J. Rubb. Res., **18(2)**, 72–86

Efficiency of Commercial Biological Compounds as Anticoagulant Agents in Natural Rubber Latex

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This work studied the effect of streptomycin sulphate and surfactin on the stability of field natural rubber (NR) latex. Field NR latex was treated with streptomycin sulphate and surfactin in the absence and presence of ammonia. The stability of NR latex was determined by formation of volatile fatty acid (VFA), enumeration of the bacterial population, measurement of NR latex alkalinity and pH of the latex. Streptomycin sulphate caused significant reduction in bacterial population and VFA formation with an additional advantage as OH^- ion stabiliser. Surfactin on the other hand was not suitable as NR latex preservative agent in the absence or presence of ammonia. Although instability of NR latex still appeared, combination of streptomycin sulphate and surfactin showed better control in terms of bacteria and VFA number compared to 0.3% ammoniated NR latex.

Keywords: Biological based agent; microorganisms; natural rubber latex

Latex preservation systems are widely studied since the early years of concentrated latex found its application in the rubber products industry. Preservation of natural rubber (NR) latex is vital in order to avoid spontaneous coagulation and putrefaction that occur immediately after tapping[1-4]. In the rubber industry, preservative agents are used to maintain the viscosity of latex concentrate for long-term storage purposes. To date, ammonia is the best preservative for NR latex due to its strong alkalinity and acts as a bactericidal agent for preventing bacterial growth. However, prolonged exposure to ammonia causes negative impact to the environment and workers health. An alternative chemical for latex preservation, tetramethyl thiuram disulphide/zinc oxide (TMTD/ZnO) is known for its carcinogenic effects[5].

The awareness in environmental and consumer issues is rising. Therefore, the future of current chemical based agriculture and processing systems must be discussed from an environmental viewpoint. Environmentally sound technologies need to be developed and the use of chemicals be reduced to be more compatible with nature. This necessitates

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the inclusion of natural processing in future research agenda that utilise biological resources to prepare products free of chemical pollutants, ensure human and animal health, preserve the natural resource base of ecosystems and enhance environmental quality.

A biological alternative to the chemical-based preservative system is the microbial derived bio-based compound. In this study, preliminary work on the stability and suitability of two commercially available bioactive compounds namely streptomycin sulphate and surfactin (Sigma Aldrich, USA) as latex anticoagulant agents were carried out. Analyses on the stability of field NR latex in the absence and presence of bioactive compounds were performed based on enumeration of microbial population, level of volatile fatty acid (VFA) and alkalinity of the NR latex.

MATERIALS AND METHODS

Materials

Natural Rubber Latex. Samples of field NR latex were obtained from Rubber Research Institute of Malaysia (RRIM) in Sungai Buloh, Kuala Lumpur (3.1586º N, 101.5594º E). Five rubber trees were chosen randomly. Before tapping, the tapping panel was cleaned using alcohol wipe to remove any coagulated latex string. The rubber tree was tapped using sterile tapping knife and the field latex was collected into steriled conical flask (autoclaved at 121ºC for 15 min). After 2 h, the conical flask was immediately covered with aluminium foil and placed into cooler box at 4ºC to avoid spontaneous coagulation.

Preservatives. Streptomycin sulphate and surfactin were purchased from Sigma Aldrich, USA.

Preparation of Field NR Latex Preserved with Streptomycin Sulphate or Surfactin in a Single Preservative System.

In order to prepare streptomycin sulphate solution with 5% (w/v) concentration with pH 7.5, 5 g of streptomycin sulphate was dissolved in 100 mL of distilled water. As for surfactin, suspension stock with 1 mg/mL (w/v) was prepared by mixing 10 mg surfactin powder with 5 mL of distilled water and 5 mL of ethanol. Surfactin was prepared in acidic condition at pH 6.5 due to the presence of ethanol which was weakly acidic. Formulations of field NR latex in the presence of streptomycin sulphate or surfactin are summarised in *Table 1*. In a single preservative system, field NR latex preserved with 0.7% ammonia was prepared as a positive control. All treated field NR latex was stored in a transparent incubator (Stuart Scientific) at room temperature until flocculation of NR latex was detected.

