SYNTHESIS OF PALM OIL-DERIVED BIOCOMPATIBLE POLYMERIC SURFACTANTS FOR NATURAL RUBBER LATEX APPLICATION

YVONNE LING TZE QZIAN

FACULTY OF SCIENCE
UNIVERSITI MALAYA
KUALA LUMPUR

2022

SYNTHESIS OF PALM OIL-DERIVED BIOCOMPATIBLE POLYMERIC SURFACTANTS FOR NATURAL RUBBER LATEX APPLICATION

YVONNE LING TZE QZIAN

DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
UNIVERSITI MALAYA
KUALA LUMPUR

2022

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of candidate: **YVONNE LING TZE QZIAN**

Matric No: **17021909/1**

Name of Degree: **MASTER OF SCIENCE**

Title of Dissertation (“this Work”):

SYNTHESIS OF PALM OIL-DERIVED BIOCOMPATIBLE POLYMERIC SURFACTANTS FOR NATURAL RUBBER LATEX APPLICATION

Field of Study: **POLYMER CHEMISTRY**

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract form, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya (“UM”), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidature’s Signature Date: 9-Nov-2022

Subscribed and solemnly declared before,

Witness’s Signature Date: 9-Nov-2022

Name:

Designation:

SYNTHESIS OF PALM OIL-DERIVED BIOCOMPATIBLE POLYMERIC SURFACTANTS FOR NATURAL RUBBER LATEX APPLICATION

ABSTRACT

Surfactant is important for NR latex applications. It serves as latex preservative to preserve latex and promote good NR film formation. However, some surfactants are toxic and skin irritant. Hence, this study aims to develop alternative green, specifically bio-based surfactant from palm oil for natural rubber latex applications. The objectives of this study are to (i) synthesize a series of biocompatible palm oil-derived polymeric surfactants and characterize the physicochemical properties of the surfactants, (ii) evaluate the *in vitro* cytotoxic and antimicrobial properties of surfactants, (iii) compound surfactants into natural rubber (NR) latex and investigate the rheological properties of the latex, and (iv) study the mechanical properties of NR latex films incorporated with the synthesized palm oil-derived polymeric surfactants. Four palm oil-derived polymeric surfactants, namely palmitic acid anionic surfactant (PAS), stearic acid anionic surfactant (SAS), oleic acid anionic surfactant (OAS) and oleic acid non-ionic surfactant (ONS), were synthesized using polyesterification method. The chemical structures of the surfactants were then confirmed using FT-IR and ^{1}H-NMR. ONS experienced the highest extent of polyesterification, followed by PAS, SAS and OAS. Consistent with the extent of reaction completion, ONS exhibited the highest M_n and M_w values among all four surfactants. One of the highlights of this project is to develop a polymeric surfactant which is biocompatible for NR latex application. It is therefore crucial to evaluate the cytotoxicity of the developed product. All four surfactants, PAS, SAS, OAS, and ONS were subjected to cytotoxicity study using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. SDS and Tween 80 were included in the study as controls. The cytotoxicity of these four surfactants were evaluated in terms of cell viability against four cell lines, including human keratinocytes (HaCaT), mouse fibroblasts (3T3), mouse hepatocytes (H2.35) and canine kidney cells (MDCK). Comparative cytotoxicity test revealed that the cell viability of these four surfactants were well above 80 %, except for SAS which exhibited low cytotoxicity on fibroblasts after prolonged exposure at high concentration. Antimicrobial test was conducted on OAS and ONS using minimum bactericidal concentration (MBC) assay against bacterial strains methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *K. pneumoniae*, and *A. baumannii*. Subsequent results revealed that OAS is the most effective surfactant in inhibiting the growth of those tested bacteria. The synthesized OAS and ONS were also compounded into NR latex at five various concentrations and subjected into rheological study. From the study, OAS exhibited better outcome with longer linear viscoelastic region (LVR), signifying its potential usage for long term latex stabilization. Mechanical properties of unaged and aged NR films compounded with OAS and ONS in five varies concentrations were also evaluated. Based on the results obtained, it is suggested that NR films incorporated with OAS could form softer films and exhibited anti-aging properties.

Keywords: Antimicrobial, cytotoxicity, NR latex, palm fatty acids, polymeric surfactant

SYNTHESIS OF PALM OIL-DERIVED BIOCOMPATIBLE POLYMERIC SURFACTANTS FOR NATURAL RUBBER LATEX APPLICATION

ABSTRAK

Surfaktan adalah penting untuk kegunaan susu getah asli. Ia digunakan sebagai pengawet susu dan membantu pembentukan filem getah asli. Namun, sesetengah surfaktan adalah toksik dan perengsa kulit. Oleh demikian, kajian ini bertujuan untuk membangunkan biosurfaktan yang bersifat hijau yang dapat dihasilkan daripada minyak sawit untuk kegunaan susu getah asli. Objektif kajian ini adalah untuk (i) mensistesis satu siri biosurfaktan polimer berasaskan minyak sawit dan mencirikan sifat-sifat fizikokimia surfaktan, (ii) menilai sifiat-sifat sitotoksik dan antimikrob surfaktan polimer, (iii) menambah surfaktan ke dalam susu getah asli (NR) dan menyiasat sifat-sifat rheologi susu getah dan (iv) mengkaji sifat-sifat mekanikal filem susu getah asli yang dihasilkan. Empat surfaktan polimer berasaskan minyak sawit, iaitu surfaktan anionik asid palmitic (PAS), surfaktan anionik asid stearik (SAS), surfaktan anionik asid oleik (OAS) dan surfaktan asid oleik bukan ionik (ONS), telah disintesiskan dengan penggunaan kaedah poliesterifikasi. Struktur kimia surfaktan polimer disahkan dengan menggunakan analisis FT-IR dan ^{1}H-NMR. ONS menunjukkan darjah poliesterifikasi tertinggi, diikuti dengan PAS, SAS dan OAS. Selaras dengan tahap penyelesaian reaksi, ONS mempunyai nilai Mn dan Mw tertinggi. Salah satu sorotan projek ini adalah untuk membangunkan surfaktan polimer biokompatibel untuk aplikasi susu getah asli. Oleh itu, penilaian kesitotoksikan terhadap produk adalah penting. Keempat-empat surfaktan, PAS, SAS, OAS dan ONS tertakluk kepada kajian sitotoksisiti dengan menggunakan ujian 3-(4,5-dimetitiazol-2-yl)-2,5-difenilltetrazolium bromida (MTT). Natrium dodesil sulfat (SDS) dan Tween 80 telah dimasukkan ke dalam kajian berfungsi sebagai kawalan eksperimen. Sitotoksisiti keempat-empat surfaktan ini dikaji atas empat jenis sel, termasuk sel keratinosit manusia (HaCaT), sel fibroblas tikus (3T3), sel hepatosit tikus (H2.35) dan sel buah pinggang anjing (MDCK). Keputusan ujian sitotoksisiti mendedahkan bahawa kehidupan dan pertumbuhan bagi keempat-empat surfaktan adalah melebihi 80%, kecuali SAS menunjukkan sitotoksisiti rendah terhadap sel fibroblas tikus setelah pendedahan lama pada kepekatan tinggi. Ujian antimikrob juga telah dijalankan antara OAS dan ONS melalui kaedah kepekatan bakteria minimum (MBC) terhadap strain bakteria *S. aureus* (MSSA yang sensitif methicillin), *S. aureus* (MRSA) yang tahan methicillin, *K. pneumoniae*, dan *A. baumannii*. Keputusan seterusnya mendedahkan bahawa OAS adalah paling berkesan dalam menghalang pertumbuhan kelima-lima bakteria yang diujiankan. Kemudian, OAS dan ONS dicampurkan ke dalam susu getah asli dalam lima kepekatan yang berlainan untuk kajian rheologi. Daripada kajian rheologi, OAS menunjukkan hasil yang lebik baik dengan bahagian viskoelastik (LVR) yang panjang serta linear, menandakan potensinya untuk menstabilkan susu getah. Sifat mekanikal bagi NR filem yang mempunyai OAS dan ONS juga telah dinilaikan. Keputusan yang diperoleh menunjukkan bahawa NR filem yang mempunyai OAS membentuk filem yang lebih lembut dan menunjukkan sifat anti-penuaan.

Kata kekunci: Antimikrob, sitotoksisiti, susu getah asli, asid lemak sawit, surfaktan polimer

ACKNOWLEDGEMENTS

First and foremost, a deepest appreciation expressed to whomever for helping me from all of the aspects to accomplish this research project on time. Particularly express my profound gratitude towards Dr. Lee Siang Yin, one of the supervisors of this research project, for spending her precious time in guiding me for the selection of the title of the research project.

I would like to appreciate Dr. Desmond Ang Teck Chye, my dearest supervisor for guiding me with tons of patience throughout the research. He had helped me to understand the purpose of doing this research, the flow of the procedure as well as precaution required to be taken while conducting this research.

In addition to that, I would like to highly indebt Dr. Koh Ryun Yan and Dr. Liew Yun Khoon, the biologist and microbiologist from International Medical University (IMU), for providing all the cell lines and hospital acquired bacteria so that my research project can be carried on fluently.

Apart from that, I would like to show my grateful to my teammate, which is Heng Yi Xin for supporting me and helping with each other throughout the research project in managed to complete the research on time.

Last to mention, I would like to appreciate my parents for their financial support as well as infinity encouragement given to me to complete the research as well as my research thesis. Once again, thanks for their full support and the accomplishment would not be achieved without them.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

^{1}H-NMR	:	Proton Nuclear Magnetic Resonance
AFM	:	Atomic force microscopy
ASTM	:	American Society for Testing and Materials
CHDM	:	Cyclohexanedimethanol
CLSI	:	Clinical Laboratory Standards Institute
CMC	:	Critical micelle concentration
CO_2	:	Carbon dioxide
DMEM	:	Dulbecco’s Modified Eagle’s medium
DMPA	:	Dimethylolpropionic acid
DMSO	:	Dimethyl sulfoxide
DSC	:	Differential scanning calorimetry
FBS	:	Fetal bovine serum
FT-IR	:	Fourier transform infrared spectroscopy
GPC	:	Gel permeation chromatography
HA latex	:	High ammoniated latex
LVR	:	Linear viscoelastic region
MBC	:	Minimum bactericidal concentration

MePEG	:	Methoxy poly(ethylene glycol)
MFFT	:	Minimum film formation temperature
MHA	:	Mueller Hinton agar
MHB	:	Mueller Hinton broth (MHB)
MRSA	:	Methicillin resistant *Staphylococcus Aureus*
MSSA	:	Methicillin sensitive *Staphylococcus Aureus*
MTT	:	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay
NASS	:	Sodium styrene sulfonate
NH_3	:	Ammonia
NR	:	Natural rubber
NRL	:	Natural rubber latex
OA	:	Oleic acid
O/W	:	Oil in water
PA	:	Palmitic acid
SA	:	Sebacic acid
SDS	:	Sodium dodecyl sulfate
SME	:	Sulfonated methyl esters

StA	:	Stearic acid
TGA	:	Thermogravimetric analysis
TMP	:	Trimethylolpropane
TSC	:	Total solid content
Tween 80	:	Poly(ethylene glycol) sorbitan monooleate
VOCs	:	Volatile organic compounds
ZDMC	:	Zinc dibutyl dithiocarbamates

CHAPTER 1: INTRODUCTION

1.1 Research background

Natural rubber (NR) is a natural occurring polymer, particularly obtained from *Hevea brasiliensis* species. It is a vital agricultural commodity which is engaged in a wide range of applications, and it has been used most extensively in latex-dipped products, especially in rubber glove manufacturing. Amongst the known benefit of NR is its protective quality, ease of accessibility, and good elasticity and mechanical strength (Yip & Cacioli, 2002; Vijayaram, 2009).

Generally, NR is commercially available in latex concentrates or dry rubber forms. NR latex is a milky fluid that can be obtained naturally from the sap of rubber tree. It can be processed into gloves, condoms, catheters, rubber thread etc. by dipping, extruding, or coating. Production of rubber articles from NR latex concentrate are usually via dipping or coating. Dry NR on the other hand refers to rubber produced from NR latex via the use of organic acid for coagulation to form dried or milled sheets of rubber. Field coagula also accounts for up to 20% of the dry rubber produced. The products formed by dry NR involve processes such as compression, molding, and extrusion (Naphon et al, 2018). Examples of products produced from dry rubber are syringe pluggers, tires, vial stoppers, and so on (Boo, 2014).

Rubber gloves sector is the largest contributor to the Malaysian rubber industry. The export of rubber gloves amounted to 56,522 tonnes in November 2021 as reported by Department of Statistics Malaysia (DOSM) (Xia, 2021). Despite the impact from COVID-19 pandemic, the Association of Natural Rubber Producing Countries (ANRPC) anticipated the recovery of world NR production in January 2021 (Premadasa, 2021). This trend has indirectly demonstrated a high demand for good quality gloves, particularly those of NR, in major emerging markets. Despite competition from synthetic rubber, demand for NR glove remains strong due to the unique and intrinsic properties of the NR article, and this includes its outstanding elasticity, softness, and high elongation at break %.

NR latex mainly composed of two major components, 1,4-cis-polyisoprene which is the rubber, as well as non-rubber components constituted by a mixture of carbohydrates, lipids and proteins which retain the NR in an aqueous serum phase (Puskar et al., 2014). Fresh NR latex has high tendency to undergo spontaneous coagulation after it was collected. This occurs naturally as the non-rubber components are subjected to bacterial attack, leading to a decrease in latex pH and subsequently coagulation of rubber particles (Stamberger, 1939). Thus, a preservative is generally required to be added into the NR latex and this is especially important for industries or applications that require the NR latex to remain fluid. The most common preservatives used in NR latex is ammonia (NH_3) because it is effective in inhibiting the growth of bacteria due to its relatively high pH, and due to its ability to act as a stabilizer to maintain the colloidal system of the NR latex (Riyajan & Santipanusopon, 2010). Nevertheless, there are some concerns relating to the use of NH_3 due to its high volatility, odor, and toxicity (Ahonen et al., 2009; Quinn et al., 2015; Yari et al, 2016; Lee et al., 2021).

Surfactants are usually added into the NR latex. They are molecules comprising hydrophilic heads and hydrophobic tails which are capable to lower the free energy of a phase boundary to make two immiscible phases miscible. It can also be defined as a surface-active agent which has the tendency to adsorb at interfaces, and it is a very important ingredient in the NR latex compounding. To date, there are numerous studies that reported the benefits and importance of introducing surfactants into NR latex (Manroshan, 2007; Ardvani et al., 2019; Lim & Misni, 2018). Not only does surfactant helps to maintain the stability of the latex, but it also affects the properties of film produced from the latex, including the film permeability and mechanical properties (Steward et al., 2000).

Latex film formation is a process where the particle in an aqueous latex comes to the closest packing and eventually form a polymer film due to particle deformation from the continuous drying process. Depending on the compatibility between surfactant and particles, surfactant will dissolve into the polymer, remain in the interfaces between particles, or phase-separate from the polymeric solution and migrate to the interfaces (Kientz & Holl, 1993). Hence, the distribution of surfactants after coalescence of latex films is important in determining the performance of the film. Less than optimal latex formulation, including the use of unsuitable amount and type of surfactant may eventually results with higher percentage of products with defects or poor mechanical strength. One example of main defect in dipped products is pinholes formation. Single dips are prone to defects like pinhole, and this has been a major problem for products which require thin film formation. Pinhole is a very small hole which resulted from bubbles formation in the latex tank, and this usually happens during latex dipping process (Joseph, 2013). Surfactant in the latex could reduce surface tension and ensure even spreading of latex on ceramic former to reduce the occurrence of such defect.

1.2 Problem statement

There are allergy concerns pertaining to the use of latex glove, and those allergic responses are usually due to the presence of latex allergens or latex additives added during compounding of NRL. Commonly, delayed Type IV hypersensitivity is caused by chemical additives used. Examples of chemical additives including accelerators, antioxidant, vulcanizing agents, stabilizers and coagulants. Delayed Type IV hypersensitivity, generally induce contact dermatitis allergy to users. On top of that, some of the chemical additives used are carcinogens, which might release nitrosamine compounds that induce human health hazard concerns. (Crowther et al., 1998; Lushniak, 2000; Ruhida & Amir-Hashim, 2012). On top of that, another notable problem is the over-reliance on petroleum-based raw materials in the production of NR latex additives is always a global trend where many industries have shifted to utilize natural or bio-based raw materials in the development of their products. Henceforth, development of biocompatible or bio-based additive is advisable.

To produce a natural rubber latex glove, the natural rubber obtained from sap of rubber tree need to maintain in aqueous dispersion medium form then subject to dipping process. However, spontaneous coagulation of filed latex after it leaves the rubber tree is a well-known fact. The airborne bacterial tend to attack the cell membrane of the natural rubber particles, lowering down the pH of the latex by producing acid, subsequently neutralize the negative charges of rubber latex and coagulation occurred. As such, ammonia is the most common stabilizer used by industry to preserve and stabilizer the natural rubber latex in dispersion phase. Nevertheless, the use of ammonia presents several concerns. One of the major challenges is the gradual evaporation of highly volatile ammonia over a period of preservative time. This requires repetitive addition of ammonia in order to maintain the pH of the latex system at the desired value. On top of this, ammonia can induce health hazard associated problems as it is irritant and corrosive (Bousquet et al., 2006). Therefore, synthesis of a biocompatible bio-based surfactant as a potential additive to preserve and stabilize the natural rubber latex system is worth investigating.

There are several defects that may occur in the manufacturing of gloves which affect the quality of the gloves being manufactured and lead to rejection of the products. Defects will lead to production loss which is undesired. Therefore, minimization of defects is an important factor ensuring the quality of products. Defects including beading defects, torn-tearing, sticky defects, weak spots and latex coagulum have occasionally seemed (Kirti, 2016). Nonetheless, one of the major problems for products which require thin film formation is pinholes, particularly for single dip products. A pinhole is a very small hole which resulted from bubbles formed in the latex tank, especially happened during latex dipping process (Joseph, 2013). Conventional surfactant is one of the key factors resulting in porous film, particularly attributed to its foaming properties (Lim & Misran, 2013). Conventional organic surfactants are prone to leaching out from the film and thus induce environmental concerns (Zhu et al., 2014). As such, development of a polymeric surfactant seems promising as it has longer chain length which postulate to entangle with the rubber particles during curing process and not subjected to migration, blooming and leaching out from cured films.

The present study aims to develop alternative green additives, specifically bio-based surfactant from palm oil for natural rubber latex application. Rheology tests is carried out to study the flow behavior and linear viscoelastic properties of NRL compounds and mechanical properties is to identify the quality of the films incorporated with the bio-based polymeric surfactant synthesized. Taken together, the results obtained could provide a solid foundation for follow-on studies on the potential biocompatible palm oil-based polymeric surfactants for natural rubber latex applications.

1.3 Research objectives

Developing latex additives synthesized entirely from renewable materials presents an attractive alternate option to address the above-mentioned concerns on environmental and toxicity issues associated with the use of conventional additives and petroleum-derived chemicals in NR latex. This project aims to develop bio-based polymeric surfactants from palm oil for NR latex application, and the breakdown of the project objectives include:

1. To synthesize and characterize a series of biocompatible palm oil-derived polymeric surfactants from entirely bio-sourced materials.
2. To compound surfactants into NR latex and investigate the rheological properties of the latex.
3. To study the mechanical properties of NR latex films incorporated with the synthesized palm oil-derived polymeric surfactants.

1.4 Thesis outline

Chapter 1: Introduction

This chapter describes the background of this research project, comprising of the introduction to subject matter and problem statement of this research study. The objectives of the study are clearly spelt out in this chapter.

Chapter 2: Literature review

The general introduction and historical development of NR, NR latex film formation process, types of surfactants, classification and applications, surfactants derived from natural products, polymeric surfactants as well as surfactants in rubber industry are discussed in detail in this chapter.

Chapter 3: Experimental

In Chapter 3, the chemicals and materials involved in the study are listed out, and the methodology adopted in the synthesis of palm oil-derived polymeric surfactants as well as characterization techniques are elaborated in detail. Some of the instrumental characterization of the surfactants include Fourier transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and gel permeation chromatography (GPC). In addition to that, general procedures for the cytotoxicity and antimicrobial tests performed on the synthesized palm oil-derived polymeric surfactants are included as well. Lastly, procedure on compounding of NR latex with the surfactants, rheological study of NR latex compounds, preparation of NR latex films and evaluation on film characteristics are also included.

Chapter 4: Results and discussions

In this chapter, the outcome from the synthesis of four palm oil-derived polymeric surfactants (PAS, SAS, OAS and ONS) were discussed. The structures of synthesized palm oil-derived polymeric surfactants were corroborated with the results of FTIR and ^{1}H-NMR. Other characterization results discussed include those from DSC, GPC and TGA analysis. Biocompatibility of the synthesized polymeric surfactants were evaluated using cytotoxicity test against four cell lines (HaCaT, 3T3, H2.35 and MDCK), while the antimicrobial properties of synthesized polymeric surfactants were tested against four bacterial strains (MSSA, MRSA, *K. pneumoniae*, and *A. baumannii*). This chapter also presents the results from the rheological study of the NR latex compounded with the polymeric surfactants. Lastly, the mechanical properties of the unaged and aged dipped films prepared from compounded NR latex were presented in detail.

Chapter 5: Conclusion

This chapter summarizes the significant findings of this research project.

CHAPTER 2: LITERATURE REVIEW

2.1 Natural rubber (NR) and film formation process

Historically, the earliest use of NR can be tracked back to the era of the Aztecs, 1,600 BC, where rubber was extracted from the tree and formed into a ball solely using heat energy from hands and the rubber ball was used during Aztec ceremonial game. This was reported by Christopher Columbus during his second voyage to the New World in 1496 (Umar et al., 2017). It is only until the 17th century when the first scientific report on rubber was published by a Frenchman named Charles Marie de La Condamine (Reisz, 2007). Then, in 1763, Macquer and Herissant reported the use of turpentine as solvent to dissolve rubber and they found that the texture of rubber products behaved differently when subjected to distinct environment (Porritt, 1919). Seven years later, Joseph Priestley, an English chemist, gave the name “rubber” when he found it could be used to rub out pencil marks, who unintentionally become the first person who added carbon black in rubber (Yikmis & Steinbuchel, 2012). In 1820, Thomas Hancock discovered mastication and the use of rubber in gloves, shoes and stockings. However, the final landmark in the historical development of rubber was the discovery of vulcanization process by Charles Goodyear in 1839 and patented in year 1844, making a big leap in rubber manufacturing and contributes to commercial production of NR (Guise-Richardson, 2010).

Natural rubber (NR) (also known as cis-polyisoprene) is of plant origin which can be obtained from families of Euphrobiacea, Apocynacea, Moracea and many more. From the Euphrobiacea family, *Hevea brasiliensis* species originating from Brazil is the one that is being exploited for commercial purposes (Tanaka & Sakdapipanich, 2001). NR obtained from *Hevea brasiliensis* is practically consists of more than 99 % of cis-1,4 isoprene units.

NR latex tapped from rubber tree is a colloidal suspension of rubber particles and can be treated differently depending on the intended applications of the rubber (Verhaar, 1959). NR latex can either undergo coagulation with acids and compressed into dried sheets to produce dry rubber products, or the NR latex can be stabilized using ammonia (NH_3) to maintain its colloidal liquid form to produce latex products by dipping process (Sakdapipanich & Roirythai, 2012).

Apart from the isoprene units, NR also constituted of approximately 6 % of non-isoprene structural units including protein, phospholipids, glycolipids, fatty acids, and carbohydrates (Ho et al., 1996; Tarachiwin et al., 2003). Parts of these non-rubbery constituents, particularly proteins and lipids serve as a protective layer on the surface of rubber particles by forming negative charge on it to preserve the latex stability (Sansatsadeekul et al, 2011). However, when in contact with air, bacteria tend to attack and neutralize the protective layer, leading to coagulation and deterioration of the NR latex. Thus, ammonia is often used in the industry to maintain the colloidal suspension of the latex. Maximum of 0.7% of ammonia is used to produce HA latex (high ammoniated latex) or 0.2% ammonia in combination with other secondary stabilizers such as dithiocarbamates (Haldemann, 1984), zinc oxide or tetramethylthiuram disulfide (Santipanusopon & Rivajan, 2009) to produce LA latex (low ammoniated latex) (Rivajan & Santipanusopon, 2010).

One of the main applications of NR is to produce rubber glove. In fact, rubber gloves export is one of the largest commercial opportunities for Malaysia rubber industry due to the abundant rubber resources available in the country and vicinity (Ibragimov & Arshad, 2017). It was analyzed that the growth in healthcare industry worldwide is one of the driving key factors for the exponential growth of rubber gloves industry. Recently, Malaysian Rubber Glove Manufacturers Association (MARGMA) has reported that Malaysia supplied 63 % of the global demand of rubber gloves, amounting to RM 18.8 billion for the year 2018, and rubber glove make up more than 70 % of all the rubber goods export (Televisory, 2019). The rubber glove industry is expanding in both terms of production capacity as well as manufacturing processes to meet the growing market demand.

Many are not aware that the manufacturing of the seemingly simple and primitive rubber glove in fact involves a series of complex processes, and many aspects of the processes must be fine-tuned to produce a good pair of gloves. Good understanding of the rubber film formation process from the latex is important to ensure that the film produced is of desirable quality. Film formation is a process that involves transformation of particles in latex dispersion that meets each other, fuse, and forms a continuous film through evaporation of the water medium. Film formation process is also an important aspect in many other industries and applications including paints, coatings, adhesive, sealants and caulks etc. (Keddie, 1997). Over the years, many studies were conducted to better understand the film formation process (Chen et al., 2011; Felton, 2007), and various techniques have been employed for the study. This includes the use of atomic force microscopic (AFM) (Lin & Meier, 1996), scanning electron microscopy (SEM) (Setua et al., 2010) and small angle neutron scattering (Chen et al., 2011). It was reported that the physical and mechanical properties of the films produced are mainly dependent on minimum film formation temperature (MFFT) (ASTM, 1993; Myers & Schultz, 1994), which is the temperature limit at which coalescence of the particles in the system can take place. It is noteworthy that film formation arises from the inter-diffusion and coalescence of the particles in the system.

