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Microbiological Degradation of Hevea Latex and its Control

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Chemicals which are able to react with carbohydrates, enzyme poisons and bactericides and which can be used as secondary preservatives for field and concentrated latices were investigated. Good control of bacterial proliferation and volatile fatty acids (VFA) build-up in field latex were obtained for two to three days, in the presence of 0.2% ammonia and a secondary preservative such as hydrazine hydrate, 1-(3-chlorallyl) 3,5,7 triazy-l-azoniadamantane chloride, hydroxylamine neutral sulphate, 1,2,-benzisothiazoline-3-one, tetramethyl thiuram disulphide and 2,2, methylene bis (4-chlorophenol).

Ammoniated latex treated with a small amount of hydrazine hydrate, or hydroxylamine neutral sulphate, or tetramethyl-thiruram disulphide prior to and immediately after centrifugation gave concentrates with better properties than those obtained by a single treatment of the same chemical at higher concentrations after centrifugation. Early addition of the secondary preservative also helps to give clean concentrates even from field latex containing a high level of biochemically degradable substrates and/or ammonia-resistant bacteria.

Natural rubber latex is sterile within the tree (McMULLEN, 1951), but becomes contaminated by bacteria soon after tapping. The bacteria multiply rapidly at the expense of the non-rubber substrates (ARCHER *et al.*, 1969) in latex causing its deterioration. The principal substrate readily available for bacterial breakdown is sucrose present at 0.1% to 1.0% in latex obtained from alternate daily tapping or even at higher concentrations in latex obtained from daily tapping (BEALING AND CHUA, 1972). Latex also contains a variety of enzymes (AUDLEY AND MOIR, 1975) of which invertase is the most important from the point of view of substrate breakdown. Invertase which increases with tapping intensity (TUPY, 1973) hydrolyses sucrose into glucose and fructose, both of which are utilised by latex bacteria, producing mainly volatile fatty acids (VFA) (JOHN, 1966b). These carbohydrates can also complex with amino-acids or organic acids present in latex enhancing VFA build-up (LOWE, 1961; JOHN, 1966a).

As smallholdings are generally tapped daily, the latex obtained contains a high concentration of sucrose and invertase which make the latex less stable; this probably explains the need for a higher concentration of anti-coagulants (CHEONG AND ONG, 1974). Should the latex be used for concentrate production, the build-up of VFA need be arrested early because of a limit of VFA and total acid content imposed on latex concentrate. An ideal anti-coagulant/preservative, therefore, should be a good bactericide, an enzyme poison and one which can complex with the non-rubber substrates in the latex to give a material not readily broken down by bacteria. Control of acid formation for a limited period in latex is normally achieved by adding 0.3% to 0.5% (w/w) ammonia. But under certain conditions of collection and seasonal influences which

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result in higher substrate levels and/or higher bacterial population, concentration of ammonia of up to 1.0% is needed. An unduly high level of ammonia is undesirable for economic reasons, and on account of the practical difficulties involved in processing the resulting highly alkaline skim.

Malaysia produced about 200 000 tons of dry rubber as latex concentrate in 1975, representing about 16% of the total rubber production. About 85% of this was produced as HA concentrate containing 0.7% ammonia and 10% as LA concentrate containing 0.2% ammonia and a secondary preservative such as boric acid (BA), sodium pentachlorophenate (SPP) or zinc diethyldithiocarbamate (ZDC).

HA concentrate has the disadvantage of possessing an offensive odour of ammonia and requires deammoniation before processing. The SPP system may have toxicity problems, excessively high mechanical stability time (MST) and films with low wet gel strength and poor colour. The BA system has poor MST and chemical stability, and a high KOH number. It is further unsuitable for the preparation of prevulcanised latex and unacceptable in certain countries due to its toxidity. The ZDC-preserved latex has low MST, poor storage stability and a tendency to pink. Thus alternative systems of preserving latex have been investigated.

ANGOVE AND PILLAI (1964, 1965a, 1965b and 1965c) studied the use of various oxines, organo-zinc compounds and rubber accelerator as secondary preservatives but these have not been commercially developed for a variety of reasons. This paper reports the results of a series of investigations of promising chemicals which include bactericides, enzyme poisons and substrate - complexing materials.

