THE EFFECTS OF FILLERS ON THE SURFACE MORPHOLOGY, MECHANICAL PROPERTIES AND PROTEIN CONTENT IN NATURAL RUBBER LATICES

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THE EFFECTS OF FILLERS ON THE SURFACE MORPHOLOGY, MECHANICAL PROPERTIES AND PROTEIN CONTENT IN NATURAL RUBBER LATICES

ABSTRACT

The purpose of adding fillers into rubber products is not only as a cheapening aid but also to improve the properties of the end products. In this study, the untreated and stearic acid (SA)-treated calcium carbonate ($CaCO_3$) were used as a filler into the mixed clones natural rubber latex concentrate (MCLC). The MCLC was prepared by processing the monoclones field latex, namely RRIM2023, RRIM2024 and RRIM2025, at a similar weight ratio. The untreated filler loadings mixed into MCLC varied from 10 to 40 parts per hundred rubber (pphr). The $CaCO_3$ treated with SA at various concentrations of 0.5%, 1.0%, 3.0%, 4.0%, 5.0% and 7.0% by % (*w/v)* in chloroform was mixed into MCLC at 30 pphr filler loading. The MCLC films at different untreated filler loadings were evaluated for their properties chemically and physically. Results obtained were evaluated and compared to observe the occurrence of chemical interferences, if any, on the determination of extractable proteins (EP) content. The addition of the SA-treated $CaCO_3$ at various concentrations of SA was to improve the filler-rubber interaction and to reduce the amount of EP content extracted from the MCLC films. The SA-treated $CaCO_3$ was characterised by using Fourier-Transform Infra-Red (FTIR) and Thermogravimetric analysis (TGA) to identify the presence of stearic acid on the surfaces of $CaCO_3$ particles. The percentage of nitrogen (N) and EP contents were measured using the Automatic Analyser and UV Microplate Reader, respectively. The EP content of MCLC films was also measured before and after exposure to ozone at 10 parts per hundred million (pphm) concentration, and the surface morphology of MCLC films was monitored before and after exposure to ozone. Their tensile and tear strengths were determined as well as their total, combined and free sulphur contents. The results of latex properties such as dry rubber content (DRC), total solids content (TSC), alkalinity (NH_3) and volatile fatty acid number (VFA No.) showed that the MCLC prepared from MCFL is clean, stable and free from foreign matters and non-polymer impurities. The cross-link concentration of samples was also being measured by the Flory-Rehner equation to identify that the presence of $CaCO_3$ in samples has the ability to influence the cross-link concentration. It was found that the addition of the filler below 20 pphr affected the cross-link concentration. The percentage of combined sulphur of unaged samples decreased from 22% until 37% and aged samples from 24% until 43%. The tensile strength of the unaged sample decreased from 10% to 20% and aged samples from 10% to 29%. Therefore, decreasing the percentage of combined sulphur led to a reduction of the tensile strength of MCLC films. The total protein contents on the MCLC films at different filler loadings showed that the values were higher in the unaged samples as compared to aged samples (70 ºC for 7 days). From TGA analysis, the presence of stearic acid can only be detected at filler treated at 3.0% concentration of stearic acid. FTIR analysis showed that the absorption peak at 875 cm^{-1} indicated that the stearic acid might be attached to the $CaCO_3$. The nitrogen (N) content of the unaged and aged samples at 3.0% stearic acid treated $CaCO_3$ was similar. However, all corrected and non-corrected EP contents for unaged samples were lower compared to the Control sample (untreated $CaCO_3$). The highest value of corrected EP content was 417.4 μg/g obtained from latex mixed with 3.0% treated filler. The results of the EP contents were reported as non-corrected and corrected values. In the corrected method, the copper reagent was replaced by water in an alkaline tartrate solution in the colour development stage during the protein assay. The results of corrected and non-corrected EP contents for the aged, treated samples were found to be inconsistent and was attributed to the heating and ageing processes. Contrary to expectation, the non-corrected EP content was lower compared to the corrected EP content. The tensile strength of unaged SA-treated $CaCO_3$ results shows an increase from Control 1 to 0.5% SA-treated before being reduced from 1.0% to 7.0%. Nevertheless, the MCLC films with the SA-treated $CaCO_3$ showed no significant difference in tensile strength after ageing but higher than the Control 1 (untreated $CaCO_3$). The tear strength of unaged samples was higher as compared to aged samples.

Keywords: Natural rubber, calcium carbonate, SA-treated $CaCO_3$, total protein contents, mechanical properties.

KESAN-KESAN PENGISI PADA MORFOLOGI PERMUKAAN, SIFAT KEKUATAN MEKANIKAL DAN PROTEIN DI DALAM LATEKS GETAH ASLI

ABSTRAK

Penggunaan pengisi getah telah diketahui dapat menjimatkan kos dan penambahbaik sifat produk getah. Dalam kajian ini, kalsium karbonat yang tidak dirawat dan dirawat telah diguna sebagai pengisi ke dalam MCLC. Lateks pekat ini di proses dan disediakan daripada lateks ladang klon RRIM2023, RRIM2024 dan RRIM2025 pada nisbah berat yang sama. Pengisi yang tidak dirawat telah divariasikan daripada 10 pphr sehingga 40 pphr manakala pengisi yang dirawat dengan kepekatan asid stearik (SA) pada 0.5%, 1.0%, 3.0%, 4.0%, 5.0% dan 7.0% dengan kadar % *(w/v)* di dalam larutan kloroform pada kepekatan pengisi 30 pphr sahaja. Sifat fizikal dan kimia di dalam film MCLC yang tidak dirawat pada kepekatan yang telah divariasikan diuji untuk memastikan gangguan kandungan kimia terhadap kandungan ekstrak protein (EP). Sementara itu, pengisi yang dirawat pada pelbagai kepekatan SA di dalam pelarut kloroform ditentukan untuk meningkatkan interaksi di antara zarah getah-pengisi dan juga mengurangkan kandungan EP yang terdapat di dalam sampel. Pengisi yang dirawat kemudiannya di analisis dengan menggunakan Fourier-Transform Infra-Red (FTIR) dan Thermogravimetric analysis (TGA) untuk mengenalpasti kehadiran asid stearik pada permukaan zarah pengisi. Peratusan kandungan nitrogen (N) dan EP telah ditentukan dengan menggunakan Automatic Analyser dan UV Microplate Reader. Penentuan kandungan EP didalam filem lateks MCLC juga telah ditentukan sebelum dan selepas pendedahan terhadap ozon pada kepekatan 10 parts per hundred million (pphm) dan morfologi permukaan sampel juga telah dipantau. Sifat kekuatan tegangan dan lusuh serta kandungan keseluruhan sulfur, kandungan gabungan sulfur dan kandungan bebas sulfur juga di uji dan di tentukan. Keputusan daripada ujian lateks iaitu kandungan getah kering (KGK), kandungan bahan

pepejal (TSC), kealkalian (NH_3) dan kandungan asid meruap (VFA) telah menunjukkan bahawa lateks pekat (MCLC) yang di sediakan daripada lateks ladang (MCFL) adalah bersih, stabil dan bebas daripada bendasing. Kehadiran $CaCO_3$ sebagai pengisi didapati boleh mempengaruhi kepekatan getah-silang, dimana pengurangan kepekatan getah-silang berkaitan penambahan kandungan pengisi. Kepekatan getah-silang ke atas filem lateks MCLC telah dikira dengan menggunakan persamaan Flory Rehner. Peratusan sampel kandungan gabungan sulfur tanpa penuaan mengurang daripada 22% sehingga 37% manakala sampel penuaan mengurang daripada 24% sehingga 43%. Pengurangan kekuatan sampel tanpa penuaan berkurang daripada 10% sehingga 20%, untuk sampel penuaan mengurang daripada 10% sehingga 29%. Oleh itu, pengurangan peratusan kandungan gabungan sulfur lebih menyurus kepada pengurangan kekuatan regangan filem lateks MCLC. Kandungan keseluruhan protein di dalam filem lateks MCLC pada pelbagai kepekatan pengisi telah menunjukkan bahawa sampel tanpa penuaan lebih tinggi berbanding sampel yang mengalami penuaan (70 ºC selama 7 hari). Hasil daripada analisis TGA, komposisi SA hanya boleh dikesan bagi pengisi yang dirawat lebih daripada kepekatan 3.0%. Analisis FTIR pula menunjukkan kehadiran puncak 875 cm^{-1} adalah kehadiran SA pada permukaan pengisi. Peratusan kandungan nitrogen (N) untuk sampel sediada dan penuaan pada pengisi berkepekatan 3.0% mempunyai nilai yang hampir sama. Manakala, kesemua kandungan EP yang diperbetulkan dan tidak diperbetulkan untuk sampel sediada lebih rendah berbanding dengan sampel kawalan (30 pphr). Kandungan ekstrak protein (EP) yang diperbetulkan dan paling tinggi nilainya adalah 417.4 µg/g daripada pengisi yang dirawat pada kepekatan 3.0%. Keputusan nilai kandungan EP adalah dilaporkan sebagai yang diperbetulkan dan tidak diperbetulkan. Keputusan EP yang diperbetulkan adalah di mana larutan kuprum telah digantikan dengan air suling dicampur dengan larutan alkali tartrate semasa proses pewarnaan assay protein. Keputusan kandungan EP yang tidak dan diperbetulkan untuk sampel penuaan

didapati adalah tidak konsisten. Ini adalah juga di sebabkan daripada proses penuaan dan pemanasan ke atas sampel. Walaubagaimana pun, kandungan EP yang tidak diperbetulkan adalah lebih rendah dibandingkan dengan kandungan ekstrak protein (EP) yang diperbetulkan. Ujian fizikal didapati kekuatan regangan bertambah daripada pengisi tanpa di rawat (Control 1) ke 0.5% pengisi yang di rawat sebelum kekuatan regangan berkurangan daripada 1.0% sehingga 7.0% pengisi yang di rawat. Namun begitu, kekuatan regangan filem latek MCLC dengan pengisi yang dirawat menunjukkan tiada perbezaan dengan keputusan kekuatan regangan selepas proses penuaan tetapi lebih tinggi dibandingkan dengan pengisi tanpa dirawat (Control 1). Nilai kekuatan lusuh sampel tanpa proses penuaan ada lebih tinggi dibandingkan dengan sampel yang mengalami proses penuaan.

Kata kunci: Getah asli, kalsium karbonat, pengisi dirawat, jumlah kandungan protein, kekuatan mekanikal.

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Λ	:	extension ratio
F	:	force applied
T_g	:	glass transition temperature
W	:	strain energy density in the legs
T	:	tearing energy
t	:	test piece thickness
E	:	the cross-linking efficiency values
U	:	total strain energy in the central region
h_o	:	unstrained
w	:	width
NH_3	:	alkalinity
NH_4SO_4	:	ammonium sulphate
$Ca(Mg.Fe)(CO_3)_2$	:	ankerite
AP	:	antigenic protein
BBOT	:	2,5-(Bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene
$CaCO_3$	:	calcium carbonate
CST	:	chemical stability time
CRT	:	cathode ray tube
$CHCl_3$	:	chloroform
CLC	:	commercial latex concentrate
Wingstay L	:	complex phenol-alkanes
Cu	:	copper
DRC	:	dry rubber content
DSC	:	Differential Scanning Calorimetry

$CaMg(CO_3)_2$: dolomite

DPNR : deproteinized natural rubber

EP : extractable protein

EV : efficient vulcanisation

FESEM : field emission scanning electron microscope

FTIR : Fourier Transform Infrared

GCC : ground calcium carbonate

HA : high ammoniated

ICP-OES : Inductive Coupled Plasma-Optical Emission Spectrometry

Fe : iron

LA : low ammoniated

Mg : magnesium

Mn : Manganese

MST : mechanical stability time

XA : medium ammoniated

MBT : 2-mercaptobenzothiazole

MC : Microcentrifuge

MCFL : mixed clones field latex

MCLC : mixed clones latex concentrate

NR : natural rubber

HNO_3 : nitric acid

$Ca(NO_3)_2$: calcium nitrate

NOx : nitrogen oxides

NDIR : non-dispersive infrared

pphr : part per hundred rubbers

pphm : parts per hundred million

PBS : phosphate buffered saline

PTA : phosphotungstic acid

KOH : potassium hydroxide

K : Potassium

KL : potassium laurate

PCC : precipitated calcium carbonates

SEM : Scanning Electron Microscope

Semi-EV : semi-efficient vulcanisation

SDS : sodium dodecyl sulfate

SA : stearic acid

H_2SO_4 : sulphuric acid

TMTD : tetramethylthiuram Disulfide

TGA : Thermogravimetric Analyser

TSC : total solids content

TCA : trichloroacetic acid

VFA No. : volatile fatty acid number

ZnO : zinc oxide

ZST : zinc oxide stability time

ZOV : zinc oxide viscosity

ZBuD : zinc dibutyl dithiocarbamate

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CHAPTER 1: INTRODUCTION

1.2 Background of the study

Natural rubber (NR) is a complex cytoplasmic system that consists of rubber and non-rubber particles. The non-rubber particles are suspended in an aqueous serum phase and contains protein, amino acids, fatty acids, sterols, triglycerides, and other neutral lipids, phospholipids, glycolipids, carbohydrates, and inorganic salts. However, part of these non-rubber substances that are water-soluble are removed during the centrifugation process when processing field latex into latex concentrate (Zhang et al., 2020). After centrifuging, the dry rubber content of the concentrated latex is about 60% compared to the initial value of about 30% to 40% in field latex (Ariavie & Ayeke, 2020; Chen, 1988).

The use of commercial fillers such as calcium carbonate ($CaCO_3$) in producing natural rubber latex (NRL) dipped products, particularly NR latex gloves, is increasing. The main reasons for adding fillers into NR products are to improve its properties and to reduce the cost of production. The addition of fillers also helps to resolve the problems of latex shortage in Malaysia. Fillers added can be categorised as reinforcing, semi-reinforcing and non-reinforcing. A typical dry NR rubber compound contains fillers and other additives that may include curatives, plasticisers, and coupling agents. Thus, it is important to consider the interactions between each ingredient added as each ingredient can affect the properties of the vulcanisate compound that may, in turn, affects the performance and service life of the end product.

Carbon black and other inorganic minerals such as clay, calcium carbonate, and silicate are generally added into rubber compounds to improve the mechanical properties of vulcanised rubbers. Carbon black, as a reinforcing filler, offer excellent reinforcement due to its strong interaction with the rubber molecules. However, at higher loading, it decreases the processability of rubber compounds. Therefore, it is essential to understand the behaviour of reinforcement of rubber by fillers such as carbon black, clays and calcium carbonate. These fillers were added to rubber formulation to optimise the properties needed for service application.

Meanwhile, the mineral fillers such as clay and calcium carbonate are considered as useful fillers in rubber compounding due to their low cost and ability to influence the dynamic properties of rubber vulcanisate, such as morphology, particularly particle size and particle size distribution, as well as shape and distribution of filler aggregates and surface energy. It is well known from prior research that fillers play a significant role in the modification of properties of rubber products. Most fillers used offer some functional benefit that contributes to the processability or utility of rubber products.

1.3 Problem statement

The extractable proteins (EP) or residual proteins from NR latex remaining in a finished product may cause some individuals (Hasma & Amir Hashim, 1997; Wiriyanantawong et al., 2020; Yip & Cacioli, 2002). This is attributed to proteins in the aqueous serum phase of the field latex that was inadequately or not completely removed in the centrifuging process during the production of latex concentrate. Generally, the total amount of proteins present in latex concentrate is relatively constant, at about 1.6% - 2.0% by weight of rubber with functional benefits that contribute to the processability or utility of rubber products (Subramaniam, 1995). Based on earlier studies by other researchers, the effect of addition of nano-sized calcium carbonate fillers in NR latex gloves at 5 parts per hundred rubber (pphr) had little or no significant effect on the extractable protein (EP) and the antigenic protein (AP) content of gloves (Amir Hashim et al., 2004). However, the amount of fillers added into the NR latex compounds does not tally with the reported usage of fillers in NR gloves production by the gloves manufacturers in Malaysia. As protein allergy is closely related to NR latex products, especially NR gloves, there is a need to carry out a systematic study on the effect of calcium carbonate fillers on the total protein content of latex products (Pailhories, 1993). This would provide an indication of the allergic potential of the NR latex products if there were an increased value of extractable protein (EP). It is known that $CaCO_3$ dispersion tends to aggregate easily due to their small particle size, large specific surface area and high surface free energy. In addition, the surface of $CaCO_3$ particles has hydroxyl groups that exhibit polarity and have characteristics of hydrophilicity and oleophobicity, making it difficult to disperse evenly in the natural rubber latex. Treating the surface of $CaCO_3$ particles also improve the dispersion of $CaCO_3$ particles and interface compatibility with the organic matrix (Yu et al., 2020). The novelty of this work by replacing the SA-treated $CaCO_3$ with untreated $CaCO_3$ into MCLC after optimising the condition of EP content and tensile strength at the highest values. The other factors were considered are the results of the combined and free sulphur. This result can indicate the agglomerate of untreated filler in MCLC films and surface morphology that has been monitored.

1.4 Previous research work

Many work-related to non-reinforcing fillers such as kaolinite clays and calcium carbonate have been carried out in the latex industries. The study on the effect of the addition of nano-sized calcium carbonate on the swelling index and mechanical properties such as tensile and tear strengths of pre-vulcanised natural rubber latex films before and after ageing have been documented. Tensile strength was reported to increase with increasing filler loading and then decreased again at loadings greater than 10 parts per hundred rubbers (pphr) (Amir Hashim et al., 2004; Cai et al., 2003). Other researchers found that increasing filler loading would decrease the vulcanisate time due to the interaction between the filler and the rubber matrix during the mixing process. Surface morphology from micrographs indicated that agglomeration of filler occurred as the filler content increases (Manroshan & Baharin, 2005; Roslim & Amir Hashim, 2010). Other researchers have also observed that the usage of calcium carbonate affects the properties of NR latex films, such as tensile strength and force at break. These properties were affected at filler loadings higher than 5% in NR latex films. Based on images obtained by scanning electron microscope (SEM), surface smoothness of NR latex films appeared to be affected at about 20% filler loading (Mohamad Akmal et al., 2013).

1.5 The problem associated with fillers in NR latex films

The use of calcium carbonate in NR latex is widely adopted in the NR dipped products industry, particularly in the production of NR latex gloves despite some problems still faced by the rubber manufacturers. A significant problem is a reduction in tensile strength that could lead to failure to meet the product specification. In addition, other researchers have reported on the reduction of mechanical properties and surface morphology of NR latex films. However, not much information is available on the effect of $CaCO_3$ or other fillers on the total protein content present in NR latex films and its relation to latex allergy (Amir Hashim et al., 2004).

1.6 Research objectives

The overall aim of this research project is to study and investigate the effect of $CaCO_3$ before and after being treated with SA in chloroform medium on the surface morphology, mechanical properties and protein content of MCLC films at different filler loadings and various SA concentrations. It is known that a typical NR latex compound mixed with untreated $CaCO_3$ dispersions has a common problem, especially on the filler distribution. However, this study developed the stearic acid-treated $CaCO_3$ by treating $CaCO_3$ with stearic acid (SA) in a chloroform medium before producing the SA-treated $CaCO_3$ dispersion. Therefore, the usage of SA-treated $CaCO_3$ dispersion in NR latex compounds can be applied, and the extractable protein (EP) contents in NR latex films were reduced without affecting the mechanical properties. The procedures adopted to meet the specific objectives of the research are as follows:

i. To prepare and evaluate the properties of natural rubber field latex of different clones (RRIM2023, RRIM2024 and RRIM2025), mixed clones of field latex (MCFL) and mixed clones latex concentrate (MCLC) before compounds with rubber chemicals, untreated and SA-treated $CaCO_3$.

ii. To determine the content of $CaCO_3$ (untreated and SA-treated) in MCLC films for monitoring the filler distribution in MCLC films. Then, the total sulphur, combined sulphur and free sulphur contents of MCLC films were measured to investigate the effect of fillers on the cross-link concentrations. The cross-link concentrations of untreated MCLC films are also being measured by the equilibrium swelling method. The reason is to confirm that the presence of $CaCO_3$ in MCLC films has influenced the cross-link concentrations.

iii. To determine the contents of nitrogen (N) and extractable protein (EP) in MCLC films on the addition of untreated and treated $CaCO_3$ as well as after exposure to ozone at 100 parts per hundred million (pphm) concentration. This is to approve that the EP content of MCLC films filled with SA-treated $CaCO_3$ has a tendency to be reduced. The effect of $CaCO_3$ and chemicals interferences on the EP content in MCLC films was measured and investigated the occurrence of $CaCO_3$ in protein assay.

iv. To observe the surface morphology of MCLC films filled with untreated and SA-treated $CaCO_3$ as well as after exposure to ozone at 10 pphm concentration. By replacing the untreated $CaCO_3$ with SA-treated $CaCO_3$, the glass transition temperature (T_g) and mechanical properties such as tensile and tear strengths of MCLC films filled with untreated and SA-treated $CaCO_3$ were also being measured. It also identifies that the MCLC films filled with SA-treated $CaCO_3$ should not affect the mechanical properties.