TABLE 1. FORMULATION FOR FIELD NR LATEX PRESERVED WITH DIFFERENT CONCENTRATION OF STREPTOMYCIN SULPHATE OR SURFACTIN

	Treatments								
	A	B	C	D	E	F	G	H	I
Ammonia (%)	0.7	-	-	-	-	-	-	-	-
Streptomycin sulphate (%)	-	0.1	0.3	0.7	1	-	-	-	-
Surfactin (%)	-	-	-	-	-	0.01	0.05	0.1	0.5

Preparation of Field NR Latex Preserved with Low Ammonia (0.3%) in the Presence of Streptomycin Sulphate or Surfactin in a Secondary Preservative System

Field NR latex preserved with 0.3% ammonia in the presence of streptomycin sulphate or surfactin as a secondary preservative agent were prepared based on the formulations shown in *Table 2*.

Volatile Fatty Acid Test (VFA)

The VFA number was determined according to the *ISO 506:1992(E)*[7]. The amount of volatile fatty acids in latex is expressed by the VFA number, the amount of potassium hydroxide in grams required to neutralise the volatile fatty acids in a latex sample containing 100 g of total solids. The volatile fatty acid number was obtained using *Equation 1*:

$$\text{VFA No.} = \left[\frac{134.64cV}{m\text{TSC}}\right] \times \left[50 + \frac{m(100-\text{DRC})}{100\,\rho}\right] \quad \ldots 1$$

where, *c* is the actual concentration, expressed in moles per cubic decimetre of the barium hydroxide solution; *V* is the volume in cubic centimetre of the potassium hydroxide solution required to neutralise the distillate; *m* is the mass in grams of the test portion; DRC is the dry rubber content, expressed as a percentage by mass of the latex concentrate; TSC is the total solid content expressed as a percentage by mass of the latex concentrate; ρ is the density in mega grams per cubic metre of the serum and 134.64 is a factor derived from the relative molecular mass of potassium hydroxide.

Enumeration of Bacterial Population

Preparation of Bacterial Growth Media. The composition of the media was based on the manufacturer's recipe, provided by DIFCO, USA. The media was prepared by dissolving 40 g TSA, in 1 L of distilled water. The pH of the media was adjusted to 7.3 ± 0.2 pH unit and was autoclaved at 121ºC for 15 minutes.

Bacterial Count. The bacterial count was performed as described previously by John (1977)[6]. Bacterial inoculums from the treated latex were obtained by collecting the serum. The latex samples were centrifuged at 20,000 rpm (40 mm rotor) for 30 min (Beckman Coulter Optima L-100K Ultracentrifuge, Rotor 21). Under this centrifugation condition, a clear separation of the rubber hydrocarbon and the serum was obtained. The serum was further diluted by using saline solution (Ringers solution, Sigma Aldrich) in a 100 fold dilution series (10^2-10^{10}). From each dilution series of latex serum, 0.5 mL was spread on the surface of growth medium (Trypticase Soy Agar, HiMedia) in duplicate. The plates were incubated for 24 h at 30ºC before counting. The number of microorganisms in each plate were counted

TABLE 2. COMPOSITION OF DIFFERENT PRESERVATIVE SYSTEMS FOR FIELD NR LATEX

Preservatives	Treatments
0.3% NH_3	T1
0.3% NH_3 + 0.3% streptomycin sulphate	T2
0.3% NH_3 + 0.5% surfactin	T3
0.3% streptomycin sulphate + 0.5% surfactin	T4

and the overall bacterial enumeration was calculated based on *Equation 2*:

$$\text{Bacteria number (cfu/mL)} = \frac{(\text{number of colonies}) \times (\text{dilution factor})}{\text{Volume pipette into the plates (mL)}} \quad \ldots 2$$

Determination of Field Natural Rubber Latex Alkalinity

The alkalinity of NR latex was determined based on in-house method. Latex with amount of 10 mg was diluted in 300 mL distilled water. The mixture was titrated against 0.1 N sulphuric acid (H_2SO_4) by using bromothymol blue as indicator. Initially 3-4 drops of indicator was added followed by 2-3 drops near the endpoint as indicated by the colour change from green to yellow. The alkalinity in field NR latex was calculated in *Equation 3*:

$$X = \frac{0.017 \times 100 \times T \times N}{W} \quad \ldots 3$$

where, N is normality of H_2SO_4; W is weight of latex taken and T is volume of H_2SO_4.

