NOVEL PALM OIL BASED POLYMERIC SURFACTANT IN NATURAL RUBBER LATEX FILMS

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ABSTRACT

A series of novel biocompatible polymeric surfactants, anionic and nonionic surfactants were synthesized in-house by copolymerization of palm oil with fully bio-sourced and renewable starting materials. With the aim to innovate the use of these bio-materials and in line with the trend of green chemistry, the properties of these synthesized surfactants are being evaluated to discover their potential in dipping applications. Results of cytotoxicity, antimicrobial, foaming and mechanical test have provided a brief ideas of their capability. Cytotoxicity of these surfactants was determined by MTT assays with SDS and Tween 80 served as the commercial references. Comparative cytotoxicity test confirmed that these surfactants were biocompatible and non-noxious against human keratinocytes (HaCaT), mouse fibroblasts (3T3), mouse hepatocytes (H2.35) and canine kidney cells (MDCK) in both dose- and time-dependent manners. Both surfactants demonstrated excellent anti-foaming properties and this justified their potential anti-foaming applications. When these surfactants were compounded into commercial HA latex, the optimum tested mechanical properties on the thin films were recorded as 27 MPa for tensile strength, 1.0 MPa for modulus at 500% and 1150% for elongation at break. This means that our surfactants are able to modify the films to become softer without scarifying the mechanical strength of the thin films. There were also no pin holes detected on the dipped films. According to Minimum Bactericidal Concentration (MBC) assay, the bacteria growth inhibition screening or MBC assay, anionic surfactant showed antibacterial efficacy at low concentration for S. aureus isolates including methicillin-resistant S. aureus. The same inhibitory effect of anionic surfactant was also observed for A. baumannii. However, high concentration of anionic surfactant was needed in order to inhibit the growth of K. pneumonia. On the other hand, nonionic surfactant did not show growth inhibition for the tested bacteria for each tested concentration except A. baumannii. These findings highly recommend consideration of the novel, bio-based palm polymeric surfactants have high potential to be utilized as bio-based stability modifiers for improved latex film formation in the preparation for aqueous-based latex formulation for dipped-good product applications.

INTRODUCTION

In view of the increasing global interest in sustainable raw material supplies, polymeric surfactants synthesized entirely from bio-sourced materials have been presented an attractive option.[1-4] Surfactants are not chemically bonded to rubber particles during film formation. Therefore, they can migrate through films and be leached out. Leaching out of surfactants has induced worldwide concerns on the surfactants toxicity, biodegradability and the level of surfactant pollution which jeopardize to aquatic organisms. The bio-based polymeric surfactants are expected to offer favorable biocompatibility properties and more cost-effective than those produced using petroleum-based raw materials.

Furthermore, surfactants with anti-foaming properties are thus preferably used to ensure the production of continuous films on the former and maintain film integrity during drying and vulcanizing stage. Notwithstanding a number of studies on the polymeric surfactant, bio-based polymeric surfactants with anti-foaming properties have less in examined. Thus, it is worthy to investigate the relationship between the chemical structures and the resultant physicochemical characteristics in detail to establish their potentials as bio-based polymeric surfactants with anti-forming properties.

Our work on novel palm oil-based polymeric surfactants have been synthesized successfully from bio-sourced starting materials as branched polyesters. It has been proved that these polymeric surfactants exhibited very promising surface activity and anti-foaming properties. The findings in this work can serve as a solid foundation for follow-on studies of the potential polymeric surfactants as the anti-foaming agents whenever the renewable and biocompatible of anti-foaming agents or surfactants are considered. The technology described in the manuscript is going to be useful for manufacturing the quality rubber glove product to sustain in the global market.

EXPERIMENTAL

Preparation of NR thin films. The compounded latex was prepared with typical NR glove formulation as reported previously.[5] stated in Table 1. The compounded latex was left overnight for maturation. The films were cured in the oven (Memmert UFB 400) under 100°C for 30 minutes.