1.7 Scope of work

In this study, field latex of three different clones, RRIM2023, RRIM2024 and RRIM 2025, were collected from Stesen Penyelidikan Kota Tinggi. These clones are recommended by the Malaysian Rubber Board for rubber planting due to their favourable characteristics. All the field latex clones were mixed at a similar weight ratio before processing them into latex concentrate. The latex properties such as dry rubber content (DRC), total solids content (TSC), alkalinity (NH_3) and volatile fatty acid number (VFA No.) of all chosen clones of field latex and MC field latex (MCFL) were evaluated prior to preparation of the MCLC according to MS 466 (1987). Ammonium laurate of 0.0025% was added to enhance latex stability, and it was determined based on the mechanical stability time (MST) following ISO 35 (2004) test method. Then, the stable latex concentrate is ready to be compounded with other rubber chemicals such as potassium hydroxide, surfactant, accelerator, curing agent, antioxidant, and protective agent. The MCLC properties such as DRC, TSC, alkalinity, VFA No. MST, pH, potassium hydroxide (KOH) number, viscosity, sludge content, coagulum content, metallic content, zinc oxide viscosity (ZOV), zinc stability time (ZST) and chemical stability time (CST) were determined and checked against the ISO 2004 (2017) specifications for quality purpose. Then, the MCLC was compounded with the $CaCO_3$ dispersion and other rubber chemicals. The MCLC films were prepared at different filler loadings by varying filler from 10 parts per hundred rubbers (pphr) to 40 pphr.

Then, the MCLC films were evaluated for their physical and chemical properties by TGA, FTIR, Inductive Coupled Plasma-Optical Emission Spectrometry (ICP-OES), DSC, FESEM, Automatic Analyser (AA), and UV Microplate Reader. Polymer identification and the amount of filler contained in the samples before and after creping were determined by FTIR and TGA, respectively. Meanwhile, the metallic content in samples were determined by ICP-OES spectroscopy. The total protein content based on total nitrogen (N) and extractable protein (EP) content by ASTM 5712 (2015) was measured before and after exposure to ozone at 10 pphm concentration. The T_g, mechanical properties such as tensile and tear strength, as well as the surface morphology of samples, were determined and monitored. The tensile and tear strengths of the MCLC films were evaluated before and after ageing at 70 °C for 7 days to gauge the performance of the samples. The tear strength of samples was performed according to ISO 34-1 (2015) test method. The cross-link concentrations were measured according to the Flory Rehner equation, whilst the total and combined sulphur contents were analysed by using an Automatic Analyser. The samples were extracted with acetone for 16 hours before combined and free sulphur contents were determined.

The $CaCO_3$ filler was blended in stearic acid (SA) of various chloroform medium concentrations before drying in the oven. Then, it was prepared in dispersion form before compounding with MCLC and other rubber chemicals. Several dispersions were prepared by treating the $CaCO_3$ with SA at 0.5%, 1.0%, 3.0%, 4.0%, 5.0% and 7.0% (*w/v*) in chloroform ($CHCl_3$) (Mihajlović et al., 2009). The dried SA-treated $CaCO_3$ was ground before preparing the SA-treated $CaCO_3$ dispersion. The SA-treated $CaCO_3$ was characterised by using FTIR and TGA. The surface morphology of powdered treated $CaCO_3$ was observed by using FESEM. Then, the SA-treated $CaCO_3$ at various concentrations was compounded at 30 pphr filler loadings into the MCLC together with rubber chemicals to produce the SA-treated MCLC films. The metallic, total nitrogen (N), EP, total sulphur contents, T_g and mechanical properties such as tensile and tear strengths of the MCLC films filled with untreated and SA-treated $CaCO_3$ were evaluated and compared.

1.8 Thesis outline

This thesis is divided into 5 chapters. Chapter 1 represent the introduction on the background of the study, problem statement, previous research work, the problems associated with the fillers in NR latex films, research objectives, the scope of work and the outline. Chapter 2 explained the literature review. All the sub-topic discussed in this chapter is on the natural rubber latex and its types, processing techniques, NR latex properties, previous work on the NR latex properties, filler properties and its types, calcium carbonate and its types, selection of fillers, filler modification, treated filler, application of fillers, sulphur vulcanisation, protein content, EP, nitrogen content, methods of protein reduction, method of EP and nitrogen contents, mechanical properties of NR latex films. Chapter 3 highlight the methodology of the research work. The materials used in this study, experimental works and characterisation techniques are such as spectroscopy, thermal, microscopy and mechanical properties are explained in detail. Chapter 4 represent results and discussion. Five main sub-topics are discussed in this chapter were focused on the untreated and SA-treated $CaCO_3$. Firstly, the evaluation of MCLC properties before preparing latex compounds. Secondly, on the thermal analysis and identification of SA-treated $CaCO_3$ and MCLC films filled with untreated and SA-treated $CaCO_3$ by TGA, DSC and FTIR. Thirdly, the surface morphology of MCLC films and then measuring the total protein content in MCLC films based on the percentage of nitrogen, extractable protein content. The extractable protein (EP) content after exposure to ozone is also being measured. Finally, on the and mechanical properties of the MCLC films such as tensile and tear strength. The cross-link measurement by Flory Rehner equation and the total sulphur, combined and free sulphur, is also being determined. Chapter 5 concludes the thesis with some suggestions for further works.

CHAPTER 2: LITERATURE REVIEW

2.1 Natural rubber latex

Field latex from the *Hevea brasiliensis* tree is the starting material of the rubber latex industry. Therefore, a proper understanding of the behaviour of the concentrated latex and its many varied uses requires knowledge of its physical and chemical characteristics, its method of preparation from field latex to latex concentrate, and its storage as well as handling history. NR field latex is a white fluid consisting mainly of rubber hydrocarbon, protein, lipids, carbohydrates, inorganic salts, and water. These compounds are distributed in three principal phases, namely a rubber phase, aqueous phase, and bottom phase. The rubber phase contributes 35% of the latex weight, the aqueous phase contributes 45% to 55% and the bottom fraction of lutoid particles at about 10% - 20% of the latex weight. However, another particulate has also been identified in latex, which is called the Frey-Wyssling complex and contains β-carotene in minor quantities. Physically, the NR latex is a white liquid with a density of 0.980 g/cm^3. Generally, the main basic constituents of the field latex are as shown in Table 2.1 (Chen, 1979; Chin & Mohinder Singh, 1980; Vaysse et al., 2012). The traditional preservative that is commonly used is ammonia at a level of 0.2% - 0.4% of the weight of latex.

Table 2.1: Typical parameter of freshly tapped field natural rubber latex (Chen, 1979)

Parameter	Percentage (%)
Total solids content	22 – 48
Dry rubber content	20 – 45
Proteinaceous substances	1.5
Resinous substances	2
Carbohydrates	1
Inorganic matter	0.5

NR can be obtained in the form of latex or dry rubber with almost 100% cis-1,4-polyisoprene rubber particles, as shown in Figure 2.1.

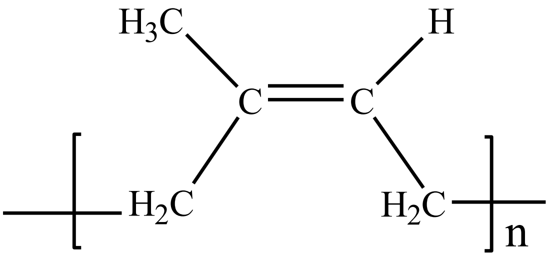


Figure 2.1: cis-1,4-polyisoprene (Hamann, 1993).

As mentioned earlier, the field latex obtained from rubber trees comprises rubber hydrocarbon and non-rubber substances, including lipids, protein, amino acids, fatty acids, sterols, triglycerides, and other neutral lipids, phospholipids, glycolipids, carbohydrates, and inorganic salts. According to Nurul Hayati et al. (2015), the field latex contains about 6% w/w non-rubber components, including protein, lipids and other contents, resulting in latex allergy in users of end products. It is generally known that these non-rubbers contain some substances that are water-soluble that can affect the properties of latex concentrates. However, part of these non-rubber substances is removed during the processing of field latex into latex concentrate. The rubber hydrocarbon content of field latex is about 30% to 35%, while for latex concentrate, it is about 60%. The latex concentrate prepared through the centrifugation process was used for producing dipped goods such as gloves, condoms, teat, and catheter (Hamann, 1993; Ohm, 1990; Simchareon et al., 2012).

2.2 Production of concentrated natural rubber latex

Natural rubber latex exudes from the tree with a dry rubber content (DRC) of around 30%. The field latex is typically concentrated to 60% DRC and gives uniform processing properties with partial removal of the non-rubber constituents during the concentration process. The field latex is concentrated to 60% DRC for economic and technical reasons (Muniandy, 1998).

2.2.1 Method of concentrating latex

Three methods of concentrating latex are usually practised in Malaysia and other countries, namely centrifugation, evaporation, and creaming (Promsung et al., 2021). In Malaysia, out of the total production of about 180000 tonnes (dry rubber weight), about 88% is produced by centrifugation, 6% by creaming and 6% by evaporation. Production of latex concentrate by centrifugation is commonly used in Malaysia and other countries (Ariavie & Ayeke, 2020; Gorton, 1972).

2.2.1.1 Centrifuged latex

The basic principle of the centrifugation process is to break up the rotating mass of latex into a number of conical shells using a high-speed rotating bowl containing a series of conical discs, as shown in Figure 2.2. The concentrate and the skim latex are then led through separate channels to two stationary gullies, where they are collected individually at the same time, as shown in Figure 2.3. In the centrifugation process, ammonia preserved blended field latex is fed into a centrifuge bowl rotating at a speed of about 7000 rpm. The design of the machine is such that under these conditions, an average centrifugal force equivalent to about 8000 *g* is exerted on the latex particles and causes the latex to separate into two fractions: a rubber-rich less dense concentrate and denser skim latex. The setting on the centrifuge is adjusted so that the concentrate contains a minimum of 60% dry rubber content, and the skim latex contains about 4% to 5% dry rubber content. The centrifugation technique is the most widely adopted method for the production of latex concentrate in Southeast Asia, if not in the world. More than 90% of all the latex concentrate is produced via this technique. In the evaporation method, only water is removed from field latex, whilst in the creaming and centrifugation methods, a fair amount of non-rubber constituents is removed together with water (Chin, 1979). The three major types of latex concentrate, namely high ammoniated (HA), low ammoniated (LA), and medium ammoniated (XA), differ in properties, as shown in Table 2.2.

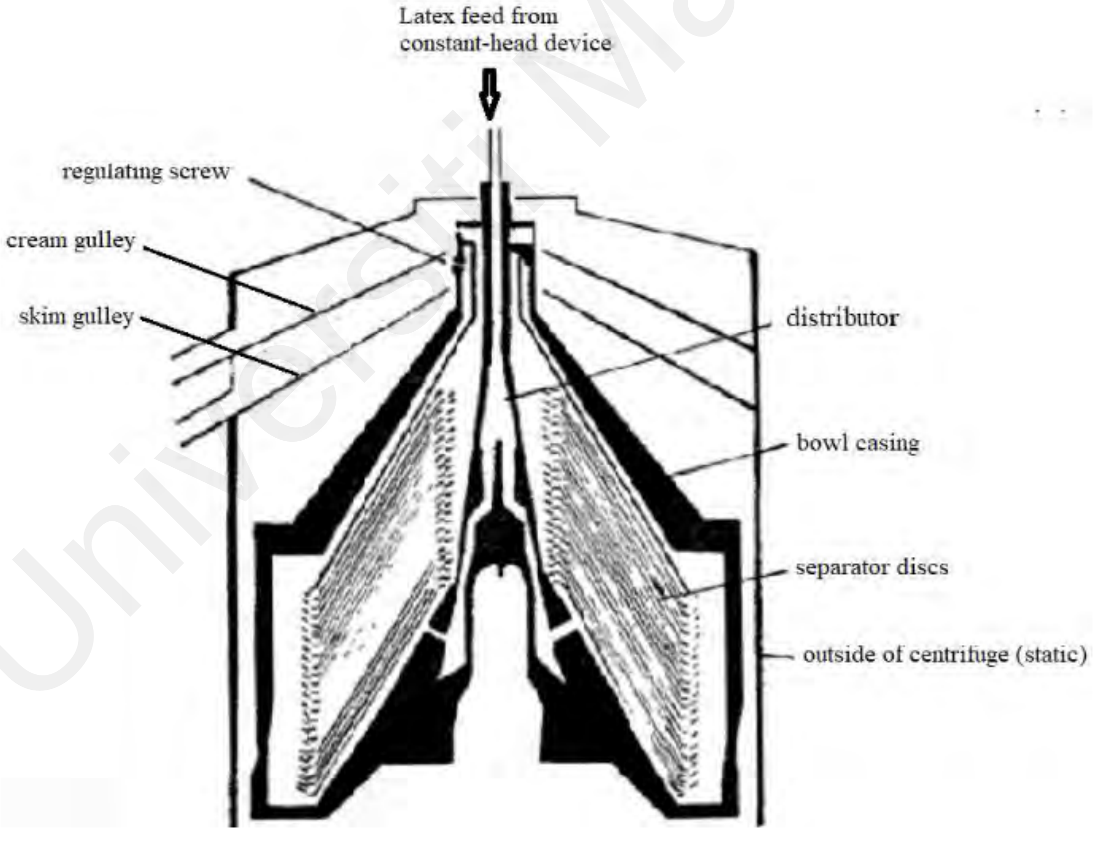


Figure 2.2: Diagrammatic section through the bowl of de Laval centrifuge (Lau, 1974).

Figure 2.3: The small-scale centrifuge machine used to separate the centrifuged and skim latex (Photo source from www.lgm.gov.my).

Table 2.2: Specification limits for the different types of NR latex concentrate according to ISO 2004:2017

Characteristic	Type HA	Type LA	Type XA[c]	Type HA Creamed	Type LA Creamed	Method of test
Total solids content, min % (by mass)	61.0 or as agreed between the two parties			65.0	65.0	ISO 124
Dry rubber content, min % (by mass)	60	60	60	64	64	ISO 126
Non-rubber solids, max[a], % (by mass)	1.7	1.7	1.7	1.7	1.7	-
Alkalinity (as NH_3), calculated with respect to the latex concentrate, % (by mass)	0.60 min	0.29 max.	0.30 to 0.59	0.55 min	0.35 max	ISO 125
Mechanical stability, min [b], Seconds	650	650	650	650	650	ISO 35
Coagulum content, max., % (by mass)	0.02	0.02	0.02	0.02	0.02	ISO 706
Copper content, max, mg/kg of total solids	8	8	8	8	8	ISO 8053
Manganese content, max., mg/kg of total solids	8	8	8	8	8	ISO 7780
Sludge content, max., % (by mass)	0.06	0.06	0.06	0.06	0.06	ISO 2005
Volatile fatty acid (VFA) number, max	0.06 or as agreed between the two parties					ISO 506
KOH number, max	0.70 or as agreed between the two parties					ISO 127

[a] The difference between the total solids content and the dry rubber content

[b] The mechanical stability time normally stabilises between 21 and 35 days when the latex is fully

[c] XA is equivalent to medium ammonia (MA) latex

2.2.1.2 Creamed latex

In the creaming process, the ammoniated field latex is treated with a fatty acid soap and a creaming agent such as ammonium alginate, filtered, warmed to 40°C and stored in bulk creaming tanks. After a few weeks, the rubber-rich cream layer is separated from the serum, and the cream can be run off separately and homogenised to give a concentrate of 66% to 68% solids content. Creamed latex concentrate is usually used for latex thread manufacturing (Anon, 1958; Gorton, 1972; Ochigbo et al., 2011; Suksup et al., 2017).

2.2.1.3 Evaporated latex

In the evaporation process, field latex is usually stabilised with soap and potassium hydroxide in preference to ammonia. Water is removed from the latex in film evaporators under high temperature and low pressure. The resulting latex possesses a high total solids content (72% or more) and high rubber content (68% or more). Evaporated latex is used for impregnation, casting, adhesive, as well as for preparing rubberised road surfaces (Anon, 1958; Blackey, 1997; Gorton, 1972)

2.2.2 Natural rubber latex properties

The properties of the natural rubber latex concentrate produced is tested to confirm whether its quality meets the agreed specification and to determine the pricing of NR latex for trading purposes. The analytical test results will also provide a better understanding of the physical and chemical nature of the latex, particularly on the colloidal stability that influences the properties of the end product, either tensile and tear strengths or extractable proteins (EP) content.

2.2.2.1 Total solids content

The total solids content (TSC) of the latex or a latex compound is defined as the percentage by mass of the whole, which is non-volatile under specified conditions of drying in an open atmosphere at an elevated temperature. The quantities of total solids content (TSC) are useful to measure the proportion of the latex or latex compound, which is non-volatile, usually water that volatile component (Blackley, 1997). The latex was dried to constant mass under specified conditions, either at atmospheric pressure or under vacuum for 2 hours at 110 °C or 16 hours at 70 °C. In the case of NR field latex, the TSC can be used to verify the presence of a considerable amount of adulterants, which are non-volatile but water-soluble. The TSC of latex is easily and accurately determined and is the basis on which the amounts of inorganic metallics such as K, Mg, Ca, Cu, Mn, and Na in the latex is expressed (Wong, 1988).

2.2.2.2 Dry rubber content

The dry rubber content (DRC) is a measurement of the percentage weight of latex that is coagulated by acetic acid under closely defined conditions (Blackley, 1997; ISO 126, 2005). It is important to note that the percentage of DRC slightly exceeds the percentage of rubber hydrocarbon content as small quantities of various non-rubber constituents are co-precipitated with the rubber. The procedure involves dilution of a known weight of latex, coagulation of the latex by acetic acid, rolling off the coagulum into a thin sheet. Then, followed by drying the rubber film in an oven at 70 °C ± 5 °C for 16 hours until constant weight. The difference between TSC and DRC will give the non-rubber content of the latex. Non-rubber solids defined as the fraction of the mass of the latex, which is non-volatile under the specified conditions of drying. The rubber content of field latex is typically about 25% to 35%, but it sometimes can be up to 43% by weight (Gorton, 1972; Ruhida, 2012).

2.2.2.3 Alkalinity

Latex usually undergoes spontaneous coagulation within a few hours after tapping because of bacterial and enzymatic action on the non-rubber constituents that lead to the development of acidity and destabilisation of the latex. Preservatives such as ammonia, formaldehyde, and sodium sulphite are commonly used to prevent degradation. Alkalinity is defined as the free alkali content of latex and expressed as the percentage of ammonia in the latex (ISO 125, 2020; Lai, 1988; Ng, 1979).

2.2.2.4 Mechanical stability time

During the preparation, storage, transportation, and processing, the latex concentrate was subjected to violent shearing stresses produced by mechanical treatment such as pumping and stirring, which led to the destabilisation of the latex. The stability of latex concentrate is measured by its mechanical stability time (MST). It represents the time in seconds required for the coalescence of rubber particles to be initiated when the latex is subjected to high-speed stirring under defined conditions. A test portion of latex concentrate is diluted to 55% (m/m) total solids content and stirred to high speed, and the time required to initiate visible flocculation is recorded, this being regarded as a measure of the mechanical stability according to ISO 35 (2004) test method. By performing the MST test, the stability of latex can be monitored since the value is related to the VFA number and viscosity of latex (Davies & Pendle, 1992). The stability of latex is assumed unstable when MST value is lower than 650 seconds.

2.2.2.5 Coagulum content

The coagulum content is the material that is retained on a mesh of 180 ± 10 μm size under specified conditions of filtration. The material that is retained comprises pieces of polymer coagulum, skin and coarse foreign matter. The coagulum content is determined by diluting a known weight of latex with a 5% aqueous solution of an appropriate surface-active substance such as potassium oleate or ammonium laurate at pH 10. After thorough mixing, the diluted latex is filtered through a clean stainless-steel wire cloth at 180 ± 10 μm that has been dried to constant mass at 100 °C ± 5 °C according to ISO 706 (2004) test method. The purpose of characterising the coagulum content is to measure the impurities that are present in latex (Wong, 1992).

2.2.2.6 Sludge content

Similar to the coagulum test, the sludge is referred to as non-polymer impurities in latex such as dirt, sand, fragments of tree bark, and magnesium ammonium phosphate, which tend to sediment under the influence of gravity (Wong, 1992). In this method, a known mass of latex is centrifuged at a specific speed to separate the rubber particles from the sediment. After removal of the rubber component, the residue is repeatedly centrifuged with an ethanol-water-ammonia mixture until the supernatant liquid is clear. The residue is then dried at 70 °C ± 5 °C until the loss in mass is less than 1 mg. The sludge content is expressed as a percentage (m/m) of the initial mass of the latex according to ISO 2005 (2014) test method.

2.2.2.7 Volatile fatty acid number

The volatile fatty acids in latex is produced by the action of microorganisms upon some of the serum carbohydrates in the latex. The amount of volatile fatty acids in latex is expressed as the number of grams of potassium hydroxide required to neutralise the volatile fatty acids in a latex sample containing 100 grams of total solids. The testing involves the extraction of latex serum with ammonium sulphate and steam distillation of a known quantity of the extracted serum after acidification. Finally, the VFA number is measured by the titration of the distillate with barium hydroxide (Blackley, 1997;

ISO 506, 2020). A high VFA number indicates an inadequate amount of preservatives in the latex. The carbohydrates and certain amino acids in the latex are metabolised by bacteria and are converted to volatile fatty acids, mainly acetic, formic and propionic acids. The VFA number is used as an indicator to measure the degree of bacterial decomposition that the latex has undergone. An increase in the amount of salts of VFA in latex causes a decrease in the mechanical stability of the latex (Chen, 1988).

2.2.2.8 pH

Although pH is a parameter that is not included in the ISO specifications, it is an important test as the pH of latex has been shown to have a significant influence on the stabilising efficiency of all fatty acid soaps and the solubility of zinc oxide that are closely correlated to latex stability. The pH measurement was performed according to ISO 976 (2013) test method.