Measurement of Field Natural Rubber Latex pH

The test was carried out in accordance to *ISO 976:2013(E)*[8]. Sufficient amount of latex was calculated based on the following formula in order to get a test sample of 50 g from total solid content as in *Equation 4*:

$$W \text{ (weight)} = \frac{50 \times 100}{TSC} \quad \ldots 4$$

where, TSC is the total solid content.

The pH electrode was rinsed with distilled water before being placed into the sample and the pH reading was recorded.

Statistical Analysis

The data obtained were analysed using IBM SPSS Statistic 20, subjected to general linear model (GLM) procedure in a multivariate design. Differences in means were compared with Tukey Test. The difference was considered significant when the P value was <0.05.

RESULTS AND DISCUSSION

Instability of NR latex is known to be due to the contamination of microorganisms from the environment. One of the by-products of bacterial activities in NR latex is the release of VFA in the system which would further reduce the latex pH and oxidise the negative charges on rubber particle surfaces[9]. Acid which consists of H^+ ion neutralises the negatively charged protein membrane and causes the protein membrane of the rubber particles to break. Subsequently, rubber particles will collide with each other and clump together to form coagulum[9–11]. Effective preservative agent must have strong bactericidal effects on these acid producing bacteria.

Based on the results tabulated in *Table 3*, increase in the VFA level was observed after 72 h of storage from field NR latex treated with 0.1% streptomycin sulphate. Natural rubber field latex mixed with 0.3% of streptomycin sulphate, showed stable formation of VFA even after five days. Changes in latex stability in the presence of 0.7% streptomycin sulphate were observed after 24 h of storage. The NR latex became cream-like, and after four days separated into two distinct layers. The upper layer of the latex remained in cream-like form whilst the bottom part was completely coagulated. Due to this condition, results on VFA number in field NR latex treated with 0.7% streptomycin sulphate could not be measured. At 1% of streptomycin sulphate, field NR latex remained stable even after

TABLE 3. EFFECT OF STREPTOMYCIN SULPHATE ON VOLATILE FATTY ACID NUMBER IN NATURAL RUBBER FIELD LATEX

Hour	VFA number				
	Ammonia (%)	Streptomycin sulphate (%)			
	0.7	0.1	0.3	0.7	1
0	0.08[c]	0.08[c]	0.08[a]	0.08 b	0.08[b]
24	0.04[a]	0.07[b]	0.04[a]	-	0.03[a]
48	0.04[a]	0.06[a]	0.04[a]	-	0.03[a]
72	0.04[a]	0.06[a]	0.04[a]	-	0.04[a]
96	0.04[a]	0.07[b]	0.04[a]	-	0.04[a]
120	0.05[a]	0.07[b]	0.04[a]	-	0.04[a]
144	0.05[b]	0.08[c]	0.04[a]	-	0.04[a]

- Not detected
a,b and c in each column indicate significant difference ($p<0.05$)

six days of storage. This indicates that, at higher concentration, streptomycin sulphate suppressed the bacteria activities more effectively. Minimal changes of VFA number were observed. The VFA numbers determined in field NR latex preserved with 0.3% and 1% of streptomycin sulphate were significantly lower than the 0.7% ammoniated latex.

Natural rubber latex preservative agent with surfactant properties could give better control to the colloidal stability of the rubber particles. The amphiphilic behaviour of a surfactant modifies the surface properties of water at the water/latex interface[19]. In this work, the effects of surfactin on NR latex stability was studied. It is assumed that surfactin could be a better control agent for colloidal stability of rubber particles, and its antimicrobial activity could also deteriorate the bacterial population.

Table 4 shows the changes of VFA numbers in field NR latex treated with different concentration of surfactin. Field NR latex preserved with 0.7% ammonia was prepared as the positive control. In the first two hours of storage, VFA numbers in all field NR latex treated with surfactin were lower than 0.7% ammoniated latex. However, in the subsequent hour, VFA levels increased and after 6 h, flocculation of NR latex was observed. This result indicated that surfactin was not effective to control VFA formation over long period of storage.