Cytotoxicity. The in vitro biocompatibility of both anionic and nonionic surfactants was assessed by measuring the viability of HaCat human keratinocytes using MTT assay. The cells were cultured in DMEM supplemented with 10% FBS medium and 1% penicillin-streptomycin. Cell suspensions were seeded into 96-well plates at a density of 1 x 10^4 cells/well and cultured for 24 hours as described above. The absorbance of each well was read at 570nm using a microplate reader (Dynex Opsys MR 24100) and the relative cell viability was calculated using *Equation 1* by comparison with control wells containing cell culture medium without dissolving surfactant polymer.

$$\text{Cell viability} = \frac{[A]_{test}}{[A]_{control}} \times 100 \qquad (1)$$

where $[A]_{test}$ is the absorbance of the test sample and $[A]_{control}$ is the absorbance of control sample.

The average cell viability obtained from triplicate determinations at each surfactant concentration was presented as a dose-response curve. Two commercial surfactants, SDS and Tween 80 were subjected to the same test and taken as the reference of anionic and nonionic surfactants for comparison purposes. The differences in the toxicity of each pair was compared using Student's t-test by Microsoft Excel 2013. Analysis outcomes with a confidence level of 95% ($p < 0.05$) were considered statistically significance. Mean value was calculated together with the standard deviation for each surfactant.

Foaming Test. About 10.0 wt/vol % of surfactant solutions were subjected to vortex for 30 seconds to create foams using IKA shaker, VORTEX 3 with the spinning speed of 6. Forming properties of immediate foam height and foam height after 5 minutes were recorded. Tests were performed three times for each sample, and average readings were taken.

Mechanical properties. Tensile tests were performed at room temperature using an Instron testing machine (model 5565). Dumbbell-shaped test samples of width 3.9 mm were cut from the rubber films using a die. The thickness of the narrow strip was determined using a thickness gauge (Mitutoyo brand). Tensile tests were carried out at room temperature according to ISO 37:2011 test method, with a 500 N loaded cell and at the cross-head speed of 500 mm/min. All measurements were performed three times and values were reported as a mean of three samples.

Antimicrobial. The agar dilution tubes were prepared according to the CLSI guideline with some modification. The control tube was MHA without surfactant but with the presence of DMSO and Tween-20. MBC values of AS1 and NS1 against some most common healthcare infectious bacteria like Gram-positive bacteria Staphylococcus aureus (S.aureus) including MRSA and MSSA; Gram-negative bacteria like Escherichia coli (E.coli), Klebsiella pneumoniae (KP) and Acinetobacter baumannii (A.baumannii) bacteria were evaluated as compared to the control tube.

RESULT AND DISUCSSION

The MTT assay is a simple calorimetric assay to measure cell toxicity, viability and proliferation. This assay measures the capacity of mitochondrial dehydrogenase enzymes in living cells to reduce the yellow, water soluble tetrazolium salt, MTT, into a water-insoluble dark blue formazan via the cleavage of the tetrazolium ring. The relative toxicity compared to controls was assessed from the amount of formazan produced which is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. In the present study, the cytotoxic activity of palm oil based polymeric

surfactants against human keratinocytes (HaCaT) was evaluated. The cytotoxicity of commercial surfactants, SDS and Tween 80 was present as a benchmark. SDS is an anionic surfactant commonly added as stabilizers in rubber industry while Tween 80 is a nonionic surfactant, a representative surfactant of pharmaceutical, food and cosmetic industry due to its high biocompatibility.