Many have carried out detailed study on the mechanism of latex film formation (Bradford & Vanderhoff, 1966; Brown, 1956; Dillon et al., 1951; Voyutskii, 1958), film modification and subsequent improvements in the quality of films produced have also been reported over the years (Frenkel, 1943; Kendall, 1982; Mason, 1973). Generally, film formation can be divided into three consecutive major physical stages, namely consolidation, compaction, and coalescence (Eckersley & Rudin, 1994; Ma et al., 2005; Steward et al., 2000). Initially, consolidation stage mainly involves higher rate of water evaporation from latex surface, followed by ordering of particles. This stage is the longest stage among the three and it will proceed until the polymer had reached around 60-70 % of volume fraction of water. Next, during compaction stage, the polymer latex sphere particles tend to undergo deformation and fill in the free void space by continuous water evaporation. Coalescence happened when further drying process proceed, allowing polymer chains to interpenetrate and finally a continuous polymer film is formed.

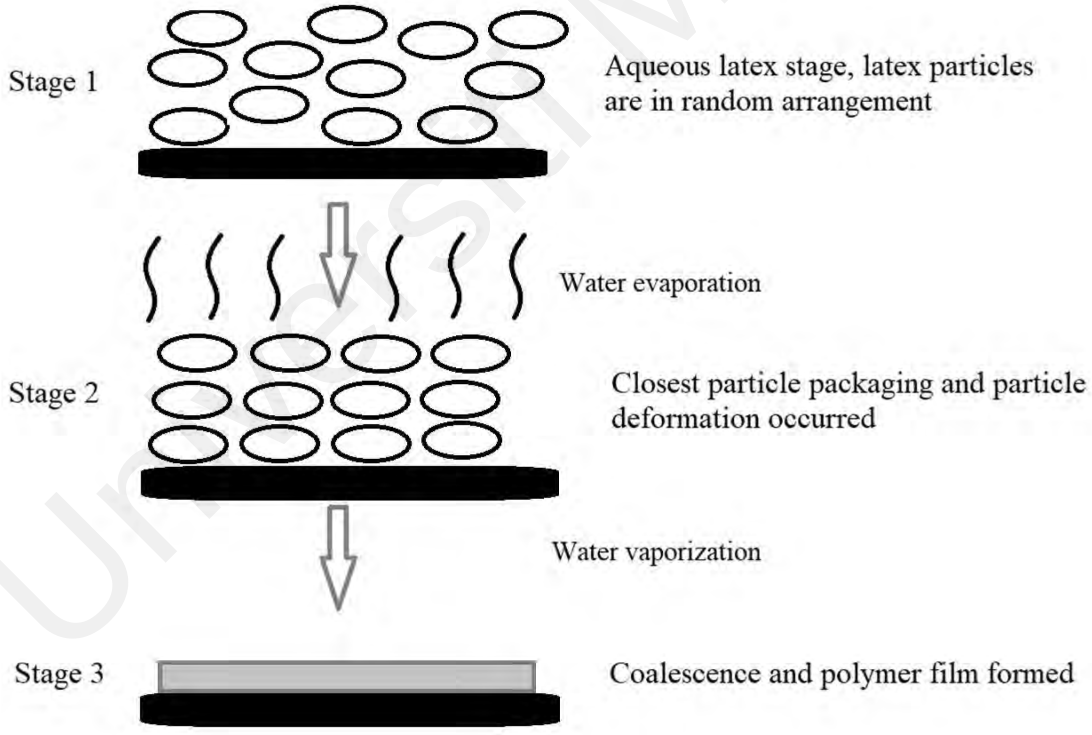

Figure 2.1: Film formation process.

2.2 Surfactants: Definition, classification, and application in rubber industry

Surfactants are surface-active substance that comprised of hydrophilic heads and lipophilic tails in a molecule, making them amphiphilic in nature. This dual affinity characteristic allows surfactants to function as emulsifier, suspending agent, stabilizer, foaming and defoaming agent, and many more in various industrial applications. Surfactant molecules have the tendency to form aggregates at critical micelle concentration (CMC), or in another term, micellization (Al-Soufi & Novo, 2021). This is an important feature that allows the formation of stable emulsion or dispersion for various applications. The global revenue of surfactants demand is expected to grow at a rate of 4.80% during the forecast period 2021-2026 (Research and markets, 2021). From the many types of surfactants, anionic surfactant accounts for about half of the production value particularly in cleaning products due to its ease and low cost of manufacturing (Farn, 2008; Gecol, 2006). Fundamentally, surfactants are classified based on the type and ionic charge that held by hydrophilic groups. Based on this classification, surfactants can be divided into four types, namely anionic, cationic, non-ionic, and amphoteric surfactants. Selective examples of the four types of surfactants are shown in Figure 2.2.

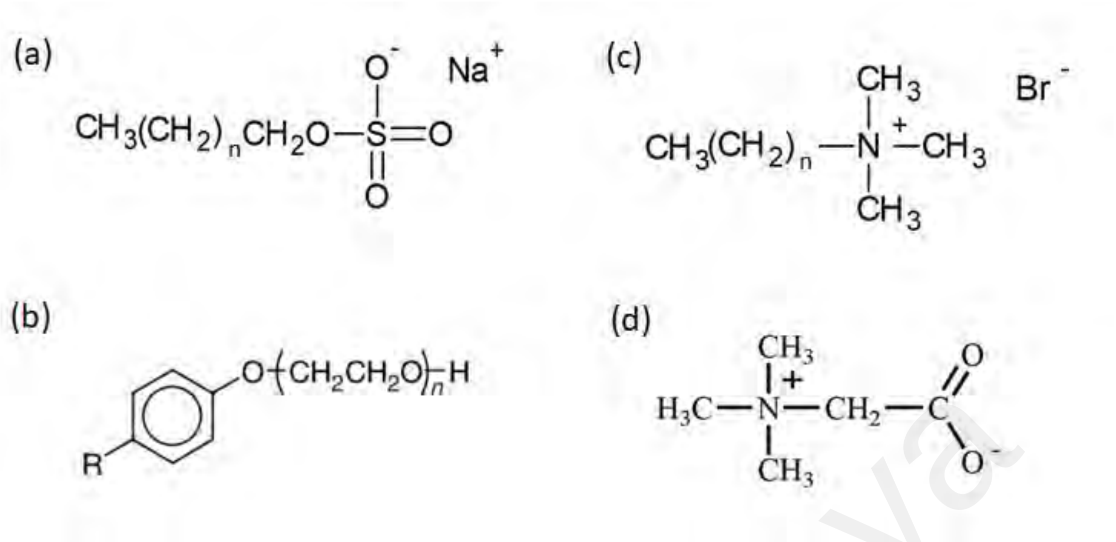

Figure 2.2: Chemical structure of selected examples of (a) Anionic surfactant, (b) Non-ionic surfactant, (c) Cationic surfactant, and (d) Zwitterionic surfactant.

Anionic surfactants carry a negative charge on their hydrophilic end. Their good cleansing power and wettability properties allow them to be the most widely used among all types of surfactants, especially in cosmetics and detergents industries (Li, 2020). Some of the commonly used anionic surfactants include sulfates, sulfonates, phosphates, and carboxylate salts (Myers, 2006). However, anionic agents are considered a potent irritant to skin, as reported by Effendy & Maibach (1995) and Effendy & Maibach (1996). They reported that sodium lauryl sulphate (SLS) and ethoxylate sodium lauryl sulphate (SLES) are found to be potentially responsible for skin irritation. Additionally. Loffler & Happle (2003) have reported similar findings in detergent application.

Non-ionic surfactants represent the second most produced surfactants by volume, and are particularly used in cleaning products (Steber, 2007). They are non-dissociable types and are often used as emulsifier agents and in detergent formulation. One of the advantages of non-ionic surfactant is its relatively low toxicity (Corazza et al., 2009). The most common non-ionic surfactants are of alkyl, fluoroalkyl or silica-based (Bajpai & Tyagi, 2010). They have less hydrophobic moieties but more varieties in hydrophilic groups, allowing them to be available in vesicle-forming surfactants.

Another ionic surfactant is the cationic surfactant which comprised of positive charge at the hydrophilic head. They are not usually present in cleaning products, but highly practical in biotechnology application (Mao et al., 2020).

Lastly, amphoteric (zwetterionic) surfactants are slightly different that the rest of the surfactants as they carry both positive and negative charges simultaneously on the same molecule. The net charge distribution of the surfactant is zero, and these charges can either be pH sensitive or non-pH sensitive (Sarkar et al., 2021). Amphoteric surfactants are not commonly used by itself, but usually used in combination with others such as ionic or non-ionic surfactants. Examples of amphoteric surfactants are betaine, amino acid, and sultaine (Sarkar et al., 2021). However, the high price of amphoteric surfactants compared to the rest, tend to limit its industrial application.

Other than classification based on charge, surfactants can also be classified based on the type of raw materials used in their production. They can be classified into petroleum-based and bio-based surfactants. The conventional surfactants are produced from petroleum-based substances and are widely used in cleaning, personal care, elastomers, plastics, oilfield chemicals, leather, paper, and paints and coatings industries. However, the depletion of non-renewable resources and environmental concern over the years have restricted their growth in the market (Das & Mukherjee, 2007; Olasanmi & Thring, 2018). These have eventually encouraged the development of bio-based surfactants. Bio-based surfactants are synthesized from renewable raw materials such as oleochemical feedstocks. They are usually produced through sustainable green chemical processes as compared to that of synthetic surfactants.

Due to their high biocompatibility, bio-based surfactants are usually safer to be formulated in cosmetics, pharmaceuticals (Bhattacharya et al., 2017), food industry (Nitschke & Costa, 2007), agriculture (Sachdev & Cameotra, 2013), petroleum industry (Silva et al., 2014), and environmental remediation (Olasanmi & Thring, 2018).

In recent years, the use of bio-based surfactants is gradually replacing the conventional petroleum-based surfactants, and this is largely attributed to some of the advantages of the former, which includes environmentally friendly, high availability, as well as low toxicity. The term "bio-based" refers to the use of ingredients derived from a living organism, for example, plants, animals or even microbes (Ana et al., 2021). Amongst the natural ingredients, palm oil, soybean oil, coconut kernel oil, castor oil, cashew nutshell oil, and fish oils are some of the more commonly used resources to prepare polymeric products encompassing polyester, polyether, polyolefin, and polyurethane (Chiellini et al., 2002; Takahashi et al., 2008; Yeoh et al., 2020). Not all bio-based materials exist as polymers naturally, but most of them are precursors to monomers and polymers. Triglycerides which are the main components present in plant oil comprise of long alkyl chains, making it suitable to be used to synthesize hydrophobic polymer. Many researchers have reported excellent properties of polymers derived from vegetable oil, and the performance of the products are not at all inferior compared to those derived from petroleum. This suggests that vegetable oil-monomers present great potential of substituting petroleum-based monomers (Miao et al., 2014).

Plant oil is one of the widely used starting materials to synthesize bio-based polymers because it is non-exhaustible and is renewable. In this study, palm oil is chosen as the renewable raw material to produce polymeric surfactant due to its abundance and easy accessibility in Malaysia. Palm oil is a type of vegetable oil and is considered a versatile raw material that can be used to produce vast range of products including coatings, foams, surfactants and many more. Palm oil is widely used in chemical industry to produce biofuels and a range of palm-based for further processing to fit their end-applications. Fatty acids present in the oil provide two sites for chemical reaction to take place, at the carboxylic group, -COOH and/or at the unsaturation, C=C. Some of the chemical reactions involving the carboxylic group include reduction, esterification, amination, and deoxygenation. Esterification is perhaps the most reported reaction involving fatty acid (Otera, 2006). Maurad et al. reported successful synthesis of palm-based alpha-sulfonated methyl esters (SME) to serve as a surfactant in detergent (Maurad et al., 2006).

Fatty acid derivatives can also be found in many other applications such as food, cosmetic, pharmaceutical, petroleum and other chemistry industry applications (Kenar et al., 2017). Particularly in the cosmetic and pharmaceutical applications, one of the primary use of fatty acid derivatives is as surfactants. In Saxena et al.'s study, they have synthesized and characterized surfactant from palm oil for enhanced oil recovery application (Saxena et al., 2017). Slamet and Pangiastika have also synthesized methyl ester sulfonate surfactant derived from crude palm oil for laundry liquid detergent application (Slamet & Pangiastika, 2017). Other than the environmentally friendly advantage, palm fatty acids were proven to be non-cytotoxic and exhibit antimicrobial properties (Roy, 2017). Rodriguez-Lopez et al. (2020) have reported a series of biosurfactants with fatty acid composition that exhibit remarkable antimicrobial properties by subjecting to cytotoxic study against mouse fibroblast cells. Zhang et al. (2014) reported effective antimicrobial properties of fatty acid sugar ester-based surfactants against gram positive bacteria. Furthermore, Karlova et al. (2010) have also reported findings consistent with the above-mentioned studies. They reported the antimicrobial properties of fatty acid fructose esters which are dependent on the chain length and concentration of the fatty acid ester.

Regardless of the types of surfactants, they have been used for various applications due to their outstanding functional diversity. Surfactants are used as emulsifiers or wetting, foaming, thickening agents for cleansing and cosmetic formulations (Milica et al., 2016). In pharmaceutical field, surfactant has been used as a solubilizer to improve water-based drug formulation (Sekhon, 2013). Savjani *et al.* (2012) have reported the use of surfactant to improve drug solubility. In the coating industry, surfactant is usually introduced to serve as emulsifier in a waterborne coating formulation. They are also added into the aqueous coating formulations to improve wetting of both substrate and dry pigment (Mahli et al., 2003; Makarewicz & Michalik, 2014). Surfactants are generally non-volatile and they remain in the coating during the drying process. Some researchers have ingeniously exploited this property and functionalized the surfactants to also serve as plasticizers for the coating, enabling the dried coating film to be softer and more flexible. Using suitable surfactants may also allow the reduction of volatile organic compounds (VOC's) in a coating formulation (Kong et al., 2015; Kronberg et al., 2014). For instance, Collins et al. (2004) have demonstrated the use of non-ionic surfactants to reduce or eliminate the need for co-solvent in the conventional coating compositions.

In glove application, addition of surfactants into latex dispersions could alter the final properties of the film, including the film terms of permeability, and mechanical properties. Surfactants serve as wetting agents to facilitate even spreading of latex on the surface of the former (Yip & Cacioli, 2001). There are several interesting findings on the interaction of surfactant with latex and the resultant rubber latex film properties (Juhue & Lang, 1993; Peethambaran et al., 1990; Tadros, 2009). Butler et al. (2003) had investigated the effect of ionic, polymeric and electrostatic stabilizers on the water-sensitive rubber latex films. Manroshan & Lim (2013) have also discussed the usage of surfactants in latex technologies. Birgin et al. (2017) also studied the effect of sodium styrene sulfonate (NaSS) concentration on emulsifier free waterborne poly(methyl methacrylate/butyl acrylate) based rubber latex and film properties. They have reported that there was a proportional relationship between film properties with the concentration of NaSS. Presence of surfactant was also found to influence the properties of the polymer film formed upon drying. Distribution of surfactants throughout the drying process can implicate the polymer latex film properties and performance (Keddie & Routh, 2010). Despite of surfactants have an overall advantage on latex dispersion and film properties, in fact, surfactants are not chemically bonded to rubber particles during film formation, they can migrate through a film and be leached out. Leaching out of surfactants has induced a worldwide concern on the surfactant's toxicity, biodegradability and the level of surfactant pollution which jeopardize to aquatic organisms. (Ivankovic & Hrenovic, 2010; Yuan et al., 2014)

Polymeric surfactants have gained much attention in recent decades (Raffa et al, 2015; Xia et al., 2008) Polymeric surfactants refer to molecules with higher structural complexity and at the same time able to promote surfactant properties (Raffa et al., 2015). They can be either natural or synthetic in origin. Unlike conventional surfactant, polymeric surfactant is the association of one or several macromolecular structures exhibiting hydrophilic and lipophilic characters, in which its physiochemical behavior can vary depending on whether the architecture of polymer is linear, branched or blocks (Kelsch et al., 2012; Kulkarni & Shaw, 2016; Qiang et al., 2014). One notable advantage or distinguished feature of polymeric surfactant is the high degree freedom to tailor its structure and chemical composition. The overall polymeric surfactant size, location of hydrophilic/hydrophobic groups, as well as distribution of those groups can be manipulated to a certain extent. This allows the polymeric surfactant to exhibit different properties and can be used for different widespread applications (Gelardi et al, 2016). Wibowo's team have successfully synthesized a polymeric surfactant from palm methyl ester and vinyl acetate that was able to reduce the oil and water interphase while increasing viscosity of the solution to fit the usage for chemical flooding in enhanced oil recovery application (Wibowo et al., 2021). Yang et al. (2017) reported the synthesis of chitosan derived polymeric surfactant with high surface activity. Polymeric surfactants are also heavily involved in emulsion polymerization process as a stabilizer, as reported in numerous studies (Asua, 2004; Lovell & Schork, 2020; Meuldijk et al., 2003; Tadros, 2013). Polymeric surfactants offer many performance advantages over conventional surfactants attract interest in different important applications not limited to agriculture, pharmaceutical, cosmetics, enhanced oil recovery, coating etc. (Mahmoud, 2018). Despite the applications of polymeric surfactants are widespread, there are less studies in natural rubber latex applications. A such, the present study is to synthesis a series of biocompatible palm oil-based polymeric surfactants and investigate its properties and explore the potential use in natural rubber latex application.

CHAPTER 3: METHODOLOGY

3.1 Materials

Palm oleic acid (OA) and palmitic acid (PA) were kind gifts from Southern Acids Industries Sdn. Bhd., Klang, Malaysia, whereas stearic acid (StA) was kindly given by Moi Foods Malaysia Sdn Bhd. Dimethylolpropionic acid (DMPA), methoxy poly(ethylene glycol) (MePEG), trimethylolpropane (TMP), cyclohexanedimethanol (CHDM), sebacic acid (SA), sodium dodecyl sulfate (SDS), polyethylene glycol sorbitan monooleate (Tween 80) and polysorbate 20 (Tween 20) were supplied by Merck Sdn. Bhd., Selangor, Malaysia. Four cell lines including HaCaT human keratinocytes, (ATCC PCS-200-010^{TM}), NIH/3T3 mouse fibroblasts (ATCC CRL 1658^{TM}), H2.35 mouse hepatocytes (ATCC CRL-1995^{TM}) and canine kidney cells MDCK (NBL-2) (ATCC CCL-34) were purchased from the American Type Culture Collection (ATCC), Manassas, USA. MTT agent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, was purchased from Biobasic, Markham, Canada. Fetal bovine serum (FBS) and dimethyl sulfoxide (DMSO) were supplied by Sigma-Aldrich Sdn. Bhd., Selangor, Malaysia. Dulbecco's Modified Eagle's medium (DMEM) was purchased from Gibco, Gran Island, New York, USA. Three types of bacteria strains (MSSA, *E. coli* and *K. pneumonia*) were provided by multi-disciplinary laboratory, International Medical University (IMU), Malaysia, while the pure culture of MRSA and *A. baumannii* were- kind gifts from Dr Vasanthakumari Neela from Universiti Putra Malaysia (UPM). High ammonia (HA) preserved NR latex with dry rubber content 60 wt % was purchased from Getahindus (M) Sdn. Bhd., crosslinking agents and compounding additives including sulfur, zinc oxide, zinc dibutyl dithiocarbamates (ZDMC), Wingstay-L were purchased from Bayer Co. Sdn. Bhd., Malaysia. These chemicals were reagent grade and were used without further treatment.

3.2 Methodology

3.2.1 Synthesis of palm oil-derived polymeric surfactants

Four palm oil-derived polymeric surfactants, PAS, SAS, OAS and ONS were in-house synthesized using poly-esterification reaction by one pot synthesis method and details were published elsewhere (Lee et al., 2018; Lee et al., 2019; Ling et al., 2021). The composition of each surfactant is listed in Table 3.1. The reaction was carried out under at 200 ± 5 °C in a three-necked reaction round bottom flask, equipped with a mechanical agitator and thermometer probe. Throughout the reaction, water produced from the condensation reaction between hydroxyl and carboxyl groups was collected with a Dean-stark decanter.

Table 3.1 Composition of palm oil-derived polymeric surfactants.

Polymeric surfactants	Code	Amount (% w/w)							
		OA	PA	StA	SA	MePEG	TMP	CHDM	DMPA
Palmitic acid surfactant	PAS	-	80.8	-	-	-	-	-	19.2
Stearic acid surfactant	SAS	-	-	80.8	-	-	-	-	19.2
Oleic acid anionic surfactant	OAS	80.8	-	-	-	-	-	-	19.2
Oleic acid non-ionic surfactant	ONS	16.0			28.0	28.0	10.0	18.0	-

At the end of reaction, the finished product was subjected to acid number test to evaluate the completion of the reaction. The extent of polyesterification (P) was calculated using Eq. (3.1).

$$P = \frac{A_0 - A_t}{A_0} \times 100 \tag{3.1}$$

where, P is the extent of the reaction, A_0 is the initial acid number taken at time zero, and A_t is the final acid number taken at the end of reaction.

3.2.2 Characterization of palm oil-derived polymeric surfactants

3.2.2.1 Fourier transform resonance spectroscopy (FT-IR)

Surfactant samples were subjected to FT-IR analysis using Thermo Scientific FTIR Nicolet 6700 series spectrometer. The FTIR spectrum and the results were recorded from 32 scans, ranging from 4,000 to 600 cm^{-1} at resolution of 2 cm^{-1}. The analysis was conducted at ambient temperature.

3.2.2.2 Nuclear magnetic resonance spectroscopy (^{1}H-NMR)

Surfactant samples were dissolved to 2 % w/v concentration in deuterated chloroform ($CDCl_3$). The ^{1}H-NMR spectra of the samples were recorded at ambient temperature using BRUKER 500 FTNMR spectrometer, with 32 scans recorded at a frequency of 500 MHz. Tetramethylsilane (TMS) was used as internal standard with peak appearing at 0 ppm, and peak from $CDCl_3$ appeared at 7.26 ppm.

3.2.2.3 Thermogravimetric analysis (TGA)

TGA analysis was carried out using Perkin Elmer Thermal Analyzer (STA) 6000. About 10 mg of sample was weighed and loaded into a sample pan and subjected to heating from 30 °C to 850 °C. A flow rate of 20 °C/ min was set, under a nitrogen atmosphere. Data analysis was then performed using Pyris software.

3.2.2.4 Differential scanning calorimetry (DSC)

The glass transition temperature (T_g) of the surfactants samples was evaluated using differential scanning calorimetry (DSC) analysis. The DSC analysis was performed using Mettler Toledo DSC 1, whereby solvent cyclohexane was used for temperature calibration. Data was then analyzed using STARe SW 13.00 software.

3.2.2.5 Gel permeation chromatography (GPC)

About 0.2 % w/v of surfactant sample solution in tetrahydrofuran (THF) was prepared for GPC analysis. The sample solution was then filtered using polytetrafluoroethylene (PTFE) filters with a pore size of 0.45 μm. About 100 μL of sample solution was then injected into a Malvern GPC Viscotek gel permeation chromatography (GPC) instrument operated with organic GPC/SEC columns, at a flow rate of 0.8 mL/min, using 3 x CLM3005-T5000 column. Calibration was performed using polyisoprene standards. The number-average molecular weight (M_n), weight-average molecular weight (M_w) and dispersity (Đ) of the samples were recorded accordingly.

3.2.2.6 Acid number determination

Acid number determination of the synthesized polymeric surfactants was carried out in accordance with ASTM D7253-16 (ASTM, 2016). Results were taken in triplicates and the average values were reported.

3.2.3 *In vitro* cytotoxicity study

The *in vitro* biocompatibility of four synthesized polymeric surfactants, PAS, SAS, OAS and ONS were assessed by measuring the cell viability of HaCaT human keratinocytes, (ATCC PCS-200-010TM), NIH/3T3 mouse fibroblasts (ATCC CRL 1658TM), H2.35 mouse hepatocytes (ATCC CRL-1995TM) and MDCK canine kidney cells (NBL-2) (ATCC CCL-34TM) using MTT assay (Meerloo et al., 2011). Initially, the cells were cultured in DMEM and were maintained in a humidified incubator at 37 °C. Subsequently, the cell suspensions were transferred into 96-well plates and cultured for 24 hours. The culture medium was then replaced with a fresh medium containing of six varied concentrations of the surfactant samples accordingly (3.125, 6.25, 12.5, 25, 50 and 100 μg/mL). The cells were then continued subject to the treatment for 24, 48 and 72 hours. At the end of each time interval, 10 μL of MTT dye solution was added to each well and incubated for another 4 hours at 37 °C. The absorbance of each well was read using microplate reader (Dynex Opsys MR 24100) at 570 nm. The relative cell viability was then calculated using Eq. (2) with comparison to the control wells containing cell culture medium without the surfactant samples. The experiment was conducted in triplicates to obtain an average value of results in both dose- and time-dependent manner. Meanwhile, SDS and Tween 80 were also subjected to this *in-vitro* cytotoxic test as controls for comparison purpose. It is noteworthy that SDS and Tween 80 are widely used surfactants and they are available commercially. The differences of cytotoxicity of each pair were compared using Student's t-test by Microsoft Excel 2016. Analysis outcomes with a confidence level of 95 % ($p > 0.05$) were considered statistically significant. Mean value was calculated together with the standard deviation for each surfactant sample.

$$\text{Cell viability} = \frac{[\text{A}]\text{test}}{[\text{A}]\text{control}} \times 100 \qquad (3.2)$$

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

3.2.4 Antimicrobial study

The agar dilution method was carried out in accordance to the Clinical Laboratory Standards Institute (CLSI) guideline with some minor modification (CLSI, 2012). After taking into consideration of the literature background studies and the state of matter of the synthesized surfactants, only two polymeric surfactants, OAS and ONS are subjected to this antimicrobial test. Initially, surfactant samples were dissolved in 9 % aqueous DMSO with Tween 20 (5 % v/v). A serial dilution was then prepared on these two surfactants and added to molten and tempered Mueller Hinton agar (MHA). The mixtures were mixed immediately and vigorously to give desired final concentrations. The control tube consisted of MHA without surfactant samples but with the presence of DMSO and Tween 20.