MATERIALS AND METHODS

Latex

Field latex was obtained from a factory bulk at about mid-day. Ammoniated latex was prepared by adding 25% ammonia solution to field latex to give a concentration of 0.2% (w/w) and was used throughout the experiment unless otherwise mentioned. Latex concentrate was prepared by centrifuging one-day-old preserved field latex and treating the resulting concentrate with additional ammonia or other preservatives. Contaminated latex was prepared by seeding field latex with about 1% (v/v) of a broth suspension of an ammonia-resistant bacterium.

Chemicals

Three types of chemicals were investigated. They were (1) chemicals able to react with carbohydrates such as hydrazine hydrate (HH), hydroxylamine neutral sulphate (HNS), boric acid (BA), semicarbazide hydrochloride (SCH), phenyl hydrazine hydrochloride (PHH), (2) enzyme poisons such as tetramethyl thiuram disulphide (TMTD), Antimucin WBR containing 16.5% phenyl-mercuric acetate (PMA), zinc oxide and (3) bactericides such as 1-(3-chlorallyl)-3, 5,7 triazy-l-azoniadamantane (Dowicil 75), 2,2 methylene bis 4 chlorophenol (Panacide) and 1,2-benzisothiazoline-3-one (Proxel CRL).

All chemicals were used as 10% aqueous solutions, except zinc oxide and TMTD, which were used as 30% dispersions prepared by ball milling for 48 h in the presence of 2% Dispersol-LR. Boric acid solution was neutralised with ammonia solution before use. Additives such as glucose, sucrose, molasses and papain were also added to latex as 10% aqeous solutions. Papain used in this investigation was obtained from Sri Lanka, prepared by drying papaya latex collected from green fruits. All chemicals were added on the weight of latex.

Storage of Samples

Latex samples were stored in sterile bottles. Where investigations required the

testing of latex at various times of storage, a fresh bottle was used after each testing.

Testing of Samples

Bacteria were enumerated in a medium of molasses/yeast-extract agar (JOHN, 1968) by pour-plating ten-fold serial dilutions of the test material, incubating at 30°C for three to five days and counting the colonies by the method of JOHN AND TAYSUM (1963). Lactices were tested for ammonia content, VFA number, KOH number and MST according to methods given in British specifications (BRITISH STANDARDS INSTITUTION, 1972).

RESULTS

Field Latex Preservation

Effect of substrate-complexing materials. BA at a concentration of 0.2% prevented VFA build-up for three days, with inhibitory effect on the bacterial population. The surviving bacteria on the third day were either not acid producers or incapable of producing acids. HH and HNS at concentration of 0.05% and 0.1% respectively, prevented VFA build-up in ammoniated latex for three days, with an inhibitory effect on bacterial population *(Table 1)*.

In a separate experiment, PHH even at a concentration of 0.3% had only a weak effect on VFA, the value on the third day being 0.51 compared to 1.07 for the control, while SCH at 0.3% had no effect on VFA build-up and on bacterial population.

Effect of enzyme poisons. PMA controlled VFA build-up for two days at 0.1% and for three days at 0.2%. A marked reduction in bacterial population was obtained at these concentrations, but 0.4% PMA was required to render the latex sterile in a day. Zinc oxide up to 0.05% controlled VFA build-up for two days, but further increase had no effect. TMTD at 0.01% prevented VFA build-up for three days, and at 0.05% reduced the bacterial population to zero in two days *(Table 2)*.

Effect of bactericides. Dowicil 75 at 0.1% controlled VFA build-up for three days. Panacide at 0.25% controlled VFA build-up for two days but 0.75% was required to control it for three days. Proxel CRL at 0.04% remarkably controlled the VFA build-up for three days. Dowicil 75 and Proxel CRL were

TABLE 1. VOLATILE FATTY ACID (VFA) AND BACTERIAL COUNT OF AMMONIATED FIELD LATEX TREATED WITH HYDRAZINE HYDRATE (HH), HYDROXYLAMINE NEUTRAL SULPHATE (HNS) AND BORIC ACID (BA)