In this study, HaCaT cells or keratinocytes was chosen as the preliminary test for cytotoxicity. Keratinocytes are the predominant cell type in the epidermis and thus are affected first by toxic substances through direct contact. The viability of HaCatT cells following exposure to anionic surfactant (AS1), nonionic (NS1) surfactant, and SDS as well as Tween 80 was compared with the viability of vehicle control (V.Ctrl) cells as shown in *Figure 1*. Cytotoxicity was rated based on the cell viability relative to controls and pursuant to ISO 10993-5, percentages of cell viability above 80% are considered as non-cytotoxicity; within 80%–60% as weak; 60%–40% as moderate and below 40% as strong cytotoxicity.[6,7] From *Figure 1*, it is clearly seen that the percentages of cell viability in all palm oil-based polymeric surfactants were well above 80% and thus these surfactants were nontoxic towards HaCat cell. This indicates that these surfactants are safe in short and prolonged skin contact. From *Figure 1,* SDS exhibited both time and dose dependent toxicity particularly at concentrations of 50 and 100 μg/mL with the difference in mean was significant ($p < 0.05$). Robinson *et al*. has reported the irritant properties of SDS, where the concentration limit of 1% is applied in products intended for prolonged contact with skin.[8] Furthermore, SDS might worsen skin problems in individuals with chronic skin hypersensitivity.[9,10] *Figure 1* confirms that Tween 80 is non-toxic upon direct contact with HaCaT cells and thus is safe for skin contact. Therefore, all palm oil-based polymeric surfactants have very high biocompatibility and safe for topical application.

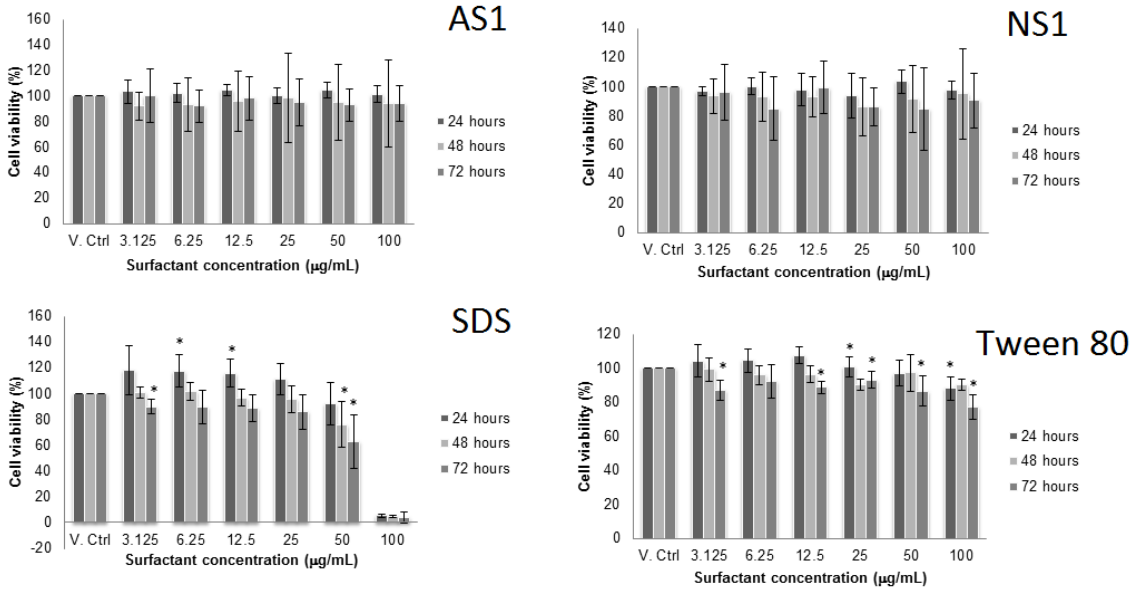

Figure 1. Effect of palm oil based polymeric surfactant and commercial surfactants on the viability of HaCaT cells after 24, 48, and 72 hours of treatment. Cell viability was assessed by MTT method and presented as percentage of absorbance with 100 % representing

vehicle control (V.Ctrl) cells. Data was expressed as mean ± SD (n=3). The symbol * denotes significant differences between control and treatment groups at p <0.05.