Before that, four different types of bacteria strains (MSSA, MRSA, *K. pneumoniae* and *A. baumannii*) were cultured in the Mueller Hinton broth (MHB) until its exponential phase at 37 °C. They were then diluted to reduce the concentration to 10^8 cfu/mL (equal to 0.5 McFarland). Next, the bacterial suspensions were further adjusted to 10^7 cfu/mL. Precisely 2 μL of the bacterial suspension was dropped on the surface of agar in each tube, followed by incubation at 37 °C for 20 hrs. Subsequently, the tubes were re-suspended thoroughly with fresh MHB and the number of bacteria in the suspension was determined by plate counting upon completion of the incubation. Minimum bactericidal concentration (MBC) of surfactant sample against each bacteria was evaluated by comparing to the control tube, where the lowest concentration of surfactant mixtures that results in ≥ 3-$\log_{10}$ reduction cfu/mL was considered as effective MBC (Ling et al., 2021).

3.2.5 Natural rubber latex compounding

Two surfactant solutions (OAS and ONS) were compounded with high ammonia (HA) NR latex at five different concentrations. The formulation for the latex compounding is shown in Table 3.2. The final total solid content (TSC) of the compound was adjusted to 30 wt %.

Table 3.2 Formulation for latex compounding.

Compounding ingredients	**Parts by weight (dry)**
HA NR latex (60 wt%)	100
Polymeric surfactant (20 wt%)	0.1, 0.3, 0.5, 0.8, 1.0
Potassium hydroxide (10 wt%)	0.3
Potassium laurate solution (20 wt%)[1]	0.2
Sulfur (S) dispersion (50 wt%)	0.5
Zinc dibutyldithiocarbamate (ZDBC) dispersion (33 wt%)	0.8
Zinc oxide (ZnO) dispersion (50 wt%)	0.3
Wingstay-L dispersion (50 wt%)	0.5

[1] Potassium laurate (20% wt) added as a commercial surfactant to act as control benchmark.

3.2.6 Rheological study of natural rubber latex

The rheological properties of NR latex compounds incorporated with OAS and ONS at five varied concentrations were measured using Discovery Hybrid Rheometer (DHR-2) from TA instrument, USA, with a concentric cylinder geometry measurement. An approximate of 23 mL of surfactant sample was used for every measurement at a set temperature of 25 ± 0.1 °C. In this study, the rheological behavior of HA NR latex, control (HA NR latex compounded with 0.3 phr of KOH), and NR latex compounded with OAS and ONS were evaluated and discussed. The average of triplicate readings was reported.

Initially, the viscosity data of the samples were obtained using a flow sweep method, with shear rate ranging from 0.001 to 1000 s^{-1}. Meanwhile, the viscoelastic properties of the NR latex compounds were evaluated using oscillation sweep analysis at an angular frequency of 10 rad/s, and the oscillation strain was set from 0.001 – 100 %. The fixed parameters were applied logarithmically with 10 points collected per decade.

3.2.7 Preparation of natural rubber latex films

The NR latex films with thickness of 0.2-0.4 mm was produced via dipping method. The films were left to dry at ambient temperature and subsequently cured in thermal oven (Memmert UFB 400) at 100 °C for 30 minutes.

3.2.8 Characterization of natural rubber latex film properties

The mechanical properties of NR latex films were evaluated in both aged and unaged forms. The aged films were obtained after treating the natural rubber latex films at 100 ℃ for 22 hours. All film samples were cut into dumbbell-shape with a width of 3.933 mm. The tensile strength of the films was measured using an Instron testing machine (model 5565) compliance to ISO 37:2011 method, with a 500 N loaded cell at the crosshead speed of 500 mm/min. The average of five readings per sample formulation was reported.

The percent retention (%) of tensile properties can be calculated using following Eq. (3.3).

$$\% \text{ Retention} = \frac{\text{Aged value}}{\text{Unaged vale}} \times 100 \tag{3.3}$$

Where aged value represents tensile value after aged, and unaged value represent tensile value before subject to aging.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Synthesis of palm oil-derived polymeric surfactants

In this study, four polymeric surfactants derived from palm fatty acids, namely PAS, SAS, OAS, and ONS were synthesized via polyesterification. This reaction involved the formation of ester linkages from the hydroxyl and carboxyl groups of the monomers, resulting with water as by-product. The water produced can be used to gauge the progression of the reaction, as well as the amount of functional groups that has reacted. The hydrophobic part of the surfactant is formed by the long alkyl hydrocarbon chains of palm fatty acid, and the proposed chemical reactions involved in the synthesis of the surfactants are shown in Figures 4.1 to 4.4. As displayed in Figures 4.1 to 4.3, the three anionic polymeric surfactants (PAS, SAS, and OAS) have very similar chemical structures. The reaction involves two molecules of palm fatty acids (PA, StA, and OA) with their primary acid groups react with alcohol functional groups of one molecule of DMPA. Meanwhile, for the synthesis of ONS in Figure 4.4, the formation of ester linkages is from the reaction between hydroxyl group of polyol monomers with carboxyl groups of sebacic acid and oleic acid. By considering the structure of the synthesized surfactants, the anionic polymeric surfactants (PAS, SAS, and OAS) comprised of distinguished hydrophilic and lipophilic parts. Contrastingly, ONS is a non-ionic surfactant with a more complex structure, where it constituted a block type hydrophilic polyester that serves as a polymer backbone which is available for grafting by both hydrophiles and lipophiles at both ends of the chain.

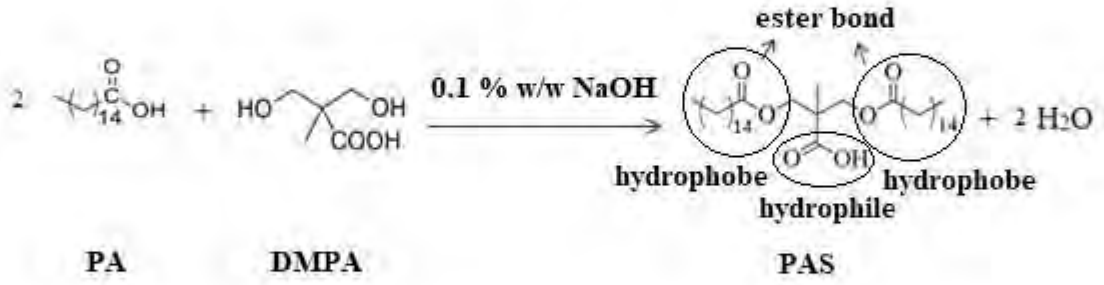

Figure 4.1: Proposed chemical reactions of palm-palmitic acid anionic polymeric surfactants (PAS).

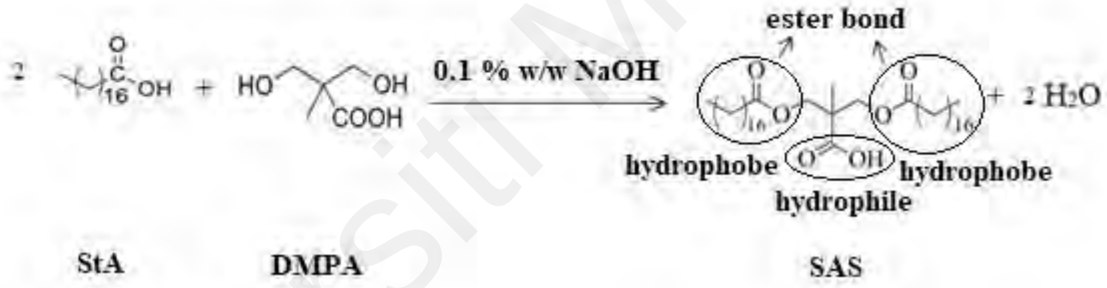

Figure 4.2: Proposed chemical reaction of palm stearic acid anionic polymeric surfactant (SAS).

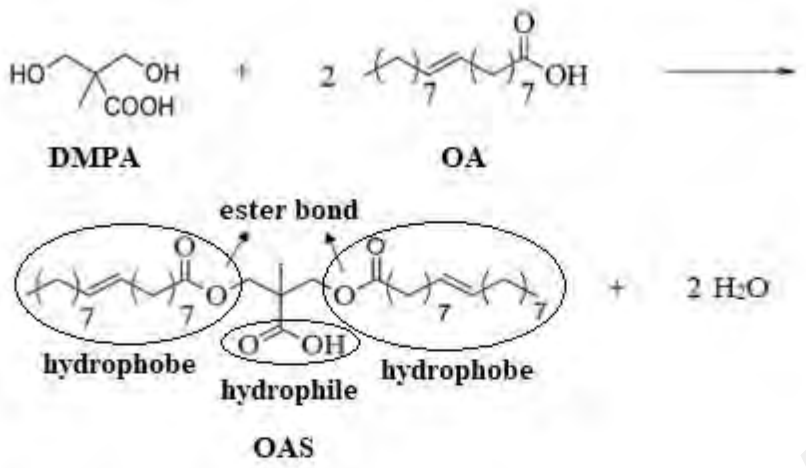

Figure 4.3: Proposed chemical reaction of palm oleic acid anionic polymeric surfactant (OAS).

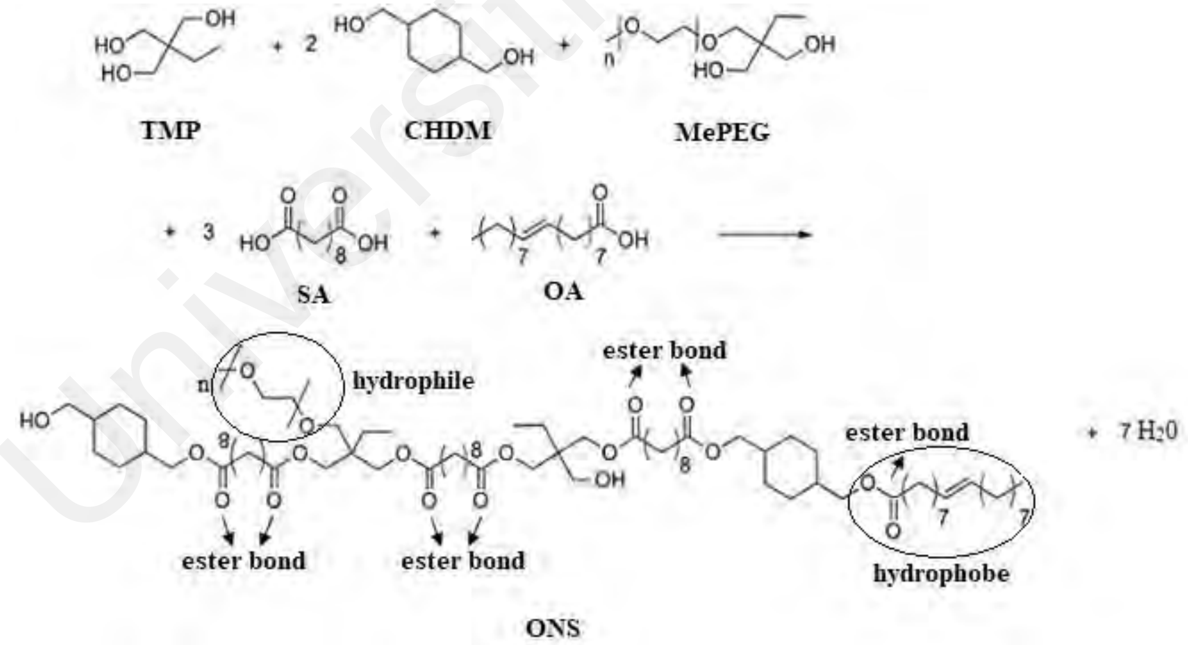

Figure 4.4: Proposed chemical reaction of palm oleic acid non-ionic polymeric surfactant (ONS).

4.1.1 Acid number determination

The extent of polyesterification (P) and acid numbers of the surfactants are recorded in Table 4.1. The ester linkages were formed from the reaction between hydroxyl groups of DMPA with carboxyl groups of palm fatty acids. The three anionic surfactants achieved equivalent extent of polyesterification, P in the range of 78 – 79 %. It is noteworthy that the P value was calculated from the changes in the acid number of the product, as per Eq. (1) in Section 3.2. The moderate P value experienced by anionic surfactants are anticipated since majority of carboxyl groups of DMPA were intentionally left unreacted. The carboxyl group serves as hydrophiles, while the hydrocarbon fatty acid chain serves as lipophiles. Presence of DMPA in their formulation explains the relatively high acid value observed in PAS, SAS and OAS. FT-IR spectra of the anionic surfactants in Figures 4.5 to 4.7 provide evidence of the esterification that took place, specifically the presence of intense peak observed in the range of 1737 – 1742 cm^{-1} which indicates the splitting of carbonyl group (C=O) of ester linkages. In contrast, an appreciably high P value of 97 % corresponding to the low acid value (6.4 mg KOH/g) was recorded for ONS. This indicates that ONS achieved nearly 100 % of the reaction conversion rate for a possible reason, in which the primary oleic acid carboxylic functional groups and primary hydroxyl groups of MePEG tend to be grafted readily to both hydroxyl and carboxylic free ends of the block-type polyester backbone. In this case, ONS is a rather linear non-ionic polymeric surfactant, with a high affinity to undergo self-assembly into an oil-in-water (O/W) micelle. The non-ionic polymeric surfactant is thus having a higher packing density with hydrophobic groups packed more tightly inside the micelle, and vice versa. This helps to improve the surfactant efficacy stability (Tan & Birge, 1996).

Table 4.1: Acid value and extent of polyesterification of palm oil-derived polymeric surfactants.

Polymeric surfactants	Extent of polyesterification, P / (%)	Acid value / (mg KOH/g)
PAS	79	80.1
SAS	78	85.3
OAS	78	88.2
ONS	97	6.4

4.1.2 FT-IR analysis of palm oil-derived polymeric surfactants

FT-IR analysis was conducted to confirm the chemical composition of the four synthesized polymeric surfactants, and the FTIR spectra of the surfactants are shown in Figures 4.5 to 4.8. Overall, the FTIR spectra of the surfactants are similar to each other except relatively more intense shoulder peaks observed in the range 1708 – 1710 cm^{-1} in the FT-IR spectra of the three anionic surfactants, OAS, PAS and SAS, and a broader band appeared at a range of 3400 – 3500 cm^{-1} in the spectrum of non-ionic surfactant, ONS. The FTIR band at 1708 – 1710 cm^{-1} in anionic polymeric surfactants is due to the carbonyl groups (-C=O) of the unreacted -COOH of DMPA which served as the hydrophilic site of the surfactants. Meanwhile, the broad band observed at 3400 – 3500 cm^{-1} in ONS is from the excess free hydroxyl group (-OH) available in the non-ionic surfactant. The characteristic band of carbonyl groups (-C=O) in ester linkage is prominent in all four polymeric surfactants, and appear as strong band at 1732 – 1742 cm^{-1}. Bands observed at 2849 – 2949 cm^{-1} correspond to aliphatic C-H stretching, whereas 1349 – 1465 cm^{-1} is from C-H bending.

Moreover, the peak observed at 1012 – 1239 cm^{-1} is from the C-O-C stretching of ester. Lastly, at 720 – 724 cm^{-1}, these are peaks that corresponds to C-H out-of-plane stretching of palm aliphatic fatty acid chains. Conclusively, these characteristic absorption bands provide further confirmation of the proposed polyester chemical structure presented in Figures 4.1 – 4.4, and reassure the success of the synthesis of the palm fatty acid ester-based polymeric surfactants.

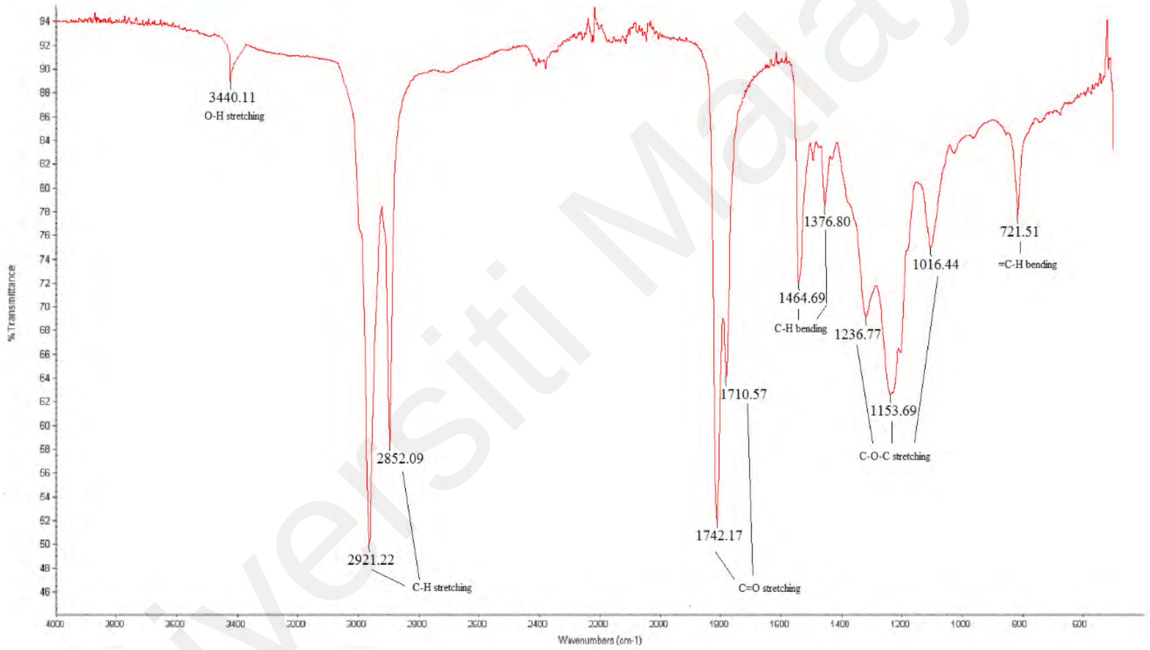

Figure 4.5: FT-IR spectrum of palm-palmitic acid anionic polymeric surfactant (PAS).

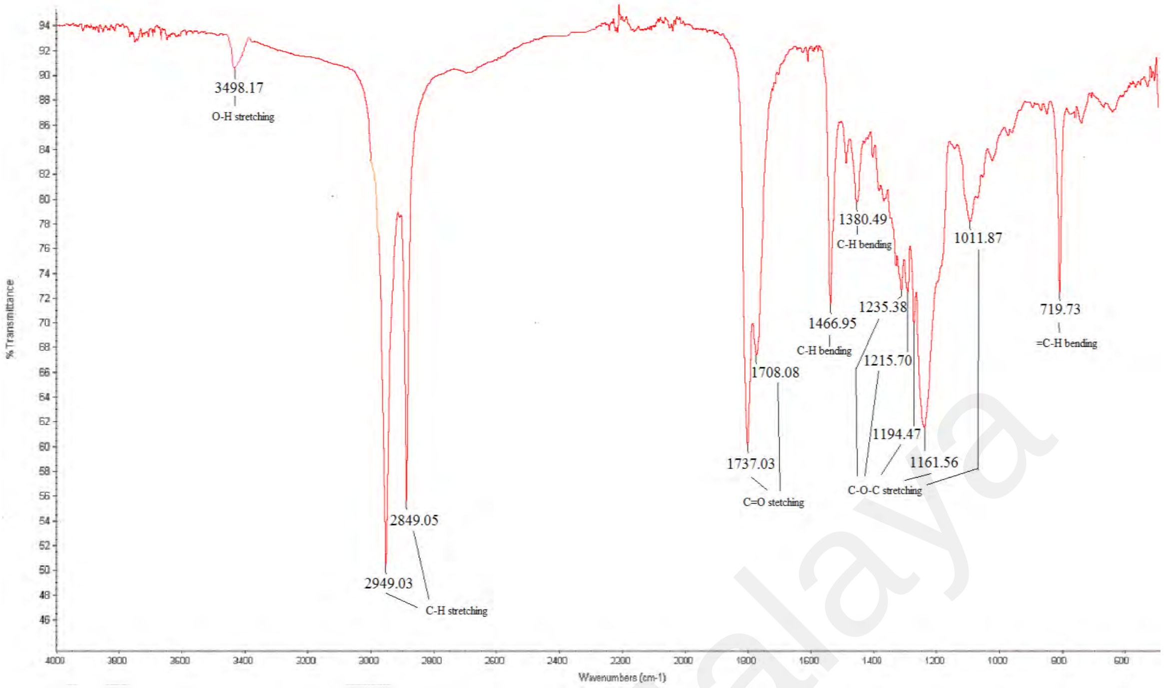

Figure 4.6: FT-IR spectrum of palm-stearic acid anionic polymeric surfactant (SAS).

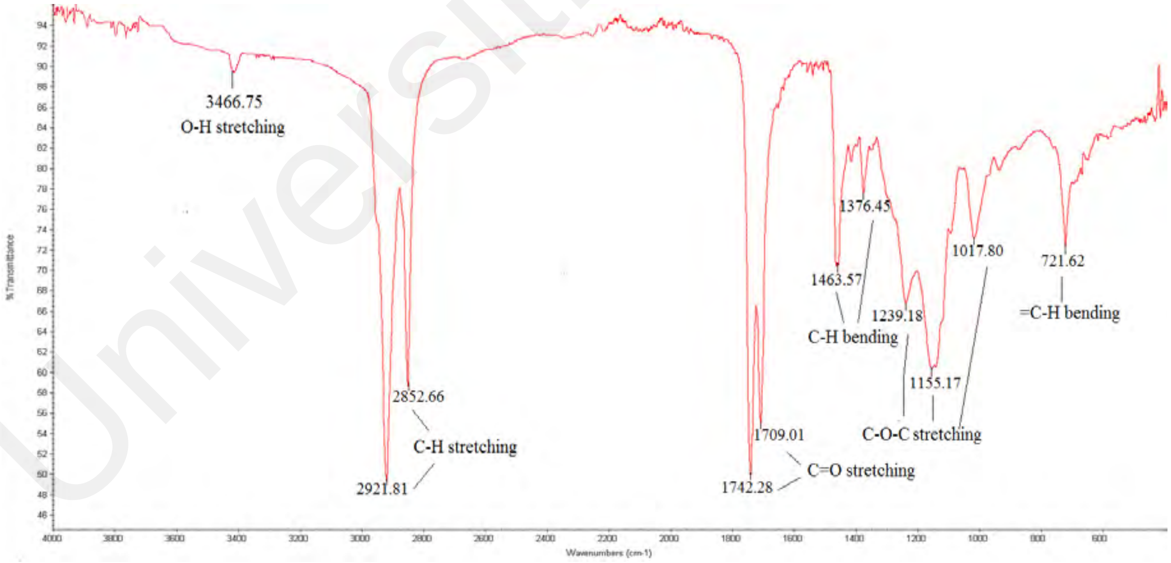

Figure 4.7: FT-IR spectrum of palm-oleic acid anionic polymeric surfactant (OAS).

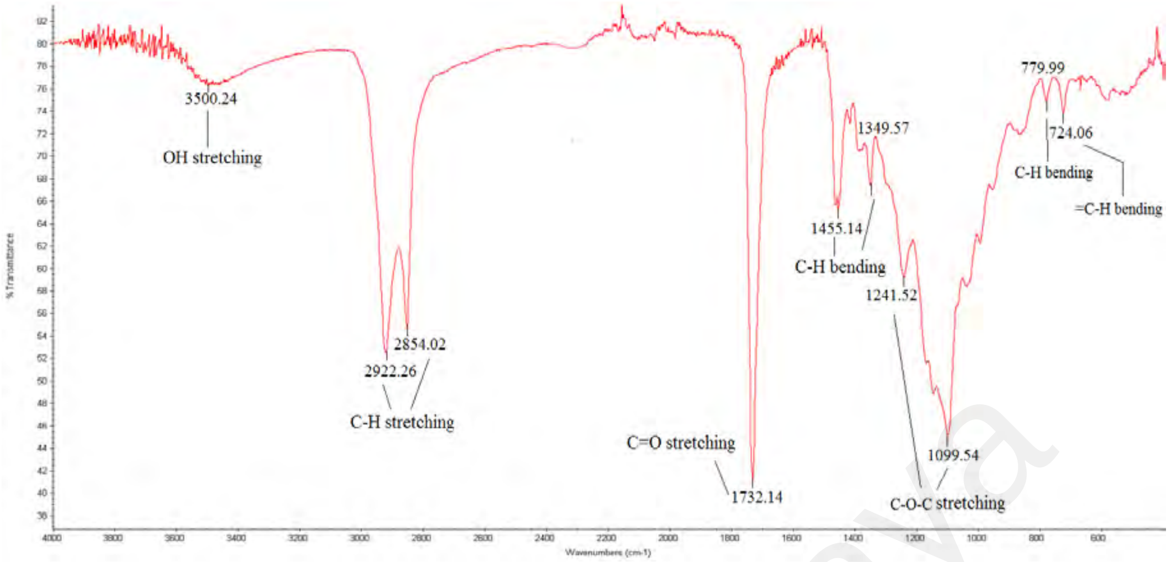

Figure 4.8: FT-IR spectrum of plam-oleic acid non-ionic polymeric surfactant (ONS).

4.1.3 ^{1}H-NMR analysis of palm oil-derived polymeric surfactants

The chemical structure of the synthesized surfactants was studied using ^{1}H-NMR, and the spectra are displayed in Figures 4.9 – 4.12. From the figures, all the surfactants have similar peaks in the chemical shifts: (i) peaks at 0.8 – 0.9 ppm that corresponds to methyl proton, -CH_3 from palm fatty acid, (ii) peaks in the region 1.3 – 1.6 ppm attributed to methylene protons, R-CH_2-R and tertiary alkyl protons, R_3-CH, (iii) peaks at 2.0 – 2.3 ppm from the methylene protons attached to the ester groups, R-CH_2COO-, and (iv) peaks in the region 4.0 – 4.2 ppm from methylene protons attached to the hydroxyl group, RCH_2OH. Peak observed at 7.3 ppm in all the spectra is from the deuterated solvent, $CDCl_3$. Detailed analysis shows that there are additional peaks appeared in the region 3.4 – 3.9 ppm in the ^{1}H-NMR spectrum of ONS, presumably from the alkyl proton, ROCH_2R which is adjacent to the ester linkages found in the chain between TMP and SA that forms the hydrophilic part of ONS. Apart from that, the vinylic protons of unsaturated fatty acid, oleic acid OAs, RCH=CHR, were found at 5.3 ppm, and are only observed in OAS and ONS. PAS and SAS were synthesized using saturated fatty acid, and this explains the absence of vinylic proton in their spectra. Taken together, result of ^{1}H-NMR analysis agrees with the proposed chemical structures in Figures 4.1 – 4.4.

