWO MONTHS' STORAGE

Serum	MST (s)	
	PR 255 RP	RRIM 730 RP
RRIM 701[a]	214	-
RRIM 730[a]	150	1 200
RRIM 804[b]	-	1 370
PR 255[b]	125	1 055

[a]Clone with high MST
[b]Clone with low MST

lipid composition though differences in the protein components of the membrane may be another possibility

Lipid Composition of Fresh Rubber Particles

Lipids in fresh *H. brasiliensis* latex have been classified into neutral lipids, glycolipids and phospholipids[6] The qualitative composition of the neutral lipids, glycolipids and phospholipids in the membrane surrounding fresh RP of the six clones studied here was similar to the lipid composition of fresh RRIM 501 latex[6]. The neutral lipids comprised triglycerides, diglycerides, free fatty acids, free and esterified sterols, free and esterified tocotrienols and fatty alcohols and their acetates *(Figure 3)*. The glycolipids consisted mainly of free and esterified steryl glucosides and mono-galactosyl and digalactosyl diglycerides while the phospholipids consisted of phosphatidyl ethanolamine, phosphatidyl choline and phosphatidyl inositol The amounts of glycolipids and phospholipids varied from 0.2% to 0.5% and 0.3% to 0.6%, respectively *(Table 2)*. The neutral lipids showed a greater variation of 0 5% to 2.3%, with the two clones with high MST, RRIM 701 and PB 28/59, showing the highest neutral lipid content. This was however not a characteristic of clones with high MST as RRIM 730 had a lower level of neutral lipids.

The high neutral lipid content of the whole latex was found to be mainly due to the prominently higher level of triglycerides compared to the other neutral lipid components *(Table 3)* The triglycerides of whole latex of RRIM 701 and RRIM 501 constituted about 1.5% - 1.7% of the rubber compared to the triglycerides of GT 1 and PR 107 which constituted only about 0.15% - 0.18% This made the neutral lipid content of the former two clones much higher than that of the latter clones. A similar distribution of triglycerides was expected to occur in the neutral lipids of the RP since the latter made up about 70% to 80% of the total neutral lipids of the whole natural rubber latex. *Figure 3* shows the prominent distribution of triglycerides in the neutral lipid composition of the RP. Further analysis on the RP of RRIM 501 revealed that a high proportion, about 48%, of the neutral lipids consisted of triglycerides. The triglyceride content in the neutral lipids of the whole latex of RRIM 501 was 63%.

Lipid Composition of Rubber Particles Stored in Ammonia

The composition of lipids associated with the RP changed on storage in ammonia. The most prominent changes were the decreasing levels of glycolipids and phospholipids (*Figures 4* and *5*) and the increasing level of free HFA in the neutral lipid fraction *(Figure 3)*.

The hydrolysis of glycolipids and phospholipids of RP with a high MST, RRIM 730 *(Figure 4)*, could be approximately divided into three stages. In *Stage I*, 0 - 12 days' storage, the hydrolysis of the polar lipids was the fastest. During this time, about 68% glycolipids and 81% phospholipids hydrolysed *Stage II*, of duration 12 - 40 days, has a slower rate of hydrolysis; a further 28% and 15% of the glycolipids and phospholipids, respectively, hydrolysed. In *Stage III*, 40 days onwards, very little hydrolysis of the polar lipids occurred. A similar pattern was observed with the hydrolysis of polar lipids of RP with low MST, RRIM 600 *(Figure 5)* This, in fact, formed a general pattern for the hydrolysis of glycolipids and phospholipids associated with RP of clones with both high and low MST, dispersed in HA serum or water. The three stages marking three different rates of hydrolysis of