Bacterial population in 0.3% ammoniated latex rapidly decreased in the first 2 h of storage (*Figure 1*). This was followed by field NR latex in the presence of 1% streptomycin sulphate. In the presence of 0.1% streptomycin sulphate, the bacterial population was reduced but started to increase again after 60 h of storage indicating that in low concentration of streptomycin sulphate the bacteriostatic potency decreased with time. The biocide activity of streptomycin sulphate was concentration dependant and the action was slower than ammonia. However, after 96 h of storage, the bacterial population in field NR latex treated with streptomycin sulphate was almost equal to ammoniated latex. In the subsequent hour, increase of bacterial population in ammoniated field NR latex was observed. This might be due to the growth of ammonia resistant bacteria. Meanwhile,

TABLE 4. EFFECT OF SURFACTIN ON VOLATILE FATTY ACID NUMBER IN NATURAL RUBBER FIELD LATEX

Hour	VFA number				
	Ammonia (%)	Surfactin (%)			
	0.7	0.01	0.05	0.1	0.5
0	0.08^{b}	0.08^{b}	0.08^{b}	0.08^{b}	0.08^{b}
2	0.05^{a}	0.02^{a}	0.03^{a}	0.02^{a}	0.02^{a}
4	0.04^{a}	0.11^{b}	0.13^{b}	0.05^{a}	0.06^{b}
6	0.04^{a}	0.35^{c}	0.27^{c}	0.10^{b}	0.14^{c}

a,b and c in each column indicate significant difference ($p<0.05$)

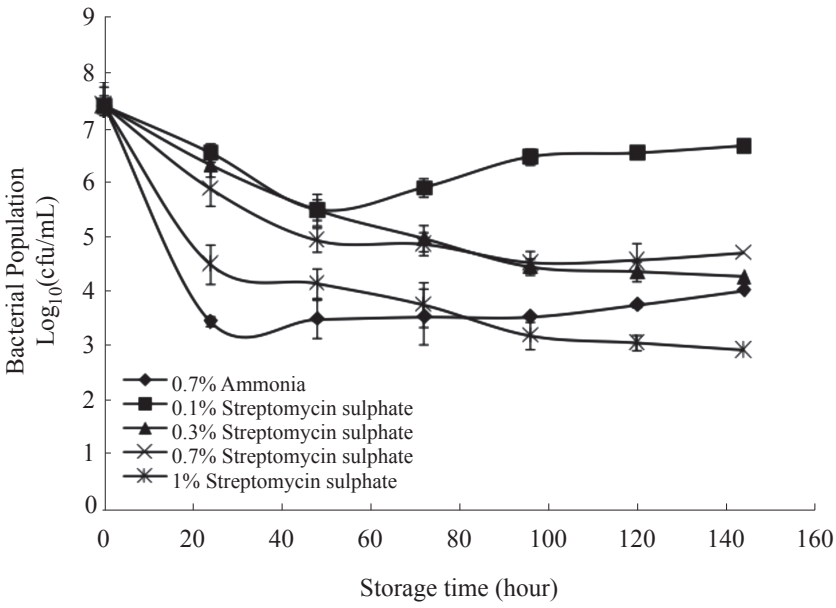

Figure 1. Effect of different concentration of streptomycin sulphate on bacterial population in field NR latex.

streptomycin sulphate gave better control in bacterial population with 42% and 60% of reduction in field NR latex with 0.3% and 1% streptomycin sulphate, respectively.

Although VFA numbers in NR latex in the presence of surfactin were high, there was a reduction in bacterial population as shown in *Figure 2*. At the highest concentration of surfactin (0.5%), 15% of bacteria were reduced in 6 h of storage from the initial population (Log_{10}7.3 cfu/mL). Increasing level of VFA in the treated field NR latex prepared in this study could be influenced by other factors. Formation of VFA merely depended on the number of bacteria, in which, better control of bacterial population will give better control in VFA number[20–22]. However, recent