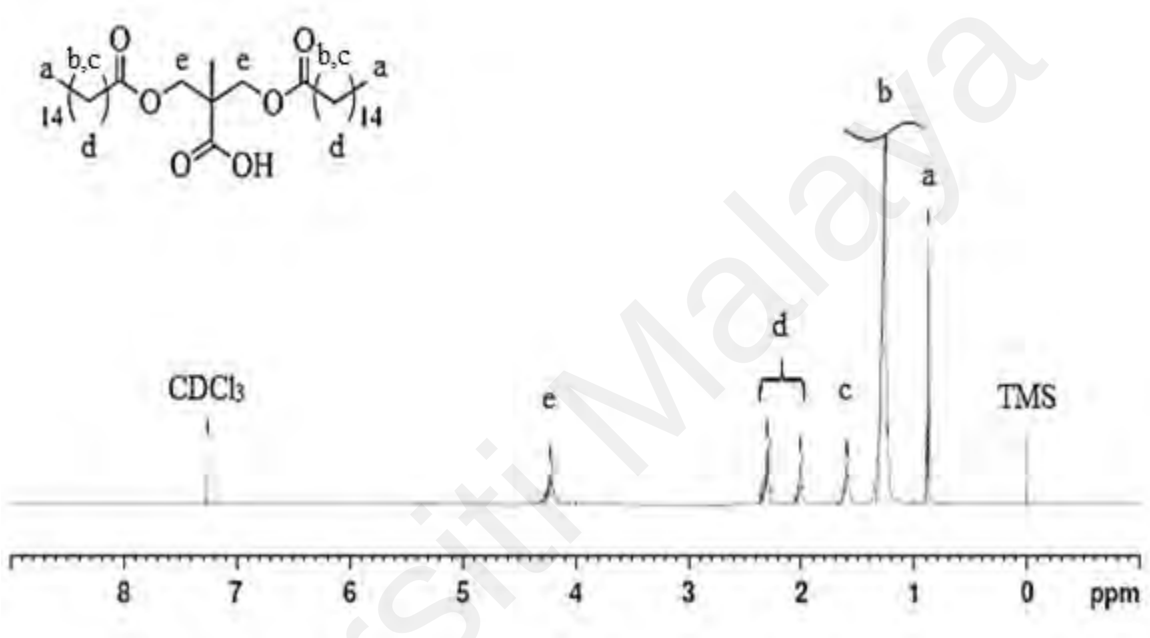

Figure 4.9: ^{1}H-NMR spectrum of synthesized palm-oil derived polymeric surfactant, PAS.

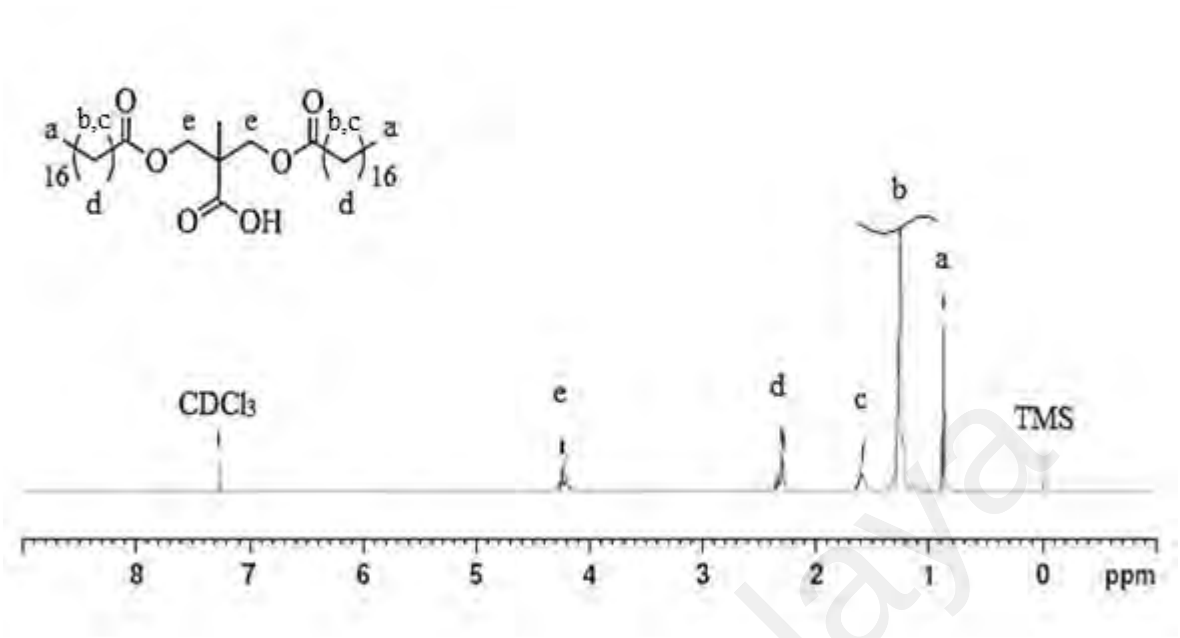

Figure 4.10: ^{1}H-NMR spectrum of synthesized palm-oil derived polymeric surfactant, SAS.

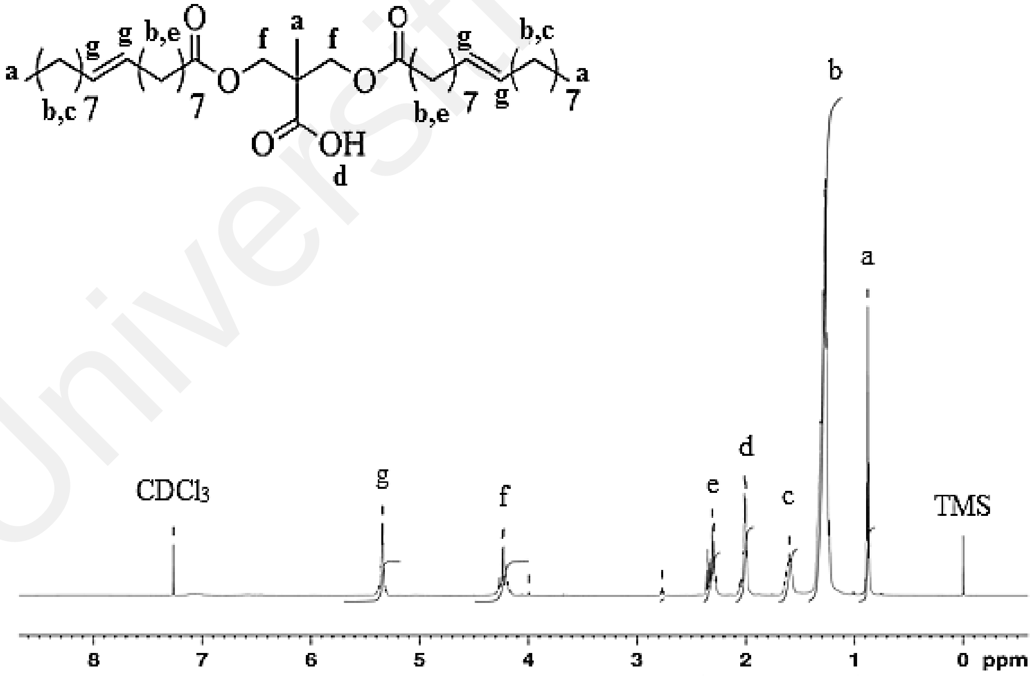

Figure 4.11: ^{1}H-NMR spectrum of synthesized palm-oil derived polymeric surfactant, OAS.

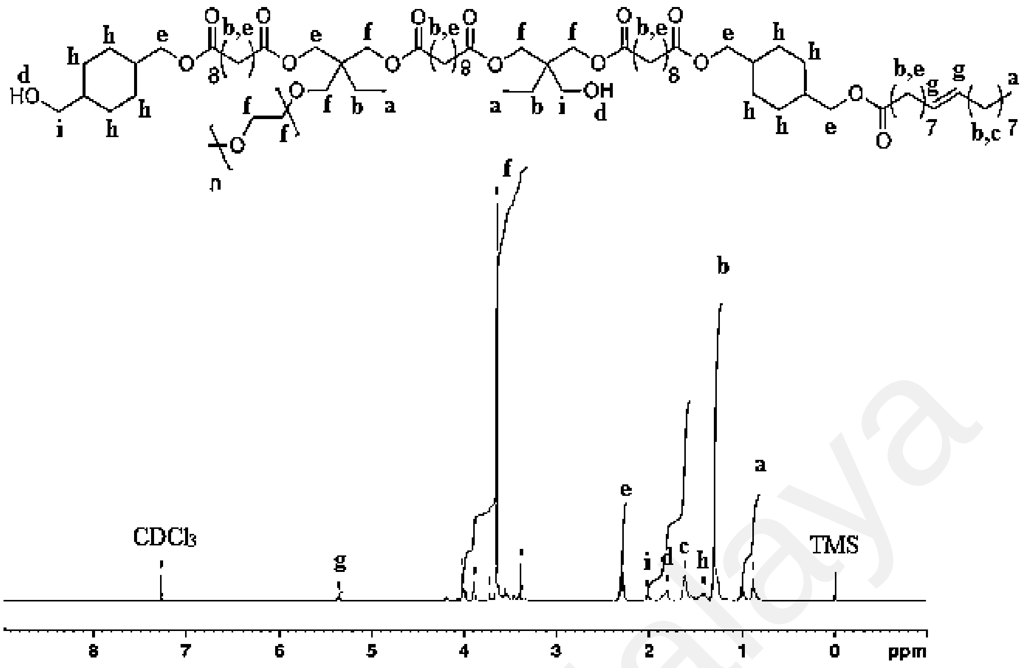

Figure 4.12: ^{1}H-NMR spectrum of synthesized palm-oil derived polymeric surfactant, ONS.

4.1.4 TGA analysis of palm-oil derived polymeric surfactants

TGA thermographs of synthesized surfactants are shown in Figures 4.13 – 4.16. In general, the TGA curves of all four polymeric surfactants displayed similar pattern of decomposition, with all of them experienced seemingly single-stage decomposition between 150 – 500 °C. However, the presence of shoulders in the derivatives curves suggests non-distinct multi-stage of thermal decomposition of the sample. The anionic surfactants (PAS, SAS and OAS) have relatively higher onset of degradation, in the range of 196 – 205 °C, while the non-ionic polymeric surfactant (ONS) on the other hand reflected a comparatively lower onset degradation temperature of 168 °C. ONS has the highest dispersity index of 2.4 among all surfactants, as shown in Table 4.2. Thus, the lower than expected onset of degradation of ONS surfactant can be attributed to the presence of low molecular weight components in the polymeric surfactant. Despite the relatively low onset of degradation shown by ONS, all four surfactants are thermally stable enough to be used for NR latex application, as the curing and processing temperatures is about 100 ºC

First derivative curves of the TGA analysis provide insight on the rate of thermal decomposition. Based on the result, the three anionic surfactants experienced the highest rate of weight loss in the region 335 – 339 ºC. Comparatively, ONS exhibited the highest weight loss peak at 432 ºC, significantly higher than those of anionic surfactant. This temperature range is presumably higher than those of anionic surfactants when bulk of the bonds in ONS are broken. This signified that ONS has the highest thermal stability compared to other anionic surfactants, which require higher amount of energy before extensive bond dissociation can take place.

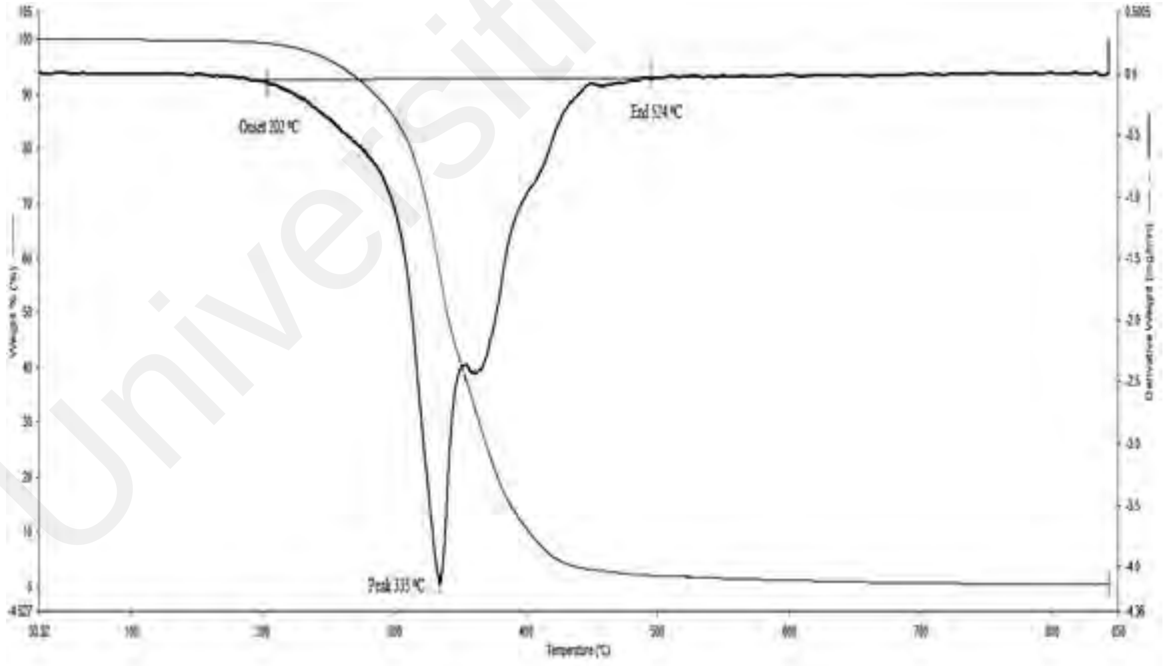

Figure 4.13: TGA thermograph of synthesized palm palmitic anionic surfactants, PAS.

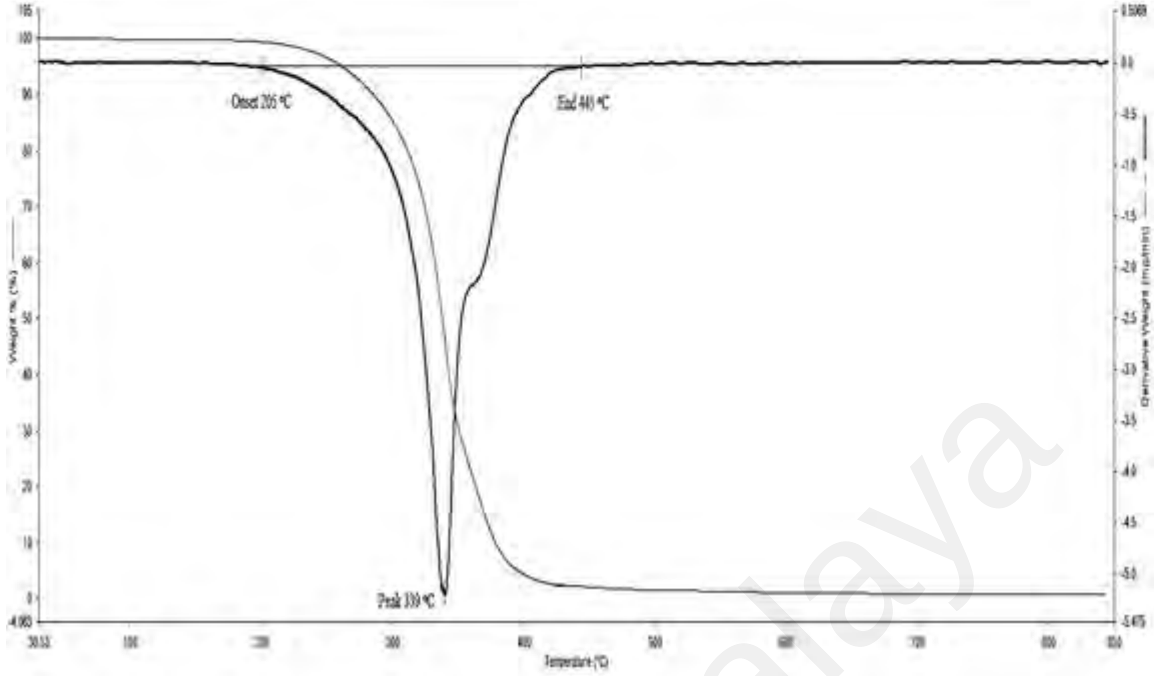

Figure 4.14: TGA thermograph of synthesized palm stearic acid anionic surfactants, SAS.

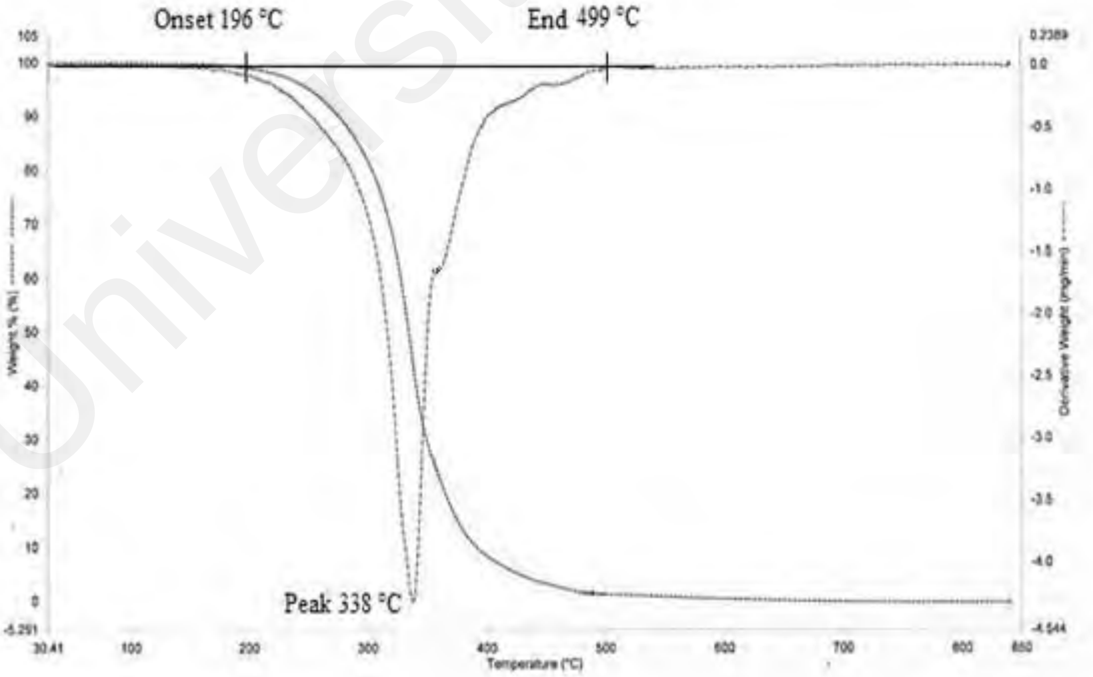

Figure 4.15: TGA thermograph of synthesized palm oleic acid anionic surfactants, OAS.

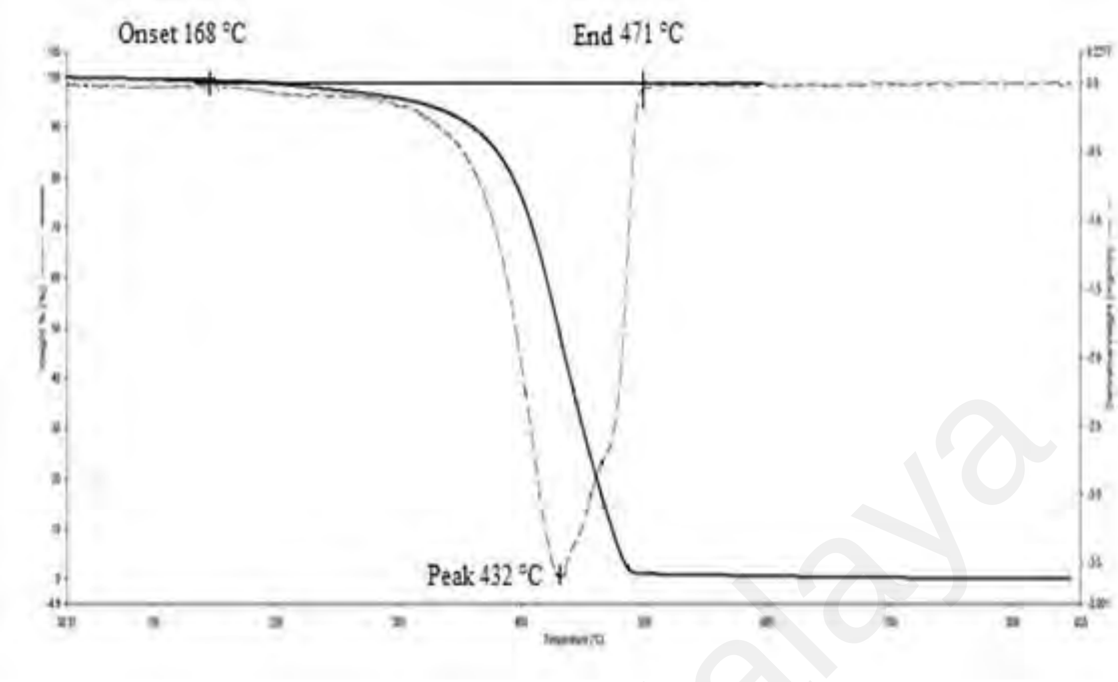

Figure 4.16: TGA thermograph of synthesized palm oleic acid non-ionic surfactants, ONS.

4.1.5 DSC and GPC analysis of synthesized palm-oil derived polymeric surfactants

The glass transition temperatures, T_g and GPC results of the synthesized palm-oil derived polymeric surfactants were tabulated in Table 4.2. From the GPC analysis, ONS exhibited the highest number-average-, M_n and weight-average molecular weight, M_w among all four surfactants. This is within expectation as the non-ionic surfactant was synthesized using more polyfunctional monomers which are capable for progressive extension of its polymer chain during the polyesterification stage. The same reason explains the relatively low molecular weight of the anionic surfactants which were largely synthesized from the reaction between monofunctional fatty acids and DMPA.

Among the surfactants, OAS has the lowest T_g value, -94.4 °C and SAS has the highest T_g value, 32.9 °C. The glass transition temperature can be affected by few factors such as molecular weight, chain stiffness, pendant groups, intermolecular forces, crosslinking, etc. (Jadhav et al., 2004). The difference in chemical structure may contribute to the difference in T_g value. In terms of anionic polymeric surfactants, SAS and PAS are saturated polymers while OAS is an unsaturated polymer. The saturated chains in the surfactants enable the molecules to experience closer packing with each other, resulting in reduced chain mobility and consequently higher amount of heat energy is needed to reach the glass transition (Barnes et al., 1988; Corkhill et al., 1987). SAS has higher T_g than PAS mainly due to the longer linear alkyl hydrocarbon chain in its polymer backbone that further restricts its mobility (Sugiyama et al., 2018).

For the non-ionic surfactant, ONS, although it has the highest molecular weight, the T_g of the surfactant is surprisingly lower than expected. The possible reason could be the presence of flexible and bulky pendant polar groups in ONS that prevents its molecules from packing closely to each other. The voids between the chains derive greater mobility and flexibility (Jadhav et al., 2004). Besides that, ONS has the largest dispersity among all four synthesized surfactants, suggesting it contains widest distribution of both small and large particles in its structure. The small particles may occupy the space between the large particles and exhibit plasticizing effect. Having high dispersity is not necessarily undesirable as the low molecular weight fraction could function as a plasticizer, while the high molecular weight fraction may induce rapid drying during the film coating. (Ngem & Mohamed, 2004; Hreczuch et al., 2016).

Table 4.2: *T_g* and GPC analysis of finished palm oil-derived polymeric surfactants, PAS, SAS, OAS and ONS.

Polymeric surfactants	T_g [1]/ °C	GPC result		
		M_n[2]	M_w[3]	Đ[4]
PAS (14)	0.5	1000	2100	2.1
SAS (16)	32.9	1000	1600	1.6
OAS	-94.4	2000	1000	2.0
ONS	-67.7	20001993	4800	2.4

[1] T_g is glass transition temperature

[2] Mn is number-average molecular weight

[3] Mw is weight-average molecular weight

[4] Đ is dispersity

4.2 *In vitro* cytotoxic study on palm-oil derived polymeric surfactants

The biocompatibility of the synthesized palm-oil derived polymeric surfactants is evaluated based on the result of the cytotoxic study using MTT assay. Four cell lines chosen for this study includes human keratinocytes (HaCaT), mouse fibroblasts (3T3), mouse hepatocytes (H2.35), and canine kidney cells (MDCK). The experiment focuses on evaluating the cell viability of synthesized surfactants against the four cell lines. SDS and Tween 80 are commercially available surfactants, and they were included in this cytotoxic study to serve as benchmark references. SDS is a conventional anionic surfactant which is often used in many cleaning and hygiene products (Arslan et al., 2018; Tadros, 2005). There are several published studies on the effectiveness of SDS in protein removal to produce deproteinized natural rubber (DPNR) latex application (Chaikumpollert et al., 2012;

Yamamoto et al., 2008; Yooyanyong et al., 2015). On the other hand, Tween 80 is a recognized biocompatible non-ionic surfactant that was found to be used extensively in the pharmaceutical field (Cristina & Lourdes, 2013). The cytotoxicity test in this study was rated according to ISO 10993-5 standard, by evaluating the cell viability relative to controls. For the interpretation of the result, percentage of cell viability above 80% is considered as non-cytotoxic; within 80 – 60 % as weakly cytotoxic, 60 – 40 % as moderately cytotoxic, and < 40% as strongly cytotoxic (Lopez-Garcia et al., 2014; ISO, 2009).