A = Fresh rubber particles

B = Rubber particles stored in ammonia for one month

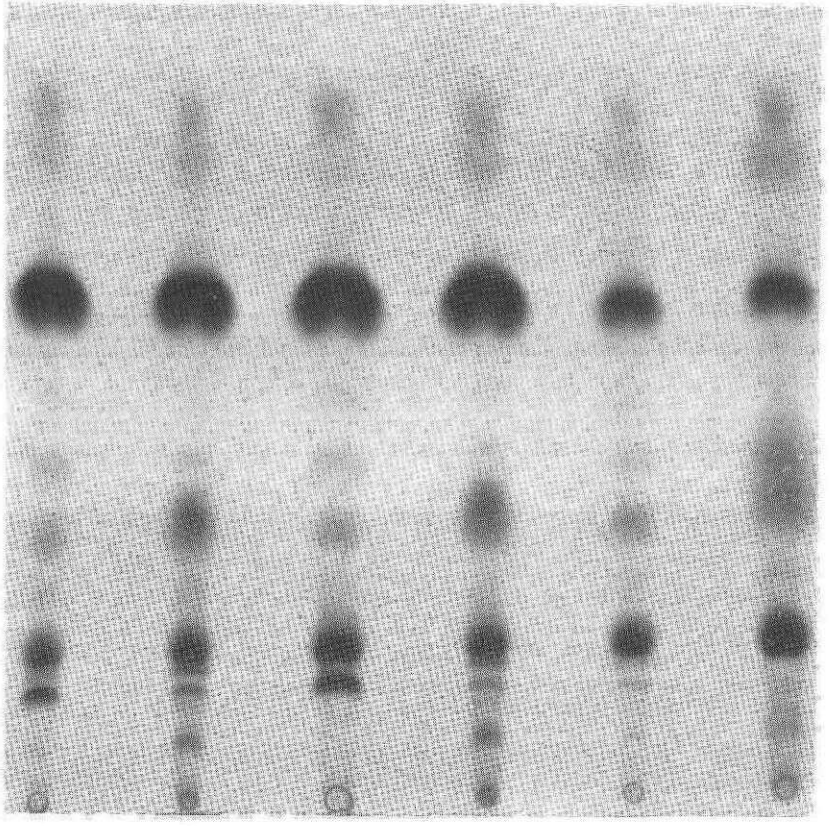

Figure 3. Thin layer chromatogram of neutral lipids associated with the rubber particles from various clonal latices.

TABLE 2 COMPOSITION OF LIPIDS FROM FRESH RUBBER PARTICLES OF SIX *HEVEA* CLONES

Clone	Composition (% dry weight of rubber)		
	Neutral lipids	Glycolipids	Phospholipids
RRIM 701	2 32	0 53	0 49
RRIM 730	0 55	0 53	0 39
PB 28/59	2 34	0 45	0 57
RRIM 600	0 45	0 30	0 58
PR 255	0 64	0 28	0 37
RRIM 804	0 92	0 41	0 54

TABLE 3 COMPOSITION OF NEUTRAL LIPIDS FROM FRESH LATICES OF FOUR *H BRASILIENSIS* CLONES

Clone	Composition (% dry weight of rubber)			
	Total neutral lipids	Esters	Triglycerides	Remaining neutral lipids
RRIM 701	2 77	0 53	1 55	0 69
RRIM 501	2 66	0 43	1 70	0 53
GT 1	1 48	0 77	0 15	0 56
PR 107	1 13	0 52	0 18	0 43

the polar lipids corresponded well with the three rates of production of HFA determined by Chen and Ng[4]

Analysis of the acyl composition of the free HFA fraction in the neutral lipids of RRIM 701 HA latex concentrate showed a high concentration of stearic, oleic and linoleic acids with smaller amounts of palmitic, palmitoleic, linolenic and furanoid fatty acids *(Table 4)*. This corresponded to the acyl composition of sterol esters, tocotrienol esters, glycolipids and phospholipids[6]. As the content of the first two acyl lipids did not reduce *(Figure 3)*, the HFA must have been derived mainly from the hydrolysis of glycolipids and phospholipids.

An interesting observation was the low concentration of furanoid fatty acid in the HFA fraction of RRIM 701 latex which was characterised by a high amount of furanoid fatty acid in its triglyceride fraction. This implied that a large proportion of this triglyceride remained unhydrolysed, possibly as a result of the location of the triglyceride deep in the membrane away from the aqueous interface making it inaccessible to the hydrolysing agents. This is not unlikely in view of the hydrophobic character of this triglyceride. The furanoid fatty acid found in the HFA fraction was thus derived mainly from the hydrolysis of glycolipids, the only other acyl lipids containing this acid[6]. The fact that the furanoid fatty acid appeared only after fourteen days of storage showed that the acylglycolipids containing this acid were less readily hydrolysed than the other acyl polar lipids except those containing palmitoleic acid, which also appeared after the same period of storage.