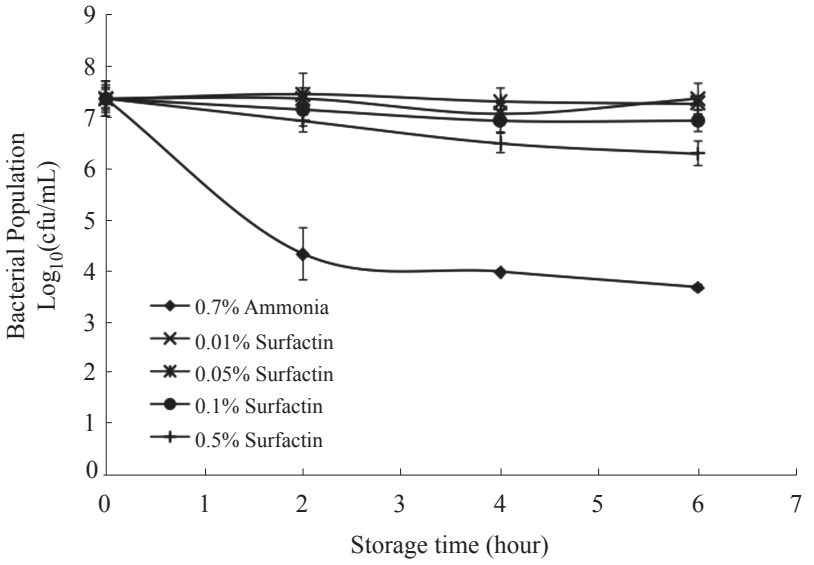

Figure 2. Effect of different concentrations of surfactin on bacterial population in field NR latex.

findings suggested that the VFA production were not only caused by bacterial activities, but also due to enzymatic reaction, thermal degradation and chemical activities[23–25]. Another possibility that caused the increment of VFA number was, enumeration of bacterial population performed in this study appear less than actual population of bacteria in the treated field NR latex. This condition might be due to the presence of surfactant activity that affects cell membrane permeability and differentiation in ionic strength which promoted flocculation of bacterial cells. Instead of being a single cell, the bacteria grew as an agglomeration of several cells on the growth media.

Despite all the changes in bacterial population and VFA number, ammonia still performed better in terms of maintaining the NR latex colloidal stability. Ammonia is the most favourable anticoagulant agent for NR latex due to its alkalinity and biocidal characteristics[11]. The high alkalinity characteristic of ammonia assists in preventing microbial growth in latex and controlling the colloidal stability of rubber particles. Ammonia in NR latex acts as an ion stabiliser by forming complexes with metal ions and helps to deposit the destabilising metal from the protective layer of the rubber particles. This justifies the stability of NR latex even though bacterial population started to increase after reaching minimum population number[26]. Streptomycin sulphate which is a water-soluble antibiotic lacks these properties. It can bind to the bacterial cell at the molecular level to inhibit new protein synthesis and ultimately deactivates the bacterial cell. However, this compound might have no effect on the colloidal stability of rubber particles. Furthermore, ammonia has immediate lethal effects that cause rapid reduction of bacterial growth while the effects of biological synthesised antimicrobial compounds from bacteria or fungi depend on the type of targeted cells (either Gram negative or Gram positive isolates).

Antimicrobial compounds produced by microorganisms vary in physiological properties and modes of actions in terms of biological toxin. Generally, the action of antimicrobial compounds can be classified in two modes, namely membrane targeting and intracellular and nuclear targeting mechanisms[13–16]. The classification however depends on the molecular weight of the compounds, ionic interaction between the compounds and the targeted bacterial cells, and surface hydrophobicity of the targeted bacterial cells, provided that the antimicrobial compounds are in pure form.

Surfactin on the other hand, might exhibit more complex mechanisms when introduced into field NR latex. It not only affects the surface tension of liquids in which it is dissolved but also exhibits antimicrobial activities. The mode of action of surfactin in field NR latex might be influenced by the presence of two different targeted cells or molecules which were the bacterial cells and the rubber particles. The hydrophobic chains of surfactant molecules tend to attach to either of these two targeted cells, depending on the strength of hydrogen bonding forces. Based on the results, surfactin in field NR latex caused reduction of bacterial population but have no significant effect on its stability.

The experimental preservation with low ammonia system was conducted to determine the effectiveness of these biological compounds as secondary preservative agent. A free ammonia system was also prepared in order to elucidate the mechanisms of action of the biological compounds in NR latex system. Concentration of streptomycin sulphate and surfactin added into field NR latex were based on experiments conducted in the earlier part of this study. From the results, 0.3% streptomycin sulphate was selected, where the bacterial population and VFA number in the treated field NR latex were significantly reduced. Although at higher concentration (1%) showed better results, additional high concentration of streptomycin sulphate was not economically effective. As for surfactin, concentration at 0.5% was authenticated as more bacteria were reduced at higher concentration. The stability of field NR latex was characterised based on the correlation between bacterial population, VFA number and NR latex alkalinity.