HaCaT cells or keratinocytes are predominant cell types that could be found in the layer of the skin epidermis. Skin is viewed as the first defense line against the penetration of foreign toxic matters into our bodies. Thus, the HaCaT cell was chosen for cytotoxicity study against the synthesized palm-oil derived polymeric surfactants as it could be affected first by toxic substances through direct contact. The effect of the surfactants and references towards the viability of HaCaT cells following exposure for 24, 48, and 72 hours were compared with the viability of vehicle control (V.Ctrl) cells and the results are shown in Figures 4.17 (a) – (f). The percentage of cell viability in PAS, SAS, OAS, and ONS well exceeded 80 %, indicating that they are all non-toxic towards HaCaT cell lines. The palm-oil derived polymeric surfactants can therefore be considered safe to be used for short and prolonged skin contact. Similar result was obtained for reference Tween 80 (Figure 4.17(f)), but drastic difference is observed for reference SDS (Figure 4.17 (e)). The result suggested that SDS exhibited strong cytotoxicity, in both time-dependent and dose-dependent manner. Figure 4.17 (e) revealed that at 50 µg/mL SDS, the cell viability decreased exponentially below 80 % particularly after 48 hours. When the concentration of SDS increased further to 100 µg/mL, the cell viability fell below 10 % following exposure for 24, 48 and 72 hours. This result is consistent with some of the reports on skin irritation caused by SDS. Marrakchi & Maibach

(2006) have reported SDS could cause chronic skin hypersensitivity issues in individuals. Likewise, study by Robinson et al. recommended maximum concentration limit of SDS of 1% is applied in products intended for prolonged skin contact (Robinson et al., 2010).

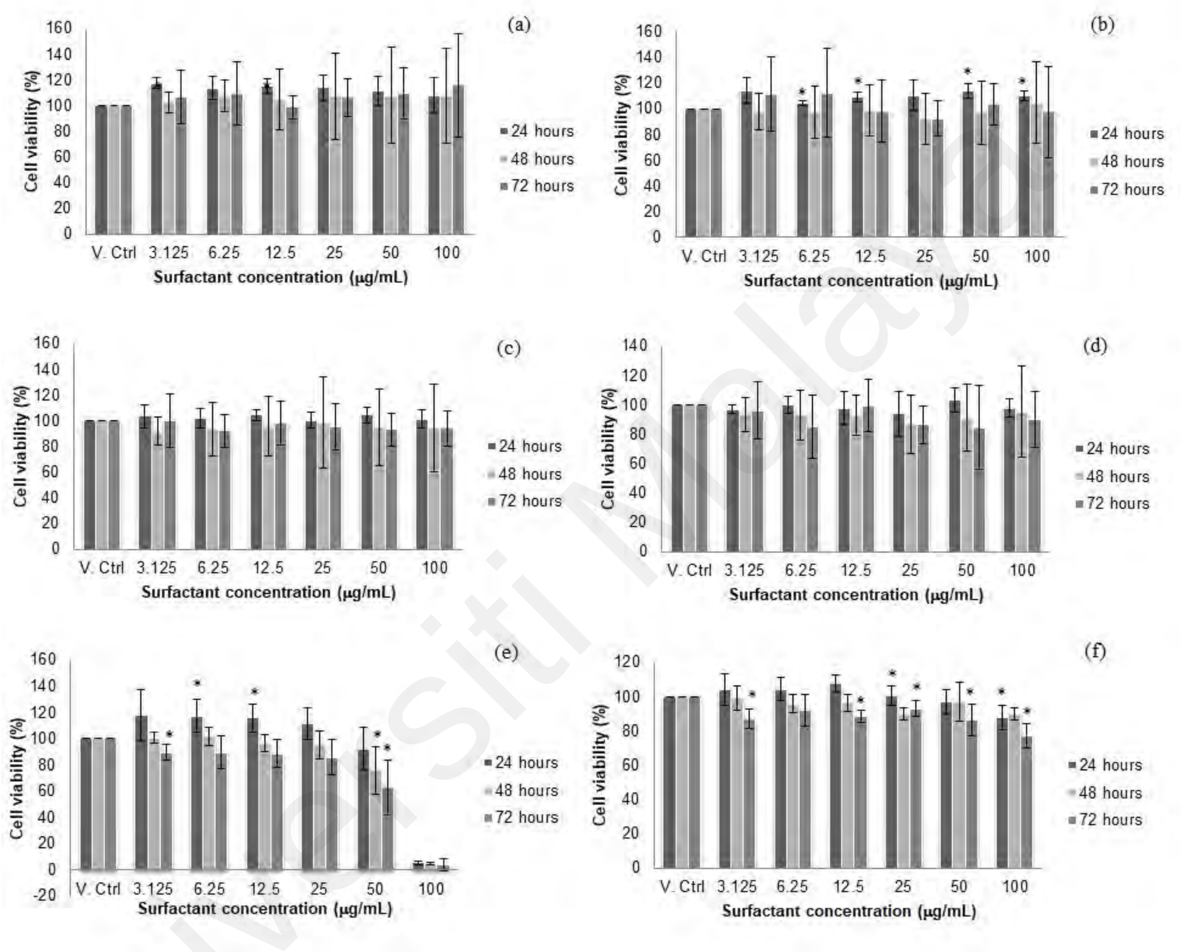

Figure 4.17: Effect of palm-oil derived polymeric surfactants (a) PAS; (b) SAS; (c) OAS; (d) ONS and reference (e) SDS; (f) Tween 80 on the cell viability of HaCaT cell lines after treatment exposure of 24, 48 and 72 hours. Cell viability was expressed in term of percentage of absorbance with 100 % representing of vehicle control cells (V.Ctrl). Result data was expressed in mean ± SD (n=3). The symbol * denotes significant differences between control and treatment group at $p < 0.05$.

3T3 was extensively used as a model system to study mitogen-related cell cycle progression (Gospodarowicz et al., 1975). The *in vitro* cytotoxicity test of the finished polymeric surfactants was also performed on 3T3 cell lines, and the results are displayed in Figures 4.18 (a) – (f). The MTT results of synthesized PAS, OAS, ONS, and reference Tween 80 are like those of HaCaT cells, where they are considered non-toxic with cell viability above 80 %. However, SAS demonstrated a significant decline in 3T3 cell viability following exposure at 100 µg/mL of surfactant concentration. This suggests that SAS exhibits moderate cytotoxicity on the fibroblasts, at an extremely high concentration of 100 µg/mL, with the cell viability remaining at 50.4 % after 72 hours exposure. There are several studies found consistent with our findings on the cytotoxic properties of stearic acids (Fermor et al., 1992; Gleeson et al., 1990). Harvey et al. (2010) have studied the growth inhibitory and pro-inflammatory of saturated fatty acids against human aortic endothelial cells and have reported that stearic acid could induce growth inhibition in human aortic endothelial cells (HAEC), and at higher concentration, it may invoke cytotoxicity. Likewise, Nordin et al. (2018) have reported that their synthesized compounds with stearic acids reflected a relatively moderate cytotoxicity against tumor cells. The results of the MTT assay for SDS (Figure 4.18 (e)) were very similar to the previous results with HaCaT cells. With these results obtained, PAS, OAS, and ONS in the present study demonstrated good biocompatible properties and it can be recommended for pharmaceutical applications, but SAS should only be used for similar application at lower concentration, preferably less than 100 µg/mL.

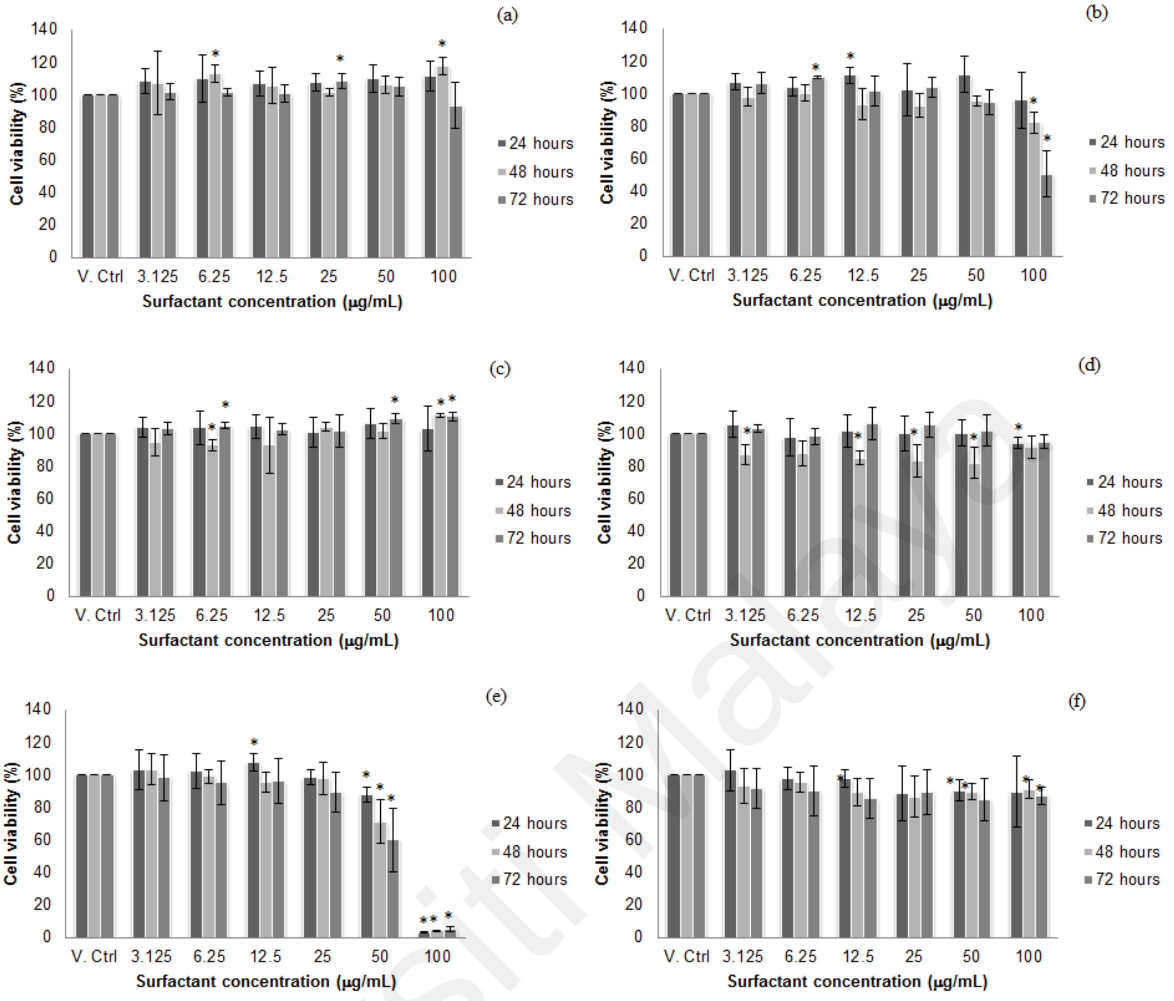

Figure 4.18: Effect of palm-oil derived polymeric surfactants (a) PAS; (b) SAS; (c) OAS; (d) ONS and reference (e) SDS; (f) Tween 80 on the cell viability of 3T3 cell lines after treatment exposure of 24, 48 and 72 hours. Cell viability was expressed in term of percentage of absorbance with 100 % representing of vehicle control cells (V.Ctrl). Result data was expressed in mean ± SD (n=3). The symbol * denotes significant differences between control and treatment group at $p < 0.05$.

The liver is one of the vital organs in the human body which has a recognized function in xenobiotic metabolism in all vertebrates. Hepatocytes (H2.35) were used to determine the hepatic toxicity of palm oil-derived polymeric surfactants. In Figures 4.19 (a) – (d), all four surfactants demonstrated high biocompatibility with cell viability above 80%. The MTT results of reference Tween 80 in Figure 4.19 (f) are equally good. As for the commercial anionic surfactant SDS shown in Figure 4.19 (e), it is highly cytotoxic against hepatocytes particularly at a concentration of 50 µg/mL and 100 µg/mL, in both time-and dose-dependent manners. At these two concentrations, cell viability fell well below 20 %. The acquired data indicates that the palm oil-based surfactants are non-toxic to liver cells and is safe for oral consumption. Therefore, all four synthesized polymeric surfactants demonstrated high potential to be utilized in oral drug formulations.

To provide comprehensive overview of the cytotoxicity of the surfactants, the last cells used in this study is canine kidney cells. Kidney is susceptible to xenobiotics due to its high blood supply and its ability to concentrate toxins. In this study, canine kidney cells MDCK (NBL-2) were used for to this cytotoxic study and served as a renal model to screen the nephrotoxicity of palm oil-derived polymeric surfactants. As shown in Figures 4.20 (a) – (d), exposing MDCK cells to the synthesized surfactants did not result in any apparent reduction in the cell viability even after 72 hours of treatment at concentration ranging from 3.125 – 100 µg/mL. As for SDS (Figure 4.20 (e)), it showed significant toxicity at high concentrations, especially at surfactant concentration of 100 µg/mL.

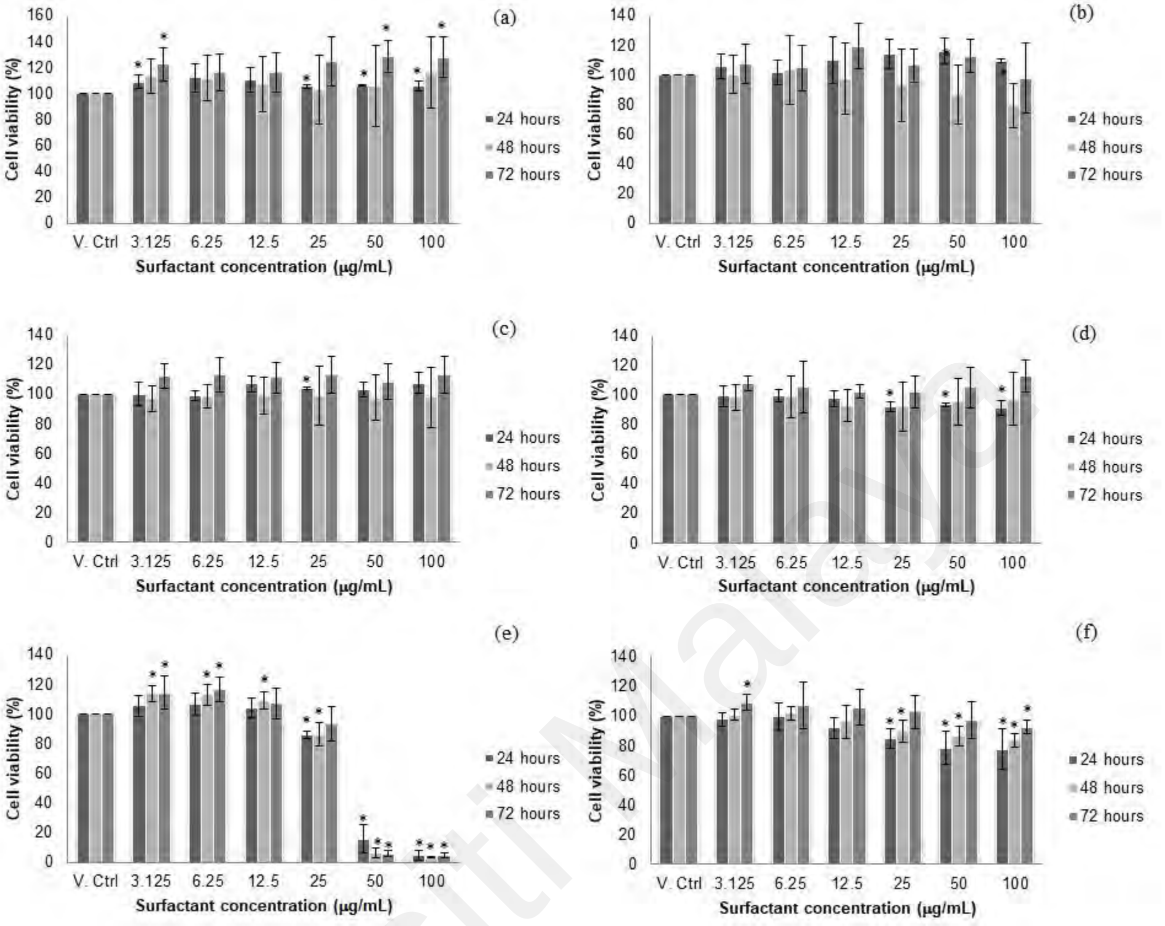

Figure 4.19: Effect of palm-oil derived polymeric surfactants (a) PAS; (b) SAS; (c) OAS; (d) ONS and reference (e) SDS; (f) Tween 80 on the cell viability of hepatocytes (H.2.35) cell lines after treatment exposure of 24, 48 and 72 hours. Cell viability was expressed in term of percentage of absorbance with 100 % representing of vehicle control cells (V.Ctrl). Result data was expressed in mean ± SD (n=3). The symbol * denotes significant differences between control and treatment group at $p < 0.05$.

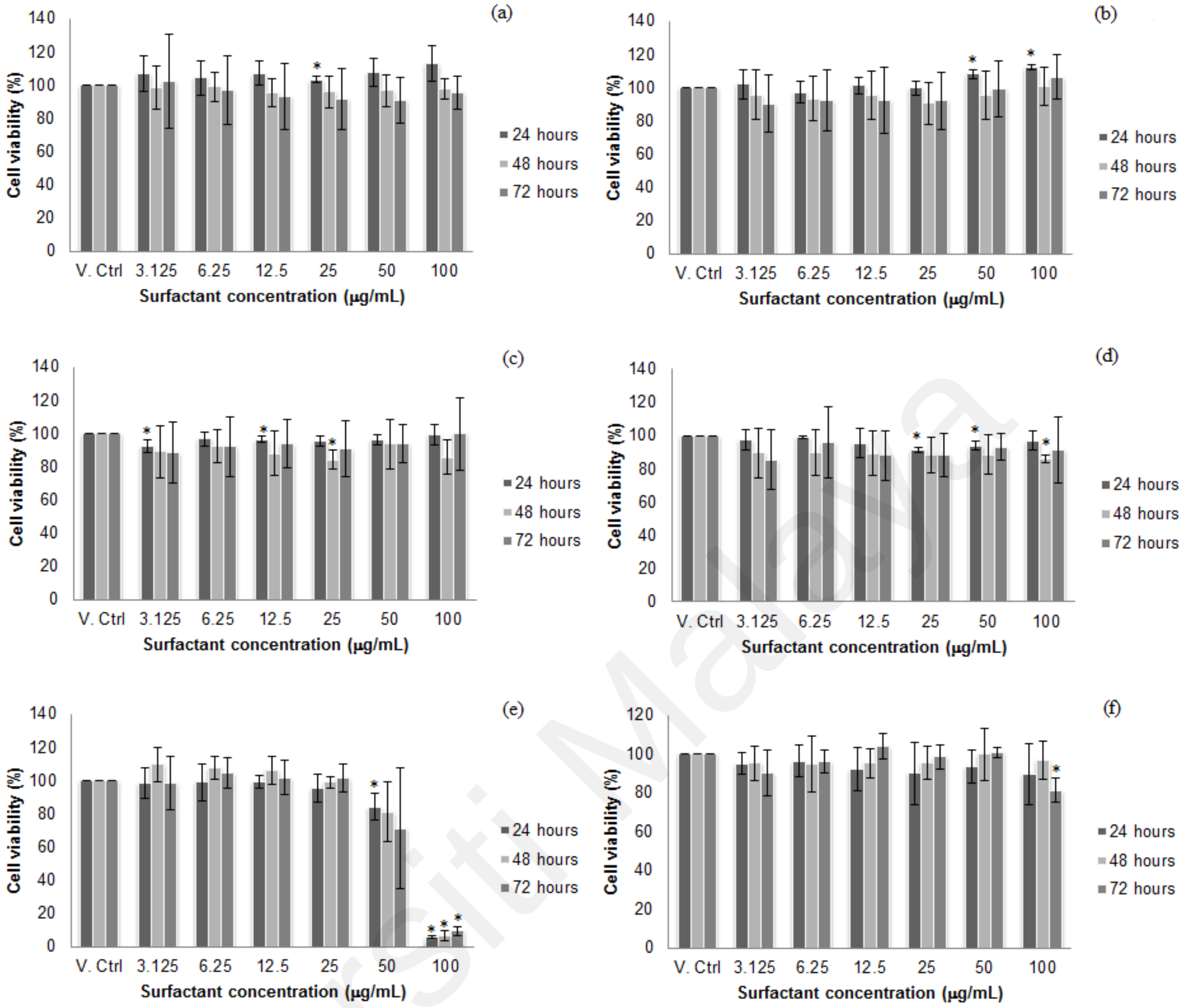

Figure 4.20: Effect of palm-oil derived polymeric surfactants (a) PAS; (b) SAS; (c) OAS; (d) ONS and reference (e) SDS; (f) Tween 80 on the cell viability of MDCK (NBL-2) canine kidney cell lines after treatment exposure of 24, 48 and 72 hours. Cell viability was expressed in term of percentage of absorbance with 100 % representing of vehicle control cells (V.Ctrl). Result data was expressed in mean ± SD (n=3). The symbol * denotes significant differences between control and treatment group at $p < 0.05$.

Taken together, the above cytotoxicity test findings against four cell lines (HaCaT, 3T3, H 2.35, and MDCK) suggests that the palm oil-derived surfactants are highly biocompatible and can be considered for applications such as additives in the pharmaceutical application, as well as for biomedical application. The use of SAS is however limited to concentration of less than 100 μg/mL. All four surfactants comprised of ester linkages which can be hydrolyzed inside the human body into non-toxic metabolites, and subsequently excreted out from the human body. This further suggests that the surfactants are harmless and reasonably safe to be used in pharmaceutical formulations.

4.3 Antimicrobial study

The antimicrobial study is conducted to explore the potential biomedical application of the palm oil-derived synthesized surfactants, particularly in medical glove application. There are numerous studies that have proven antimicrobial properties delegated by palm fatty acids (Agoramoorthy et al., 2007; Kabara et al., 1972; Knapp & Melly, 1986; Zheng et al., 2005). Desbois & Smith (2009) discussed the potential biomedical exploitation of free fatty acids. Souza et al. (2014) have also reported significant MIC result observed in fatty acids and its esters derivatives against Candida species. It is therefore interesting to investigate the antimicrobial property of the newly developed palm fatty acid-based surfactants in the current study. The antimicrobial property of two of the polymeric surfactants, OAS and ONS were evaluated using MBC assay. The other two surfactants, PAS and SAS were omitted from this study after considering the solid physical state of the surfactants at ambient temperature that may restrict their application in NR latex. According to visual observation, SAS appeared to be solid state at room temperature, with is in consistent with T_g value (32.9

°C) as shown in Table 4.2. Furthermore, PAS with T_g value of 0.5 °C appeared to be gel-like appearance and tends to be participated out upon 3 months of storage in ambient temperature.

MBC assay is a technique commonly used to determine the bactericidal activity of a particular chemical (a potential antibacterial agent) against different bacteria. A high MBC value indicates that more drug is needed to affect the organism’s function or replicability, and vice versa. Three types of bacteria were evaluated in this study, namely *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Acinetobacter baumannii* (*A. baumannii*). These bacteria were chosen because they were “superbugs” or antibiotic-resistant bacteria that could cause serious health associated infections (HAIs) and surgical site infections (SSIs) (Anestopoulos et al., 2020). The antimicrobial activities of the synthesized palm oil-derived polymeric surfactants were reported in log reduction values as clearer indication of at least 99.9% effect of reduction induced by bioactive compound (Ling et al., 2021).

Results from the antimicrobial test is summarized in Table 4.3. According to the bactericidal activity screening, OAS confers antimicrobial efficacy at concentration of 6 % v/v which significantly reduce Gram-positive bacterial *S. aureus*, including both MRSA and MSSA strains in more than 3-$\log_{10}$ reduction. Chen et al. (2011) have also indicated that anionic surfactants have antimicrobial properties towards Gram-positive bacteria which is in consistent with our findings. According to Kabara et al. (1972), fatty acid derivatives compounds demonstrate antimicrobial properties against both gram-positive and gram-negative microorganisms. Similar bactericidal effect of OAS could be observed against Gram-negative bacteria strains, including *A. baumannii* as well. However, a relatively higher concentration of about 15 % v/v of OAS was observed to adequately reduce at least 99 % of the growth of *K. pneumoniae*. Oppositely, ONS does not demonstrate antimicrobial activity for tested bacterial, except for Gram-negative *A. baumannii*, which exhibits an antimicrobial efficacy at a concentration of 2 % v/v.

Based by the MBC results observed, it can be concluded that OAS exhibits better antimicrobial properties than ONS. Despite Gram-negative bacteria have a thinner cell wall, it consists of outer lipid membrane which forms an additional protective layer, making it more resistant to be penetrated. (Kapoor et al., 2017). The MBC results showed that OAS exhibits promising inhibition of broad spectrum of bacteria, with more significant antimicrobial effect against Gram-positive bacteria (MRSA and MSSA). Yoon et al. (2018) have reported that unsaturated fatty acid with 18 carbon long chains have potent antimicrobial activity against different types of Gram-positive bacteria. Interestingly, the data highlighted that ONS exhibited antimicrobial effect against *A. baumannii* only. Oleic acid was reported to be interfere the cell membrane in an indirect manner, eventually leading to cell membrane disruption and lysis (Lee et al., 2017). The antimicrobial properties of these surfactants might presumably derived from the presence of unsaturated palm oleic acid in which constructs the hydrophobe moieties of the synthesized polymeric surfactants which potentially inhibit the growth of the tested bacteria as reported elsewhere (Anestopoulos et al, 2020; Khan et al, 2015; Perinelli et al, 2019). Taken together, the synthesized palm-oil derived polymeric surfactants might be useful in biomedical applications, particularly in the medical glove industry (Lee et al., 2021).