Table 1. Immediate foam height of 1.0 w/v % surfactant solution

Surfactant Solution	Foam height (mm)	Foam stability, R5 (%)
AS1	1.4	93
NS1	1.0	50
SDS	18.0	72
Tween 80	5.0	100

The foaming behaviour of four surfactants at room temperature was also evaluated. Foaming behavior was characterized by foamability and foam stability. From *Table 1*, both the commercial surfactants, SDS and Tween 80 had a higher foaming tendency compared to AS1 and NS1 surfactants as both SDS and Tween 80 generated foam with greater height. El-Sukkary et al. have concluded that the foaming properties were determined by the alkyl chain length of surfactant molecules, where long alkyl chain length of surfactants could inhibit the foaming ability.[11] The result tabulated in *Table 1* are thus in an agreement where AS1 and NS1 are polymeric surfactant with longer alkyl chain length than SDS and Tween 80 were capable to produce shorter foam height. Besides that, the self-coiling of long alkyl chains of polymeric surfactants might happened prior to the adsorption of surfactants at the air/water interface when they were dispersed in aqueous solutions.[12] This might also possibly happened to the long fatty acid chains of both AS1 and NS1, resulting in the ageing effect of the adsorption behaviour and causing difficulty in generating foam.

Foam stability can be evaluated by finding out the R5 values of surfactants as proposed by Lukenheimer and Malysa.[12] The R5 parameter is expressed as below:

$$R5 = \frac{h_5}{h_0} \times 100 \qquad (2)$$

Where, h_0 is the maximum (immediate) foam height and h_5 is the foam height at 5 minutes. 50% of R5 value is identified as the cut-off point between metastable and low stability foam. *Table 1* stated that foams produced by all tested surfactants were metastable as they had a R5 value greater than 50%. SDS had generated the most foam but Tween 80 had the greatest foam stability. NS1 surfactant produced less and least stable foam while there was no bubble produced after 30 seconds of the vortex in the case of AS1 surfactant. Low foaming ability of both AS1 and NS1 surfactants indicated their great potential in anti-foaming applications.

Table 2. Mechanical properties of NR latex films compounded with different surfactants

Surfactant	Tensile Strength (MPa)[a]	Modulus, M500 (MPa)[a]	Elongation at break (%)[a]
Potassium laurate	29.0	2.3	920.0
AS1	19.0	1.9	920.0
NS1	28.0	2.3	930.0
AS1:NS1=50:50	23.0	1.7	930.0

Footnote: [a] NR latex films were cured under 120°C and 30 minutes.

Table 2 shows the mechanical properties of NR latex films compounded with AS1, NS1 and potassium laurate where potassium laurate being served as a control. From *Table 2*, the tensile strength of latex films compounded with NS1 was at par with that of latex films compounded with potassium laurate. When both AS1 and NS1 were mixed in an equal ratio, the modulus of the latex films have reduced significantly. This signified that palm oil-based polymeric surfactants exhibited high potential in fabricating soft and pliable thin films. In the further work on modification of the polymeric structures of the surfactants, it was found that the mechanical properties of the latex films could reach up to the maximum values of 27 MPa for tensile strength, 1.0 MPa for modulus at 500% and 1150% for elongation at break (further details will be presented in the oral session).

In a further study to eluate the antimicrobial properties of the surfactants, MBC was also carried out to determine the antimicrobial properties of the surfactants against some of the most common healthcare infectious bacteria. From the study, AS1 was found to be more effective in inhibiting >95% of Staphylococcus aureus (S.aureus), both MRSA and MSSA and Acinetobacter baumannii (A.baumannii), with the MBC value of < 5%v/v. Higher amount of AS1 needs to inhibit >95% of Klebsiella pneumoniae (KP). NS1 only exhibited significant antimicrobial properties towards A.baumannii with the MBC value of about 0.3%v/v.

CONCLUSION

The novel palm oil-based polymeric surfactants, AS1 and NS1 were successfully synthesized with high biocompatibility. These surfactants of potentially non-cytotoxic are thus safe to be added into the latex compounding as to reduce the risk of irritant or allergic contact dermatitis (Type IV hypersensitivity). These surfactants with anti-foaming properties could serve as potential anti-foaming agents in dipping mixes or other similar latex compounds to either prevent foam formation or to destroy existing foam. Latex films incorporated with these surfactants exhibited low modulus indicating the high potential in fabricating soft and pliable gloves to provide comfort and natural fit for longer hours of usage. The additional antimicrobial properties of both surfactants have

potentially offered for a further investigation in providing an additional protection layer for the latex gloves against the hospital-acquired infections.

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