2.2.2.9 Potassium hydroxide number

The potassium hydroxide number (KOH No.) of preserved latex is defined as the number of grams of potassium hydroxide equivalent to the acid radicals combined with ammonia in latex concentrate containing 100 grams of total solids (Blackley, 1997). KOH number is also a measure of the content of ammonium soaps and ammonium salts that are formed during the changes that have occurred in latex according to ISO 127 (2018) test method. Some of these changes may have been caused by the bacterial action on the non-rubber constituents prior to full ammoniation and others by the hydrolysing action of the ammonia, causing a further break-down of the proteins during storage. Therefore, KOH number is indicative of the state of preservation and the age of the latex. High KOH number indicates a poorly preserved latex, and old latex usually has a higher KOH number.

2.2.2.10 Viscosity

The viscosity of latex is measured based on the torque produced on a specific spindle that is driven by an electric motor to rotate at a constant rotational frequency and controlled rate of shear while immersed to a specified depth in the latex. The distribution of strain rate depends upon the flow characteristics of the fluid. The viscosity of a latex sample is determined by a Brookfield viscometer. The viscometer readings are affected by turbulence and by the angle of immersion of the spindle. The latex viscosity is determined at 25 °C ± 2 °C except in tropical climates, where it is determined at 27 °C ± 2 °C according to ISO 1652 (2011) test method. The apparent viscosity is obtained by multiplying the torque reading by a coefficient that depends on the rotational frequency and size of the spindle. Measurements are made on the undiluted latex or the latex after dilution to require total solids content.

2.2.2.11 Metallic content

It is well known that the content of copper and manganese in certain forms are known to catalyse the oxidative breakdown of NR. Copper and manganese contents were measured according to ISO 19050 (2015) test method. The latex is filmed, ashed, and digested in nitric acid before the absorbance of the solution is measured at a selected wavelength on the ICP-OES. The absorbance is proportional to the concentration of the metallic of interest. The predominant metallic cations present in high ammoniated latex concentrate are K, Mg, Fe, Cu, and Zn. The concentrations of K, Mg, Fe, and Cu reported are1800 ppm, 30 ppm, 5 ppm, and 3 ppm, respectively. The metallics Fe and Cu have a potentially deleterious effect that may cause the latex concentrate to oxidise, and copper is one of the metals which has a catalytic effect on the degradation of latex and rubber (Chen, 1988; Duangthong et al., 2017; Rattanadaecha, 2012).

2.2.3 Previous study on the natural rubber latex properties

Once the tree is tapped, the field latex that exudates out is susceptible to contamination by bacteria from the bark of the tree or the surrounding air. The relatively high protein and carbohydrate content of field latex provide a nutritious base for bacteria, which could tend to grow rapidly if unchecked. The addition of ammonia and other preservatives in the field latex is to minimise the bacterial growth, which could cause coagulation of the latex. It is good practice to measure the VFA content of field latex before processing it into latex concentrate. It is the best indicator of the state of preservation of the latex (Blackley, 1997; Chen, 1988; Narongwongwattana et al., 2015). A VFA number, more than 0.02, can be considered good enough to turn into latex concentrate (Wong, 1988). Other tests that needs to be carried out on the field latex include DRC and the values are in the range of 25% to 40%.

According Ong (2000) the clonal origin of the natural rubber tree has a significant influence on the latex yield and properties of raw NR. Therefore, the selection of suitable rubber clones is essential. RRIM 2000 series were found to have a timber yield 3 to 5 times and latex yield three times higher as compared to RRIM 600. The latex properties such as DRC, TSC, NH_3, MST, KOH number, sludge, coagulum, viscosity, gel content, and metallics tests were comparable to those of presently used clones and the latex could be used to produce rubber products without difficulty. None of the clonal latices has a DRC below 25%, a level where the latex is unsuitable for latex concentrate preparation. The field latex was tested for ammonia content, rubber content, and VFA number before processing into latex concentrate. This is to ensure that the latex concentrate produced after processing is colloidally stable and meets the ISO 2004 (2017) specification. Therefore, it is essential to preserve natural rubber latices with ammonia. The ammonia content and VFA number in field latex should be at least 0.3% and maximum 0.03 respectively. The DRC of field latex is between 25% and 40% and is converted to a minimum 60% DRC latex concentrate using suitable skim screw adjustments. Field latex with DRC less than 25% is not economical to be processed into latex concentrate as less weight of latex concentrate is produced after the centrifugation process (Lau, 1980).

The properties of NR latex concentrate that must be evaluated after processing are DRC, TSC, VFA No. and alkalinity. Then, after two weeks, the parameters that need to be monitored are VFA No., MST, pH, KOH No., viscosity, sludge, coagulum, metallic content, nitrogen content, and gel contents. These parameters are indicative of the latex stability that could affect the quality of latex products being manufactured. TSC property was able to indicate the rubber and non-volatile residue in the natural rubber latex. Whilst DRC which is a much closer approximation to the actual rubber content than TSC. Therefore, the different between TSC and DRC was taken as an indication of non-volatile non-rubber substances in latex (Blackley, 1997; Ong, 2000; Yip, 1990). Latex properties such as NH_3 and VFA No. were determined to confirm that the natural rubber latices inadequately preserved with ammonia and to indicate the activity of microorganisms or bacteria in the latex. The alkalinity content for latex concentrate is usually in the range of 0.60% to 0.70%, and VFA No. is less than 0.06. The KOH number is a measure of the content of anions in latex. These include those of the volatile fatty acids, higher fatty acids, phosphates, carbonates, and bicarbonates. The presence of carbonates and bicarbonates may be due to the absorption of carbon dioxide during exposure of the latex to air. All the other anions are inherent components of the latex system, the contents of which may vary from clone to clone. The KOH number is also defined as the number of grams of potassium hydroxide equivalent to the acid radicals combined with ammonia containing 100 grams of total solids. Higher KOH and VFA numbers reflect inadequate preservation of latex (Yip, 1990).

Another important parameter, MST is related to the colloidal stability of latex concentrate. The value of MST less than 650 seconds implies that the latex is in an unstable condition and will easily flocculate or coagulate when exposed to shear force, temperature of storage and agitation. The minimum requirement for latex concentrate in stable condition is 650 seconds. The MST of latex concentrate of different types of RRIM clones collected from SPKT, Kota Tinggi, Malaysia is as shown in Table 2.3. All the clones had MST values more than 650 seconds indicating that the latex concentrate prepared from these RRIM clones are colloidally stable. The RRIM clones were categorised according to their mechanical stability time, which is an indicator of latex stability following as ISO 2004 (2017) specification and Ong (2000).

Table 2.3: MST of latex concentrate of different types of RRIM clones (Ong, 2000)

RRIM Clones	**Mechanical Stability Time, MST (seconds)**			
	< 650	**650 – 900**	**900 - 1200**	**>1200**
	Nil	600, 2001, 2014, 2023	2002, 2015, 2016, 2024, 2026	2008, 2009, 2025

The nitrogen content of field latex is mostly associated with the proteinaceous materials present, and it has been reported that about 30% of these materials are present in the rubber hydrocarbon phase and about 70% in the non-rubber phase. The proteins have a significant role in influencing the stability of rubber latices. Earlier work by other researchers have shown the effect of seasonal changes on the nitrogen content of clones prior to the RRIM2000 series (Ali et al., 2020; Resing, 1955). The nitrogen content in NR latex film, varied from 0.51% to 0.87% (Morris & Sekhar, 1959; Wei et al., 2020; Yip, 1990). The metallic contents that were measured in the latices were mainly Mg, P, Cu, and Mn. Both Mg and P influence latex stability, while Cu and Mn are known to enhance the oxidation of rubber polymers. However, the latter two metallics are known to be present in latex in relatively low concentrations at a range of 1 ppm to 7 ppm for copper and less than 2 ppm for manganese as compared to other metals, such as potassium, magnesium, calcium and sodium (Bateman & Sekhar, 1996; Rattanadaecha, 2012).

2.3 Fillers

In ISO 1382 (2020), fillers are defined as solid compounding ingredients in particulate form, which may be added in relatively large proportions to a rubber or rubber latex for technical or economic purposes. The application of fillers in rubber products was not only for cost reduction but also for formulating new materials specifically for better or improved properties (Amir Hashim et al., 2004; Prasopdee & Smitthipong, 2020; Roslim & Amir Hashim, 2010). It is believed that the production of fillers has gone through a sophisticated process since it depends on its usage. Fillers are added into the rubber to fill spaces between the rubber particles, and they can be modified and tailored to any application in order to influence the design of manufacture (Wypych, 2010). However, they are likely to degrade the properties of the rubber or latex to which they are added.

2.3.1 Filler properties

Filler properties are related to the end product performance because of filler characteristics such as physical state, chemical composition, particle shape, particle size, aspect ratio, particle size distribution, internal particle structure, particle-particle association, density, refractive index, colour, pH, moisture, oil absorption, thermal properties and magnetic properties. The potential application of filler in rubber material is dependent on the filler characteristics in order to gain optimum filler performance or right filler for a specific application (DeArmitt & Rothon, 2017). Table 2.4 shows the range of fillers properties (Wypych, 2010)

Table 2.4: Characteristics of fillers (Wypych, 2010)

Physical state	All materials are solids and in semi-solid form or dispersion form (water slurries)
Chemical composition	Inorganic or organic with established chemical composition. Maybe a single metallic, natural product, mixtures of different materials in unknown proportions or materials of a proprietary composition
Particle shape	Spherical, cubical, irregular, dendritic, block, plate, flake, fibre, mixtures of different shapes.
Particle size	Range from a few nanometers to tens of millimetres (nanofillers to fibres)
Aspect ratio	1 (spherical or cubical) to 1,600 (fibers)
Particle size distribution	Mono disperse, designed mixture of sizes, Gaussian distribution, irregular distribution
Particle surface area	From 1 to over 500 m^2/g. Depending on the specific surface area particles have different levels of porosity from completely non-porous and smooth to very porous with a range of pores sizes
Particle internal structure	Hollow to porous to void free solid
Particle-particle association	Singular, agglomerates, aggregates, flocculated materials
Density	From 0.03 g/cm^3 (expanded polymer beads) to 19.35 g/cm^3 (tungsten powder)
Refractive Index	Typical range from 1 (air) to 3.2 (iron oxide)
Colour	Full range of colours from colourless and transparent, with increasing opacity through white to black
pH	From 2 (carbon black) to 12 (calcium hydroxide)
Moisture	Traces (most) to 50% (slurries and fillers having crystalline water)
Oil absorption	From a few grams to over 1000 g/100 g of filler
Thermal properties	Thermal expansion coefficient and thermal conductivities vary widely
Electric and magnetic properties	Wide variations are possible between non-conductive and conductive and between magnetic and non-magnetic

In order to transform raw rubber into a useful product, it has to be mixed or compounded with other ingredients. Previously, the main reason for rubber compounding is to incorporate ingredients necessary for vulcanization. Vulcanisation is a chemical reaction that enables the formation of cross-links between long polymer chains resulting in a three-dimensional network structure (Azemi, 2009; Kohjiya & Ikeda, 2021; Morton, 2013). Appropriate compounding enables the vulcanised product to achieve the required levels of physical properties such as tensile strength, tear strength and hardness. Different types of rubber chemicals like accelerators, fillers, plasticisers, antioxidants, antiozonants, processing aids were mixed into rubber compounds to achieve the desired characteristic over the whole of its service life. It is well known that the main reason for adding fillers into latex compounds is to reduce cost without affecting the end-product properties. However, to a certain extent, fillers will affect the mechanical properties like tensile strength and modulus of the product.

The application of carbon black and other inorganic minerals such as clay, calcium carbonate, silicates, etc., in dry rubber has clearly demonstrated improvement in the mechanical properties of the rubber products (Dominic et al., 2020). For example, carbon black has offered excellent reinforcement due to its strong interaction with rubber molecules. However, at higher loading, it decreases the processability of rubber compounds. The reinforcing effects occurred from the fractal nature of filler aggregates. At high usage of filler, the surface activity and filler networks in the rubber matrix played an essential role in controlling the interaction between rubber-filler (Frida et al., 2019; Mathew et al., 2007; Valent n et al., 2010).

2.3.2 Types of fillers

Fillers can be categorised as reinforcing or non-reinforcing fillers. A rubber compound generally contains fillers and other additives such as curatives, plasticisers, and coupling agents. It is important to consider the effect of each ingredient after processing on the properties of a vulcanisate compound, which could affect the performance and service life of the product. In the case of dry rubber, carbon black and other inorganic minerals such as clay, calcium carbonate, silicates, etc., are used to improve the mechanical properties of vulcanised rubbers. Table 2.5 showed the different types of inorganic fillers available to the rubber industry (Dannenberg, 1982; DeArmitt, 2000; DeArmitt & Rothon, 2017).

Table 2.5: Different types of inorganic fillers (DeArmitt, 2000)

No	Types of inorganic filler	Name of filler
I	Ground natural products	a) Calcium carbonate- limestone, marble, chalk and oyster shells b) Calcium and magnesium silicate -talc, dolomite and wollastonite c) Amorphous silica d) Low volatile bituminous coal e) Delayed petroleum coke
II	Classified natural products	a) Clays (kaolin) b) Diatomaceous earth (silica)
III	Precipitated products	a) Calcium carbonate b) Silicas – hydrated c) Sodium aluminium silicate d) Aluminium silicate e) Calcium silicate f) Aluminium oxide – hydrated
IV	Fumed products	a) Carbon black b) Silicas – anhydrous c) Zinc oxide d) Magnesium oxide e) Aluminium oxide – anhydrous
V	Surface modified products	a) Surface treated or coated (hydrophobic) calcium carbonates, talcs, clays and silicas b) Calcined clays c) Treated clays for improved curing (amines, bases, accelerators, etc.) d) Silanised clays (coupling agents) e) Resin-grafted clay (aminoplast) f) Polymer-grafted calcium carbonate (carboxyl-terminated polybutadiene)

The non-reinforcing fillers or non-black fillers are usually used in natural rubber latex products for cost reduction purposes; semi-reinforcing filler for processing improvement and reinforcement of the rubber by increasing hardness, tensile strength, tear resistance, abrasion resistance and other properties in the production of a compound in order to achieve high-performance products. Ground calcium carbonate is widely used as filler in plastics, rubber, paper, paint and ink industry. It is relatively cheap and has superior whiteness, inertness, and incombustibility, low oil-absorbency, and water absorption as compared to other inorganic powders. It is used in the application of polymer composites in order to improve workability. The cost of the polymer composites can be decreased greatly by replacing the volume of expensive resin with an inexpensive GCC filler (Jeong et al., 2009; Rothon & Paynter, 2017).

2.3.3 Classification of fillers

Fillers can be classified according to their chemical and physical characteristics or divided into organic and inorganic groups. Each of these two groups can be further subdivided into fibrous and non-fibrous types. Protein filler is included together under organic, as shown in Figure 2.4.

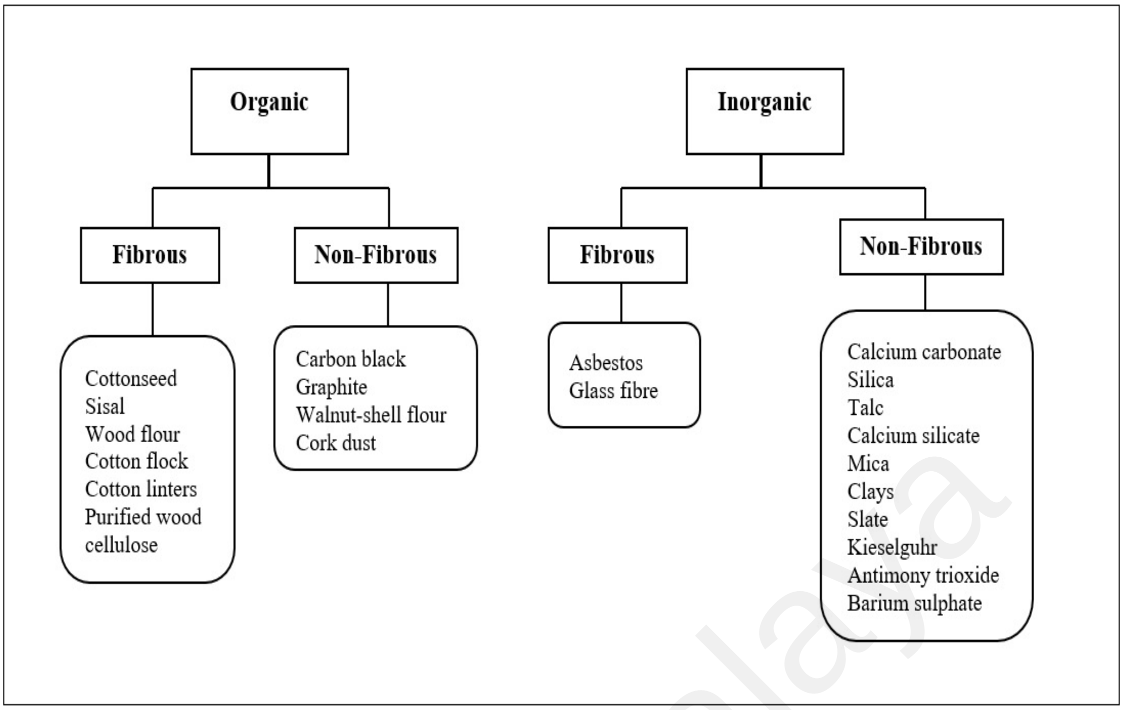


Figure 2.4: Classification of fillers (DeArmitt, 2000).

The classification of fillers is quite crucial as it can help researchers in choosing the right filler for the specific application in rubber material. For example, classification by particle size is helpful since particle size can affect the performance of the end products. The eight most important characteristics in classifying the fillers are as follows:

i. Particle size and distribution
ii. Aspect ratio
iii. Chemical composition of the surface
iv. Mechanical properties of filler particles
v. Electric and thermal conductivity
vi. The quantitative description of interactions
vii. Composition Mixtures
viii. Optical properties

Existing data has enabled us to classify fillers according to the above properties (Barbin & Rodgers, 1994; DeArmitt, 2000; DeArmitt & Rothon, 2017; Defonseka, 2020; Ritchie, 1972).

2.3.4 Calcium carbonate

Calcium carbonate ($CaCO_3$) occurs naturally as the predominant component of chalk and limestone, and also as the double carbonate with magnesium in dolomite ($CaMg(CO_3)_2$) and ankerite ($Ca(Mg.Fe)(CO_3)_2$). The two naturally occurring crystalline forms of $CaCO_3$ are aragonite (orthorhombic) and calcite (hexagonal). The latter is more stable and used in the study conducted by Ritchie (1972). At the industrial scale, powdered $CaCO_3$ can be produced by two methods, quarried and ground from naturally occurring materials. It is also made by precipitation from dissolved calcium hydroxide and carbon dioxide. The natural ground $CaCO_3$ and the synthetically prepared precipitated material compete industrially based primarily on particle size and the characteristics imparted to a product. $CaCO_3$ is one of the most versatile mineral fillers added to a wide range of products, including paper, paint, plastics, rubber, textiles, caulks, sealants, and printing inks. Commercial grades of $CaCO_3$ mined from natural sources are either calcite, aragonite, or sedimentary chalk. In most precipitated grades aragonite is the predominant crystal structure. $CaCO_3$ is listed as a food additive and is not considered a toxic material. The exposure to dust is regulated, and both natural ground and precipitated $CaCO_3$ can contain low levels of impurities that are regulated (Al Omari et al., 2016; Carr et al., 2000; DeArmitt, 2000; DeArmitt & Rothon, 2017).

2.3.4.1 Precipitated calcium carbonates

The precipitated calcium carbonates (PCC) refers to those forms of $CaCO_3$ produced by chemical means as opposed to a natural product. In the manufacturing of PCC, the process involves the interaction of calcium and carbonate ions under specific and controlled conditions, followed by separation, drying and disintegration. The grades depend on additives that may be added at appropriate stages. Other forms of production, such as conversion of soda ash to caustic soda by heating the solution with slaked lime resulted in the formation of sludge comprising precipitated $CaCO_3$ mixed with calcium hydroxide. Another method, the carbonated lime type of $CaCO_3$ is produced by calcining limestone in a kiln, slaking the lime produced and diluting it to a milling suspension. The kiln gases, which contain a high proportion of carbon dioxide, are purified by scrubbing and then fed into the suspension of slaked lime. The precipitated $CaCO_3$ is then dried under controlled temperature as irregular or uneven heating could affect the particle size distribution and properties. The precipitated $CaCO_3$ also can be prepared from calcium chloride which may be converted by reaction with sodium carbonate. However, many factors can influence the nature of the product such as temperature, reactant concentration, the flow of addition of components and speed of mixing (Abeywardena et al., 2020; El-Sherbiny et al., 2015; Erdogan & Eken, 2017; Ritchie, 1972).

2.3.4.2 Ground calcium carbonates

Ground calcium carbonates (GCC) or Limestone is obtained by surface quarrying or underground mining. All the limestone extracted subsequently undergoes some form of preparation before use. In most instances, it is necessary to reduce the stone to a state of fine subdivision. Limestone varies in colour from pure white to black, and the hardness, density, porosity, and texture, as well as degree of purity, may vary greatly even in one deposit. Finely pulverised limestone or marble of high quality is used as a pigment and filler in the plastic industry under the name of whiting. It is used in the same applications as the precipitated calcium carbonates, which are produced by chemical means as opposed to the natural product (El-Sherbiny et al., 2015; Mohamad Akmal et al., 2013; Ritchie, 1972).