Table 4.3: The MBC result of synthesized palm oleic acid polymeric surfactants, OAS and ONS.

Bacteria	**MBC[1] (% v/v)**	
	OAS	**ONS**
MSSA[2]	6.0	> 2.0
MRSA[3]	6.0	> 2.0
Klebsiella pneumonia	15.0	> 2.0
Acinetobacter baumannii	6.0	2.0

[1] MBC is based on log reduction of at least 3-log$_{10}$ CFU/mL, where each value was reported as mean of independent triplicates.

[2] MSSA is methicillin-sensitive Staphylococcus aureus

[3] MRSA is methicillin-resistant Staphylococcus aureus

4.4 Rheological behavior of natural rubber latex (NRL) concentrate incorporated with synthesized polymeric surfactants

Figures 4.21 and 4.22 are plots of shear viscosity of pure NRL, control (HA NRL compounded with 0.2 phr of potassium laurate), and NRL compounded with OAS and ONS ranging from the shear rate of 0.001 s^{-1} to 1000 s^{-1}. In general, shear-thinning behavior was observed in all the samples (HA NRL, control and NRL compounded with OAS and ONS) where the latex viscosity decreased when the applied shear rate increased gradually (Tadros, 2009). This phenomenon reflects the pseudoplastic behavior of latex. Initially, when NRL was subjected to a low shear rate, the latex particles moved in a Brownian motion of rotation and tend to collide and interfere with each other, thus contributing to high viscosity. As the shear rate increased accordingly, the rubber latex particles started to deform and align with the direction of the flow. Hence, less interference between the rubber latex particles and a decrease trend in viscosity was observed. The addition of surface-active agents can modify the flow properties of latex, and this is crucial in latex applications since viscosity of the latex could greatly affects the properties of the final product (Peethambaran et al., 1990; Correa et al., 2015).

NRL compounded with OAS, particularly at higher concentration of 0.8 and 1.0 phr, displayed a comparatively higher viscosity than HA NRL in low shear rate. This could be attributed to the stronger intermolecular forces of attraction experienced between the particles (Franks et al., 2000). However, when shear stress was applied, the increasing shear force pushed the charged particles closer together, inducing a repulsive force that repel the particles to a larger separation distance, and Van der Waals forces becomes the dominant force. This explained latex exhibited more liquid-like behavior and lower viscosity, implied that NRL compounded with surfactants were easily disturbed under applied stress (Franks et al., 2000).

On the other hand, NRL compounded with ONS offered less shear thinning behavior where the reduction in viscosity is rather small with increasing shear rate as compared to those in OAS-compounded latex. Without charge, the repulsive forces between the particles at high shear rate is expected to be significantly lower and this explains the minimal reduction in the viscosity of the latex compounded with the non-ionic surfactant. The inter-molecular forces of attraction derived from its complex chemical structure could have made ONS-compounded latex to have higher shear-resistance as well, leading to insignificant viscosity loss at high shear rate. The shear thinning behavior of the ONS-compounded latex is however comparable to the control. In summary, the viscosity behavior as displayed in Figures 4.21 and 4.22 implied that a more fluid-like behavior could be observed in NRL with the addition of the palm oil-derived polymeric surfactants. Between the two, NRL incorporated with OAS demonstrated a more pseudo-plastic behavior than that with ONS, particularly at higher surfactant loading.

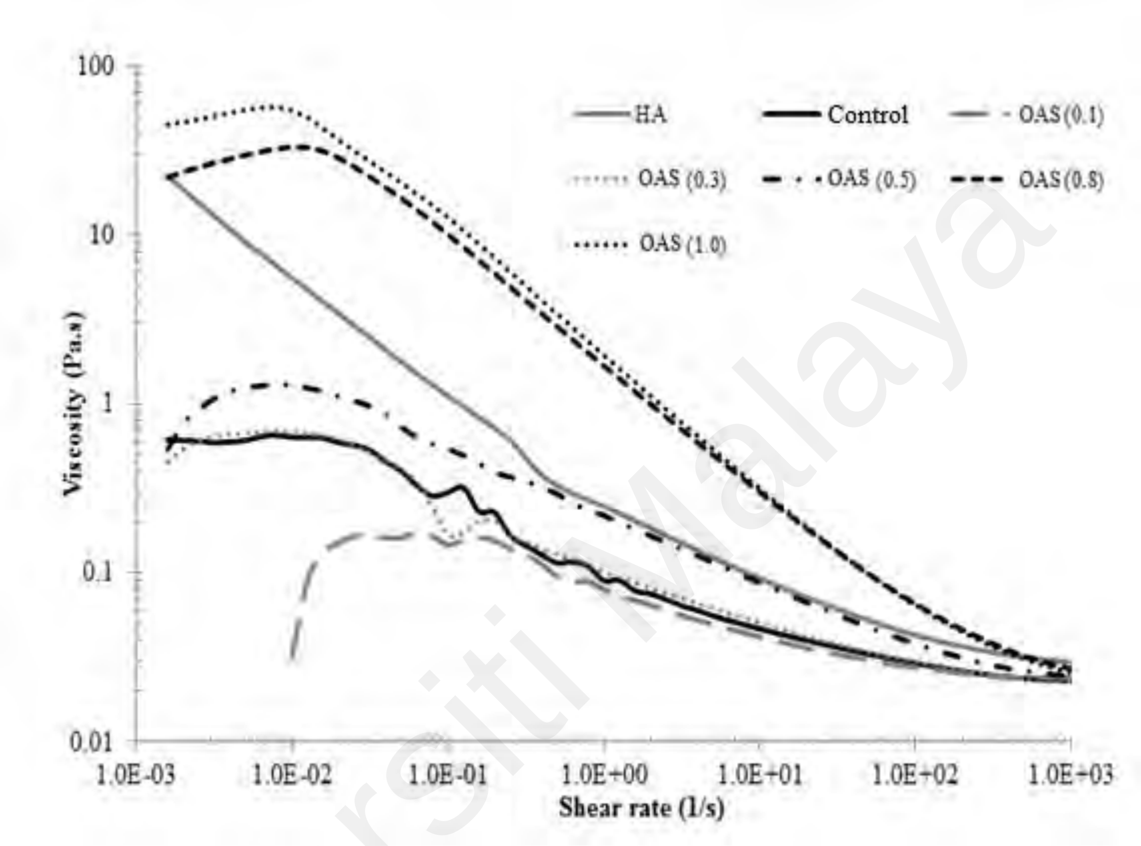

Figure 4.21: Flow sweep analysis of NRL compounds, HA NRL; control as reference and HA NRL compounded with synthesized palm oleic acid anionic polymeric surfactant, OAS, at concentration of 0.1, 0.3, 0.5, 0.8 and 1.0 phr.

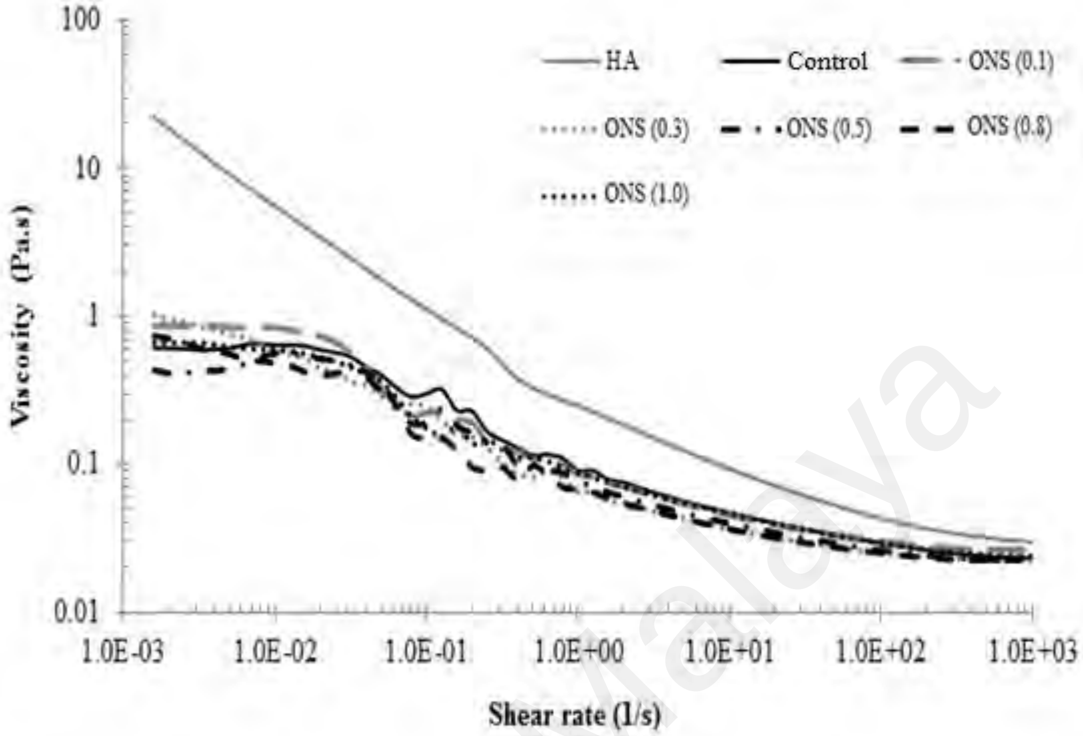

Figure 4.22: Flow sweep analysis of NRL compounds, HA NRL; control as reference and HA NRL compounded with synthesized palm oleic acid non-ionic polymeric surfactant, ONS, at concentration of 0.1, 0.3, 0.5, 0.8 and 1.0 phr.

The plots of oscillation sweep analysis of pure HA NRL, control and NRL compounded with OAS and ONS in five varied concentrations are illustrated in Figures 4.23 and 4.24. Generally, almost all NRL compounds showed dominancy of loss modulus (G") values throughout the entire strain ranges, suggesting that these NRL compounds were easily disturbed and exhibited liquid-state behaviour. However, NRL compounded with OAS, particularly at 0.8 phr and 1.0 phr behaved contrastingly, where the compounded latex demonstrated viscoelastic behaviour. As shown in Figure 4.23, NRL compounded with 0.8 and 1.0 phr OAS displayed dominancy of G' over G" values instead. The circumstances have recommended that NRL compounded with higher content of OAS experienced a more solid-like behaviour. OAS is an anionic surfactant which was anticipated to have strong electrostatic interactions with surrounding which facilitate its retention and integration within the latex system and contributed to the formation of network structure of ordered chain segments. This network structure subsequently stabilizes the latex system until a yield stress is achieved, which is the value where there is a crossover between G’ and G”. Eventually, inducing strain greater than critical yield stress will destroy the internal structures, leading to an abrupt change of the latex properties.

On top of that, the linear viscoelastic region (LVR) was found to be lengthen in NRL incorporated with OAS, particularly with increased content of OAS. Basically, LVR is the measurement of stability and usually is at the region where stress and strain waves are set at such low values that stress is proportional to strain. By referring to both figures, comparison made across pure HA NRL and control, NRL incorporated with OAS were found to have more prolonged stability, especially with a higher content at 0.8 phr and 1.0 phr. In contrast, NRL compounded with ONS observed insignificant difference in the length of LVR across all five concentrations. The LVR of latex compounds containing ONS were almost in similar length as the LVR of latex compounds with low content of OAS. As ONS is a non-ionic surfactant, it is expected that it has less interactions with surrounding compared to OAS. This resulted the formation of less network structure of ordered network structure within the latex system.The analysis suggested that NRL samples incorporated with OAS, especially at a higher concentration have shown better viscoelastic properties and higher stability, and these outcomes are desirable especially for NRL processing and applications.

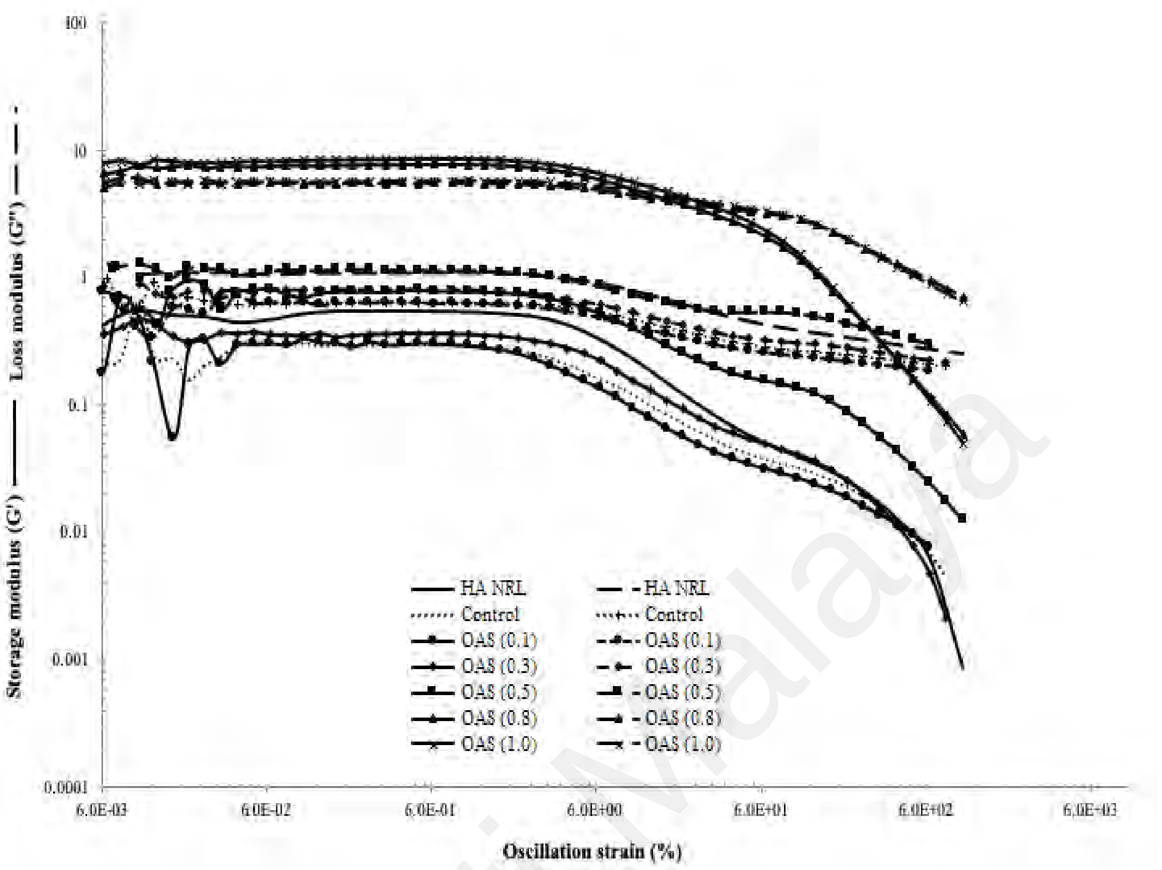

Figure 4.23: Oscillation sweep analysis of NRL compounds, HA NRL; control as reference and HA NRL compounded with synthesized palm oleic acid anionic polymeric surfactant, OAS, at concentration of 0.1, 0.3, 0.5, 0.8 and 1.0 phr.

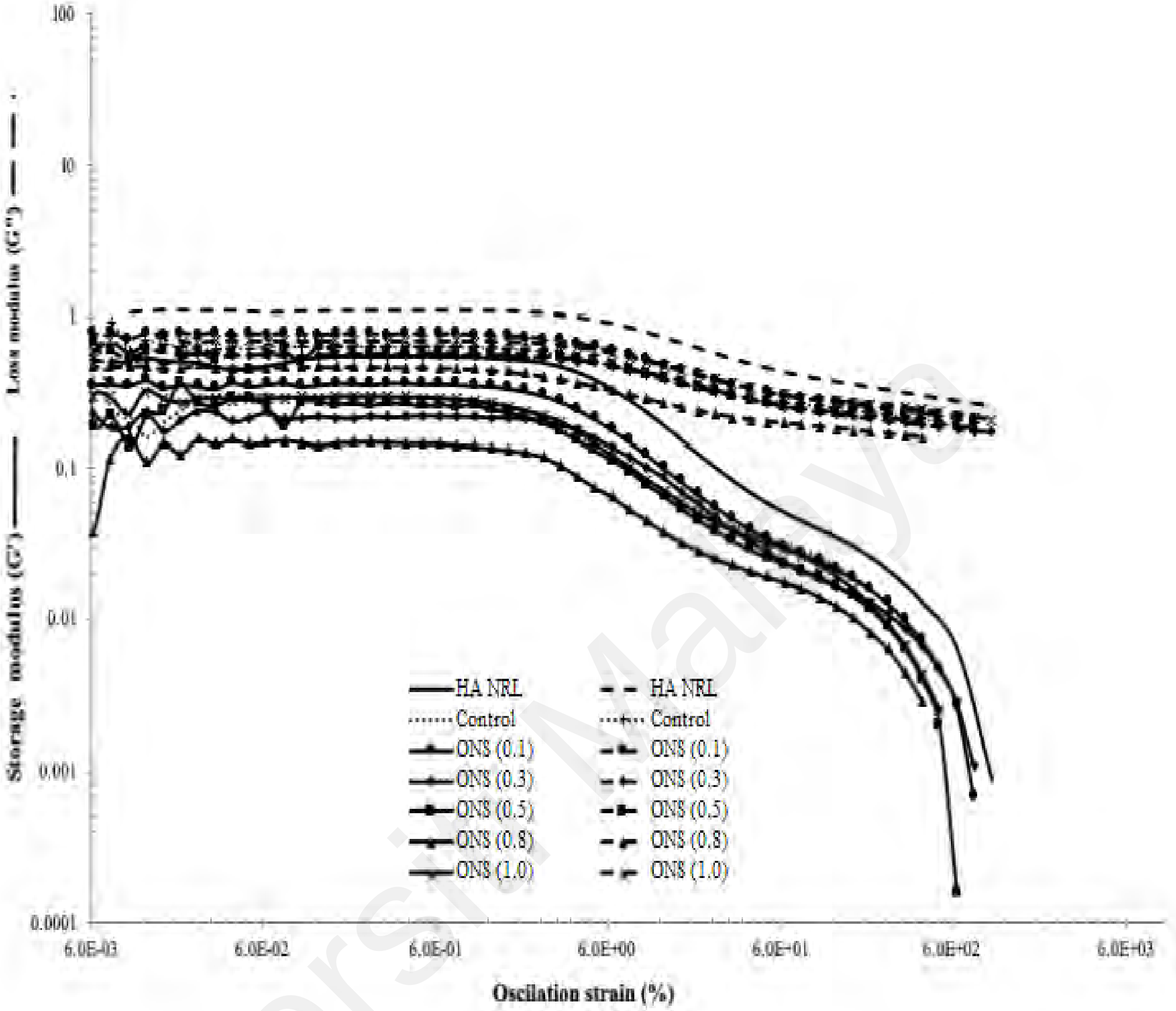

Figure 4.24: Oscillation sweep analysis of NRL compounds, HA NRL; control as reference and HA NRL compounded with synthesized palm oleic acid non-ionic polymeric surfactant, ONS, at concentration of 0.1, 0.3, 0.5, 0.8 and 1.0 phr.

4.5 Mechanical properties of NR films incorporated with palm oleic-acid polymeric surfactants (OAS and ONS)

The mechanical properties including tensile strength, elongation at break (EB), modulus 500 (M500) and tear strength of NR films incorporated with OAS or ONS at five different concentrations under unaged curing condition were studied, and the results were tabulated in Tables 4.4 and 4.5 and sketched in Figure 4.25. Generally, tear strength is to measure the ability of a material to withstand from a tearing effect. EB is to measure how far a film can be stretched before it breaks, while tensile strength measures the force needed to break the film.

As displayed in Table 4.4 & Figure 4.25, NR films compounded with OAS in unaged curing condition exhibited a significant overall reduction in mechanical properties as compared to control. The reduction in tear strength, tensile strength and M500 can be clearly observed in all five different surfactant loadings. These might be due to the plasticizing effect exhibited by OAS that made the NR film softer. This phenomenon is dictated by the mechanism governing the film formation of the NR latex film. During firm formation stage, the latex particles tend to align to form ordering arrangement and undergo deformation and compaction to eventually form a coherent film. However, addition of surfactants can disturb this orderly packing arrangement and creates some free voids in the structure (Geurts et al., 1996; Steward et al., 2000). In addition to the void, the presence of OAS molecules in the system may also weakens cohesive bonds between the latex particles throughout the film formation process (Heng et al., 2021). Consequently, reduction in mechanical properties of the OAS compounded NR film was observed. However, the mechanical properties improved with the increasing concentration of OAS. This indicated that the presence of hydrophilic material (ionic surfactant) could facilitate film drying (Feng, 1997) and thus higher concentration of OAS could have presumably improved the coalescence of films, leading to slightly increase in mechanical properties at 1.0 phr of OAS.

Interestingly, as shown in Table 4.5 & Figure 4.25, NR films incorporated with ONS revealed an insignificant difference in mechanical properties as compared to the control throughout the five varied concentration of surfactant loading, particularly 0.3 phr and 0.5 phr. The high molecular weight ONS could have presumably undergone physical entanglement with the latex particles, and this might compensate some losses in mechanical strength that could arise from the weaker cohesive bond between latex particles (Namsheer & Chandra, 2021). At the same time, no significant improvement was observed as the system could have already achieved optimum crosslinking to derive the maximum strength to the films (Dazylad et al., 2007; Roslim et al., 2018; Sasidharan et al., 2005).

Tables 4.6, 4.7 and Figure 4.26 demonstrated the mechanical properties of aged NR films compounded with OAS and ONS, respectively. In the context of material science, aging can be perceived as a degradation process that takes place when a product or material is subjected to high storing temperature, and the change on the product is usually reflected on its mechanical properties (Ngolemasango, et al., 2008; Woo & Kim, 2006). Referring to the mechanical properties of the aged and unaged control NR films incorporated with KOH in Tables 4.4 to 4.7, there is apparent reduction in tear strength, M500 and slightly decrease in tensile strength of the film after aging. This clearly indicates that there was a loss of strength and structural integrity of the control NR films, presumably from the oxidative degradation (Li & Koenig, 2005) that took place when stored under high temperature for prolonged period. NR films compounded with OAS, particularly at 0.1 – 0.8 phr however revealed higher tensile strength after it was aged. The slight increase in tensile properties of NR films loaded with OAS could be due to the formation of new cross-linkages during thermal aging with available of free sulfur in the latex system. This phenomenon also known as post-curing (Manaila, et al., 2018).

Table 4.4: Mechanical properties of unaged NR films incorporated with control, and palm oleic-acid polymeric surfactants (OAS) in five varies loading (phr).

OAS loading (phr)	Tear strength (N/mm)	EB (%)	M500 (MPa)	Tensile strength (MPa)
Control	19.0 ± 0.3	920 ± 2	2.3 ± 0.1	29.0 ± 0.6
0.1	12.0 ± 0.6	860 ± 1	1.9 ± 0.1	18.0 ± 0.8
0.3	15.0 ± 0.4	860 ± 1	2.0 ± 0.1	18.0 ± 0.6
0.5	13.0 ± 0.2	920 ± 1	1.9 ± 0.1	19.0 ± 0.3
0.8	12.0 ± 0.3	900 ± 2	2.0 ± 0.2	21.0 ± 0.6
1.0	12.0 ± 0.2	920 ± 1	2.1 ± 0.1	23.0 ± 0.2

[1] Unaged curing condition at 100 °C for 30 minutes; EB is elongation at break; M500 is modulus 500.

Table 4.5: Mechanical properties of unaged NR films incorporated with control, and palm oleic-acid polymeric surfactants (ONS) in five varies loading (phr).

ONS loading (phr)	Tear strength (N/mm)	Elongation at break, EB (%)	Modulus 500, M500 (MPa)	Tensile strength (MPa)
Control	19.0 ± 0.3	920 ± 2	2.3 ± 0.1	29.0 ± 0.6
0.1	17.0 ± 0.1	930 ± 3	1.7 ± 0.1	21.0 ± 0.3
0.3	18.0 ± 0.4	920 ± 1	2.1 ± 0.1	28.0 ± 0.5
0.5	18.0 ± 0.3	930 ± 2	2.3 ± 0.1	28.0 ± 0.8
0.8	18.0 ± 0.2	930 ± 1	2.1 ± 0.1	25.0 ± 0.3
1.0	17.0 ± 0.8	920 ± 2	2.3 ± 0.1	26.0 ± 0.3

[1] Unaged curing condition at 100 °C for 30 minutes; EB is elongation at break; M500 is modulus 500.