Streptomycin and surfactin are commonly used as an antibiotic. The mode of action of antibiotics is affected by pH[16]. Both compounds exhibit different preference in different pH. Therefore, it was interesting to study the effect of these compounds on pH in field NR latex. Propagation of microorganisms is also affected by the pH of its surrounding. Most of the acid producing bacteria are unable to survive under alkaline condition[1]. Ammonia, the most favourable NR latex preservative agent assists in increasing the stability of NR latex by increasing the negative surface charge of the rubber particles[27]. Being alkaline in nature, ammonia also helps to retard microbial growth and increase the alkalinity (pH) of NR latex. From four treatments prepared in this work, three treatments namely T1, T2 and T3 have pH above ten (alkaline) and showed very minimal changes in pH ranging from 0.1 to 0.5. This indicates that in the presence of streptomycin sulphate or surfactin with low ammonia, the alkalinity of the latex could be maintained (*Figure 3*).

The alkalinity of field NR latex preserved with combination of streptomycin sulphate or surfactin with low ammonia (Treatment T2 and T3) were stable for a week (*Figure 4*). Further storage showed that these preserved field NR latex did not require extra doses of ammonia. In order to maintain the alkalinity of the latex, ammonia is still needed in minimum amount at the early stage of storage. Therefore, the usage of ammonia in field NR latex can

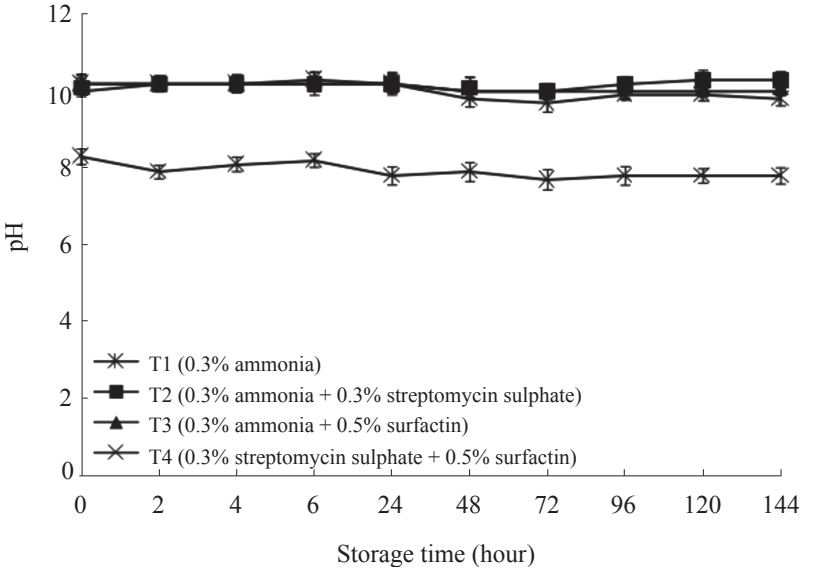

Figure 3. Measurement of pH of field NR latex preserved with 0.3% ammonia (T1), 0.3% ammonia+ 0.1% streptomycin sulphate (T2), 0.3% ammonia+ 0.5% surfactin (T3) and 0.1% streptomycin sulphate+0.5% surfactin (T4).

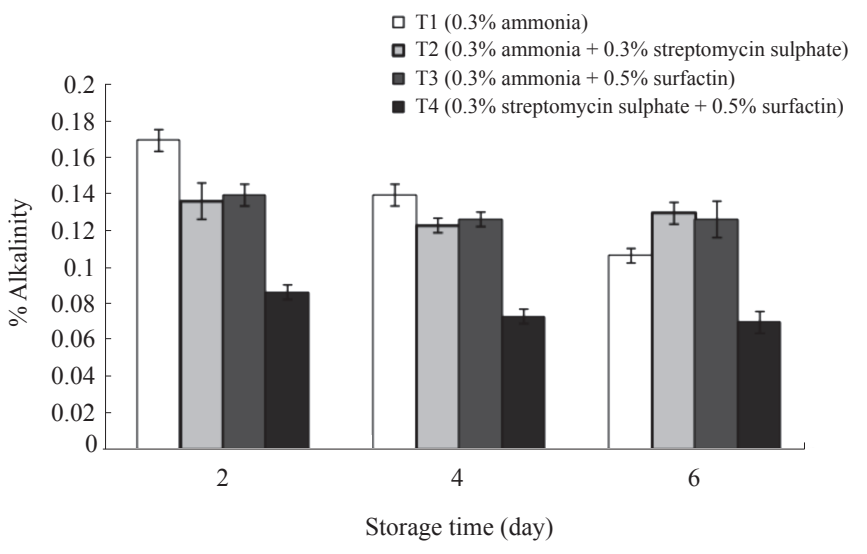

Figure 4. Alkalinity of field NR latex preserved with 0.3% ammonia (T1), 0.3% ammonia+ 0.1% streptomycin sulphate (T2), 0.3% ammonia+ 0.5% surfactin (T3) and 0.1% streptomycin sulphate+0.5% surfactin (T4).

be reduced to a minimum by integrating these biological mixtures into NR latex.