2.3.5 Selection of fillers

The selection of fillers for a specific application is unquestionably governed in the first instance by their chemical constitution, availability, and price. The limit of their usefulness as filling materials is dependent on their physical characteristics. Therefore, the ideal filler would have to comply with a wide range of both chemical and physical requirements. An indication of the requirements needed to achieve an ideal filler as the following list (DeArmitt & Rothon, 2017; Defonseka, 2020; Ritchie, 1972; Wypych, 2010) :

i. Maximum improvement in the desirable physical properties of the polymer compound
ii. Low moisture absorption
iii. Specific gravity appropriate for the application
iv. Good wetting characteristic
v. Freedom from deleterious chemical impurities and objectionable physical effects
vi. Low cost and good availability
vii. Non-inflammability
viii. Absence of odour
ix. Good colour, retained during processing without effect on other colours
x. Good chemical resistance
xi. Good heat resistance
xii. Availability in controlled particle size
xiii. Good dispersion characteristics
xiv. Low solubility in water and environmental solvent

Meanwhile, the most important physical properties of fillers are as follows:

i. Particle size, shape and distribution
ii. Surface area
iii. Oil absorption
iv. Bulk density
v. Density
vi. Refractive index, opacity and colour
vii. Hygroscopic and moisture content
viii. Hardness.

However, these properties are usually interdependent.

2.3.6 Filler surface modification

Smaller particles are needed to maximise the effect of the filler in a polymer. Surface energy of the particles increases with decreasing particle size. The failure of filler dispersibility has been attributed to the agglomeration of particles at high surface energy. Therefore, it is necessary to treat the filler with surface modifying agents to improve the dispersibility, water resistance, mechanical properties and reinforcement in a polymer system. Fatty acid, surfactant, resin etc., are often used as surface modifying agents for calcium carbonate. An examination of the properties of surface-modified filler is necessary to gauge its effectiveness component, physical properties, including fluidity, adhesion and flow ability. Therefore, the surface properties such as contact angle measurement, hydrophobically and dispersive of treated and untreated filler need to be characterized (Krysztafkiewicz et al., 1994; Li et al., 2020).

Besides economic reasons, the purpose of modifying or treating the fillers that are added into the polymer or rubber composites is to impart some characteristics such as stiffness, electrical insulation, thermal stability and optical properties. However, it has been observed that in most cases, a loss in mechanical properties such as tensile strength and tenacity is observed due to a lack of affinity between the polymer and the filler resulting in low adhesion. The difference in surface energy between the components in the composite is believed to be the main reason for the weak affinity between filler and polymer. Some fillers can be treated with organic compounds that act as coupling agents to resolve the weak affinity. The coupling agents may reduce the surface energy and improve interfacial adhesion. The weak affinity can be overcome to a certain when filler particles are extremely small, as in nanocomposites structures. For example, the filler, montmorillonites, when added into composites at (1 to 5 wt.%), enhanced the physicomechanical thermal and microphase properties. The high surface area is one of the most attractive characteristics of nanoparticles as it creates a great amount of interphase in a composite. It is well documented that intermolecular interaction takes place between the polymer and nanoparticles. Another significant factor is the uniform distribution of nanoparticles over polymer matrix that helps to improve nanoparticles and matrix interaction (Kamal et al., 2012; Rudawska & Frigione, 2020).

The importance of calcium carbonate as filler in composite materials is well recognised, and its application in the polymer industry is increasing. However, the incompatibility between the calcium carbonate having high energetic hydrophilic surfaces and the low-energy hydrophobic surfaces of the polymer is a problem that needs to be resolved before calcium carbonate can be used as a functional filler. In order to improve the dispersibility of calcium carbonate in polymeric medium, its surface needs to be rendered organophilic. A variety of surface modifiers such as silanes, phosphate, titanates and others are available. The most widely used calcium carbonate surface coating is the treatment with stearic acid, in which the monolayer of hydrophobic organic molecules is attached to the mineral surface. The structure and properties of such organic film have a strong influence on the final properties of the composites because this film represents the interface between the two phases of the heterogeneous material. Besides, it is well known that when the fatty acids are absorbed onto calcium carbonate surface, they initially form a monolayered array of alkyl chains oriented so that the carboxylic groups are adjacent to the mineral surface (Ghosh et al., 2020; Mihajlović et al., 2009; Roy et al., 2016; Yu et al., 2020).

2.4 Treated filler

As mentioned earlier, polymers are often mixed with various particulate additives and fillers to produce a new class of materials, termed polymeric composites. This combination of materials leads to new desirable properties. Mineral fillers are added to the polymer matrix to improve mechanical properties, surface hardness, etc. (Zaman & Khan, 2021). The thermogravimetric method is commonly used to study the absorption of organic material by natural minerals, for example, filler treated with a fatty acid such as stearic acid (Mihajlović et al., 2009). In a study on calcite surfaces treated with different amounts of stearic acid in toluene, thermogravimetry was used to detect the organic contaminants on the calcite surface and to determine the optimal amount of surfactant necessary to treat it with an alkyl monomer (Yousefi et al., 2020). The researchers reported that thermal analysis could differentiate between chemisorbed, intercalate (local bilayer), and free acid molecules, which may be present on the surface of calcite fillers (Ersoy & Köse, 2020; Mihajlović et al., 2013). In the solution method, the calcite surface is usually treated with an acid solution in a nonpolar solvent. Other researchers have also had reported that the calcite wetted with distilled water before adding the n-decane plus stearic acid solution could alter the adsorption of acid on the surface of the mineral (Hansen et al., 2000).

2.4.1 Toughening mechanism of treated filler

Several models of the toughening mechanism have been proposed to explain the impact performance of particulate-filled polymer nanocomposites. It was accepted that a weak interface is needed to promote debonding between rigid particles and the polymer matrix, allowing for micro void formation. The micro voids are required to generate the energy-absorbing mechanism arising from extensive plastic deformation in the remaining matrix ligaments between neighbouring voids. In nanoparticles study, it is well-known that the particles tend to aggregate during compounding with the polymer matrix or even before its production and storage. The aggregation of nanoparticles is connected to their small particle size since the equilibrium between adhesion and separation forces is strongly dependent upon this parameter. For small particles, separation forces are smaller than adhesion forces. If the formation of large agglomerates is allowed during the various stages of the processing of polymer composites, brittle behaviour will result. Large agglomerates, at limited stresses, can either debond from the matrix or break inside with the formation of cracks that propagate through an aggregate, and this leads to the formation of many cracks and other defects responsible for fracture (Cao et al., 2016; Mihajlović et al., 2013; Shi et al., 2010; Yang et al., 2019).

Stearic acid is one of the fatty acids that is commonly used to treat or coat filler. Stearic acid was chosen in this study for particular reasons. The stearic acid can act as a compatibiliser to bridge the boundary between the rubber particle matrix and filler particles. Besides, stearic acid is not only a suitable surface modifier for calcium carbonate but also a good dispersant for the dispersion of filler in the rubber matrix. It is a typical surfactant for PCC particles (Shi et al., 2010). Stearic acid is categorised as a universal, inexpensive surfactant and is used to improve $CaCO_3$ hydrophobic properties. Many studies have been done on the effect of stearic acid surface treatment on the mechanical properties and thermal behaviour (Cao et al., 2016; Mihajlović et al., 2009; Patti et al., 2021; Shi et al., 2010).

2.4.2 Application of fillers in rubbers

A remarkable phenomenon in polymer science is the reinforcement of rubber by fillers such as carbon black, clays and calcium carbonate. These fillers added to rubber formulation in order to optimise the properties needed for particular applications. Mineral fillers such as clay and $CaCO_3$ have been considered as useful fillers in rubber compounding due to their low cost (Jeon et al., 2018; Phuhiangpa et al., 2020; Roslim & Amir Hashim, 2010). The filler also was added in rubber compounding due to its ability to influence the dynamic properties of rubber vulcanisate. The ability to influence dynamic vulcanisate properties is its morphology, particularly particle size and particle size distribution as well as shape and distribution of filler aggregates and surface activity, particularly surface energy. It is well known from prior research that fillers often play a significant role in the modification of properties of rubber products because most fillers used to offer some functional benefit that contributes to the processability or utility of rubber products (Ghari & Shakouri, 2012; Ippolito et al., 2021; Kamal et al., 2012; Mohamad Akmal et al., 2013).

One usage of fillers in natural rubber is in the production of NR gloves. The use of NR gloves are well established in the healthcare sector. The gloves act as a barrier for the prevention of the transmission of contagious diseases. The superior attributes of natural rubber latex gloves are easy donning, comfort and fit, high elongation and low modulus, high tensile properties, superior tear and puncture strength (Amir Hashim et al., 2004; Mohamad Akmal et al., 2013; Roslim & Amir Hashim, 2010). Latex gloves with varying content of $CaCO_3$ have been produced and exported to other countries from Malaysia. Hence, a study must be made to establish an acceptable level of $CaCO_3$ that can be incorporated into latex gloves. It is known that there are many types of $CaCO_3$, such as ground limestone, ground chalk and precipitated whitings. The differences between the types are their particle size, material cost and effect on the physical properties. For example, ground limestone is an off-white powder with particle size below 100 mesh and used for low cost concerned, high loadings which used to give moderate hardness.

On the other hand, the ground chalk or whiting is a low-cost compound, able to give a moderate hardness and relatively high resilience at high loadings but with weak tensile strength and tear resistance. Precipitated whiting by-products from water-softening processes or produced by precipitation from solutions of calcium salts have a particle size ranging from about 20 µm to 50 µm (Ciullo et al., 1999). It is used as semi-reinforcing fillers whereby high loadings can be incorporated in mechanical goods and proofing to give low-cost products of excellent appearance with moderate hardness and better physical properties than the ground material (Ghari & Shakouri, 2012; Idrus et al., 2011; Wijesinghe et al., 2016). The active fillers like carbon black, zinc oxide, kaolin, etc., when added to latex, either lower the strength of latex vulcanisates or yield compounds, the strength of which falls far short of the values found for compounds made by the usual energy-consuming method of addition of fillers to the dry rubber on rolls or in mixers (Dannenberg, 1982; Dogadkin et al., 1958; Fan et al., 2020; Vellinger, 1933).

The usage of treated filler or other additives in rubber compounds might be different in nature and physical form (Jeon et al., 2018; Krysztafkiewicz et al., 1994; Nakatsuka et al., 1985; Papirer et al., 1984). However, the main objective is to achieve optimal properties as required by a specific application. Advances in filler and reinforcement technology in filled polymer composites are widely distributed across performance level parameters like physical properties (Katueangngan et al., 2016). Surface treatment of fillers that are generally classified as inert extenders or reinforcing agents helps reduce the use of the resin and improve specific physical properties (Ari et al., 2019; Krysztafkiewicz et al., 1994; Mihajlović et al., 2009; Papirer et al., 1984). The addition of fillers typically enhances physical properties such as flexural strength, flexural modulus, heat deflection temperature and overall stiffness and resistance of the composite to greater loads and at higher use temperatures. However, the disadvantage aspect of filler addition is loss in impact strength and development of plastic composites that are more challenging to process. Surface modifiers and treatments that function as processing aids or coupling agents are now entering a period of re-intensified development. New products for filler surface treatment that help to increase the extender loading and decrease the final cost of the resin composite have met several obstacles. For example, in mineral-filled thermoplastic composites, the high cost of the surface modifiers, their exotic chemistry and handling complexities act as deterrents to their usage. It is known that polymer matrices are mostly hydrophobic, and the surfaces of most fillers are hydrophilic in nature (Othman et al., 2017). So, it is difficult to achieve a strong interfacial bond between the continuous polymer phase and the discontinuous filler reinforcement phase. The optimum properties of polymeric compounds are achieved when the chemical and physical forms are present in a stable condition, and filler dispersion is homogeneous in the compounds.

Other types of filler sources such as biofiller were added into natural rubber latex compound and latex glove film formation via dipping process. A previous report by Tangboriboon et al. (2013) stated that water extractable protein was removed by using eggshell dissolved into hydrochloric acid to form calcium chloride solution. Meanwhile, Ye et al. (2000) have mentioned that calcium ions (Ca^{2+}) from calcium chloride ($CaCl_2$) also influenced the adsorption behaviour of proteins at the oil-water interfaces as well. Other reasons for this work are to reduce allergic reactions to latex and to increase mechanical properties of NRL films (Tangboriboon et al., 2019).

2.5 Sulphur vulcanisation of natural rubber latex.

Vulcanisation is a chemical process which cross-links the rubber chains to produce a three-dimensional network structure with enhanced strength and elastic properties (Azemi, 2009; Wei et al., 2019). The chemistry and technology of sulphur vulcanisation of NR have been investigated in great detail over many years. Almost all the fundamental investigation into the chemistry of sulphur vulcanisation of NR have been carried out using the polymer in bulk form or solution (Joseph et al., 2015; Lewis, 1986). Figures 2.5 and 2.6 show a schematic representation of a typical network structure of cross-link. This network structure consists of different type of cross-link depending on the types of vulcanisation system used (Sriring et al., 2020).

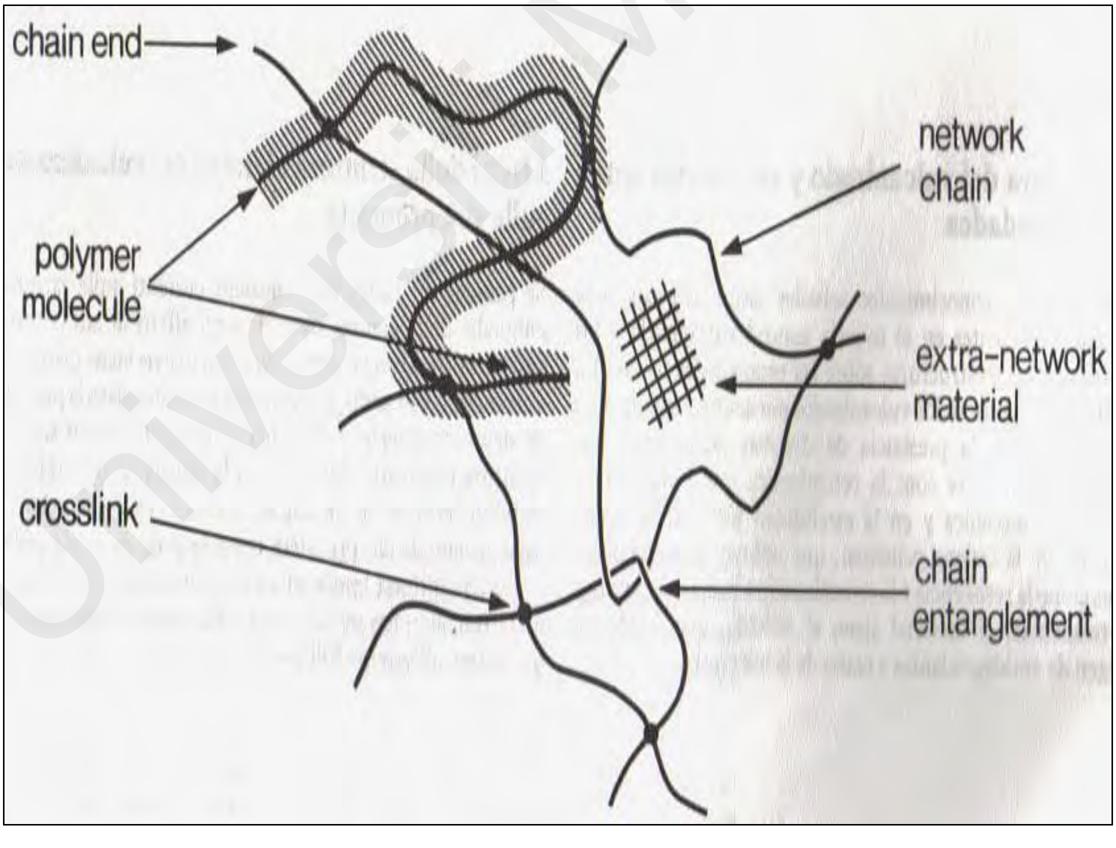


Figure 2.5: Molecular view of rubber vulcanisate (Lewis, 1986).

In vulcanisation system with sulphur alone, the process is very slow, and it takes a long time to cure. Adding accelerators such as zinc dibutyl dithiocarbamate (ZDBC), 2-mercaptobenzothiazole (MBT) and activators such as ZnO and stearic acid was found to reduce the cure time (Joseph et al., 2015; Blackley, 1997; Hofmann, 1967; Selker & Kemp, 1949). Sulphur vulcanisation systems can be grouped into conventional, efficient vulcanisation (*EV*) and semi-efficient vulcanisation (Semi-*EV*) as shown in Table 2.6. The cure system differs from each other in terms of sulphur to accelerator ratio. The conventional system has higher sulphur to the accelerator and in contrast, *EV* has low sulphur, high accelerator (Hofmann, 1967; Lewis, 1986). They are ranked by means of crosslinking efficiency, *E*, that can be defined as the number of network-combined sulphur atoms present per physically-effective chemical cross-link (Chapman & Porter, 1988).

(i) The conventional system consists of a high proportion of polysulphidic and disulphidic cross-links that resulted in excellent tensile strength, resilience, fatigue, and abrasion to the end products. However, this system has weak reversion resistance and poor resistance to ageing, set and stress-relaxation.

(ii) Efficient Vulcanisation or *EV* system can be defined as a curing system that maximises the use of sulphur for crosslinking. *EV* system produces mainly monosulphidic cross-links. Monosulphidic produces lower tensile strength, tear strength and abrasion resistance. However, it gave a better reversion resistance and resistance to ageing, set and stress relaxation than polysulphidic cross-links. This attributed to the higher bond energy of monosulphidic (84 kcal/mol) than polysulphidic (64 kcal/mol).

(iii) Semi-*EV* is an intermediate system between the conventional and *EV* system. Semi-*EV* provides a compromise between conventional and *EV* system to balance the mechanical strength and heat ageing resistance.

Table 2.6: Classification of sulphur vulcanisation systems (Lewis, 1986)

Cure system	**Sulphur Concentration (pphr)**	**Accelerator Concentration (pphr)**	**Cross-link type**	**Approximate range of *E* values obtained**
Conventional	2.0 to 3.5	1.0-0.5	Polysulphidic	10-25
EV	0.3 to 1.0	6.0 to 2.0	Monosulphidic	1.5-4
Semi-*EV*	1.0 to 2.0	2.5 to1.2	Mixed types	4-8
Very inefficient	3.5 to 10	0.5 to 0	Polysulphidic + cyclic sulphides	25-100

E – The crosslinking efficiency values

The main types of sulphur cross-link that occurred in a sulphur vulcanisation system can be classified as polysulphidic cross-link, monosulphidic cross-link and disulphidic cross-link, as shown in Table 2.6 and Figure 2.6. Polysulphidic (c) cross-links is a bridging in which two polymer chains are linked by a chain of three or more sulphur atoms. This polymer chain is weak and labile and is not thermally stable. However, it has a high influence on mechanical properties. Disulphidic cross-links (b) refer to cross-link where there are two sulphur atoms bridging the two polymer chains. The proportion of disulphidic cross-links is seldom more than 20% or 30% in both conventional and *EV* systems. Monosulphidic cross-links (a) refer to cross-link where the two polymer chains are bridged by one sulphur atom. It is usually thermally stable and has a high influence on reversion resistance and ageing. Cyclic sulphide (d) is an intra cross-link and formed on the same polymer chain. It is also known as main chain modification. This type of cross-link is not so important and can be formed by the thermal decomposition of polysulphidic cross-link. These cross-links are responsible for high resistance to low-temperature crystallisation. Other type of sulphur cross-link that can occur in a vulcanisation system are conjugated diene and triene groups (h, g) and pendent accelerator groups (f). Conjugated diene and triene groups are hydrogenated main chain modifications. It is formed by thermal decomposition of polysulphidic cross-links and they have little effect on the physical properties. In the case of pendent accelerator groups, it is formed from initial rubber bound pendent groups in which disulphide or polysulphide chains are terminated by accelerator fragments. It imparts polarity that arises from accelerator fragments and producing a weak secondary interaction between neighbouring polymer chains. This type of group can affect rebound resilience and heat-build up (Chapman & Porter, 1988; Krejsa & Koenig, 2020; Lewis, 1986).

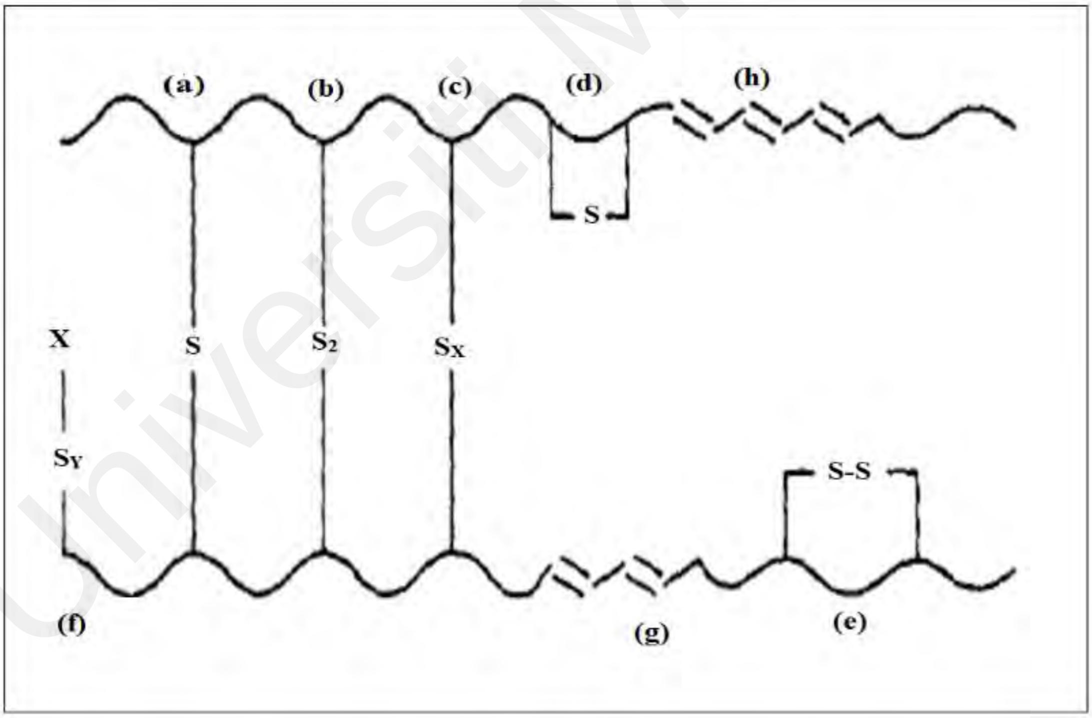


Figure 2.6: Structural features of an accelerated sulphur vulcanisate of natural rubber.(x = accelerator fragment, x≥ 3, y≥1) (Lewis, 1986).