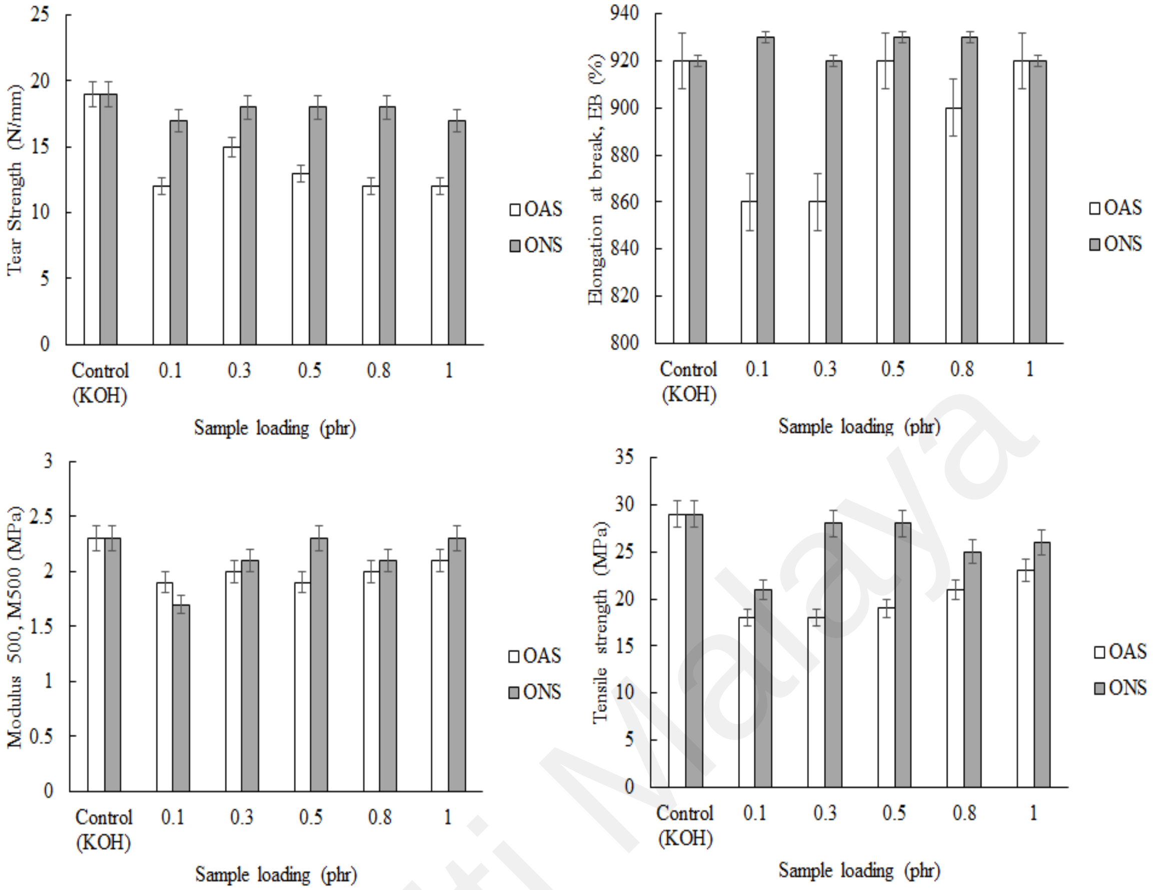

Figure 4.25: Mechanical properties (a) Tear strength, (b) Elongation at break (EB), (c) Modulus 500 (M500), and (d) Tensile strength of unaged natural rubber (NR) films incorporated with control, and palm oleic-acid polymeric surfactants, OAS and ONS in five varies loading (phr).

Table 4.6: Mechanical properties of aged NR films incorporated with control, and palm oleic-acid polymeric surfactants (OAS) in five varies loading (phr).

OAS loading (phr)	Tear strength (N/mm)	EB (%)	M500 (MPa)	Tensile strength (MPa)
Control	10.0 ± 0.2	1080 ± 1	1.7 ± 0.1	28.0 ± 0.6
0.1	9.0 ± 0.3	1060 ± 1	1.5 ± 0.1	23.0 ± 0.4
0.3	9.0 ± 0.2	1030 ± 1	1.5 ± 0.1	23.0 ± 0.3
0.5	8.0 ± 0.2	1050 ± 2	1.3 ± 0.1	22.0 ± 0.6
0.8	10.0 ± 0.1	1060 ± 2	1.4 ± 0.1	23.0 ± 0.3
1.0	8.0 ± 0.2	990 ± 2	1.6 ± 0.1	21.0 ± 0.6

[1] Aged curing condition at 100 °C for 22 hours; EB is elongation at break; M500 is modulus 500.

Table 4.7: Mechanical properties of aged NR films incorporated with control, and palm oleic-acid polymeric surfactants (ONS) in five varies loading (phr).

ONS loading (phr)	Tear strength (N/mm)	EB (%)	M500 (MPa)	Tensile strength (MPa)
Control	10.0 ± 0.2	1080 ± 1	1.7 ± 0.1	28.0 ± 0.6
0.1	10.0 ± 0.3	1070 ± 2	1.5 ± 0.1	28.0 ± 0.2
0.3	10.0 ± 0.3	1090 ± 2	1.5 ± 0.1	27.0 ± 0.2
0.5	10.0 ± 0.4	1020 ± 3	1.6 ± 0.1	26.0 ± 0.3
0.8	11.0 ± 0.1	1080 ± 3	1.5 ± 0.1	27.0 ± 0.5
1.0	10.0 ± 0.3	1010 ± 2	1.7 ± 0.1	27.0 ± 0.6

[1] Aged curing condition at 100 °C for 22 hours; EB is elongation at break; M500 is modulus 500.

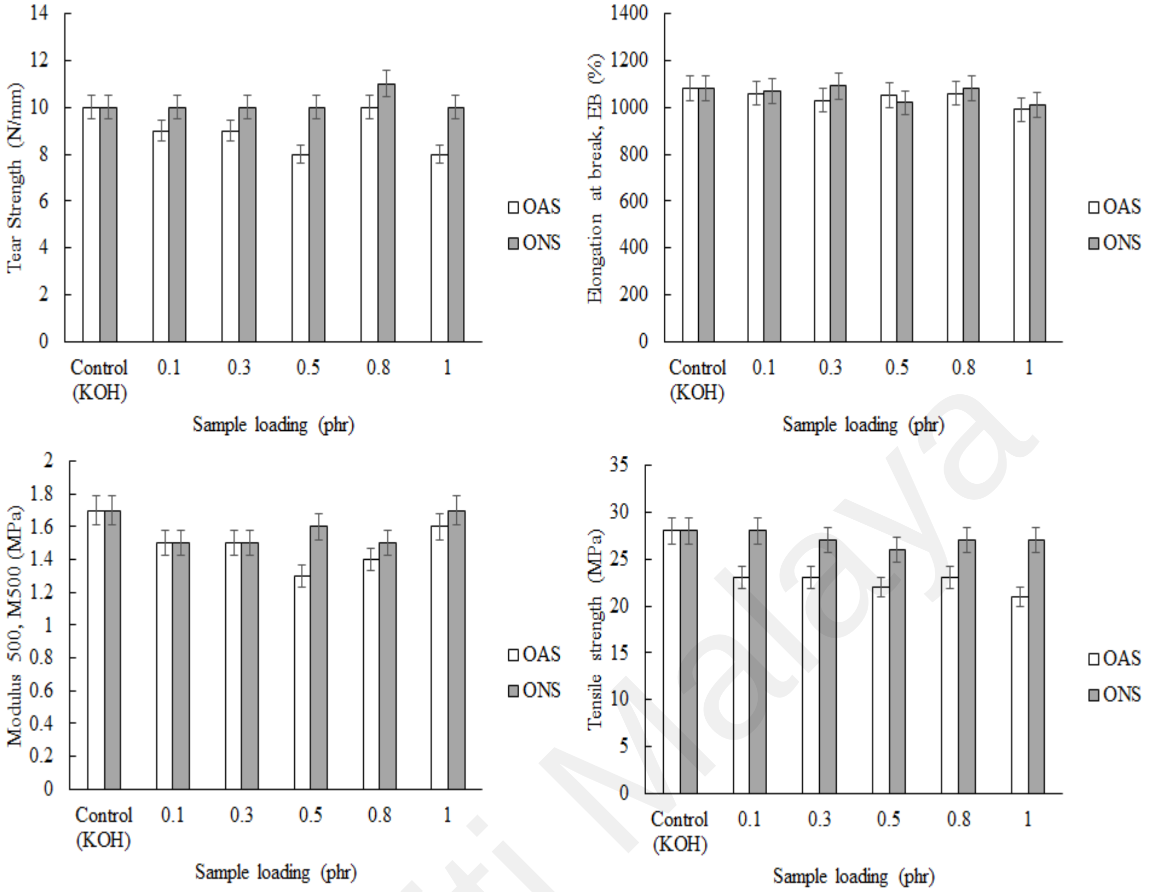

Figure 4.26: Mechanical properties (a) Tear strength, (b) Elongation at break (EB), (c) Modulus 500 (M500), and (d) Tensile strength of aged natural rubber (NR) films incorporated with control, and palm oleic-acid polymeric surfactants, OAS and ONS in five varies loading (phr).

The percent retention (%) of tensile properties was calculated and recorded in Figure 4.27 to further quantify the performance of NR films incorporated with OAS and ONS upon subjection to thermal aging for 22 hours. At 22 hours, NR films compounded with OAS exhibited a remarkable higher retention value throughout five varies loading concentrations as compared to ONS, indicating that OAS demonstrated a better aging resistance property than ONS. This is more likely due to enhance rubber particle integration during the aging process (Ramli et al., 2014). The presence of unsaturated alkyl chain of the oleic fatty acid in the surfactant could have contributed to the improved integration during the aging process. The result suggests that OAS may derive some anti-aging properties to the NR films. There is however no significant difference in the mechanical properties of the NR films compounded with ONS as compared to the control in Table 4.7 & Figure 4.26, indicating there is less aging resistance properties derived from incorporating ONS.

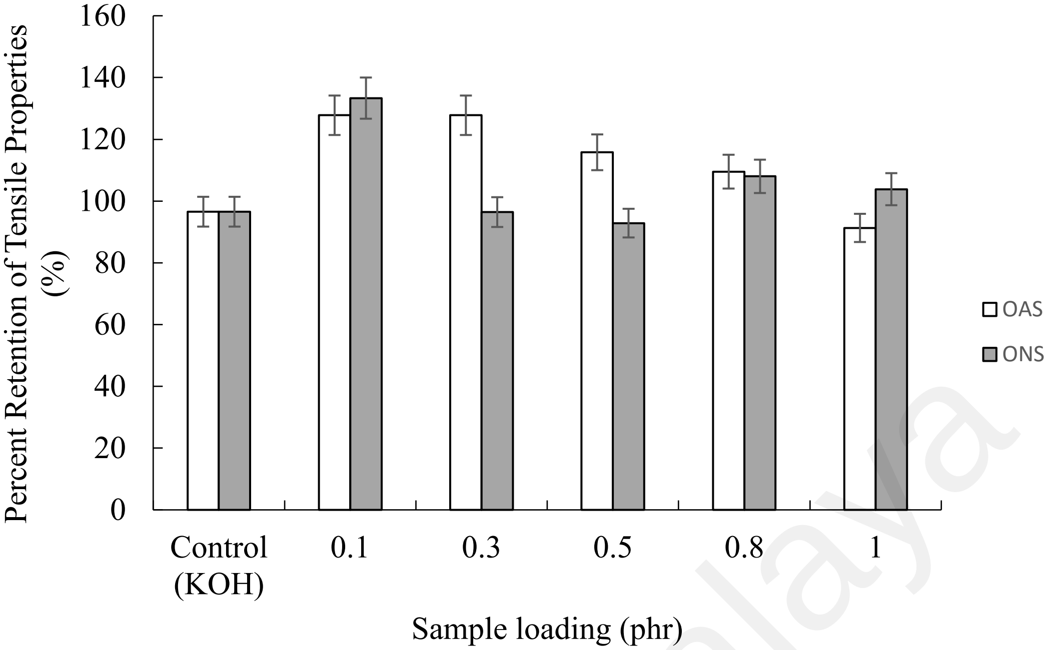

Figure 4.27: **The percent retention (%) of tensile properties of NR films incorporated with control, and palm oleic-acid polymeric surfactants, OAS and ONS in five varies loading (phr).**

CHAPTER 5: CONCLUSION AND FUTURE WORK

In a nutshell, all polymeric surfactants in this work have been synthesized from naturally sourced starting materials, specifically palm fatty acids. PAS, SAS, and OAS have similar chemical structures as confirmed by FT-IR and ^{1}H-NMR analysis. ONS tends to have a block-type polyesters backbone which is readily grafted by hydrophiles and lipophiles at both ends of the chain. ONS exhibited the lowest glass transition temperature and has the highest prospect to be used as a plasticizer, whereas SAS with the highest glass transition temperature can then be used to produce a hard film. The cytotoxic study revealed all synthesized polymeric surfactants have high biocompatibility and non-toxic against many cell types in both doses- and time-dependent manners. Thus, all of them are highly considered safe to be used in biomedical applications. However, it is recommended that the concentration of SAS shall not exceed 100 µg/mL if it were to be used for pharmaceutical applications. Apart from that, antimicrobial study showed that OAS presented better antimicrobial properties than ONS surfactant. The rheological study suggesting that NRL compounded with OAS tends to lengthen the LVR region and presented a better viscoelastic behavior and stability which beneficial to natural rubber latex (NRL) application. Mechanical properties of the NR films incorporated with OAS indicated that the film produced is relatively softer with some extent of anti-aging property.

This study has achieved the desired objectives of producing a series of biocompatible polymeric surfactants from entirely bio-sourced starting materials. The subsequent characterization that has suggested their potential to be exploited for biomedical application. The softness and better stability of OAS compounded NR latex glove tackle the glove common defects and toxicity concerns of chemical additives used in NR latex application greatly presented the potential of the synthesized polymeric surfactants to be used in natural rubber latex application.

Nevertheless, it is worth investigating the potential usage of the synthesized surfactants for synthetic rubber gloves as well. There is notable shift in demand from NR gloves to synthetic ones following the limitation in the NR latex supply. The switch from NR to synthetic rubber has been particularly evident in medical gloves. Another factor that driven the switch in medical glove is to avoid the risk of type I hypersensitivity associated with NR. Apart from that, it is a known fact that synthetic rubber such as NBR exhibit better abrasion resistance and solvent resistance. However, it tends to have lower flexibility which reduces the user's tactility and dexterity. To address this, the investigation on the incorporation of synthesized palm oil-derived polymeric surfactants into those synthetic rubbers is of interest and highly relevant.

REFERENCES

Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V., & Hsu, M. J. (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India, *Brazilian Journal of Microbiology*, *38*, 739-742.

Ahonen, E. Q., Lopez-Jacob, M. J., Vazquez, M. L., Porthé, V., Gil-González, D., García, A. M., … Project, ISTAL. (2009). Invisible work, unseen hazards: The health of woman immigrant household service workers in Spain. *American Journal of Industrial Medicine*, *53*, 405-416.

Al-Soufi, W., & Novo, M. (2021). A surfactant concentration model for the systematic determination of the critical micellar concentration and the transition width. *Molecules*, *26*, Article#5339.

American Society of Testing and Materials. (2016). ASTM D7253-16: Standard test method for Polyurethane raw materials: Determination of acidity as acid number for polyether polyols. https://www.astm.org/Standards/D7253.htm

Ana, B. M., Lorena, R., Myriam, R., Alejandro, L., Xanel, V., & Jose, M. C. (2021). Synthetic and bio-derived surfactants versus microbial biosurfactants in the cosmetic industry: An overview. *International Journal of Molecular Sciences*, *22*, 2371-2394.

Anestopoulos, I., Kiousi, D. E., Klavaris, A., Galanis, A., Salek, K., Euston, S. R., … Panayiotidis, M. I. (2020). Surface active agents and their health-promoting properties: Molecules of multifunctional significance. *Pharmaceutics*, *12*, 688.

Arayapranee, W., & Rempel, G. L. (2013). Effects of polarity on the filler-rubber interaction and properties of silica filled grafted natural rubber composites. *Journal of Polymers, 2013*.

Ardyani, T., Mohamed, A., Bakar, S. A., Sagisaka, M., Umetsu, Y., Mamat, M. H., … Eastoe, J. (2019). Surfactants with aromatic headgroups for optimizing properties of graphene/natural rubber latex composites (NRL): Surfactants with aromatic amine polar heads. *Journal of Colloid and Interface Science*, *545*, 184-194.

Arslan, A., Yopkaya, E., Bingol, D., & Veli, S. (2018). Removal of anionic surfactant sodium dodecyl sulfate from aqueous solutions by O3/UV/H2O2 advanced oxidation process: Process optimization with response surface methodology approach. *Sustainable Environment Research*, *28*, 65-71.

Asua, J. M. (2004). Emulsion polymerization: From fundamental mechanisms to process developments. *Journal of Polymer Science Part A: Polymer Chemistry*, *42*, 1025-1041.

Bajpai, D., & Tyagi, V. K. (2010). Nonionic surfactants: An overview. *Tenside Surfactants Detergents*, *47*, 190-196.

Bakand, S., Winder, C., Khalil, C., & Hayes, A. (2005). Toxicity assessment of industrial chemicals and airborne contaminants: Transition from in vivo to in vitro test methods: A review. *Inhalation Toxicology*, *17*, 775-787.

Bakand, S., Winder, C., Khalil, C., & Hayes, A. (2006). An experimental in vitro model for dynamic direct exposure of human cells to airborne contaminants. *Toxicology Letters*, *165*, 1-10.

Barnes, A., Corkhill, P. H., & Tighe, B. J. (1988). Synthetic hydrogels: 3. Hydroxyalkyl acrylate and methacrylate copolymers: Surface and mechanical properties, *Polymer*, *29*, 2191–2202.

Bhattacharya, B., Ghosh, T. K., & Das, N. (2017). Application of bio-surfactants in cosmetics and pharmaceutical industry. *Scholars Academic Journal of Pharmacy*, *6*, 320-329.

Birgin, S., Tomovska, R., & Asua, J. M. (2017). Effect of ionic monomer concentration on latex and film properties for surfactant-free high solids content polymer dispersions. *European Journal Polymer*, *93*, 480-494.

Boo, J. M. (2014). The difference between "latex", "natural rubber", "natural rubber latex" and "dry natural rubber". Retrieved from https://www.westpharma.com/en/blog/2014/December/The-Difference-Between-Latex-Natural-Rubber-Natural-Rubber-Latex-and-Dry-Natural-Rubber

Bousquet, J., Flahault, A., Vandenplas, O., Ameille, J., Duron, J., Pecquet, C., … Annesi-Maesano, I. (2006). Natural rubber latex allergy among health care workers: A systematic review of the evidence. *The Journal of Allergy and Clinical Immunology*, *118*, 447-454.

Bradford, E. B., & Vanderhoff, J. W. (1966). Morphological changes in latex films. *Journal of Macromolecular Chemistry*, *1*, 335-360.

Brown, G. L. (1956). Formation of films from polymer dispersions. *Journal of Polymer Science*, *22*, 423-434.

Butler, L. N., Fellows, C. M., & Gilbert, R. G. (2003). Effect of surfactant systems on water sensitivity of latex films. *Journal of Applied Polymer Science*, *92*, 1813-1823.

Chaikumpollert, O., Yamamoto, Y., Suchiva, K., & Kawahara, S. (2012). Protein-free natural rubber. *Colloid and Polymer Science*, *290*, 331-338.

Chen, C. H., Wang, Y., Nakatsuji, T., Liu, Y. T., Zouboulis, C. C., Gallo, R. L., … Huang, C. M. (2011). An innate bactericidal oleic acid effective against skin infection of methicillin-resistant Staphylococcus aureus: A therapy concordant with evolutionary medicine. *Journal of Microbiology and Biotechnology*, *21*, 391-399.

Chen, X., Fischer, S., & Men, Y. (2011). Temperature and relative humidity dependency of film formation of polymeric latex dispersions. *Langmuir, 27*, 12807–12814.

Chiellini, A., Chiellini, E., & Cinelli, P. (2002). Polymers from renewable resources. In Scott, G. (Eds.). *Degradable Polymers* (pp. 163-233). Dordrecht: Springer.

Clinical and Laboratory Standards Institute. (2012). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard—9th edn. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.

Collins, M. J., Martin, R. A., & Stockl, R. R. (2004). The use of surfactants as plasticizers to reduce volatile organic compounds in water-based polymer coating compositions (E.U. Patent No. EP1003819B1). European Patent Office. https://patents.google.com/patent/EP1003819B1/en

Corazza, M., Lauriola, M. M., Zappaterra, M., Bianchi, A., & Virgili, A. (2009). Surfactants, skin cleansing protagonists. *Journal of European Academy of Dermatology and Venereology, 24*, 1-6.

Corkhill, P. H., Jolly, A. M., Ng, C. O., & Tighe, B. J. (1987). Synthetic hydrogels: 1. Hydroxyalkyl acrylate and methacrylate copolymers - water binding studies. *Polymer*, *28*, 1758–1766.

Correa, H., Maria, F. A., & Furtado, C. (2015). Natural rubber latex: Determination and interpretation of flow curves. *Polimeros*, *25*, 365-370.

Cristina, P., & Lourdes, C. (2013). Performance of the biocompatible Tween 80, for the formation of microemulsion suitable for new pharmaceutical processing. *Journal of Applied Chemistry*, *2013*, 1-10.

Crowther, B. G., Lewis, P. M., & Metherell, C. (1998). Compounding. In Roberts, A. D. (Eds.). *Natural rubber Science and technology* (pp. 225). United Kingdom: Oxford University Press.

Das, K., & Mukherjee, A. K. (2007). Comparison of lipopeptide biosurfactants production by Bacillus subtilis strains in submerged and solid state fermentation systems using a cheap carbon source: Some industrial applications of biosurfactants. *Process Biochemistry*, *42*, 1191–1199.

Dazylah, D., Mazam, M. S., & Amir, H. M. Y. (2007). Changes in the micro-structure and tensile properties of peroxide prevulcanised natural rubber latex films during thermal oxidative ageing. *Journal of Rubber Research*, *10*, 222-234.

Desbois, A. P., & Smith, V. J. (2009). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*, *85*, 1629-1642.

Dillon, R. E., Matheson, L. A., & Bradford, E. B. (1951). Sintering of latex particles. *Journal of Colloid Science*, *6*, 108-117.

Eckersley, S. T., & Rudin, A. (1994). Drying behavior of acrylic latexes, *Progress in Organic Coatings*, *23*, 387-402.

Effendy, I., & Maibach, H. I. (1995). Surfactants and experimental irritant contact dermatitis, *Contact Dermatitis*, *33*, 217-225.

Effendy, I., & Maibach, H. I. (1996). Detergent and skin irritation. *Clin Dermatol*, *14*, 15-21.

Eric, H. L. T., & Robert, R. B. (1996). Correlation between surfactant/micelle structure and stability of bacteriorhodopsin in solution, *Biophysical Journal*, *70*, 2385 – 2395.

Farn, R. J. (2008). *Chemistry and technology of surfactants*. United State: John Wiley & Sons.

Felton, L. A., (2007). Characterization of coating systems. *American Association of Pharmaceutical Scientists*, *8*, Article#112.

Feng, J. (1997). Molecular and environmental aspects of latex film formation. Retrieved from https://tspace.library.utoronto.ca/handle/1807/11739

Fermor, B. F., Masters, J. R. W., Wood, C. B., Miller, J., Apostolov, K., & Habib, N. A. (1992). Fatty acid composition of normal and malignant cells and cytotoxicity of stearic, oleic and sterculic acids in vitro. *European Journal of Cancer*, *28*, 1143-1147.

Franks, G. V., Zhou, Z., Duin, N. J., & Boger, D. V. (2000). Effect of interparticle forces on shear thickening of oxide suspensions. *Journal of Rheology*, *44*, Article#759.

Frenkel, J. (1943). Viscous flow of crystalline bodies under the action of surface tension. *Journal of Physics (USSR)*, *9*, 385-398.

Gecol, H. (2006). The basic theory. In Richard, J. F. (Eds.). *Chemistry and technology of surfactans* (pp. 24-39). United States: Blackwell Publishing Ltd.

Gelardi, G., Mantellato, S., Marchon, D., Palacios, M., Eberhardt, A. B., & Flatt, R. J. (2016). Chemistry of chemical admixtures. In Pierre-Claude, A. & Flatt, R. J. (Eds.). *Science and technology of concrete admixtures* (pp. 149-218). United Kingdom: Woodhead publishing.

Geurts, J. M., Lammers, M., & German, A. L. (1996). The effect of bimodality of the particle size distribution on film formation of latices. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *108*, 295-303.

Gleeson, R. P., Ayub, M., Wright, J. T., Wood, C. B., Habib, N. A., Soutter, W. P., … White, J. O. (1990). Fatty acid control of growth of human cervical and endometrial cancer cells. *British Journal of Cancer*, *61*, 500-503.

Gospodarowicz, D., Lindstrom, J., Rudland, P., & Benirschke, K. (1975). Fibroblast growth factor: Its localisation, purification, mode of action, and physiological significance. *Advances in Metabolic Disorders*, *8*, 301-335.

Guise-Richardson, C. (2010). Redifining vulcanization: Charles Goodyear, patents and industrial control, 1834-1865. *Technology and Culture*, *51*, 357-387.

Haldeman, L. J. (1984). Stabilizer for low ammonia natural rubber latex compounds (U.S. Patent No. 4455265). Bridgestone Firestone Inc. https://patents.google.com/patent/US4455265A/en

Harvey, K. A., Walker, C. L., Xu, Z., Whitley, P., Pavlina, T. M., Hise, M., … Siddiqui, R. A. (2010). Oleic acid inhibits stearic acid-induced inhibition of cell growth and pro-inflammatory responses in human aortic endothelial cells, *Journal of Lipid Research*, *51*, 3470-3480.

Heng, Y. X., Ling, Y. T. Q., Lee, S. Y., Ang, D. T. C., & Gan, S. N. (2021). Natural rubber latex film in the presence of renewable vegetable oil nanoemulsion. *Polymer Bulletin, 78,* 2103-2120.

Ho, C. C., Kondon, T., Muramatsu, N., & Ohsima, H. (1996). Surface structure of natural rubber latex particles from electrophoretic mobility data. *Journal of Colloids and Interface Science*, *178*, 442-445.

Hoh, A., & Maier, K. (1993). Comparative Cytotoxicity Test with Human Keratinocytes, HaCaT Cells, and Skin Fibroblasts to Investigate Skin-Irritating Substances. In Bernd, A., Bereiter-Hahn, J., Hevert, F., & Holzmann, H. (Eds.), *Cell and tissue culture models in dermatological research* (pp. 341-347). Berlin: Springer.

Hreczuch, W., Dabrowska, K., Chrusciel, A., Sznajdrowska, A., & Materna, K. (2016). 2-Ethylhexanol derivatives as nonionic surfactants: Synthesis and properties. *Journal of Surfactants and Detergents, 19*, 155-164.

Ibragimov, A., & Arshad, F. M. (2017). Exploring relationship between rubber productivity and R&D in Malaysia. *Outlook on Agriculture*, *46*, 28-35.

Ivankovic, T., & Hrenovic, J. (2010). Surfactants in the environment. *Archives of Industrial Hygiene and Toxicology, 61*, 95-110.

Jadhav, N. R., Gaikwad, V. L., Nair, K. J., & Kadam, H. M. (2009). Glass transition temperature: basics and application in pharmaceutical sector, *Asian Journal of Pharmaceutics*, *3*, 82-89.

Joseph, R. (2013). *Practical Guide to Latex Technology*. England: Smithers Rapra Technology.