Concentration (%)	HH				HNS				BA			
	VFA No. (day)		Bacterial count[a] (log/ml) (day)		VFA No. (day)		Bacterial count[b] (log/ml) (day)		VFA No. (day)		Bacterial count[c] (log/ml) (day)	
	1	3	1	3	1	3	1	3	1	3	1	3
Nil	0.06	1.52	5.6	6.6	0.04	1.07	6.2	6.3	0.11	1.06	7.0	8.6
0.025	0.03	0.39	5.5	6.3	0.03	0.19	5.4	6.4	-	-	-	-
0.05	0.04	0.04	5.8	5.4	0.01	0.07	4.6	5.2	0.05	0.57	6.8	8.2
0.10	0.02	0.02	4.8	4.1	0.02	0.03	4.4	4.5	0.04	0.56	5.8	7.8
0.20	0.03	0.02	4.6	3.9	0.02	0.02	4.3	4.2	0.03	0.04	5.9	4.8

[a]Initial population = 5.3 (log/ml)
[b]Initial population = 6.2 (log/ml)
[c]Initial population = 6.1 (log/ml)

TABLE 2. EFFECT OF TETRAMETHYL-THIURAM DISULPHIDE (TMTD), PHENYL-MERCURIC ACETATE (PMA) AND ZINC OXIDE ON VOLATILE FATTY ACID (VFA) AND BACTERIAL COUNT OF AMMONIATED FIELD LATEX

Concentration (%)	*TMTD*						*PMA*						*Zinc oxide*					
	VFA No. (day)			Bacterial count[a] (log/ml) (day)			VFA No. (day)			Bacterial count[b] (log/ml) (day)			VFA No. (day)			Bacterial count[c] (log/ml) (day)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0.00	0.04	0.63	0.92	5.2	3.9	5.2	0.43	1.16	coag.	5.3	7.5	7.4	0.04	0.60	0.83	5.2	5.6	5.2
0.01	0.02	0.02	0.02	2.5	3.1	2.4	0.28	1.12	-	5.4	7.5	8.3	-	-	-	-	-	-
0.025	0.02	0.02	0.03	2.2	1.6	1.3	-	-	-	-	-	-	0.03	0.09	0.35	4.5	3.0	2.6
0.05	0.02	0.02	0.02	2.0	nil	nil	0.03	0.71	1.29	5.1	5.7	6.7	0.02	0.05	0.08	5.3	3.5	1.5
0.10	0.03	0.03	0.03	nil	nil	nil	0.03	0.03	0.27	4.6	4.5	4.4	0.02	0.05	-	4.3	3.0	-
0.20	0.02	0.03	0.02	nil	nil	nil	0.04	0.04	0.05	4.5	4.0	3.9	0.03	0.04	0.11	4.2	3.3	1.6

[a] Initial population = 6.3 log/ml
[b] Initial population = 6.4 log/ml
[c] Initial population = 6.3 log/ml

TABLE 3. VOLATILE FATTY ACID (VFA) AND BACTERIAL COUNT OF AMMONIATED FIELD LATEX TREATED WITH BACTERICIDES

Bactericide (%)	VFA No. (day) 1	2	3	Bacterial count (log/ml) (day) 0	1	2	3
Dowicil 75							
0.00	0.41	0.88	1.17	6.5	5.4		5.3
0.05	0.03	0.03	0.13		3.5	2.2	2.3
0.10	0.02	0.02	0.02		2.9	1.8	1.0
0.20	0.03	0.03	0.04		2.5	1.5	Nil
Panacide							
0.00	0.07	1.04	1.52	6.1	-	5.4	5.9
0.25	0.02	0.03	0.91		-	5.2	3.9
0.50	0.02	0.02	0.80		-	4.2	3.8
0.75	0.02	0.02	0.03		-	4.1	3.3
Proxel CRL							
0.00	0.02	0.05	1.33	6.2	6.2	6.1	6.8
0.02	0.03	0.11	0.72		6.2	6.2	6.8
0.04	0.02	0.02	0.03		5.5	4.4	1.8
0.08	0.02	0.04	0.04		4.8	3.6	1.0

good bactericides while Panacide was moderately so *(Table 3)*.

Effect of carbohydrates. Sucrose, glucose and molasses, when added to ammoniated latex, increased VFA *(Table 4)*, molasses giving the highest value.