Streptomycin is a class of antibiotic derived from sugars and contain some sugar or substituted sugar moiety[28]. It consists of diguanido group that is linked to a nitrogen-containing disaccharide-like compound[29]. In nature, streptomycin is a strong base with three basic functional groups[30]. The optimum antimicrobial reaction of streptomycin sulphate occurs in slightly alkaline condition at pH 8. In acidic condition, between pH 6.6 to pH 5.9, the bacteriostatic potency of streptomycin sulphate is decreasing[31]. Therefore, in T2, there was a possibility that in the presence of streptomycin sulphate, numbers of hydroxide ion (OH^-) increased, thus, maintaining the alkalinity of the field NR latex.

As for bacterial indomitability, numbers of bacteria were able to survive in pH above eight which showed that most of these bacteria were able to tolerate an alkaline condition. Increment of bacterial population might be due to the propagation of resistant bacterial strains. Taysum[20] claimed that the number of ammonia-loving bacteria from latex treated with high ammonia is successfully isolated. This indicates that the bacteria had acclimatised themselves to the environment. Shum and Wren[32] had successfully isolated bacteria from field NR latex and ammoniated latex from several sources. A bacterial growth media, molasses yeast extract agar (MYEA) gave good viability indication on the viable count of bacterial population in field NR latex but certain bacteria were unable to propagate well on MYEA. Growth of ammonia-resistant bacteria was observed on specialised media containing ammonia at certain dilution series. However, no bacterial growth was detected on MYEA. Interestingly, population of bacteria in treatments T2 and T4 significantly decreased by almost 30% (*Table 5*). Although the biological compounds have weak alkalinity, these compounds gave an extra advantage to the normal bactericidal effect of ammonia.

Based on *Table 5*, treatments T2 and T4 have the lowest level of VFA formation that decreased proportionally with the bacterial population. After six days of storage, both treatments recorded VFA numbers at 0.04 with bacterial population at $Log_{10}2.82$ cfu/mL in treatment T2, and $Log_{10}1.73$ cfu/mL in treatment T4. Meanwhile, after six days of storage VFA number in field NR latex preserved with 0.3% ammonia (T1) and in field NR latex preserved with 0.3% ammonia and 0.5% surfactin (T3), increased from 0.047 to 0.065 and from 0.050 to 0.067 respectively. These were followed by the increase of bacterial population in the respective treatments. Despite the increase in VFA number and bacterial population, field NR latex in treatments T1, T2 and T3 did not coagulate after six days. On the other hand, field NR latex in treatment T4 was partially coagulated although significant reduction of bacterial population was recorded.

Streptomycin sulphate has bactericidal effect against both Gram negative and Gram positive bacteria. It causes detrimental effect to the bacterial cells at the molecular level by binding to the 30S ribosomal subunit that lead to misreading of the genetic code by inhibiting protein synthesis important for bacterial cell growth[31]. Meanwhile, surfactin with its surface active properties could act as sequestering agent to remove metal ions and affect bacterial cell membrane permeability. Streptomycin sulphate performs better in alkaline condition, while surfactin being an anionic biosurfactant shows efficiency in reducing the surface tension of a substance in acidic condition with minimal effect to pH changes[33].

As an experimental ammonia-free system in treatment T4, the effect of these biological compounds in NR latex could be postulated.