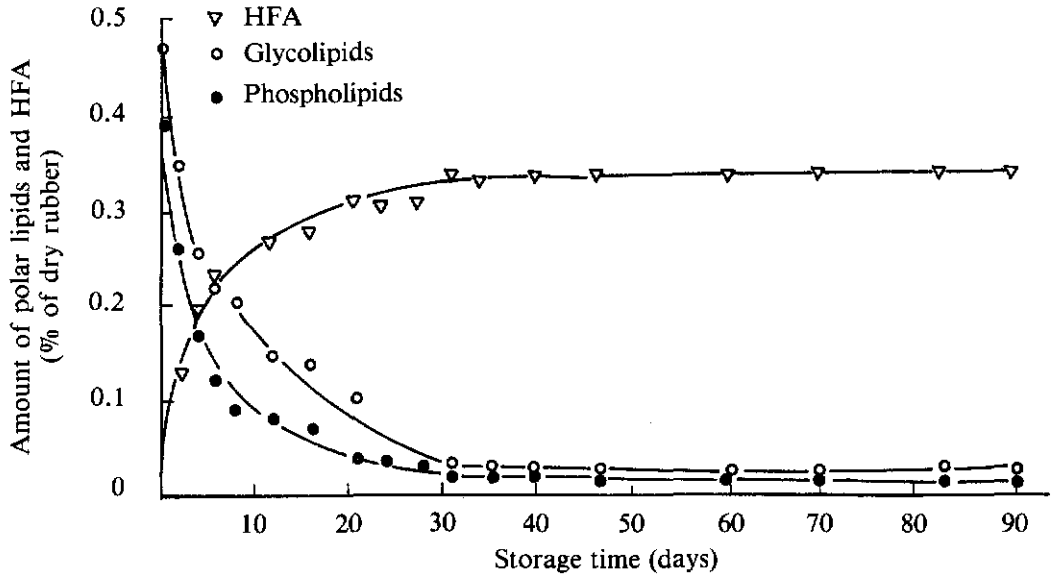

Figure 4. Effect of storage time on the amount of glycolipids, phospholipids and HFA associated with 0.7% ammoniated rubber particles of a clone with high MST, RRIM 730.

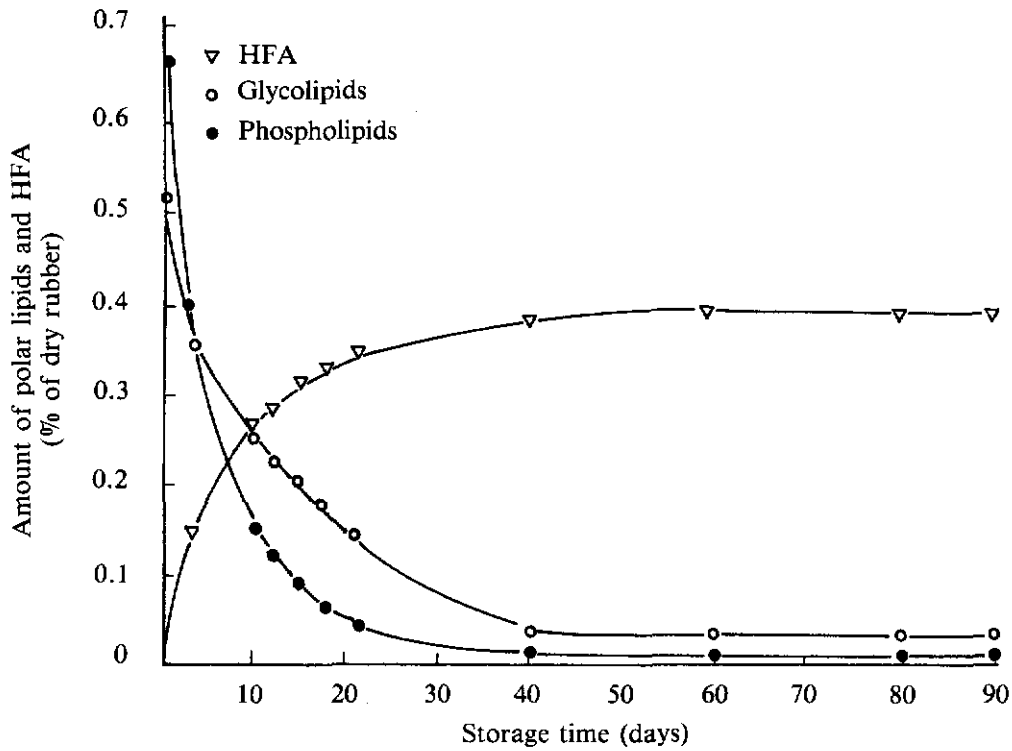

Figure 5. Effect of storage time on the amount of glycolipids, phospholipids and HFA associated with 0.7% ammoniated rubber particles of a clone with low MST, RRIM 600.