The mechanism of sulphur vulcanisation in NR latex within the rubber particles is similar to that of dry rubber. Generally, the sulphur vulcanisation mechanism involves three main stages. In the first stage, the vulcanisation ingredients form sulphurating complexes with the natural activators in the latex. In the second stage, these complexes are assimilated into the rubber particles, and in the third stage, the rubber hydrocarbon reacts with the sulphurating complexes to form a network of cross-links in the rubber particles. However, the time needed for these reactions to occur depends on the types and level of chemical additives that are added into the latex and the temperature of the reactions. In latex vulcanisation, the network structure within each particle depends on the extent of vulcanisation that occurs in the rubber particle. The rubber particle that is highly vulcanised usually consists of a denser network of cross-link as compared to lightly vulcanised rubber particle (Krejsa & Koenig, 2020). Therefore, to extend more filler loadings will reduce the tensile properties of NR latex films because addition more than 40 pphr, the fillers will agglomerate and affect the polysulphidic cross-link concentration (Ruhida, 2020). Figure 2.7 shows the overall course of accelerated sulphur vulcanisation.

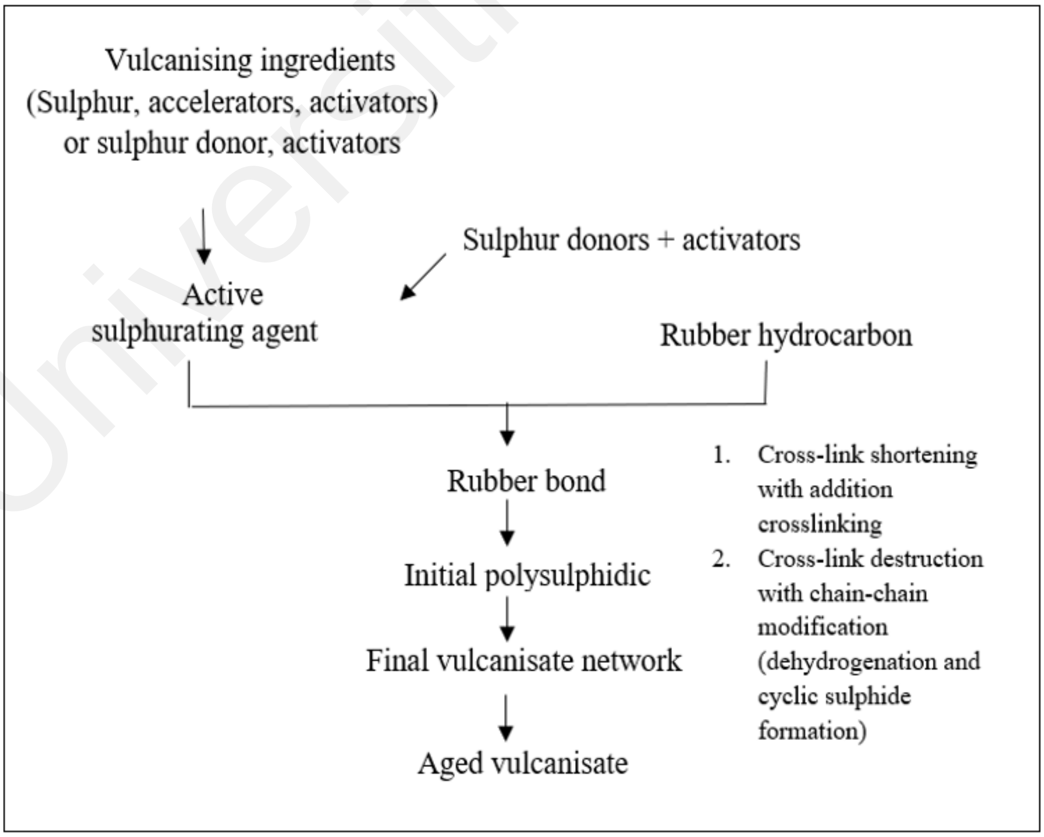
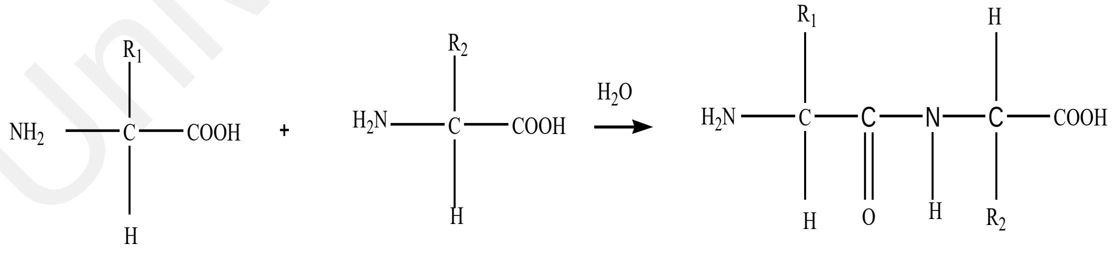


Figure 2.7: The pathway of sulphur vulcanisation (RH is natural rubber hydrocarbon and X is the accelerator fragment) (Chapman & Porter, 1988).

2.6 Protein content

2.6.1 Non-rubbers content in natural rubber latex

Natural rubber latex contains less than 2% of natural non-rubber materials like natural protein. Although the amount of protein is small or relatively low as compared to the rubber phase, the effect caused by the presence of non-rubber materials like protein on the processability, vulcanisation characteristic and physical properties of NR products is quite significant (Blackley, 1997). The non-rubber components of about 5% in natural rubber have been shown to affect the properties of vulcanised and un-vulcanised natural rubber. Protein is one of the predominant non-rubber components (Baker & Fulton, 2012; Theamsawade et al., 2020). Proteins are made up of various combinations of the twenty naturally occurring amino acids. One structural unit of amino acid has one amine, one carboxyl and one R side chain group. The R group influences the reactivity of the amino acid and consequently the protein complex when two or more of the amino acids combine through the formation of peptide bonds as shown in Figure 2.8 (Moolsin et al., 2021; Norhayati, 2005). A protein complex thus consists of one terminal amine and one carboxyl group.



$$NH_2-C(R_1)(H)-COOH + H_2N-C(R_2)(H)-COOH \xrightarrow{H_2O} H_2N-C(R_1)(H)-C(=O)-N(H)-C(H)(R_2)-COOH$$

Figure 2.8: Formation of a peptide bond (Norhayati, 2005).

The protein behaviour on rubber particles surfaces in the latex stage is vital in conferring the anionic charges on the surface of the particles, thus acting as barriers preventing the rubber particles to coalescence. The repulsion due to the similar anionic charges on each of the rubber particles prevents the rubber particles from combining and is believed to be one of the mechanisms of maintaining colloidal stability in NR latex. Protein is amphoteric and can behave as anions, cations or both depending on the presence of either basic or acidic groups in its R substituents. In an alkaline medium such as in NR latex, the acid functions of the proteins were ionised to become anions, leaving the basic portion unchanged and the reverse happens when the proteins are in the acidic medium (Berthelot et al., 2016; Norhayati, 2005).

2.6.2 Proteins in natural rubber latex

Proteins which constitute about 1% to 1.5% of natural rubber latex consist of a wide range of components that are water-soluble and water-insoluble. It is the water-soluble proteins eluted from latex products which have been found to be the primary source of immediate hypersensitivity reactions such as contact urticaria and anaphylaxis (Allergy Type 1). The nature of the extractable proteins, however, could differ from the total latex proteins as various steps are involved in processing the latex to latex products. In fresh latex, the proteins are distributed into three major fractions, the rubber phase at 27%, the β-serum at 25% and the C-serum at 48%. The major proportion of water-soluble proteins in fresh latex are derived from the β- and C-sera. The proteins in the rubber fraction are mainly associated with the interfacial layer surrounding the rubber particles. They are mostly water-insoluble and are only extractable by detergents (SDS) or organic solvents (chloroform-methanol). Only a small fraction of the rubber particle proteins (1 mg/g rubber) are water-soluble and extractable by ammonia (Abdul Aziz, 1993).

Research have shown that only two major proteins of molecular weights 14 kD and 24 kD can be extracted from the rubber particles in the latex. More proteins can be extracted from the clear serum (C-serum) and the bottom fraction (B-serum) than the rubber particles (Bottier, 2020; Hasma & Amir Hashim, 1997). Ammoniation to preserve fresh latex tends to denature the proteins with an increase in low molecular weight proteins observed on storing ammoniated NR latex. Proteins on the rubber particles tend to bind strongly on the surface of the particles. This help in maintaining the colloidal stability of the rubber particles at the latex stage due to the anionic nature of these proteins in NR latex. These surface-active proteins will remain fused with the rubber particles when the rubber particles cohere to form latex films as the serum phase was removed (Amir Hashim, 1993; Hasma, 1992; Hasma & Amir Hashim, 1997; Abdul Aziz, 1993; Sriring et al., 2020).

2.6.3 Latex allergy and extractable protein contents in NR latex

In the late 1980s, natural rubber latex allergy was identified as an increasingly significant health care problem, However, the issue seemed to phase out in the last several years. In the United State, about 150,000 to 1 million healthcare workers and over 15 million people worldwide were still reported to suffer from latex allergy (Kahn et al., 2016). The starting material for all-natural rubber latex-dipped products, the latex concentrate, is derived from latex of the *Hevea brasiliensis* trees when tapped. Like the Hevea field latex, the latex concentrate also has a milky appearance, comprising of a dispersion of rubber particles in an ambient serum. Its rubber hydrocarbon content is 60% (Than-ardna et al., 2019). Besides the non-rubber substances, it also contains 1.5% - 2% of proteins, that are present in the rubber particles. Not all of these proteins can be extracted once the latex is processed into latex gloves or products. The amount of extractable proteins is low, usually not more than a few per cent of the total present. It is this small fraction that constitutes the residual extractable fraction containing the allergens that are implicated in the allergy reaction. So far, it is still unclear how many allergens are consistently present in the final products, since changes are likely to occur during the manufacturing process. Nonetheless, a number of them have recently been identified in the latex products, three of which by the RRIM. The presence of such allergens in a latex product gives rise to its allergenic potential (Hill, 2019; Pailhories, 1993; Sofian et al., 2015; Yip, 1998).

It is well established that residual proteins from NR latex remaining in a finished product may cause allergic reactions in some individuals (Henry et al., 2020). This arises from the fact that only part of the non-rubbers in the aqueous serum phase of the latex has been removed completely. In general, the total amount of protein that is present in latex concentrate is relatively constant, about 1.6% - 2.0% by weight of rubber (Subramaniam, 1995; Yip, 2003). Amir Hashim et al.(2004) has studied the effect of nano-sized calcium carbonate fillers in NR latex gloves and found that the filler does not affect the extractable and antigenic protein contents of gloves at a filler loading of 10%.

2.6.4 Protein reduction of NR latex

In addressing the latex protein allergy issue, much effort have been made to reduce the final total protein contents of the NR latex products such as the use of low protein latices, enzyme treatment, chlorination, leaching protocols and polymer coating (Howe, 2007; Lim & Amir Hashim, 2014; Mahendra et al., 2021; Neils, 1992; Perrella & Gaspari, 2002; Yip, 1990; Yip et al., 1997).

2.6.4.1 Chlorination process

Chlorination of gloves is yet another method employed for reducing extractable proteins from natural rubber latex articles. In general, the chlorination process involves attachment of chlorine atoms onto the backbone of natural rubber molecules on the surface of the latex films. Free chlorine atoms required can be produced by either dissolving chlorine gas in water or by reacting hypochlorite solution with acid to release the chlorine. According to Manhart et.al, (2020) during the chlorination, the double bonds of polyisoprene surface are saturated by chlorine and both surface roughness and surface hardness lead to distinctive decrease in surface friction.

The chlorination process occurs during post-production leaching step, although it may be conducted as a part of the on-line leach bath treatment. The surface of the glove is altered, and part of the processing powders is removed. The powder-free gloves produced through this chlorination process has a typical thin layer of chlorinated NR. The modified rubber surface is smooth and non-tacky. The gloves can be easily donned without the aid of dusting powder (Nor Aisah, 1994; Norhanifah et al., 2015). These steps have reduced the total and extractable protein contents in the glove, either by denaturing the protein or by leaching but have not eliminated the protein allergy problem (Howe, 2007; Manhart et al., 2020; Meleth, 2012; Preece et al., 2021).

2.6.4.2 Polymer coated gloves

Use of polymer as coating material began in the 1960s when the hydrogel technology for medical devices was introduced. Specific requirements need to be considered for polymer coating of medical devices such as the coating must adhere to the underlying rubber latex substrate and can offer durability and donnability characteristics. The coating must also be resistant to chlorination and the post-forming process including rinsing, leaching and drying. Another requirement is that the coating must not degrade after the sterilisation process, especially for surgical gloves. Many types of polymer coating materials are available such as hydrogels, polyurethane, silicone, acrylic and nitrile. The benefits of polymer coating are enhanced lubricity, excellent biocompatibility, hydrophilicity hardness and permeation resistance to certain chemicals and solvents, as well as softness and flexibility, and high tensile strength and tear resistance (Ambegoda et al., 2021; Manhart et al., 2020; Norhanifah et al., 2015; Preece, 2021).

2.6.4.3 Deproteinised NR latex

Deproteinised NR latex (DPNR) is prepared by treating fresh field with urea. The use of fresh field latex will reduce the cost of production. The fresh latex is incubated with urea concentration ranging from 0 to 1.0% in the presence of surfactant either sodium dodecyl sulphate or potassium laurate at a concentration ranging from 0.6% to 1.0% for an hour. The reaction is carried out at room temperature to 70 °C. In another study it was reported that urea at 0.6% and surfactant 0.6% to 1.0% resulted in low EP, antigenic protein (AP) and nitrogen (N) content. The study also reported that films of latex treated with 0.8% of potassium laurate and 0.06% urea at a temperature of 40 °C resulted in the highest percentage of proteins and nitrogen reduction (Ichikawa et al., 1993; Jayadevan et al., 2018; Nguyen et al., 2020; Norhanifah et al., 2015; Nurul Hayati, 2009).

2.6.5 Determination of extractable protein contents

Five test methods can be used for the measurement of extractable protein (EP) contents, namely RRIM Modified Lowry, ASTM D 5217, EN 455-3 and ISO 12243. In a comparative study of all the five test methods, it was observed that the RRIM Modified Lowry and ISO 12243 test methods were the most sensitive and versatile (Rosmahani et al., 2002). The EP from NR examination gloves which are known to have the potential of eliciting Type 1 latex allergy, are generally determined by ASTM D 5712:1995 and widely used by glove manufacturers. This test method gave a lower EP value compared to the revised version, ASTM D 5712-15 test method. However, the difference occurred from the variable protocols specified in the two standard assays, as shown in Table 2.7 (ASTM D5712, 2015; Hasma et al., 2006; McMahan et al., 2015; Ruhida et al., 2020; Yip, 1997).

Table 2.7: Differences in ASTM D 5712-95 and ASTM D 5712-15 protocols

Protocols	ASTM D 5712-95	ASTM D 5712-2015
Test sample size	7 cm x 7 cm	Whole glove
Extraction medium	Distilled water	25 mM PBS
The ratio of sample to extraction medium	1 g to10 ml	1 g to 5 ml
Extraction conditions	37°C for 2 hours	25°C for 2 hours
Standard curve	Non-precipitated OVBM	Precipitated OVBM
NaOH concentration	0.1 M NaOH	0.2 M NaOH
Copper and Folin reagents	Commercial reagents	Freshly prepared

PBS and OVBM: Phosphate Buffered Saline and Ovalbumin, respectively

According to the previous study by a researcher has found the ASTM 5712:1995 method gave the highest per cent recovery of 89.3%, followed by ISO, EN, RRIM and lastly ASTM 5712:1999 at 88.8%, 87.6%, 84.0% and 76.8% respectively. The author also found that the ASTM 5712:1995 method was the second least sensitive amongst the modified Lowry methods and the RRIM method was the most sensitive method as shown in Figure 2.9 and this study was supported by Hasma et.al (2006) and Rosmahani et al. (2002).

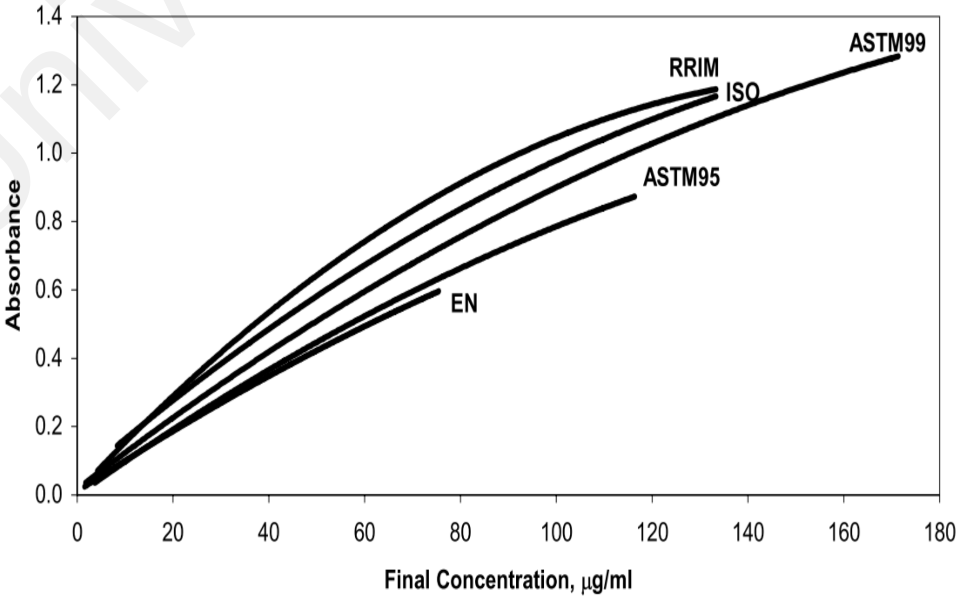


Figure 2.9: Sensitivity of Modified Lowry methods (Rosmahani et al., 2002).

The authors observed that at lower to middle concentration, all the modified Lowry methods conformed to Beer's law, as shown in Figure 2.10. However, the ASTM 5712:1999 and RRIM methods showed a polynomial fit at higher concentrations. Based on the study of NR gloves, the RRIM method was found to give the highest mean and median values with the widest range of EP content, i.e., between 9 µg/g to 369 µg/g. EN method, on the other hand, was found to give the lowest mean and median values and having the narrowest range. RRIM and ISO methods were found to be the most sensitive and versatile method. The ISO method demonstrated the least effect of interferences. The methods can be ranked as ISO method better than RRIM followed by ASTM 5712:1999, ASTM 5712:1995 and EN 433 test methods.

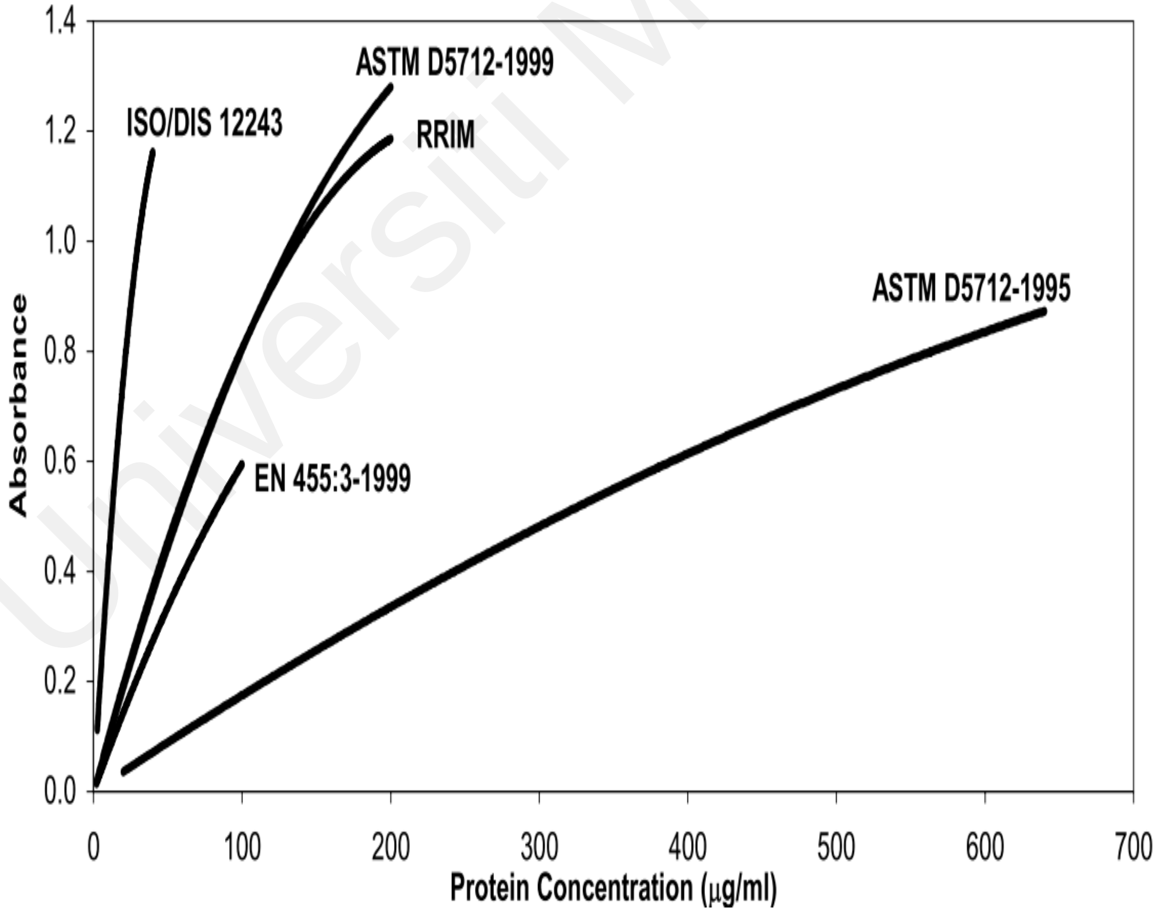


Figure 2.10: Standard calibration curves for five modified Lowry assays (Rosmahani et al., 2002).