Effect of secondary preservatives on latex treated with sucrose. When 0.5% sucrose was added to latex treated with 0.2% ammonia, coagulation of the latex occurred on the second day. To control VFA build-up and bacterial population it was necessary to increase the concentration of ammonia to 0.5% or to use secondary preservatives such as TMTD, HNS, HH or Dowicil 75, up to 0.1%, or PMA and BA up to 0.2% and 0.4% respectively *(Table 5)*.

Effect of secondary preservatives on latex treated with papain. To find out the effect of the secondary preservatives in controlling the VFA build-up in latex containing nitrogenous substrates such as peptides and amino acids, ammoniated field latex containing 0.05% papain and 0.05%

TABLE 4. VOLATILE FATTY ACID (VFA) OF AMMONIATED FIELD LATEX SEEDED WITH CARBOHYDRATES

Treatment		VFA No. (day) 1	2	3
Control[a]	-	0.05	0.60	0.88
Sucrose	0.1%	0.05	0.96	1.14
Sucrose	0.2%	0.04	0.98	1.34
Glucose	0.1%	0.05	0.90	1.19
Glucose	0.2%	0.05	1.08	1.42
Molasses[b]	0.2%	0.14	0.98	1.47
Molasses	0.4%	0.13	1.28	1.46

[a]Initial VFA number 0.02
[b]Sugar content about 50%

TABLE 5. VOLATILE FATTY ACID (VFA) AND BACTERIAL COUNT OF LATEX TREATED WITH SUCROSE AND SECONDARY PRESERVATIVES

Ammonia (%)	Substrate/ Preservative (%)	VFA No. (day) 1	2	3	Bacterial count (log/ml) (day) 1	2	3
0.1S	-	0.07	1.04	1.52	5.4	5.9	Thickened
0.1S	S	0.10	C	C	5.3	C	C
0.5O	S	0.03	0.03	0.03	4.5	2.6	4.9
0.1S	S + TMTD, 0.05	0.03	0.02	0.02	4.0	nil	nil
0.1S	S + PMA, 0.2	0.04	0.04	0.04	4.3	3.5	nil
0.1S	S + BA, 0.4	0.02	0.04	0.07	6.2	6.0	Thickened
0.1S	S + HNS, 0.05	0.03	0.03	0.02	5.9	4.3	-
0.1S	S + HNS, 0.1	0.03	0.02	0.03	5.9	6.1	-
0.1S	S + HH, 0.05	0.03	0.02	0.03	-	-	-
0.1S	S + Dowicil 75, 0.1	0.04	0.04	0.02	3.1	1.8	1.6

S = 0.5% Sucrose
C = Coagulated

ammonium laurate was treated with various secondary preservatives and tested for VFA and bacterial population for five days *(Table 6)*. Addition of papain increased the VFA build-up and bacterial population. However, TMTD, PMA, zinc oxide and HH effectively controlled VFA build-up and reduced the bacterial population, the latex was rendered sterile by PMA and TMTD on the second and fifth day respectively.

Effect of secondary preservatives on latex seeded with ammonia-resistant bacteria. The effect of the addition of secondary preservatives to 0.2% ammoniated latex seeded with ammonia-resistant bacteria is given in *Table 7*.

Even 0.5% ammonia did not preserve the latex beyond the second day, but the secondary preservatives gave satisfactory control of VFA, with PMA and TMTD

TABLE 6. VOLATILE FATTY ACID (VFA) AND BACTERIAL COUNT OF AMMONIATED LATEX TREATED WITH PAPAIN AND SECONDARY PRESERVATIVES

Treatment	VFA No. (day) 2	3	5	Bacterial count[a] (log/ml) (day) 2	3	5
Control	0.21	0.83	1.32	4.4	2.8	2.5
P	1.06	1.43	C	5.4	5.5	4.6
P + TMTD, 0.05%	0.03	0.05	0.05	1.9	2.6	Nil
P + PMA, 0.2%	0.06	0.06	0.05	Nil	Nil	Nil
P + ZnO, 0.05%	0.02	0.03	0.07	2.7	2.6	2.5
P + HH, 0.05%	0.04	0.03	0.04	4.4	4.2	2.1