TABLE 4 COMPOSITION OF FREE FATTY ACIDS OF HA LATEX CONCENTRATE OF RRIM 701 AFTER DIFFERENT STORAGE TIMES IN AMMONIA

Days of storage	Relative fatty acid composition (%)						
	16 0	16 1	18 0	18 1	18 2	18 3	F_2
0	13 2	-	25 2	29 1	22 8	9 7	
9	13 8	-	15 4	27 6	37 6	5 5	
14	6 4	3 3	18 3	23 3	29 4	5 3	14 1
21	7 3	2 4	20 1	21 3	31 0	5 2	12 6
28	3 4	2 2	10 4	23 5	31 8	9 1	19 6

F_2 = Furanoid fatty acid

Besides the free HFA content, the other lipid component which appeared only after storage in ammonia was the component more polar than the diglyceride fraction *(Figure 3)*. Based on the *Rf* values obatained on thin-layer chromatograms[8] and the infra-red spectrum of the isolated fraction, the component was deduced to be monoglycerides The presence of monoglycerides could result from incomplete hydrolysis of glycolipids and phospholipids to free HFA. The production of monoglycerides was confirmed by separate hydrolysis of glycolipids and phospholipids of fresh natural rubber latex in 0.7% ammonia for about a week. This gave monoglycerides as well as HFA and diglycerides at equivalent *Rf* values to those of the neutral lipid components from the ammoniated RP

Role of Lipids in Mechanical Stability of Latex Concentrate

The membrane surrounding the RP stored in ammonia can be visualised to contain extra negative charge imparted by the hydrolysis products of glycolipids and phospholipids, the free HFA, located at the aqueous interface. The additional charge could render the RP more stable to externally applied mechanical forces However, from the results presented in this paper, the HFA present in the membrane could be argued to be not the major contributing factor to the rise in MST as widely thought. This could be explained through the rates of production of HFA, calculated from the hydrolysis of glycolipids and phospholipids as depicted in *Figures 4* and *5* which did not correspond well with the rise in MST of the HA redispersed RP except that of RP of RRIM 730 *(Figure 1)*. At the initial period of fast production of HFA, the RP of RRIM 730 gave a fast rise in MST of over 1000 s. The MST of PB 28/59 increased sharply during the second stage of slow production of HFA while the remaining clonal RP showed a fast rise in MST after forty days when the HFA production would presumably have ceased. In fact, after forty days of storage when the latices were expected to contain equal HFA levels as indeed shown by Chen and Ng[4], the MST values differed significantly. This means that there is no correlation between the rise in MST and increase in HFA.

There are reports that the rise in MST caused by HFA depends on their chain lengths[9]. The present work however does not show the dependence of rise in MST on the fatty acid chain lengths of C_{14} to C_{20}. The fatty acid composition in the HFA fraction derived from the hydrolysis of phospholipids and glycolipids of four clonal latices *(Table 5)* was about the same, yet the MST of RRIM 701 and GT 1 differed from those of PR 107 and RRIM 600. RRIM 701 and GT 1 are clones with high MST while PR 107 and RRIM 600 are clones with low MST. Besides this, the addition of HFA, normally in the form of ammonium laurate, does not guarantee an equal increase in the MST of all clonal latex concentrates; the MST of some clones remains unaffected[1].

TABLE 5. FREE FATTY ACID COMPOSITION OF FOUR CLONAL LATICES

Clone	Relative fatty acid composition (%)								
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	F_2
RRIM 701	0.5	9.4	2.5	16.1	15.8	29.2	2.9	0.7	23.0
GT 1	0.5	8.6	1.6	17.7	23.6	42.5	2.2	-	3.3
RRIM 600	0.4	9.0	1.4	18.0	17.9	49.7	3.2	-	0.4
PR 107	0.6	8.1	1.1	17.1	16.4	48.3	4.9	-	3.5

F_2 = Furanoid fatty acid

Free fatty acid content alone cannot be considered as the main factor controlling the rise in MST of NR latex on ammoniation. This can be further deduced from the effects of serum on the MST of HA latex concentrates. As stated earlier, serum did not inhibit the hydrolysis of glycolipids and phospholipids of RP with both high and low MST. Thus, RP of the two groups of clones dispersed in serum fraction would contain comparable amounts of HFA. The fact that the MST of the two groups of RP still differed when the latter were dispersed in serum *(Figure 2)* clearly shows that other factors, and not HFA alone, inherent in the RP are more responsible for the mechanical stability of natural RP.

These studies were carried out using three samples of each clone. Since the composition of NR latex is known to vary greatly with clones it will be necessary to study more clones to confirm the observations described in this paper.

CONCLUSION

This rise in MST of HA natural latex concentrates was controlled mainly by changes in the membrane surrounding the RP. These changes were affected by the serum constituents which were found to inhibit the rise in MST of clonal latex with low MST, but not the rise in MST of clonal latex with high MST. This pointed to possible differences in the composition of the membrane surrounding the two groups of RP. The lipid membrane components do not show the expected differences. Although on storage in ammonia the glycolipids and phospholipids hydrolysed to HFA, the increasing presence of which had been broadly linked to the rise in MST, the formation of these fatty acids does not explain the different rates of rise in MST of different clones. Other substances present in the membrane must be involved in controlling the rise in MST of natural latex concentrates.

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