2.6.6 Nitrogen content

2.6.6.1 Kjeldahl method

Nitrogen occurs in natural raw rubber mainly as protein has been widely used as an index of protein content, and its value can be used to indicate the protein content (Faridah Hanim et al., 2018; Mekkriengkrai et al., 2006). Although the proportion of nitrogen varies in different types of proteins the formula, protein is equal to 6.25 times nitrogen content is generally accepted but should be not regarded as a precise indication of the protein content. For example, skim rubber contains a notably high amount of nitrogen (Van Der Bie, 1949). The Kjeldahl method is universally regarded as the reference test method for nitrogen determination (Singh et al., 2020). In the semi-micro Kjeldahl procedure, the rubber is oxidised by heating with a catalyst mixture and concentrated sulphuric acid (H_2SO_4), thereby converting nitrogen compounds into ammonium hydrogen sulphate from which, after making the solution alkaline, the ammonia is removed by steam distillation. The liberated ammonia is absorbed in a boric acid solution and titrated with standard acid. The percentage of nitrogen can calculated according to Equation 2.1 (Faridah Hanim et al., 2018; Promsung et al., 2021; Nurul Hayati et al., 2015).

$$\text{Percentage of Nitrogen} = \frac{(V_1 - V_2)N * 0.0140}{W} * 100 \tag{2.1}$$

where,

V_1 = millimetres of H_2SO_4 for titration of the contents of receiving a flask

V_2 = millimetres of H_2SO_4 required for titration of the blank

N = Normality of the H_2SO_4

W = weight of the sample in grams

However, the Kjeldahl test method is time consuming which is a disadvantage for industrial applications.

2.6.6.2 Dumas method

Another test method that can be used to estimate total protein content is by determining the nitrogen (N) content through UPB/P/04 (2007) and Dumas method (Chaikumpollert et al., 2012; Saint-Denis & Goupy, 2004; UPB/P/04, 2007). Therefore, in this study, the nitrogen was measured based on the Dumas principle and method by using an Automatic Analyser (Colson, 1950; Faridah et al., 2008; Parks et al., 1954). With the automatic analyser, more samples can be analysed within a shorter period with similar precision and accuracy as compared to the Kjeldahl method. The Automatic Analyser can also be used to determine nitrogen, carbon/nitrogen, and carbon/hydrogen/nitrogen in organic matrices. The instrument provides results within 5 minutes for all metallics being determined (Mohd Noor et al., 2021; Saint-Denis & Goupy, 2004).

2.6.6.3 Principle of the automated Dumas method

A test sample is weighed inside a ceramic crucible and placed in a high-temperature furnace at 1050°C in an atmosphere of oxygen. The sample is combusted with high purity oxygen, converting all the metallics into oxides, namely O_2, CO_2, H_2O, N_2 and nitrogen oxides. The NOx gases are collected and passed through several traps. All other gases will be removed except nitrogen and nitrogen oxides. The gases that were produced by the sample were carried by a carrier gas (Helium) towards a copper catalyst whereby the nitrogen oxides are reduced into nitrogen. The mixture is then carried into the thermal-conductivity cells that emit an electrical signal proportional to the nitrogen content. The final result is calculated from a calibration curve plotted using known reference standards and expressed as a percentage of the initial sample weight. Figure 2.11 shows the diagram of the Automatic Analyser (Saint-Denis & Goupy, 2004).

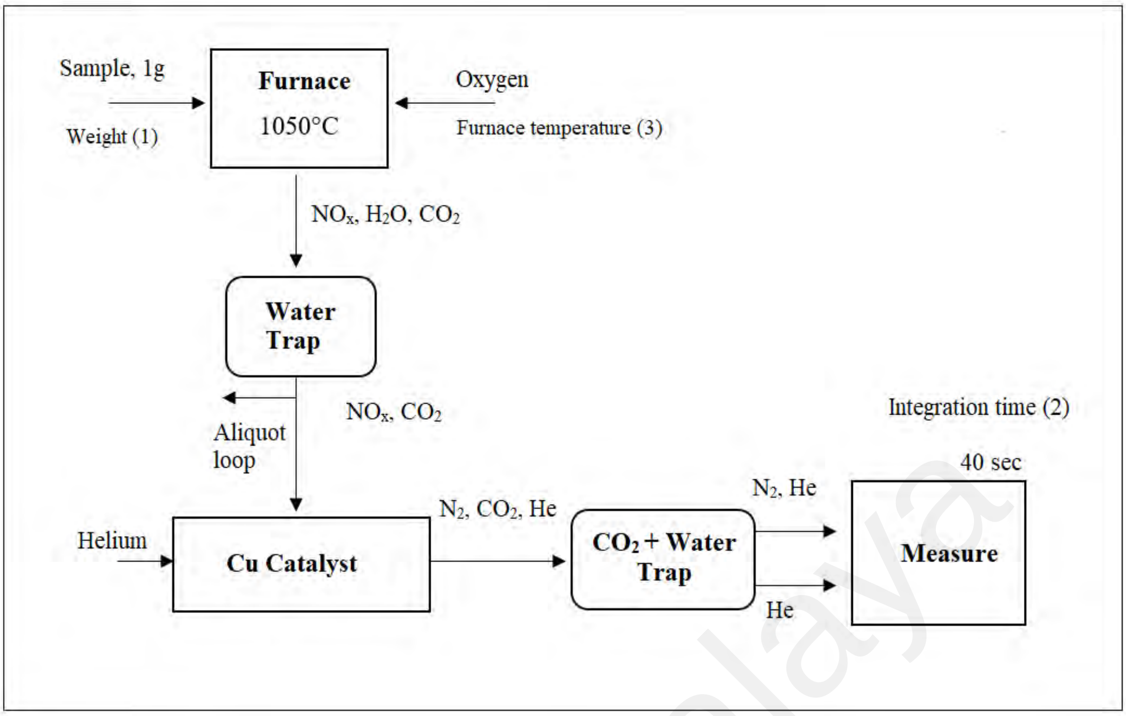


Figure 2.11: Schematic Diagram of the Automatic Analyser (Saint-Denis & Goupy, 2004).

2.7 Mechanical properties of NR latex films

2.7.1 Tensile strength of NR latex films

The tensile strength of rubber film can be defined as its resistance to rupture under tension. Tensile strength is very sensitive to impurities, flaws, state of cure and level of dispersions of compounding ingredients. The presence of impurities and flaws act as a stress raiser and initiate failure, which decreases tensile strength. The tensile test can be used to assess the quality of rubber compounds in terms of stress-strain characteristics such as ultimate elongation and tensile strength (Amnuaypornsri et al., 2009; Roland, 2005; Wei et al., 2020). Tensile properties can also provide a basis for the comparison of compounding ingredients. An under cured vulcanisate and poorly dispersed compounding ingredients decrease tensile strength. NR latex films made from the vulcanised NR latex generally produce or possess higher tensile strength and tear strength as compared to synthetic latex articles.

In a study conducted by MRB, it was observed that the addition of calcium carbonates up to 20 pphr into compounded natural rubber latex produced a film with slight improvement in strength and stiffness. Although an improvement in the strength of NR latex film was observed with the filler loading, a drastic reduction in film elasticity was observed. In a study by Muhamad Akmal et al. on the effect of filler loading on physical properties and surface morphology, they reported that the smoothest appearance based on surface morphology was obtained at 20% filler content (Mohamad Akmal et al., 2013; Roslim & Amir Hashim, 2010). In another study, it was reported that ultrafine calcium carbonate and bio fillers have improved mechanical properties and modulus of films (Cai et al., 2003; Ghani et al., 2019; Jayathilaka et al., 2020).

2.7.2 Tear strength of NR latex films

Tearing is the term used to indicate the fracture of vulcanised rubber. Tear strength is the force to produce tearing per unit thickness under unstrained conditions. It is well established that the tear resistance of vulcanised rubber is very much affected by the type of tear failure (Azemi, 2005; Nuraya et al., 2012). Latex dipped products such as gloves and condoms with thickness from 0.02 mm to 0.05 mm for condoms and glove from 0.10 mm to 0.45 mm is subject to failure associated with tearing when in contact with sharp objects. Therefore, the latex should possess a very high tear resistance in order to avoid tear failure during usage. There are two types of tear failure which is steady (smooth) tearing and stick-slip tearing. However, another type of tear failure, known as knotty tearing, is an extreme form of stick-slip energy (Ab Rahman et al., 2019; Hamann, 1993; Jayathilaka et al., 2020; Sirisinha et al., 2003). Tearing energy, *T* defined mathematically is as given in Equation 2.2.

$$T = \left(\frac{\delta U}{\delta A}\right) l \tag{2.2}$$

Where U is the total strain energy stored in the specimen, the partial derivative *(δ)* indicates that the specimen is considered to be held at constant length *l*, so that the external forces do no work. Tearing energy can be calculated or measured in terms of the applied forces or deformations for various shape test piece such as trousers, pure shear, angle and split, as shown in Figure 2.12 (Lake & Thomas, 1988).

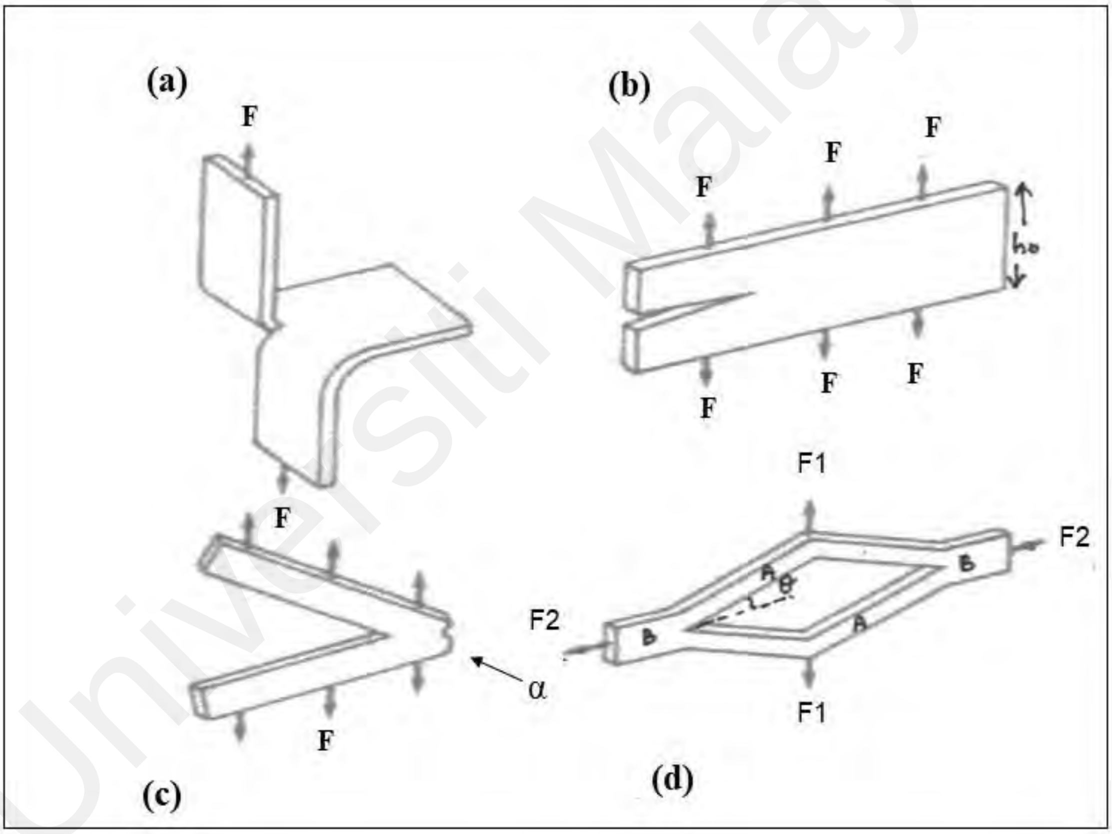


Figure 2.12: Various tear test pieces: (a) 'trousers'; (b) pure shear ; (c) 'angled';(d) 'split'(Lake & Thomas, 1988).

Rivlin & Thomas (1997) have described a detailed check on the applicability of the criterion to the tearing of natural rubber. The results of the calculations are as follows:

(i) ‘trousers’ test piece, as shown in Figure 2.12 (a).

$$T = \frac{2F\lambda}{t} - wU \tag{2.3}$$

where F is the force applied to each leg, t and w the test piece thickness and width respectively, and λ the extension ratio, U is the strain energy density in the central region and W the strain energy density in the legs.

(ii) pure shear test piece as shown in Figure 2.12 (b)

$$T = Uh_o \tag{2.4}$$

where h_o is the unstrained value of the height h and U is the strain energy density in the central region, remote from the crack, which is in pure shear.

(iii) angled test piece as shown in Figure 2.12 (c),

$$T = \frac{2F}{t} sin\,(\alpha/2) \tag{2.5}$$

where F and t the applied force and test piece thickness. The angle α is kept constant.

(iv) ‘split’ test piece as shown in Figure 2.12 (d)

$$T = [F_1\lambda_1 \sin\theta + F_2(\lambda_2 \cos\theta - \lambda_2]/t - w(U_1 - U_2) \tag{2.6}$$

where F_1, F_2 are the forces applied to the respective pairs of legs, λ_1, λ_2 and U_1 and U_2 the corresponding extension ratios and strain energy densities 2θ the angle of opening of the legs ($tan\ \theta = F_1/F_2$).

2.7.3 Effect of fillers on the mechanical properties of NR latex films

The application of fillers such as $CaCO_3$ that was mixed into NR latex compound to produce the NR latex product, especially NRL glove mainly for cost reduction and to improve the mechanical properties, tensile and tear strengths. However, the stiffening affecting elastic properties gave the product ineffective for the intended product, and the NRL glove that filled filler at the minimum requirements of standards was acceptable to the consumer. A study by other researchers on the usage of $CaCO_3$ types of PCC has found that both tensile strength and elongation at break of the NR latex films reduced with increasing $CaCO_3$ loading. It also found that ageing caused a reduction in tensile stress. However, the elongation at break and tensile strength was increased only up to 15 phr filler loading (Singh et al., 2019). The other researchers have attempted to replace the calcium carbonate with biofillers such as starch, eggshell and chitosan as mechanical properties like tear strength, tensile strength and elongation at break of the NRL films becoming focal point (Tangboriboon et al., 2019). Ghani et al. (2019) have found the optimum concentration to be at 10 phr of tacca starch, producing tensile stress at maximum load was at 18 MPa whilst the elongation at break was 650%. The tensile stress was found 2 MPa at 300 mm elongation, and tear strength was 15 N/mm.

CHAPTER 3: METHODOLOGY

3.1 Materials

The clones chosen in this study were selected from Stesen Penyelidikan Kota Tinggi (SPKT), Kota Tinggi, Johor and included those recommended by the Malaysian Rubber Board (MRB) for the industry. The clones used in this study were RRIM2023, RRIM2024 and RRIM2025. The collected field latex was weighed and placed in clean containers before ammoniation at 0.4%. All the samples were brought immediately to Academic Hevea Malaysia (AHM) laboratory, Stesen Penyelidikan Sg Buloh (SPSB), Selangor, to be processed into latex concentrate. The details of the production of MCLC latex concentrate from MCFL is as shown in Appendix A.

3.2 Chemicals

All the ingredients used for latex compounding are of commercial grade. The chemicals, sulphur dispersion that is used as a cross-linking agent, zinc oxide as an activator, accelerator Zinc dibutyl dithiocarbamate (ZBuD) and complex phenol-alkanes (Wingstay L) as an antioxidant were manufactured by Merck. While the $CaCO_3$ that was mixed into MCLC latex compounds was commercial-grade and manufactured by Zancarb. All insoluble ingredients were prepared as dispersions. These include sulphur, zinc oxide, accelerator and antioxidant.

3.3 Methods

3.3.1 Preparation of aqueous dispersion for latex compounds

The formulation for the preparation of 50% total solids content sulphur dispersion, accelerator (ZBuD) dispersion and zinc oxide dispersion are shown in Table 3.1.

Table 3.1: Formulation of 50% aqueous dispersion for sulphur, accelerator and zinc oxide

Chemicals	**Part by weight (grams)**
50% sulphur dispersion	
Sulphur	50
Dispersing aid	1
Distilled water	49
50% Accelerator dispersion	
Accelerator (Zinc dibutyl dithiocarbamate - ZBuD)	50
Dispersing aid	1
Distilled water	49
50% Zinc oxide (ZnO)	
ZnO	50
Dispersing aid	1
Distilled water	49

The dispersions were prepared in a 500 mL plastic bottle containing about 50 pebbles of various sizes. The pebbles are used to help in the dispersion and grinding of the ingredients when the bottle is shaken. Then, the other ingredients were added into the bottle. The bottle was tightened and sealed before being shaken in a Vibro-mill for 48 to 72 hours. The dispersing aid used is sodium naphthalene formaldehyde sulphonate.

3.3.2 Preparation of chemical solutions for latex compounds

The composition of the chemical solutions used to make 10% total potassium hydroxide and potassium laurate are as shown in Table 3.2 and Appendix B.

Table 3.2: Composition of chemical solutions used for latex compounds

Chemicals	**Part by weight (grams)**
10% Potassium hydroxide	
85% Potassium hydroxide	11.8
Distilled water	88.2
20% Potassium Laurate	
Part A	
Lauric acid	16.8
Distilled water	39.2
Part B	
Potassium hydroxide	4.8
Distilled water	39.2

The potassium hydroxide pellets were placed into distilled water and stirred at room temperature. The preparation of 20% of potassium laureates involved two stages. First, the lauric acid was added into warm water at about 75 ºC with fast stirring (Part A). The second stage, the potassium hydroxide, was dissolved in distilled water (Part B) and then slowly added into Part A solution with slow stirring.

3.3.3 Preparation of stearic acid-treated $CaCO_3$

In the preparation of SA-treated $CaCO_3$, the temperature and techniques need to be changed to identify the optimum condition of stearic acid (SA) compatible with the suitable solvents. Besides that, a trial on the treated $CaCO_3$ is also being identified to ensure that SA-treated $CaCO_3$ dispersion is compatible with the MCLC so that the MCLC films are produced. The SA that was used for modification of the $CaCO_3$ surface, as shown in Table 3.3. The surface of $CaCO_3$ was treated with a known concentration of stearic acid in chloroform medium at 0.5, 1.0, 3.0, 4.0, 5.0 and 7.0 by % (*w/v)* following the earlier published work by (Mihajlović et al., 2009) as guidance. Approximately 2.50 grams of SA was weighed before dissolved in 500 ml chloroform to give 0.5%

concentration, as shown in Table 3.4. Similarly, SA of varying concentration, 1.0%, 3.0%, 4.0%, 5.0% and 7.0% by (*w/v*) were prepared by dissolving in chloroform. In order to treat the $CaCO_3$ particles with SA at various concentrations in dispersion form, approximately 10 grams of 50% commercial $CaCO_3$ dispersion was weighed and mixed with 100 ml of distilled water. Then, it was placed in the water-bath for about 30 minutes at a temperature of 50 °C. An appropriate amount, about 10 ml of dissolved stearic acid in chloroform medium, was added into the $CaCO_3$ suspension. The $CaCO_3$ suspension was stirred at 4000 rpm for 15 minutes, centrifuged at 8000 rpm for 8 minutes at 9 °C before the semi-solid slurries were collected and washed with distilled water. Then, it was dried in the oven at 50 °C for 24 hours before the grinding process. The dried ground treated $CaCO_3$ was turned into a dispersion medium by mixing 20% treated $CaCO_3$ with 1.0% of dispersing agent and 79.0% of distilled water at vigorous stirring for 30 minutes, as shown in Table 3.5. The treated $CaCO_3$ dispersion was poured into a 500 ml plastic bottle containing about 50 pebbles of various sizes as aids in dispersing and grinding the ingredients when the bottle was shaken on the milling machine.