[a] Initial Count = log 5.7/ml
P = 0.05% papain
C = coagulated

TABLE 7. VOLATILE FATTY ACID (VFA) AND BACTERIAL COUNT OF AMMONIATED LATEX SEEDED WITH AMMONIA-RESISTANT BACTERIA

Preservative (%)	VFA No. (day)			Bacterial count[a] (log/ml) (day)		
	1	2	3	1	2	3
NH_3, 0.25 (control)	0.04	0.59	0.84	5.5	5.0	-
NH_3, 0.5	0.03	0.03	0.12	4.0	2.9	2.4
HNS, 0.1	0.02	0.02	0.03	5.4	4.3	3.4
HH, 0.5	0.03	0.02	0.02	5.3	3.7	3.2
BA, 0.2	0.03	0.03	0.03	5.6	4.8	4.5
TMTD, 0.05	0.03	0.04	0.04	4.3	1.9	Nil
PMA, 0.2	0.05	0.05	0.05	3.6	Nil	Nil

[a]Initial Count = log 6.3/ml

rendering the latex sterile by the second and third day respectively.

Latex Concentrate Preservation

Effect of pretreatment of field latex on HA concentrate. TMTD, HH, HNS and BA at concentrations ranging from 0.025% to 0.25% effectively controlled the bacterial proliferation and VFA build-up in 0.2% ammoniated field latex for two to three days *(Tables 1* and *2).* These chemicals were therefore added to field latex containing 0.3% ammonia, centrifuged on the following day and the ammonia content brought up to 0.7% to find out their effects on the properties of the resulting concentrate *(Table 8).*

The pretreatment of ammoniated field latex with either 0.025% TMTD or 0.05% HH improved the KOH number, giving satisfactory VFA number and MST. On the other hand, HNS and BA adversely affected

TABLE 8. PROPERTIES OF HYDRAZINE HYDRATE HA CONCENTRATE OBTAINED FROM AMMONIATED FIELD LATEX WITH AND WITHOUT SECONDARY PRESERVATIVES

Preservative in field latex (%)	VFA No. (month)		Property of HA concentrate MST (month)			KOH No. (month)	
	0	6	0	1	6	0	6
Control	0.01	0.01	105	425	940	0.42	0.63
TMTD, 0.025	0.01	0.01	90	420	1 080	0.36	0.51
Control	0.02	0.02	120	960	780	0.41	0.58
HH, 0.05	0.01	0.01	90	670	880	0.35	0.45
Control	0.01	0.03	100	765	1 470	0.37	0.49
HNS, 0.05	0.01	0.01	90	690	990	0.36	0.54
Control	0.01	0.01	155	1 110	1 290	0.31	0.49
BA, 0.20	0.01	0.01	155	535	870	0.46	0.59

the KOH number, though well within the specification, presumably because of their sulphate and borate ions respectively.

Effect of pretreatment of field latex on LA concentrate. Ammoniated field latex treated with 0.025% TMTD, 0.05% HH, or 0.05% HNS was centrifuged on the following day after which the ammonia content of the concentrate was adjusted to 0.2% and it was re-treated with 0.025% TMTD, 0.025% HH or HNS *(Table 9)*.

Two-stage treatment with 0.025% TMTD gave a better latex concentrate than the one obtained with 0.1% TMTD added to the concentrate. The addition of 0.05% ammonium laurate further improved the MST of the concentrate, which was superior to that of the HA concentrate prepared from the same bulk of field latex by standard methods. Addition of 0.1% HH to the LA concentrate preserved it satisfactorily for six months, but two-stage treatment with 0.05% and 0.025% HH gave better results, though the MST values were not too satisfactory. Satisfactory VFA values and unsatisfactory MST and KOH numbers were obtained with HNS at 0.2% on concentrate but all properties except MST were improved by two-stage treatment with 0.05 HNS.