Juhue, D., & Lang, J. (1993). Effect of surfactant postadded to latex dispersion on film formation: a study by atomic force microscopy. *Langmuir*, *9*, 792-796.

Kabara, J. J., Swieczkowski, D. M., Conley, A. J., & Truant, J. P. (1972). Fatty acids and derivatives as antimicrobial agents. *Antimicrobial Agents and Chemotherapy*, *2*, 23-28.

Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology, Clinical Pharmacology*, *33*, 300-305.

Karlova, T., Polakova, L, Smidrkal, J., & Filip, V. (2010). Antimicrobial effects of fatty acid fructose esters. *Crezh Journal of Food Sciences*, *28*, 146-149.

Keddie, J. L. (1997). Film formation of latex. *Materials Science and Engineering: R: Reports*, *21*, 101-170.

Keddie, J. L., & Routh, A. F. (2010). Fundamentals of latex film formation. Germany: Springer.

Kelsch, A., Tomcin, S., Rausch, K., Barz, M., Mailander, V., Schmidt, M., … Zentel, R. (2012). HPMA copolymers as surfactants in the preparation of biocompatible nanoparticles for biomedical application. *Biomacromolecules*, *13*, 4179-4187.

Kenar, J. A., Moser, B. R., & List, G. R. (2017). Fatty acids: Naturally occurring fatty acids: source, chemistry and uses. In Moghis, U. A. (Eds.). *Fatty Acids* (pp. 23-82). United State: Academic Press and AOCS press.

Kendall, K., & Padget, J. C. (1982). Latex coalescence. *International Journal of Adhesion and Adhesives*, *2*, 149-154.

Khan, M. F., Zepeda-Velazquez, L., & Brook, M. A. (2015). Tunable, antibacterial activity of silicone polyether surfactants. *Colloids and Surfaces B: Biointerfaces*, *132*, 216–224.

Kientz, E., & Holl, Y. (1993). Distribution of surfactants in latex films. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *78*, 255-270.

Kirti, S. (2016). Defects reduction using root cause analysis approach in gloves manufacturing unit. *International Research Journal of Engineering and Technology*, *3*, 173-183.

Knapp, H. R., & Melly, M. A. (1986). Bactericidal effects of polyunsaturated fatty acids. *The Journal of Infectious Diseases*, *154*, 84-94.

Kong, Y. Y., Hu, B. J., Choy, K., Li, X. Y., & Chen, G. D. (2015). Study of miniemulsion formulation containing 1-octyl-3-methylimidazolium hexafluorophosphate for its application in low-emitting coating products. *Soft Matter*, *11*, 1293-1302.

Kronberg, B., Holmberg, K., & Lindman, B. (2014). *Surface chemistry of surfactants and polymers*. United States: John Wiley & Sons.

Kulkarni, V. S., & Shaw, C. (2016). *Essential chemistry of formulators of semisolid and liquid dosages*. United State: Acedemic Press.

Lee, J. K., Kim, D. B., Kim, J. I., & Kim, P. Y. (2000). In vitro cytotoxicity tests on cultured human skin fibroblasts to predict skin irritation potential of surfactants. *Toxicology In Vitro*, *14*, 345-349.

Lee, S. Y. (2018). Palm based polymeric surfactants for improved latex film formation. In: 9th Internal Rubber Glove Conference and Exhibition (IRGCE), Kuala Lumpur Convention Centre (KLCC), Kuala Lumpur, Selangor.

Lee, S. Y., Ng, A., Singh, M. S. J., Liew, Y. K., Gan, S. N., & Koh, R. Y. (2017). Physiochemical and antimicrobial properties of natural rubber latex films in the presence of vegetable oil microemulsions. *Journal of Applied Polymer Science, 134,* Article#44788.

Lee, S. Y., Chan, E., Chan, H. H., Li, C. C. K., Ooi, Z. H., Koh, R. Y., & Liew, Y. K. (2021). Antimicrobial agents and anti-adhesion materials for medical and surgical gloves. *Rubber Chemistry and Technology*, *94*, 1-23.

Lee, S. Y., Ling, Y. T. Q., & Heng, Y. X. (2019). An environment-friendly palm fatty acid-based polymeric surfactants for coating applications: Physiochemical, surface tension and low-foaming properties, *Journal of Polymers and the Environment*, *27*, 2707-2719.

Lee, S. Y., Heng, Y. X., Ling, Y. T. Q., Ang, D. T. C., & Mok, K. L. (2021). Non-cytotoxic palm-based polymeric surfactant as a green stabilizer in natural rubber latex. *Journal of Rubber Research*, *24*, 423-433.

Li, G., & Koenig, J. L. (2005). A review of rubber oxidation. *Rubber Chemistry and Technology, 78,* 355-390.

Li, Z. (2020). Modern Mild Skin Cleansing. *Journal of Comestics, Dermatological Sciences and Applications*, *10*, 85-98.

Lim, H. M., & Misni, M. (2018). Effect of sodium dodecyl sulphate and polyoxyethylene dodecyl ether on the rheological behavior and stability of natural rubber latex. *ASM Science Journal*, *11*, 44-55.

Lim, H. M., & Misran, M. (2013). Effect of sodium dodecyl sulfate, brij 35 on natural rubber latex properties. *Journal of Rubber Research*, *16*, 162-168.

Lin, F., & Meier, D. J. (1996). Study of latex film formation by atomic force microscopy. 2. Film formation vs rheological properties: Theory and experiment. *Langmuir*, *12*, 2774-2780.

Ling, Y. T. Q., Heng, Y. X., Chan, H. H., Yap, Y. J., Lee, S. Y., Koh, R. Y., … Mok, G. L. (2021). Evaluation of biocompatible palm-based polymeric surfactants for potential natural rubber latex stabilization applications, *Journal of Rubber Research*, *24*, 369-380.

Ling, Y. T. Q., Yap, Y. J., Heng, Y. X., Lee, S. Y., Koh, R. Y., Ang, D. T. C., … Gan, S. N. (2021). Physiochemical and in vitro cytotoxicity properties of biocompatible palm fatty-acid based polyesters, *Sains Malaysiana*, *50*, 395-407.

Loffler, H., & Happle, R. (2003). Profile of irritant patch testing with detergents: sodium lauryl sulphate, sodium laureth sulphate and alkyl polyglucoside. *Contact Dermatitis*, *48*, 26–32.

Lovell, P. A., & Schork, F. J. (2020). Fundamentals of emulsion polymerization. *Biomacromolecules*, *11*, 4396-4441.

Lushniak, B. D. (2000). Occupational skin diseases. *Primary Care*, *27*, 895-916.

Ma, Y., Davis, H. T., & Scriven, L. E. (2005). Microstructure development in drying latex coatings, *Progress in Organic Coatings*, *52*, 46-62.

Mahli, D. M., Steffenhagen, M. J., Xing, L., & Glass, J. E. (2003). Surfactant behavior and its influence on the viscosity of associative thickeners solutions, thickened latex dispersions, and waterborne latex coatings. *Journal of Coatings Technology*, *75*, 39-51.

Mahmoud, A. A. (2018). A comprehensive review on applications of polymeric surfactants for chemical enhanced oil recovery. *Recent Advance in Petrochemical Science*, *4*, 47-49.

Makarewicz, E., & Michalik, A. (2014). Research on the influence of the type of surfactant and concentrator in aqueous dispersion of pigments. *Journal of Surfactants and Detergents*, *17*, 773-784.

Manaila, E., Stelescu, M. D., & Craciun, G. (2018). Degradation studies realized on natural rubber and plasticized potato starch based eco-composites obtained by peroxide cross-linking. *International Journal of Molecular Sciences, 19*, 2862-2879.

Manroshan, S. (2007). The effect of hydrophobically modified inulin on the properties of natural rubber latex concentrates, *Journal of Rubber Research*, *10*, 156-165.

Manroshan, S., & Lim, E. H. M. (2013). Surfactants and their use in latex technology. *MRB Rubber Technology Development*, *13*, 33-36.

Mao, X., Auer, D. L., Buchalla, W., Karl-Anton, H., Maisch, T., Hellwig, E., … Cieplik, F. (2020). Cetylpyridinium chloride: mechanism of action, antimicrobial efficacy in biofilms, and potential risks of resistance. *Antimicrobial Agents and Chemotherapy*, *64*, e00576-20.

Marrakchi, S., & Maibach, H. I. (2006). Sodium lauryl sulfate-induced irritation in the human face: Regional and age-related differences. *Skin Pharmacology and Physiology*, *19*, 177-180.

Mason, G. (1973). Formation of films from latices a theoretical treatment. *British Polymer Journal*, *5*, 101-108.

Maurad, Z. A., Ghazali, R., Siwayanan, P., Ismail, Z., & Ahmad, S. (2006). Alpha-sulfonated methyl ester as an active ingredient in palm-based powder detergents, *Journal of Surfactants and Detergents*, *9*, 161-167.

Meerloo, J., Kaspers, G. J., & Cloos, J. (2011). *Cell sensitivity assays: The MTT assay*. Germany: Springer Science + Business Media.

Meuldijk, J., Kemmere, M. F., Lima, de, S. V. W., Reynhout, X. E. E., Drinkenburg, A. A. H., & German, A. L. (2003). Some key factors in emulsion polymerization process development. *Polymer Reaction Engineering*, *11*, 259-276.

Miao, S., Wang, P., Su, Z., & Zhang, S. (2014). Vegetable-oil-based polymers as future polymeric biomaterials. *Acta Biomaterialia*, *10*, 1692-1704.

Milica, L., Ivana, P., & Snezana, S. (2016). An overview of novel surfactants for formulation of cosmetics with certain emphasis on acidic active substances. *Tenside Surfactants Detergents*, *53*, 7-19.

Myers, D. (2006). *Surfactant Science and Technology*. United States: John Wiley and Sons.

Namsheer, K., & Chandra, S. R. (2011). Conducting polymers: a comprehensive review on recent advances in synthesis, properties and applications. *RSC advances*, *11*, 5659-5697.

Naphon, P., Wiriyasart, S., & Naphon, N. (2018). Thin rubber sheet drying curve characteristics of fresh natural rubber latex. *International Journal of Applied Engineering Research*, *13*, 8447-8454.

Ngem, N. A., & Mohamed, A. S. (2004). Surface and thermodynamic properties of diquaternary bola-form amphiphiles containing an aromatic spacer. *Journal of Surfactants and Detergents*, *7*, 23-30.

Ngolemasango, Bennett, F. E., M., & Clarke, J. (2008). Degradation and life prediction of a natural rubber engine mount compound, *Journal of Applied Polymer Science*, *110*, 348-355.

Nitschke, M., & Costa, S. G. V. A. O. (2007). Biosurfactants in food industry. *Trends in Food Science and Technology*, *18*, 252-259.

Nordin, N., Yahaya, B. H., & Yusop, M. R. (2018). Electrochemical synthesis and in vitro cytotoxicity study of copper(II) carboxylates with different fatty acid alkyl chain lengths. *New Journal of Chemistry*, *42*, 15127-15135.

Olasanmi, I. O., & Thring, R. W. (2018). The role of biosurfactants in the continue drive for environmental sustainability. *Sustainability*, *10*, 4817-4829.

Otera, J. (2006). *Esterification: Methods. Reactions and Applications* (2nd ed.). Germany: Wiley-VCH.

Peethambaran, N. R., Kuriakose, B., Rajan, M., & Kuriakose, A. P. (1990). Rheological behavior of natural rubber latex in the presence of surface-active agents. *Journal of Applied Polymer Science*, *41*, 975-983.

Perinelli, D. R., Petrelli, D., Vitali, L. A., Bonacucina, G., Cespi, M., Vllasaliu, D., … Palmieri, G. F. (2019). Quaternary ammonium leucine-based surfactants: The effect of a benzyl group on physicochemical properties and antimicrobial activity. *Pharmaceutics*, *11*, Article#287.

Porritt, B. D. (1919). The rubber industry- past and present. *Journal of Royal Society of Arts*, *67*, 252-267.

Premadasa, R. B. (2021). Monthly overview of NR economy. Retrieved from http://www.anrpc.org/

Puskas, J. E., Chiang, K., & Barkakaty, B. (2014). Natural rubber (NR) biosynthesis: Perspectives from polymer chemistry. In S. Kohjiya & Y. Ikeda (Eds.), *Chemistry, Manufacture and Applications of Natural Rubber* (pp. 30-67). United Kingdom: Woodhead Publishing.

Qiang, T., Bu, Q., Huang, Z., & Wang, X. (2014). Structural and interfacial properties of hyperbranched-linear polymer surfactant. *Journal of Surfactants and Detergents*, *17*, 959-965.

Quinn, M. M., Henneberger P. K., Braun, B., Delclos, G. L., Fagan, K., Huang, V., … Zock, J. (2015). Cleaning and disinfecting environmental surfaces in health care: Toward an integrated framework for infection and occupational illness prevention. *American Journal of Infection Control*, *43*, 424-434.

Raffa, P., Wever, D. A. Z., Picchioni, F., & Broekhuis, A. A. (2015). Polymeric surfactants: synthesis, properties, and links to applications. *Chemical Reviews*, *115*, 8504-8563.

Ramesh, P. B., Kevin, O. C., & Ramakrishna S. (2013). Current progress on bio-based polymers and their future trends. *Progress in Biomaterials*, *2*, Article#8.

Ramli, R., Jaapar, J., Singh, M. S. J., & Yatim, A. H. M. (2014). Physical properties and fatigue lifecycles of natural rubber latex gloves. *Advances in Environmental Biology*, *8*, 2714-2722.

Reisz, E. (2007). Curiosity and rubber in the French Atlantic. *Atlantic Studies*, *4*, 5-26.

Research and markets. (2021). Global Surfactant Market Outlook & Forecasts 2021-2026 – Increasing Consumption of Surfactants by Cosmetics & Personal Care, Booming Agrochemical Industry & Growing Food and Beverage Industry. Retrieved from https://www.globenewswire.com/en/news-release/2021/07/27/2269416/28124/en/Global-Surfactant-Market-Outlook-Forecasts-2021-2026-Increasing-Consumption-of-Surfactants-by-Cosmetics-Personal-Care-Booming-Agrochemical-Industry-Growing-Food-and-Beverage-Indust.html

Riyajan, S., & Santipanusopon, S. (2010). Influence of ammonia concentration and storage period on properties field NR latex and skim coagulation, *KGK Rubberpoint*, *2010*, 240-245.

Robinson, V. C., Bergfeld, W. F., Belsito, D. V., Hill, R. A., Klaassen, C. D., Marks, J. G., … Andersen, F. A. (2010). Final report of the amended safety assessment of sodium laureth sulfate and related salts of sulfated ethoxylated alcohols. *International Journal of Toxicology*, *29*, 151S-161S.

Rodriguez-Lopez, L., Lopez-Prieto, A., Lopez-Alvarez, M., Perez-Davila, S., Serra, J., Gonzalec, P., … Moldes, A. B. (2020). Characterization and cytotoxic effect of biosurfactants obtained from different sources. *ACS Omega*, *5*, 31381-31390.

Roslim, R., Tan, K. S., & Jefri, J. (2018). Study on morphological structures and mechanical properties of natural rubber latex films prepared at different prevulcanisation and drying temperatures. *Journal of Rubber Research, 21*, 1-16.

Roy, A. (2017). A review on the biosurfactants: Properties, types, and its applications. *Journal of Fundamentals of Renewable Energy and Applications*, *8*, 248-252.

Ruhida, A. R., & Amir-Hashim, M. Y. (2012). Improving natural rubber latex film softening due to oil, *Journal of Rubber Research*, *15*, 81-95.

Sachdev, D. P., & Cameotra, S. S. (2013). Biosurfactants in agriculture. *Applied Microbiology and Biotechnology*, *97*, 1005-1016.

Sakdapipanich, J. T., & Rojrythai, P. (2012). Molecular structure of natural rubber and its characteristics based on recent evidence. In Sammour, R. (Eds.). *Biotechnology* (pp213-238). Croatia: InTech.

Sansatsadeekul, J., Sakdapipanich, J., & Rojruthai, P. (2011). Characterization of associated proteins and phospholipids in natural rubber latex. *Journal of Bioscience and Bioengineering*, *111*, 628-634.

Santipanusopon, S., & Rijayan, S. (2009). Effect of field natural rubber latex with different ammonia contents and storage period on physical properties of latex concentrate, stability of skim latex and dipped film. *Physics Procedia*, *2*, 127-134.

Sarkar, R., Pal, A., Rakshit, A., & Saha, B. (2021). Properties and applications of amphoteric surfactant: A concise review. *Journal of Surfactants and Detergents*, *24*, 709-730.

Sasidharan, K. K., Joseph, R., Palaty, S., Gopalakrishnan, K. S., Rajammal, G., & Pillai, P. V. (2005). Effect of the vulcanization time and storage on the stability and physical properties of sulfur-prevulcanised natural rubber latex. *Journal of Applied Polymer Science, 97*, 1804-1811.

Savjani, K. T., Gajjar, A. K., & Savjani, J. K. (2012). Drug solubility: Importance and enhancement technique. *ISRN pharmaceutics*, *2012*, Article#195727.

Saxena, N., Pal, N., Dey, S., & Mandal, A. (2017). Characterizations of surfactant synthesized from palm oil and its application in enhanced oil recovery. *Journal of the Taiwan Institute of Chemical Engineers*, *81*, 343-355.

Sekhon, B. S. (2013). Surfactants: Pharmaceutical and medicinal aspects. *Journal of Pharmaceutical Technology, Research and Management*, *1*, 43-68.

Setua, D. K., Awasthi, R., Kumar, S., Prasad, M. & Agarwal, K. (2010). Scanning electron microscopy of natural rubber surfaces: quantitative statistical and spectral texture analysis using digital image processing. In Méndez-Vilas, A. & Díaz, J. (Eds.). *Microscopy: Science, Technology, Applications and Education* (pp. 1642-1652). Badajoz: Formatex.

Silva, R. D. C. F. S., Almeida, D. G., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2014). Applications of biosurfactants in the petroleum industry and the remediation of oil spills. *International Journal of Molecular Sciences*, *15*, 12523-12542.

Slamet, M. I., & Pangiastika, P. W. (2017). Synthesis of methyl ester sulfonate surfactant from crude palm oil as an active substance of laundry liquid detergent. *AIP Conference Proceedings*, *1904*, Article#020058.

Sourav, D., Susanta, M., Aniruddha, G., Rumpa, S., & Bidyut, S. (2015). A review on natural surfactants. *RSC Advances*, *5*, 65757-65767.

Souza, J. L. S., Silva, A. F. D., Carvaiho, P. H. A., Pacheco, B. S., Pereira, C. M. P. & Lund, R. G. (2014). Aliphatic fatty acids and esters: Inhibition of growth and exoenzyme production of Candida, and their cytotoxicity in vitro: Anti-candida effect and cytotoxicity of fatty acids and esters. *Archives of Oral Biology*, *59*, 880-886.

Stamberger, P. (1939). Spontaneous coagulation of rubber latex. *Nature*, *143*, Article#520.

Steber, J. (2007). The ecotoxicity of cleaning product ingredients. In Ingegard, J., & Somasundaran, P. (Eds.). *Handbook for cleaning/decontamination of surfaces* (pp. 721-746). Nederland: Elsevier.

Steward, P. A., Hearn, J., & Wilkinson, M. C. (2000). An overview of polymer latex film formation and properties. *Advances in Colloid and Interface Science*, *86*, 195-267.

Sugiyama, F., Kleinschmidt, A. T., Kayser, L. V., Rodriquez, D., III, M. F., Alkhadra, M. A., Wan, J. M. H., … Lipomi, D. J. (2018). Effects of flexibility and branching of side chains on the mechanical properties of low-bandgap conjugated polymers. *Polymer Chemistry*, *9*, 4354-4363.

Tadros, T. (2009). Polymeric surfactants in disperse systems. *Advance Colloid Interface Science*, *147-148*, 281-299.

Tadros, T. (2013). Polymeric Surfactant. In Tadros, T. (Eds.) *Encyclopedia of Colloid and Interface Science* (pp. 964-995). Berlin: Springer.

Tadros, T. F. (2005). *Applied Surfactants: Principles and Applications*. Weinheim, Germany: Wiley-VCH.

Takahashi, T., Hirayama, K., Teramoto, N., & Shibata, M. (2008). Biocomposites composed of epoxidized soybean oil cured with terpene-based acid anhydride and cellulose fibers. *Journal of Applied Polymer Science*, *108*, 1596–1602.

Tan, E. H. & Birge, R. R. (1996). Correlation between surfactant/micelle structure and stability of bacteriorhodopsin in solution. *Biophysical Journal*, *70*, 2385 – 2395.

Tanaka, Y., & Sakdapipanich, J. T. (2001). Chemical structure and occurrence of natural polyisoprenes. In Koyama, T. & Steinbüchel, A. (Eds.), *Biopolymers, Polyisoprenoids* (pp. 1-25). Germany: Wiley-VCH.

Tarachiwin, L., Sakdapipanich, J., & Tanaka, Y. (2003). Gel formation in natural rubber latex:2. Effect of magnesium ion. *Rubber Chemistry and Technology*, *76*,1185-1193.

The American Society for Testing and Materials. (1993). ASTM O 2354-68: Standard Test Method for Minimum Film Formation Temperature of Emulsion Vehicles. https://www.astm.org/DATABASE.CART/HISTORICAL/D2354-10.htm

Televisory. (2019). Retrieved from: https://www.televisory.com/blogs/-/blogs/malaysian-rubber-glove-industry-an-upda-2

Thomas, F. G., Amanda, M., & Rafael, L. Q. (2016). Bio-based polymers with potential for biodegradability. *Polymers*, *8*, Article#262.

Umar, H. Y., Giroh, D. Y., Agbonkpolor, N. B., & Mesike, C. S. (2017) An overview of world natural rubber production and consumption: An implication for economic empowerment and poverty alleviation in Nigeria. *Journal of Human Ecology*, *33*, 53-59.

Verhaar, G. (1959). Natural latex as colloidal system. *Rubber Chemistry and Technology*, *32*, 1627–1659.

Vijayaram, T. R. (2009). A techincal review on rubber. *International Journal on Design and Manufacturing Technologies*, *3*, 25-37.

Voyutskii, S. S. (1958). Amendment to the papers by bradford, brown, and co-workers: "Concerning mechanism of film formation from high polymer dispersions". *Journal of Polymer Science*, *32*, 528-530.

Wibowo, A. D. K., Tiani, P., Aditya, L., Handayani, A. S., & Christwardana, M. (2021). Synthesis and characterization of polymeric surfactant from palm oil methyl ester and vinyl acetate for chemical flooding. *Reaktor*, *21*, 65-73.

Woo, C. S., & Kim, W. D. (2006). Heat-aging effects on the material properties and fatigue life prediction of vulcanized natural rubber, *e-Journal of Soft Materials*, *2*, 7-12.

Xia, H. (2021). Malaysia's natural rubber production falls 33.5 pct in April. Retrieved from http://www.xinhuanet.com/english/asiapacific/2021-06/15/c_1310009116.htm

Xia, H., Wang, D., Wang, G., & Wu., J. (2008). Effect of polymer solution viscoelasticity on residual oil. *Petroleum Science and Technology*, *26*, 398-412.

Yamamoto, Y., Nghia, P. T., Klinklai, W., Saito, T., & Kawahara, S. (2008). Removal of proteins from natural rubber with urea and its application to continuous processes. *Journal of Applied Polymer Science*, *107*, 2329-2332.

Yang, X., Zhang, B., Li, P., Guo, C., Li, J., & Li, Q. (2017). Synthesis of a high surface active polymeric surfactant based on chitosan and characteristics of complexation with cetyltrimetylammonium bromide. *Journal of Dispersion Science and Technology*, *39*, 1485-1491.

Yari, S., Asadi, A. F., & Varmazyar, S. (2016). Assessment of semi-quantitative health risks of exposure to harmful chemical agents in the context of carcinogenesis in the latex glove manufacturing industry. *Asian Pacific Journal of Cancer Prevention*, *17*, 205-211.

Yeoh, F. H., Lee, C. S., Kang, Y. B., Wong, S. F., Cheng, S. F., & Ng, W. S. (2020). Production of biodegradable palm oil-based polyurethane as potential biomaterial for biomedical applications. *Polymers*, *12*, 1842-1868.

Yikmis, M., & Steinbuchel, A. (2012). Historical and recent achievements in the field of microbial degradation of natural and synthetic rubber. *Applied and Environmental Microbiology*, *78*, 4543-4551.

Yip, E., & Cacioli, P. (2002). The manufacture of gloves from natural rubber latex. *Journal of Allergy and Clinical Immunology*, *110*, S3-14.

Yoon, B. Y., Jackman, J. A., Valle-Gonzalez, E. R., & Cho, N. J. (2018). Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications. *International Journal of Molecular Sciences*, *19*, 1114-1153.

Yooyanyong, A., Danwanichakul, D., & Danwanichakul, P. (2015). The effect of remaining SDS on protein release in later leaching steps of deproteinized natural rubber. *KKU Engineering Journal*, *43*, 41-46.

Yuan, C., Xu, Z. Z., Fan, M. X., Liu, H. Y., Xie, Y., & Zhu, T. (2014). Study on characteristics and harm of surfactants. *Journal of Chemical and Pharmaceutical Research*, *6*, 2233-2237.

Zakharova, L. Y., Pashirova, T. N., Fernandes, A. R., Doktorovova, S., Martins-Gomes, C., Silva, A. M., & Souto, E. B. (2018). Self-assembled quaternary ammonium surfactants for pharmaceuticals and biotechnology. In Grumezescu A. M. (Eds.). *Organic Materials as Smart Nanocarriers for Drug Delivery* (pp. 601-618). United States: William Andrew Publishing.

Zhang, X., Song, F., Taxipalati, M., Wei, W., & Feng, F. (2014). Comparative Study of Surface-Active Properties and Antimicrobial Activities of Disaccharide Monoesters. *PLoS One*, *9*, Article#e114845.

Zheng, C. J., Yoo, J., Lee, T., Cho, H., Kim, Y., & Kim, W. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids, *FEBS letter*, *579*, 5157-5162.