Table 3.3: Characteristic of stearic acid (Mihajlović et al., 2009)

Name	**Stearic acid**
Molecular formula	$CH_3(CH_2)_{16}COOH$
Molecular weight, g/mole	284.47
Density, g/ml	0.847
Dissociation constant, pKa	5.7
Solubility in water, g/100 ml	0.34 at 25°C and 0.1 at (37°C)
Solubility in alcohol, g/100 ml	2.5
Solubility in chloroform $CHCl_3$	Very soluble
Thermal analysis	341°C, 319°C

Table 3.4: The amount of stearic acid used for treated $CaCO_3$ dispersions

Concentration of stearic acid (%)	Weight of stearic acid (grams)
0.5	2.50
1.0	5.0
3.0	15.0
4.0	20.0
5.0	25.0
7.0	35.0

Table 3.5: The preparation of 20% SA-treated $CaCO_3$ dispersion

20% SA - Treated $CaCO_3$	Parts by weight (grams)
Treated $CaCO_3$	20
Anchoid	1
Soft water	79

3.3.4 Properties of MCFL and MCLC

Several test methods exist for the determination of the latex properties of field latex and latex concentrate. In this study, the Malaysian Standard (MS) and ISO standard test methods were used to evaluate the latex properties. The properties of each clone of field latex and mixed clones field latex (MCFL) such as DRC, TSC, alkalinity and VFA number were evaluated according to the MS 466 (1987) test methods before processing and centrifuging into mixed clone natural rubber latex concentrate (MCLC) according to ISO 2004 (2017) specification. It is important to have the composition of MCFL by determining the DRC of MCFL before centrifuging the MCFL into MCLC for selecting the right screw for the machine. At the early stage, the essential test parameters that are used to evaluate the properties of latex for each clone of field latexes and MCFL are DRC, TSC, alkalinity and VFA No. For MCLC latex and commercial latex concentrates (CLC), the test parameters that are used to determine their properties are similar as for MCFL with the addition of other test parameters such as mechanical stability time (MST), potassium hydroxide number (KOH No.), pH, viscosity, sludge and coagulum contents. The test procedures that were used to measure the properties of each clone of field latex, MCFL and MCLC were as follows:

3.3.4.1 Determination of dry rubber content

The dry rubber content (DRC) of field and latex concentrate was measured by weighing 10 grams of the latex and coagulating with 2% acetic acid. The procedure involves dilution of latex, coagulation of the latex by addition of acetic acid, heating on the steam bath for 10 to 15 minutes until a clear serum is obtained as indicating complete coagulation. The coagulated rubber rolled into a thin sheet at 2 mm thickness before drying in the oven for 16 hours at 70 °C. The DRC is calculated by following Equation 3.1 according to ISO 126 (2005) test method.

$$\mathrm{DRC} = \frac{m_1}{m_o} \text{x } 100\% \tag{3.1}$$

where,

m_o is the mass, in grams of the test portion

m_1 is the mass, in grams of the dry sheet

3.3.4.2 Determination of total solids content

The total solids content (TSC) of field and latex concentrate was measured by pouring 2 grams of latex into a petri dish and drying to a constant mass for 16 hours at 70 °C in the oven according to ISO 124 (2014) test method. The TSC is calculated by following Equation 3.2.

$$\text{TSC} = \frac{m_1}{m_0} \text{ x } 100\% \tag{3.2}$$

where, m_o is the mass, in grams, of the test portion before drying

m_1 is the mass, in grams, of the final dried material

3.3.4.3 Determination of alkalinity

Alkalinity (NH_3) refers to the total alkalis in latex. The amount of alkalis in latex is determined electrometrically by using 0.10 M sulphuric acid (H_2SO_4) with a pH meter. The alkalinity is calculated according to ISO 125 (2020) test method by following Equation 3.3.

$$\text{Alkalinity (as NH}_3\text{)} = \frac{F_1 cV}{m} \tag{3.3}$$

where,

F_1 is a factor 3.4
c is the actual concentration, expressed in mole H_2SO_4 per cubic decimetres of acid used
V is the volume, in cubic centimetres, of acid used
m is the mass, in grams, of the test portion

3.3.4.4 Determination of the volatile fatty acid number

The volatile fatty acid number (VFA No.) is expressed as the number of grams of potassium hydroxide required to neutralise the volatile fatty acids in a latex sample containing 100 grams of total solids. Extraction of latex serum was carried out by adding ammonium sulphate $(NH_4)_2SO_4$ into latex concentrate and heated on the water bath at 70ºC until the sample coagulated. The coagulated latex was pressed using a stainless-steel rod in order to collect the serum. The collected serum was filtered through a filter paper and acidified with 5 ml of 50% H_2SO_4 solution before steam distillation. Then, the distillate was titrated with barium hydroxide. The VFA number was calculated according to Equation 3.4 by following ISO 506 (2020) test method.

$$\text{VFA Number } = \left(\frac{134.64\, c\, V}{m\ \text{TSC}}\right) \text{x } 50 + \frac{m\,(100 - \text{DRC})}{100\rho} \tag{3.4}$$

where, *c* is the actual concentration, expressed in moles per cubic decimetre, of the barium hydroxide solution

V is the volume, in cubic centimetres, of barium hydroxide solution required to neutralise the distillate

m is the mass, in grams, of the test portion

DRC is the dry rubber content, expressed as a percentage by mass, of the latex concentrate

TSC is the total solids content, expressed as a percentage by mass, of the latex concentrate

p is the density, in megagrams per cubic metre, of the serum, 134.64 is a factor derived from the relative molecular mass of potassium hydroxide, its equivalence to barium hydroxide and those parts of the serum acidified and distilled

3.3.4.5 Determination of viscosity

The viscosity of the latex was measured by using Brookfield Viscometer instrument with a specific spindle driven by an electronic motor to rotate at a constant rotational frequency and a controlled rate of shear while immersed to a specified depth of the latex according to ISO 1652 (2011) test method.

3.3.4.6 Determination of mechanical stability time

The mechanical stability time (MST) of latex was measured according to the ISO 35 (2004) test method, where the actual TSC of latex needs to be diluted to 55% at 35 °C to 37 °C prior to the determination. The diluted latex was stirred at 14000 rpm by using the Klaxon machine, and the time was recorded in seconds as the first permanent floc of latex appeared on the surface of distilled water in a petri dish.

3.3.4.7 Determination of pH and potassium hydroxide number

The pH of latex was measured by using a digital pH meter brand Mettler Toledo. The potassium hydroxide number (KOH No.) of latex can be defined as the number of grams of potassium hydroxide equivalent to the acid radicals combined with ammonia in latex concentrate containing 100 grams of total solids. The measurement of KOH No. can be performed by following the ISO 127 (2018) test method.

3.3.4.8 Determination of coagulum content

A stainless-steel wire cloth with aperture width of 180 ± 10 μm was washed with 200 ml of 5% of potassium oleate and dried in the oven at 100 °C ± 5 °C to a constant mass. The test filter was clamped firmly between stainless steel rings and wetted with 5% potassium oleate before filtering 200 grams of latex of through. Wash the residue on the wire cloth with the same surfactant solution until it is free from latex. Then, the wire cloth with coagulum was heated in the oven for 30 minutes at 100 °C ± 5 °C before weighing. The coagulum content was calculated according to Equation 3.5 by following ISO 706 (2004) test method.

$$\text{Coagulum content} = \frac{m_2 - m_1}{m_0} \text{ x } 100\% \tag{3.5}$$

where,

m_o is the mass in grams of the test portion

m_1 is the mass in grams of the wire cloth

m_2 is the mass in grams of the wire cloth and dried coagulum

3.3.4.9 Determination of sludge content

A latex sample of about 40 grams was centrifuged for 20 minutes at 12000 m/s^2. Then, the cream layer at the upper layer was scooped off by using a pipette and carefully draw off the supernatants liquid to approximately 10 mm above the top of the sludge. Then, the tube was filled up with an ammonia-alcohol solution before re-centrifuged for 25 minutes before pipette off the supernatant liquid to approximately 10 mm above the top of the sludge. This procedure repeated until the supernatant liquid was clear after centrifuging. Finally, the decant supernatant solution to the 10 mm mark and the sludge was transferred into a heat resistant beaker and dried in the oven at 70 ºC ± 2 ºC until the loss in mass less than 0.001 gram in 30 minutes. The sludge content was calculated according to Equation 3.6 by following ISO 2005 (2014) test method.

$$\text{Sludge content} = \frac{m_1}{m_o} \text{ x } 100\% \tag{3.6}$$

where, m_1 and m_o are the mass of the test portion and the dried sludge, respectively.

3.3.4.10 Determination of metallic content

About 10 grams of latex sample was poured into the petri dish and dried in the oven at 70 °C for 16 hours. The latex films were creped at least 10 times by using a micro-mill to ensure that the samples were homogenous. A test sample of 5 grams from the milled latex film were finely cut and weighed in a porcelain crucible and wrapped with the ashless filter paper. Then, the sample was placed in a muffle furnace at 550 °C for 4 hours to allow all the organic matter and carbonaceous residue in the sample to be ashed. The ash was then treated with 1 ml of 65% nitric acid (HNO_3) and heated on a hot plate at 100 °C until dried. The reason for adding the concentrated nitric acid was to suppress the precipitation of metallics. Then, 10 ml of 1.6% HNO_3 was added to the sample in a porcelain crucible and transferred to the hot plate for 30 minutes until the sample was completely disintegrated. The samples were allowed to cool down for about 30 to 60 minutes before filtering into a 50 ml volumetric flask. The solution was made to volume with 1.6% HNO_3, and the metallic content was determined by ICP-OES Optima 4300DV according to the MRB test method (UPB/P/058, 2015).

3.3.5 Natural rubber latex compounding

3.3.5.1 Preparation of natural rubber latex compound

Untreated calcium carbonate filler was used in formulating the natural rubber latex compound (as shown in Table 3.6) in this study and served as Control. The details of a compound of MCLC with other rubber chemicals are as shown in Appendix B.

Table 3.6: Formulation of MCLC compound with $CaCO_3$ dispersions of untreated $CaCO_3$ at various concentrations

Components	Concentration (pphr)
Latex concentrate, 60%	100
Potassium hydroxide, 10%	0.1
Potassium laurate, 20%	0.5
Sulphur dispersion, 50%	1.5
Zinc dibutyldithiocarbamate (ZBuD), 33.3%	1.0
Zinc oxide, 50%	0.5
Wingstay L, 40%	1.0
Calcium carbonate dispersion, 50%	10, 20, 25, 30, 35, 40

The MCLC was compounded with each of the rubber chemicals and dispersions at a specific concentration in pphr, as stated in Tables 3.6, 3.7 and 3.8. All the compounds were prepared at 30% TSC. The composition of the coagulant solution used for dipping purposes is as shown in Table 3.9.

Table 3.7: Formulation of MCLC compound mixed with untreated $CaCO_3$ as filler at different filler loadings (part per hundred rubber)

Components	Concentration (pphr)
Latex concentrate, 60%	100
$CaCO_3$ dispersion, 50%	10, 20, 25, 30, 35, 40

Table 3.8: Formulation of MCLC compound with filler dispersions of SA-treated $CaCO_3$ at various concentrations

Components	**Concentration (pphr)**
Latex concentrate, 60%	100
Potassium hydroxide, 10%	0.1
Potassium laurate, 20%	0.5
Sulphur dispersion, 50%	1.5
Zinc dibutyldithiocarbamate (ZBuD), 33.3%	1.0
Zinc oxide, 50%	0.5
Wingstay L, 40%	1.0
Treated $CaCO_3$ dispersion, 20% (at 0.5%, 1.0%, 3.0%, 4.0%, 5.0% and 7.0% SA concentrations)	30

Table 3.9: The composition of the coagulant solution for the dipping process

Ingredient	**Parts (w/w)**
Calcium nitrate	30
Calcium carbonate	10
Vulcastab LW	0.1
Distilled water	59.9

3.3.5.2 Preparation of MCLC films

A clean glass plate was heated in the oven at 70 ºC for at least 10 minutes. The warmed plate was then immersed into the coagulant suspension that contained calcium nitrate at a concentration of 30% for 20 seconds in order to ensure that the coagulant entirely and uniformly coats the surface of the glass plate. The coated glass plate was dried in an oven at 70 ºC for 5 minutes before dipping it slowly into the latex compound. The dwell time in the latex compound was 30 seconds. Then, the latex film was allowed to gel before leaching in distilled water at 70 ˚C for one minute in order to ensure that all of the non-rubber constituents were leached out into the distilled water and to strengthen the latex film. The natural rubber film was dried in an oven at 70 ˚C for 20 minutes to eliminate excess water on the film and vulcanised at 110 °C for 15 minutes. Corn starch was applied to the dried films so that each film could be easily stripped from the glass plate. For the purpose of this study, MCLC mixed with the untreated filler at different filler loadings and filler treated with various percentages of stearic acid concentrations were prepared in a similar manner.

3.3.5.3 Solvent extraction of MCLC films

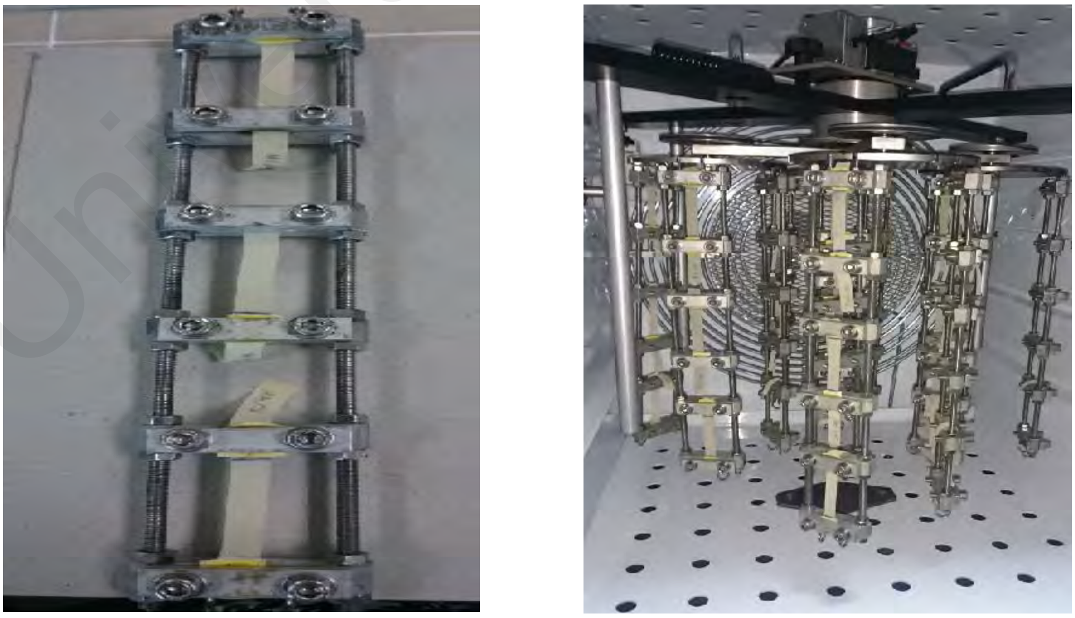
The extraction of MCLC films was carried out by using the Soxhlet apparatus with acetone as solvent. The samples were passed through a cold roll of laboratory mill following ISO 1795 (2007) test method before a test sample of about 3 to 5 grams is weighed to the nearest 0.1 mg. The weighed sample was rolled with lens cleaning tissue or placed in thimble filters in order to form loose roll from which the rubber could not fall and no contact with other part of the rubber. Then, the packet was placed in an appropriate extraction apparatus and the extraction carried out for a continuous period of 16 hours in the water bath maintained at 70 °C. Extraction of the sample must be subjected to 80 cycles of hot extractions of solvent. After 16 hours of extraction, the sample was allowed to cool. The extracted rubber sample was discarded. The receiver flask was removed and fit into a distillation head with the condenser and the bulk of the solvent distilled off into a suitable weighed flask. The weighed flask with the contents was dried for 1 hour at 70 °C in the oven and weighed again. The acetone extract of the sample was expressed as a percentage by mass calculated according to Equation 3.7 by following ISO 1795 (2007) test method.

$$\text{Acetone extract} = \frac{C - B}{A} \times 100\% \qquad (3.7)$$

where A, B, and C are the mass of the test portion, empty receiver flask and receiver flask plus the extract after drying, respectively.

3.3.5.4 Characterisation of MCLC films after ozone exposure

The MCLC films containing different filler loadings were characterised after ozone exposure according to ISO 1431 (2012) test method. The test pieces of MCLC films were placed in tension jigs at 10% strain inside the ozone chamber at 10 pphm concentration with temperature 40ºC for a certain period of time, as shown in Figure 3.1. The ozonised oxygen generated in the ozonised by UV quartz lamp collected in a separate chamber where the samples were exposed. After ozone exposure for 3, 6, 9 and 24 hours, the samples were analysed for total nitrogen and extractable protein contents and surface morphology.



(a) **(b)**

Figure 3.1: (a) Test pieces of MCLC films placed in tension jigs at 10% strain and (b) the test pieces of MCLC latex film hung in the ozone chamber.

3.3.6 Characterisation of $CaCO_3$ and SA-$CaCO_3$ treated in MCLC films

The characterisation of

i. $CaCO_3$ in MCLC films at various filler loadings from Control to 40 pphr,
ii. $CaCO_3$ treated with SA at various concentrations (0.5, 1.0, 3.0, 4.0, 5.0 and 7.0) in chloroform by (% *w/v)* in powdered and dispersion form and
iii. $CaCO_3$ treated with SA in MCLC films at 30 pphr filler loading were evaluated by using TGA, FTIR, DSC and FESEM.

3.3.6.1 TGA measurements of $CaCO_3$ and SA- treated $CaCO_3$ contents in MCLC films

Thermogravimetric analysis (TGA) of the samples were carried out by using TGA in a slow stream of nitrogen and oxygen. In this study, two types of samples were analysed, namely unmilled and milled. The milled samples were creped by a small-scale milling machine at least 6 times before weighing at about 0.015 to 0.020 grams in the crucible by a semi microbalance. Then the crucible was heated inside the TGA from 50 °C to 850 °C in nitrogen with a similar heating rate of 20 °C/min. The analysis ended at about 850 °C in oxygen, where no further decrease in weight. All the $CaCO_3$ and treated $CaCO_3$ contents were determined by TGA under similar conditions by following the MRB test method (UPB/P/010, 2010). The details of the calculation of $CaCO_3$ as filler after TGA analysis is stated in Appendix C.

3.3.6.2 The glass temperature *(T_g)* measurement of MCLC films

The glass temperature (T_g) of MCLC films at different filler loadings of untreated and SA-treated $CaCO_3$ at various concentrations were measured by Differential Scanning Calorimetry (DSC). In this experiment, about 0.10 to 0.015 grams of the sample was weighed and sealed in 40 μL aluminium crucibles. The sample was heated from 25 °C to 110 °C with a heating rate of 110 °C/min for 2 minutes. Then, the sample was cooled to 20 °C at the heating rate of 20 °C/min for 2 minutes. After that, the sample was heated to 110 °C again at the heating rate of 10 °C/min. The parameter of T_g was estimated by DSC Mettler Toledo STARe software, and the accuracy of DSC was at ±0.2 °C/min according to the MRB test method. Based on the DSC curve, three positions were produced, namely onset, midpoint and end temperatures. The number samples before reported at three positions a mean of 2 replicates. The T_g of samples were selected and reported at midpoint temperature according to the MRB test method (UPB/P/009, 2010).

3.3.6.3 FTIR identification of untreated and SA-treated $CaCO_3$.

The identification of $CaCO_3$ in MCLC latex films at different filler loadings and SA-treated $CaCO_3$ in the form of powder, dispersions and MCLC films at various concentrations of SA were evaluated by Fourier Transform Infrared (FTIR) Nicolet iZ10 Module according to the MRB test method (UPB/P/011, 2010). The FTIR spectra were generated by the absorption of electromagnetic radiation in the frequency range 400 - 4000 cm^{-1} by organic molecules. The percentage of transmittance was plotted against wavenumber (cm^{-1}). The functional group present is identified by the frequency and intensity of the absorption peak. Each functional group and structural feature in the molecule absorb at characteristic frequencies and provide information of the structural geometry in the molecule. The intensity of the absorption peak is an indication of its bond strength.

3.3.6.4 Surface morphology of untreated and SA-treated $CaCO_3$ in MCLC films

Field-emission Scanning Electron Microscope (FESEM) JEOL JSM-840 model-6211 was used to investigate the surface morphology of MCLC films with $CaCO_3$ at different filler loadings and $CaCO_3$ treated with SA at various concentrations. The surface morphology of MCLC films before and after exposure to ozone at 10 pphm concentration was also observed. The main objective is to observe changes on the surface of the samples with and without filler. A thin layer of the sample was prepared and mounted on a metallic stub with the help of silver tape and conducting paint in the upright position. Gold of 2.5 μm thickness was sputtered on the surface of the sample prior to FESEM observation under the electron beam.

3.3.7 Total protein content measurement

3.3.7.1 Measurement of the total nitrogen content of MCLC films

About 10 grams of MCLC films was creped at least 10 times by using a milling machine to ensure that the samples were homogenised before analysis was carried out. A test sample of 0.150 grams of the creped MCLC films and reference standard material was weighed for the analysis. The reference standard material (SRM) defined as a material or artefact that has had one or more of its property values certified by a technically valid procedure, and is accompanied by, or traceable to, a certificate or other documentation which NIST issues. The reference standard material used in this experiment was 2,5-(Bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene (BBOT). This standard was used to identify the percentage of nitrogen in samples. However, for aged samples, the MCLC films were heated in an oven at 70°C for 7 days before the creping process. All the samples and standards were placed in the autosampler, where each of the samples will be transferred individually to the instrument's purge chamber directly above the furnace, eliminating the possible interference of atmospheric gases during the transfer process. Carbon, hydrogen, and nitrogen present in the sample were oxidised to carbon dioxide, water and NO_x, respectively. A thermal conductivity cell was used to detect the nitrogen content and the results displayed in weight per cent or mg/kg.