LA latex concentrate without pre-treatment. Field latex containing 0.35% ammonia was centrifuged, further treated with various levels of PMA, Panacide, Dowicil 75 or Proxel CRL and their properties up to six months compared with those of a control HA concentrate *(Table 10)*. Satisfactory VFA and KOH numbers and reasonably satisfactory MST values were obtained with PMA at 0.1%. Panacide at 0.2% gave satisfactory results, with the MST build-up being better than that of the HA control. Dowicil 75 at 0.4% - 0.5% gave good control of VFA, but MST and KOH values were not satisfactory. MST did not markedly improve even when treated with ammonium laurate. Proxel CRL at 0.03% and above gave satisfactory preservation, except in respect to MST value, which was rectified by treatment with ammonium laurate.

TABLE 9. PROPERTIES OF LA CONCENTRATE OBTAINED FROM AMMONIATED FIELD LATEX, WITH AND WITHOUT TETRAMETHYL-THIURAM DISULPHIDE (TMTD), HYDRAZING HYDRATE (HH) AND HYDROXYLAMINE NEUTRAL SULPHATE (HNS) PRETREATMENT

Preservative (%)		Properties of concentrate						
		VFA No. (month)		MST (month)			KOH No. (month)	
Field latex	Concentrate	0	6	0	1	6	0	6
TMTD, 0.025	TMTD, 0.025	0.01	0.01	45	65	260	0.35	0.52
TMTD, 0.025	TMTD, 0.025[a]	0.01	0.02	70	1 080	2 100	0.41	0.58
Nil	TMTD, 0.10	0.01	0.03	45	70	240	0.48	0.68
Nil	TMTD, 0.10[a]	0.01	0.02	90	80	1 710	0.54	0.70
Nil	HA Control	0.01	0.01	105	425	940	0.42	0.63
HH, 0.05	HH, 0.025	0.02	0.04	60	190	390	0.28	0.46
Nil	HH, 0.10	0.02	0.05	60	135	200	0.40	0.62
Nil	HA Control	0.02	0.02	120	960	1 100	0.41	0.38
HNS, 0.05	HNS, 0.05	0.01	0.02	80	170	870	0.33	0.57
Nil	HNS, 0.20	0.01	0.02	55	160	250	0.60	0.77
Nil	HA Control	0.01	0.03	100	165	1 470	0.37	0.49

[a]With 0.05% ammonium laurate

TABLE 10. PROPERTIES OF LA CONCENTRATE

Preservative (%)	VFA No. (month)			MST (month)				KOH No. (month)		
	0	3	6	0	1	3	6	0	3	6
PMA, 0.1	0.01	0.01	0.02	50	240	600	720	0.30	0.45	0.52
Control (HA)	0.01	0.02	0.02	120	1 010	1 360	1 320	0.30	0.45	0.48
Panacide, 0.2	0.02	0.02	0.02	145	1 230	1 470	1 520	0.41	0.50	0.53
Control (HA)	0.01	0.01	0.02	110	775	960	1 260	0.41	0.45	0.50
Dowicil 75, 0.4	0.01	0.07	-	90	140	85	-	0.59	0.86	-
Dowicil 75, 0.5	0.01	0.02	-	80	110	65	-	0.66	0.75	-
Dowicil 75, 0.4[a]	0.01	-	-	110	360	-	-	0.48	-	-
Control (HA)	0.01	0.01	-	110	775	960	-	0.41	0.46	-
Proxel-CRL, 0.03	0.01	0.02	-	60	90	420	-	0.44	0.53	-
Proxel CRL, 0.03[a]	0.01	0.02	0.02	95	920	1 250	1 420	0.41	0.51	0.56
Control (HA)	0.01	0.01	0.02	110	735	690	880	0.41	0.52	0.56

[a]With 0.05% ammonium laurate

DISCUSSION

The control of bacterial proliferation and the resulting VFA build-up in latex can be achieved by the use of chemicals which react with carbohydrates, enzyme poisons or inhibitors or bactericides. The principal carbohydrate substrate present in field latex which is readily broken down by bacteria to form VFA is sucrose. The deliberate addition of sugars to field latex confirms this finding *(Table 4)*. But their reactivity towards carbonyl or hydroxy groups of other inherent compounds in latex would reduce their availability for bacterial breakdown.