TABLE 5. EFFECT OF VFA NUMBER AND BACTERIAL POPULATION IN FIELD LATEX WITH DIFFERENT TREATMENTS

Treatment	Day	Volatile fatty acid (VFA)	Numbers of cells Log_{10}(cfu/ml)	Latex physical appearance after six days
T1 0.3% NH_3	1	0.047^a	4.26^a	No coagulation
	2	0.047^a	4.30^a	
	3	0.053^a	4.41^a	
	4	0.059^a	4.56^a	
	5	0.062^b	4.59^a	
	6	0.065^b	4.63^b	
T2 0.3% NH_3 + 0.3% streptomycin sulphate	1	0.040^a	5.21^d	No coagulation
	2	0.050^b	4.65^c	
	3	0.050^b	3.78^b	
	4	0.047^a	3.43^b	
	5	0.043^a	2.91^a	
	6	0.040^a	2.82^a	
T3 0.3% NH_3 + 0.5% surfactin	1	0.050^a	5.08^b	No coagulation
	2	0.053^a	4.91^b	
	3	0.053^a	4.73^a	
	4	0.057^a	4.70^a	
	5	0.063^a	4.68^a	
	6	0.067^b	4.65^a	
T4 0.3% streptomycin sulphate + 0.5% surfactin	1	0.063^c	5.25^d	Partially coagulated
	2	0.057^b	4.43^c	
	3	0.052^b	4.04^c	
	4	0.050^b	2.82^b	
	5	0.045^a	1.87^a	
	6	0.043^a	1.73^a	

a,b,c and d in each column indicate significant difference ($p<0.05$)

Without ammonia, the alkalinity of field NR latex in this treatment was expected to be more acidic. However, with the presence of free bases from streptomycin sulphate, the pH was raised to slightly alkaline at pH 8.3, until it reduced to pH 7.8 as the NR latex started to coagulate. Although there were significant reduction of bacterial population and VFA numbers, there were also possibilities that other factors might influence the stability of the NR latex. The possible reactions that may occur when these compounds are introduced into field NR latex in the absence of ammonia are:

(1) The ionic interaction between surfactin molecules with bacterial cells in NR latex was stronger than the ionic interaction between surfactin molecules with the rubber particles. Therefore, number of surfactin-bacterial cell complexes was higher and ultimately bacterial cells were killed due to mineral depletion.

(2) As an anionic biosurfactant, surfactin would probably bind to the metal ions such as Mg^{2+} or Ca^{2+} presence on rubber particles. With the removal of metal ions, electrostatic forces between particles were increased. However this reaction might not happen due to the different ionic strength exhibited by the bacterial cells and rubber particles.

(3) In environment with excess H^{+}, the anionic surfactin molecules might be bound with the H^{+} instead of the metal ions resulted in decreased of electrostatic forces between the rubber particles, and

(4) At initial time of storage, streptomycin sulphate effectively killed the bacterial cells by disrupting the synthesis of growth elements proteins. As the pH dropped, bacteriostatic potency of streptomycin was also reduced. However, in the presence of surfactin, the remaining bacterial cells probably form complexes with surfactin molecules and were killed.

CONCLUSION

In this work, streptomycin sulphate and surfactin were tested against NR field latex stability as a stand-alone preservative agent (ammonia-free system) and also as secondary preservative in the presence of 0.3% ammonia. The stability of NR field latex was characterised based on the bacterial population, VFA number formation and the alkalinity of the treated NR field latex. Streptomycin sulphate effectively controlled the putrefaction of NR field latex during short term storage by showing better control on bacterial population compared to normal bactericidal effect of ammonia. In the presence of streptomycin sulphate with ammonia, NR latex remained stable after six days of storage and showed significant reduction in bacterial population and VFA formation with an additional advantage as OH^{-} ion stabiliser. Surfactin on the other hand was not suitable as NR latex preservative agent in the absence or presence of ammonia. However, combination of streptomycin sulphate and surfactin gave better results in terms of controlling bacterial propagation and inhibiting drastic changes of VFA numbers when compared to 0.3% ammoniated NR latex. Neverthless, the effect of these compounds on rubber particles have to be further studied as field NR latex treated with these compounds in the absence of ammonia showed instability. The study concluded that biological compounds have a promising potential to function as bactericidal and sequestering agent to minimise pre-coagulation of NR latex. Increasing the concentration of streptomycin sulphate or surfactin could possibly enhance NR latex stability.

ACKNOWLEDGEMENT

The authors wish to acknowledge the technical and financial assistance given by Malaysian Rubber Board for the project. We also appreciate the advice and comments made by the Panel of the Scientific Economic and Advisory Committee (SEAC) during the proposal and monitoring stage of the study. Not to be forgotten are the capable technical work and support rendered by Hanipiah Basri and Aminah Yusuf.

Date of receipt: July 2014
Date of acceptance: January 2015

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