Ammonia, commonly used as a latex preservative is a carbonyl reagent which reacts with glucose and fructose in latex resulting in an aldehyde ammonia or a keto ammonia complex, which is less amenable for bacterial breakdown. HH and HNS are more powerful carbonyl reagents, and therefore have given better preservation *(Table 1)*; HH is more effective, as it contains two amino groups per molecule which condense with two molecules of sugars (PIGMAN, 1957). PHH and SCH are also carbonyl reagents, but are not effective in ammoniated latex as the condensation reactions do not take place under alkaline conditions (PIGMAN, 1957).

BA complexes with cis-ortho-hydroxy groups (BOESEKEN, 1949) in glucose and fructose molecules but is less effective than HH and HNS presumably because of its ability to react with quebrachitol (ANGYAL AND ANDERSON, 1959), present in large amounts in latex (BEALING AND CHUA, 1972), thus leaving less to complex with fructose and glucose.

Even if the carbohydrate level is kept low by the use of complexing chemicals, VFA may still build up in latex and become unstable due to the activities of a wide variety of enzymes such as proteases and lipases indigenously present in latex (AUDLEY AND MOIR, 1975). Further, the complete killing of bacteria at a relatively late stage in field latex does not necessarily prevent VFA build-up since enzymes capable of producing VFA can be released from the dead bacterial cells, unless they are deactivated by enzyme poisons or inhibitors.

Zinc oxide (SEKAR AND WREN, 1956), TMTD (KRISHNASWAMY, 1969) and PMA (NADARAJAH, 1965) have been used as enzyme poisons or inhibitors in this investigation. Evidence on the behaviour of enzymes was obtained by the deliberate

addition of papain, a protease, to ammoniated field latex, which markedly increased VFA; the VFA, however, was effectively controlled by the use of enzyme poisons or inhibitors *(Table 6)*.

Being a good enzyme inhibitor TMTD effectively arrested VFA build-up at 0.01%. Zinc oxide, although a good enzyme poison, is not improved at levels above 0.05%, presumably because of its reaction with ammonia in the presence of ammonium ions to form zinc amine complexes. Field latex was preserved with 0.1% PMA two days, but its relatively high mammalian toxicity excludes its use in latex *(Table 2)*.

Among the bactericides investigated as secondary preservatives for latex concentrate *(Table 10)*, Proxel CRL is very promising, requiring only 0.03% to be effective. An additional 0.05% ammonium laurate is needed to ensure good MST increase. Another promising bactericide is Panacide, somewhat identical to sodium pentachlorophenate (SPP). Both these chemicals are claimed to be far less toxic than SPP. Dowicil 75 is also a good bactericide, but it adversely affects the MST values. This may be due to the presence of bicarbonate (25% by wt) which is known to decrease MST (CALVERT AND SMITH, 1974).

Field latex is extremely susceptible to microbial degradation because of the large bacterial population and high concentration of non-rubber substrates. Hence the early preservation of field latex meant for production of concentrate is of utmost importance. Consequently, the pretreatment of field latex before centrifugation with a secondary preservative leads to a concentrate with better preservation qualities. This process of obtaining good quality HA or LA concentrate is of special value when field latex contains high levels of substrates for bacterial proliferation, such as in smallholders' latex.

Using this method, a satisfactory low-ammonia concentrate is obtained by pretreating ammoniated field latex with 0.025% TMTD and topping up the concentrate with a further addition of 0.025% TMTD and 0.05% ammonium laurate *(Table 9)*. The total amount of TMTD in the concentrate is much less than the concentration of 0.2% used by ANGOVE AND PILLAI (1965c). The use of HH and HNS has also been investigated in this manner and has given satisfactory results, although ANGOVE (1964) did not obtain satisfactory results even with 0.2% HH. The LA-HNS system may be considered for the manufacture of foam rubber and adhesives in place of the presently used HA system containing HNS at concentration of 0.05% - 0.1% (GORTON, 1974). Addition of ammonium laurate at 0.05% to HH and HNS-treated concentrate, after preparation, was found in a separate experiment, to overcome the defect of low MST values.

It is concluded that in the preparation of a LA concentrate, pretreatment of the field latex with a small amount of the secondary preservative at the earliest possibility, prior to centrifugation, followed by topping up the concentrate with a further small amount of the same preservative, ensures a better preserved concentrate than one in which the secondary preservative has been added only to the concentrate. By using this method, the overall amount of chemical required can also be reduced.

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