3.3.7.2 Measurement of the total nitrogen content of MCLC films after solvent extraction

About 3 to 5 grams of crepe samples were weighed before extraction with acetone as a solvent for 16 hours by using the Soxhlet apparatus according to ISO 1795 (2007) test method. Then, the total nitrogen content for all the samples was measured and analysed by Automatic Analyser under similar conditions as stated under sub-section 3.3.7.1.

3.3.7.3 Determination of extractable proteins content of MCLC films

Extractable proteins (EP) content were extracted from MCLC films with untreated $CaCO_3$ and SA-treated $CaCO_3$ by following the ASTM D5712 (2015) test method. The essential protocols in this method involved sample preparation, protein extraction, precipitation of proteins, solubilising of protein precipitate, and finally, protein assay. The samples were extracted with phosphate-buffered saline (PBS) buffer 0.025 M at a ratio of sample to PBS buffer. 10 ml per gram of sample at 37 °C for 2 hours with continuous agitation at 200 rpm by using a shaker. Then, the extract was centrifuged at 2000x *g*. for 15 minutes. Carefully pipette 1.0 ml of clear supernatant into the microcentrifuge tubes before adding 0.1 ml of trichloroacetic acid (TCA) and 0.1 ml of phosphotungstic acid (PTA). The MC tubes will be vortex after pipetting the chemicals into the microcentrifuge tubes in order to ensure that chemicals are mixed evenly in the sample. After 30 minutes, the extract was centrifuged at 6,000x *g* for 15 minutes, and the supernatant was decanted by inverting all the microcentrifuge tubes for at least 40 minutes in order to remove the excess liquid. The precipitation that was left inside the microcentrifuge tubes was re-dissolved in 0.1 M of sodium hydroxide. At the solubilising of protein, 0.2 ml aliquot of each dissolved protein solution was treated with 0.1 ml of reagent A (alkaline copper tartrate – BioRad Lowry DC protein assay kit) followed by

0.8 ml of reagent B (Folin reagent – BioRad Lowry DC Protein assay kit) and named as the non-corrected condition.

Nevertheless, when the reagent was replaced with distilled water before reacting with reagent B was named as a corrected condition. The dark blue colour was allowed to develop at room temperature for 20 minutes before the absorbance was measured at 750 nm by UV Microplate Reader. The results were taken against on ovalbumin calibration curve into µg/g of samples and reported as non-corrected and corrected EP content as shown in Appendix D.

3.3.8 Chemical interference measurement on the protein assay of MCLC films

The interference of chemicals on the protein assays of MCLC films was evaluated by testing the protein content in solution spiked with the chemicals or in the extracts of cured dipped films incorporated with the chemicals. The MCLC was compounded with various chemicals solution or dispersion at similar concentrations, as stated in Tables 3.7 and 3.9 under sub-section 3.3.5. In this study, the focus was on the effect of chemical interferences, particularly of the filler loadings on the protein assay in MCLC films. The latex compound was matured for 24 hours at ambient temperature before carrying out the dipping process.

3.3.9 Effect of $CaCO_3$ on the cross-link concentrations of MCLC films

3.3.9.1 Measurement of cross-link concentrations by equilibrium volume swelling method

The MCLC films containing different filler loadings were cut into a circular shape of 23 mm diameter. The test samples were weighed and then placed in a glass container and filled with about 40 ml of toluene. The samples were allowed to reach their equilibrium swelling at room temperature for 48 hours (Newell et al., 1975). The swollen test sample was removed from the toluene, lightly blotted with filter paper to remove any excess toluene on the sample's surface. Then, quickly placed in a weighing bottle and weighed. The swollen sample was dried for 1 hour at 50 ºC in an air oven and followed by another 2 hours drying in a vacuum oven in order to obtain a constant weight. The volume fraction of rubber in the swollen test sample V_r calculated by using Equation 3.8 (Ghari & Shakouri, 2012; Godbole et al., 2017). Details of the calculation are as shown in Appendix E.

$$V_r = \frac{V_R}{(V_R + V_s)} \tag{3.8}$$

where, V_R = volume of rubber

V_s = volume of solvent absorbed

The cross-link concentration of the test sample was calculated based on Flory-Rehner equilibrium swelling, as shown in Equation 3.9.

$$-\ln(1 - V_r) - V_r - \chi(V_r^2) = 2\rho\, V_o\, (\eta)\, phy\, V_r^{1/3} \tag{3.9}$$

where, $(\eta)\ phy =$ manifested cross-link concentration

$\mathrm{V_o} =$ molar volume of solvent

For toluene, $V_o = 103.11$ cm^3

$\chi = 0.37$, Huggins rubber-solvent interaction parameter

$\rho =$ Density of rubber hydrocarbon (0.919 g/cm^3)

3.3.9.2 Measurement of total, combined and free sulphur of MCLC films

The total and combined sulphur contents of MCLC films with different filler loadings before and after extraction were analysed with an Automatic Analyser. The procedure to determine the total sulphur and combined sulphur content was similar to in the determination of total nitrogen, as stated under sub-section 3.3.7.1. However, the difference is in the reference standard material used in this experiment. The reference standard material used in this experiment was coal. The details of the calculation for the total and combined sulphur contents are as shown in Appendix F.

3.3.10 Mechanical properties of MCLC films

3.3.10.1 Tensile strength

The MCLC films with different filler loadings of untreated $CaCO_3$ and SA-treated $CaCO_3$ at various concentrations of SA were cut into a dumbbell shape, as shown in Figure 3.2 for the tensile test. In this experiment, the tensile strength of 5 replicates for each sample (unaged and aged) were measured according to the ASTM D 412 (2002) with the load cell used at 500 N with the distance between grip at 20 mm and 25 mm of the extensometer. The cross-head speed used was 500 mm/min, tested by using tensile strength instrument, brand Instron, model 5564.

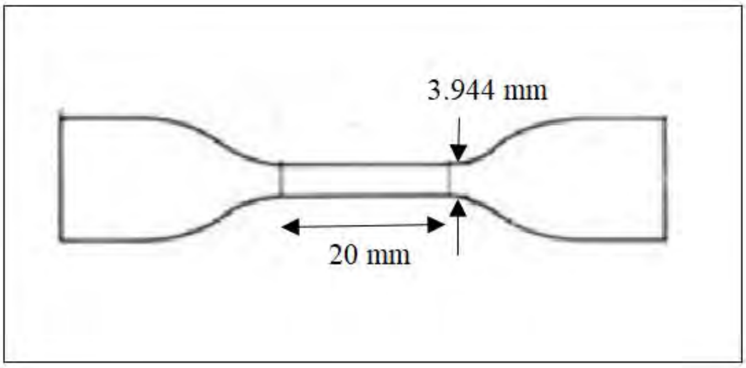


Figure 3.2: Test piece for tensile strength.

3.3.10.2 Tear strength.

In the case of tear strength measurement, the MCLC films at different filler loadings and treated $CaCO_3$ at various concentrations of SA were cut into trouser shape, as shown in Figure 3.3. The tear strength of 5 replicates for each sample (unaged and aged) were measured according to ISO 34 (2015) test method with a load cell at 500 N and cross-head speeds used at 100 mm/min, tested by using tensile strength instrument, brand Instron, model 5564.

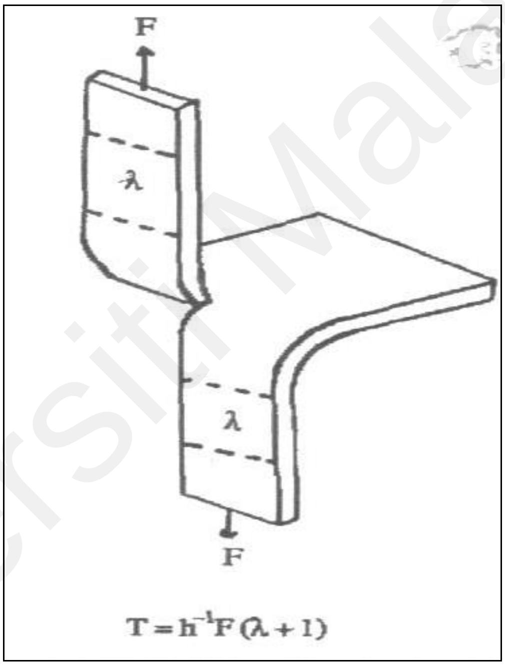


Figure 3.3: Schematic diagram showing the separation of the leg of the trouser test piece (Azemi, 1993).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Characterisation of monoclonal field latex, MCFL and MCLC films with untreated and SA-treated $CaCO_3$

The properties of monoclones, mixed clones field latex (MCFL) and mixed clone latex concentrate (MCLC) were prepared before compounds with rubber chemicals with untreated and SA-treated $CaCO_3$. The MCLC was compound with untreated $CaCO_3$ at various loadings from 10 pphr until 40 pphr whilst the SA-treated $CaCO_3$ dispersion was prepared at various concentrations such as 0.5, 1.0, 3.0, 4.0, 5.0 and 7.0 by % (*w/v)* was treated with stearic acid (SA) in chloroform ($CHCl_3$) medium before compounds with MCLC at 30 pphr loadings. The purpose of this experiment is to evaluate the MCFL and MCLC properties. Also, to investigate the SA-treated $CaCO_3$ dispersion obtained at the optimum concentration of stearic acid to be attached or coated on the surface of the rubber particles. Thermogravimetric analyser (TGA), Differential scanning calorimeter (DSC) and Fourier transform infrared spectrometer (FTIR) was used to analyse the MCLC films with untreated and SA-treated $CaCO_3$.

4.1.1 Properties of field latex of monoclones, MCFL and MCLC

Latex from three different clones, namely RRIM2023, RRIM2024 and RRIM2025, were collected from Stesen Penyelidikan Kota Tinggi is recommended by the Malaysian Rubber Board for rubber planting. Clones RRIM2023 and RRIM2024 are classified under Group 2 and have obtained the minimum latex yield of 1800 kg/ha/year with a minimum mean of clear bole volume of 0.3 m^3/tree while RRIM2025 is a clone recommended for rubber forest plantation. The RRIM2025 clone had a high mean of clear bole volume of 0.28 m^3/tree at 15 years of planting (Nurmi Rohayu et al., 2015).

These three clones of latex were mixed at a similar weight ratio. The properties of the mixed clones field latex (MCFL) were determined to confirm its dry rubber content (DRC) and to check its fitness and suitability before the preparation of the latex concentrate. The quality of the mixed clones latex concentrate (MCLC) was evaluated prior to the addition of the filler dispersion and other chemicals. This will give a better understanding of the physical and chemical nature of the colloidal latex system that influences its properties. Table 4.1 shows the results of latex properties of each of the monoclonal field latex (RRIM2023, RRIM2024, RRIM2025) and MCFL prepared from the three clones. The properties of MCLC and CLC are shown in Table 4.2. The MCFL and latex concentrates were determined according to the Malaysian Standard (MS) and the International Organization for Standardization (ISO) test methods, respectively.

Table 4.1: Properties of field latex of different clones, namely RRIM2023, RRIM2024, RRIM2025 and MCFL

Test Parameter	**RRIM 2023**	**RRIM 2024**	**RRIM 2025**	**MCFL**
Total solid content (TSC), %	41.47	41.66	39.46	42.87
Dry rubber content (DRC), %	38.98	39.59	36.92	40.39
Non-rubber content (NRC), %	2.49	2.07	2.55	2.48
Alkalinity (NH_3), %	0.27	0.27	0.31	0.38
Volatile fatty acid No. (VFA No.)	0.046	0.023	0.070	0.025
Total nitrogen content, %	0.731	0.715	0.632	0.583

Table 4.2: The properties of mixed clones latex concentrate (MCLC) and commercial latex concentrate (CLC)

Test Parameters	Mixed clones latex concentrate (MCLC)	Commercial latex concentrate (CLC)
Total solid content (TSC), %	61.78	62.26
Dry rubber content (DRC), %	60.75	60.98
Non-rubber content (NRC), %	1.03	1.28
Volatile fatty acid No. (VFA No)	0.039	0.045
Alkalinity (NH_3)	0.71	0.53
pH	10.35	10.08
Potassium hydroxide (KOH) Number	0.50	0.713
Mechanical stability time (MST), seconds	1314	1331
Viscosity (manual), Cps	85	92
Sludge content, %	0.005	0.007
Coagulum content, %	0.0121	0.0004
Nitrogen content, %	0.27	0.36
Zinc stability time (ZST), seconds	276	219
Chemical stability time (CST), seconds	251	108
Zinc oxide viscosity (ZOV), cps	56	61

The percentage of the non-rubber content of field latex of the mono-clones and MCFL were higher as compared to MCLC and CLC, as shown in Table 4.1. The non-rubber contents of the mono-clones field latex ranged from 2.07% to 2.55% and are much higher compared to that of the MCLC at 1.03%. The reduction in the non-rubber content is expected as some parts of the non-rubber components were removed during the centrifuging process. About 10% to 15% of the total rubber together with a proportion of non-rubber constituents of the field latex remains in the serum phase with 5% to 8% dry rubber content. Normally, the NR latex contains 6% non-rubbers components, including proteins, phospholipids, and inorganic metallics can affect significantly the properties of the NR products (Promsung et al., 2021; Wei et al., 2020; Zhang, H. et al., 2020).

Field latex is colloidally unstable and can undergo spontaneous coagulation within a few hours if no preservative is added. The nitrogen content in field latex has been shown to be associated mostly with the presence of proteinaceous materials. It was reported that about 30% of these materials were present in the rubber hydrocarbon phase and about 70% in the non-rubber phase. Most of these proteinaceous materials have an essential role in the stability of latex. The influence of seasonal changes on the nitrogen content of latex of earlier clones was reported by Yip (1990). Tables 4.1 and 4.2 show the results of nitrogen (N) content for all monoclonal latex and MCFL. The values ranged from 0.58% to 0.73% and are much higher compared to both the latex concentrate used (0.27% and 0.36%, respectively). The reduction in the nitrogen content is due to the removal of non-rubbers from the serum during the centrifuging process (Danwanichakul et al., 2014).

Ammonia is the common preservative mixed into field latex and latex concentrate. It prevents bacterial and enzymatic action on the non-rubber constituents that result in the development of acidity and destabilisation of the latex. Therefore, the ammonia content in latex as measured by the alkalinity test is related to latex stability. Latex is subjected to violent agitation during stirring and pumping of latex into the storage drum. Therefore, the value of mechanical stability time (MST) for MCLC and CLC of 1314 seconds and 1334 seconds, respectively, indicates that both the MCLC and CLC were in stable condition, more than 650 seconds, as shown in Table 4.2. The VFA number of latex was determined to investigate the action of microorganisms upon some of the carbohydrates in the serum phase. The low VFA number for MCFL and MCLC of 0.0250 and 0.039, respectively, indicated that there was low or minimal microbial activity. The latex concentrates were thus well preserved by the addition of ammonia as bacterial growth was hindered, which would otherwise cause coagulation of the latex. It is important for any field latex that is to be processed into latex concentrate to have a minimal level of bacteria and this is the primary reason for determining the VFA content of field latex before concentration (Pendle, 1990). Field latex with a VFA level of 0.02% to 0.04% is preferred for latex concentration production (Muniandy, 1998).

The KOH number is used to determine the content of ammonium soaps and ammonium salts formed during any changes that occurred in the latex. The changes are caused by bacterial action on the non-rubber constituents before full ammoniation and hydrolysing action causing a further breakdown of the protein during storage. The KOH number is indicative of the state of preservation and the age of latex (Blackley, 1997). The KOH No. for the MCLC was 0.5, which is less than 0.7, the maximum permissible limit according to ISO 127 (2018) test method. KOH No. higher than 0.7 indicates that the latex is poorly preserved and is of older age (Pendle & Gorton, 1978; Yip, 1990). Therefore, such latex is not recommended for use in the production of NR latex dipped products. The coagulum content is defined as the amount of material that is retained on an 85-mesh test sieve and usually contains coarse foreign matter and pieces of rubber coagulum, while the sludge content indicates non-polymeric impurities that tends to sediment under the influence of gravity. Impurities such as dust, sand, bark, fragments and magnesium ammonium phosphate make up the sludge. Table 4.2 shows that the sludge and coagulum content of both types of latex concentrates were very low based on ISO 2004 (2017) specifications. Therefore, the MCLC was assumed to be clean, stable and free from coarse foreign matter and pieces of rubber coagulum as well as non-polymeric impurities (Lai, 1988).

4.1.2 The metallical content in NR latex and MCLC films

The metallic contents of the three monoclonal field latex, MCFL, MCLC, CLC and MCLC films filled with untreated and SA-treated were analysed to find the concentration of metallics present in the rubber.

4.1.2.1 The metallical content in field latex of monoclones, MCFL and MCLC

The field latex consists of rubber hydrocarbon particles and non-rubber substances such as lipids, proteins, carbohydrates, acids, amines and inorganic constituents. Some of these non-rubbers may influence the properties of MCFL and MCLC produced. The metallic contents commonly analysed in field latex and latex concentrate are magnesium (Mg), manganese (Mn), copper (Cu), phosphorus (P), zinc (Zn) and iron (Fe). A few metallics such as Mg, Mn and Zn are known to affect latex stability. In addition, staining on the surface and defect in the end products may occur if higher than the permitted level of metallic content is present according to ISO 2004 specification (ISO, 2017).

The metallic contents present in the different types of natural rubber latex is shown in Table 4.3. It is observed that the contents of Al, Ca, Cu, Mg and P in the three monoclonal field latex is quite significant before mixing into MCFL. The difference was attributed to clonal variation since the higher content of non-rubbers that present in latex. The non-rubbers can affect the properties of latex concentrate and approximately one-third of the P in latex was found in the rubber hydrocarbon phase and the remaining in the non-rubber phase (Ruhida, 2012; Yip, E, 1990). In this study, it was found that metallics like Ca and Mg obtained a similar trend as a pervious study by Yip, (1990). K and P also indicate a similar trend, but the content of these two metallics was shown to be dependent on the fertilizer and soil condition before tapping and latex collection (Nurmi Rohayu et al., 2015; Ruhida, 2012).

Based on results shown in Table 4.3 and Figure 4.1, it was found that the potassium (K), magnesium (Mg), manganese (Mn) and phosphorus (P) contents for MCLF lower as compared to each clone of field latex. The MCFL was higher as compared to MCLC and this might be due to improper mixing of the latex contributed to the results obtained for MCFL before it was processed into MCLC. The Mn was not detected in MCLC because the values is very low and below of detection limit and this metallic prone to the oxidation on the rubber products. It was believed that the Mg ion in latex has reacted with di-ammonium hydrogen phosphate (DAHP) and settled down in the form of sludge. According to normal practice, the DAHP was added into the field latex for 24 hours to settle Mg ions in the latex. The DAHP will react with Mg ions and produce the sludge at the bottom of the container. Then, the sludge was removed, and the latex samples were then centrifuged using a centrifuge machine (Somarathna et al., 2018). Mg is an essential metallic for plant growth, and its content in field latex was reported to be influenced by clones, seasonal change, soil and fertilizer regime. Therefore, the presence of an appreciable amount of Mg in field latex could affect the latex stability during processing operations. The acceptable Mg content in natural rubber latex concentrate is below 50 parts per million (ppm). Earlier work reported that an excessive amount of magnesium reacted with surfactant in the latex compound that led to the formation of flocs. Therefore, the probabilities of defects such as pinholes occurring on the surface of NR latex films could be high. The flocculation can be trapped on the surface of NR latex films resulting in the formation of pinholes (Ruhida, 2011). Zinc oxide (ZnO) is widely used as an activator, filler or gelling agent in rubber and latex compounds. However, ZnO is classified as an unsafe compound due to its detrimental effect on the living environment and organisms (Heideman et al., 2004; Yusof, A. et al., 2006). Table 4.3 shows the result of Zn content of field latex in the different clones, MCFL and MCLC. MCLC had similar Zn content with MCFL but lower compared to all monoclones field latex. The mean Zn content of MCFL and MCLC was 5 ppm which was below 6 ppm. However, the mean Zn content of CLC (commercial latex concentrate) was 173 ppm, as shown in Figure 4.1. The high Zn content in CLC is due to the addition of tetramethyl thiuram disulphide and zinc oxide (TMTD/ZnO) in the latex concentrate to enhance the MST value within two weeks. The mixture of TMTD/ZnO with various amount of ammonia (from about 0.3% to 0.8% by weight of latex) is usually added before processing the field latex into commercial latex concentrate. These chemicals affect the chemical stability of the latex (Santipanusopon & Riyajan, 2009).

Table 4.3: The metallical contents of monoclones field latex, MCFL and MCLC

***Metallical content (ppm)**	**RRIM2023**	**RRIM2024**	**RRIM2025**	**MCFL**	**MCLC**
Calcium (Ca)	29	28	2	20	28
Copper (Cu)	4	6	3	4	1
Ferum (Fe)	6	6	7	5	4
Potassium (K)	5283	5364	4899	4308	1066
Magnesium (Mg)	525	447	254	85	4
Manganese (Mn)	0.7	0.6	0.4	0.2	ND
phosphorus (P)	735	1145	775	558	258
Zinc (Zn)	6	6	6	5	5

*All the results obtained is the mean value of 3 samples
ND (non-detected): value obtained was below the limit of detection

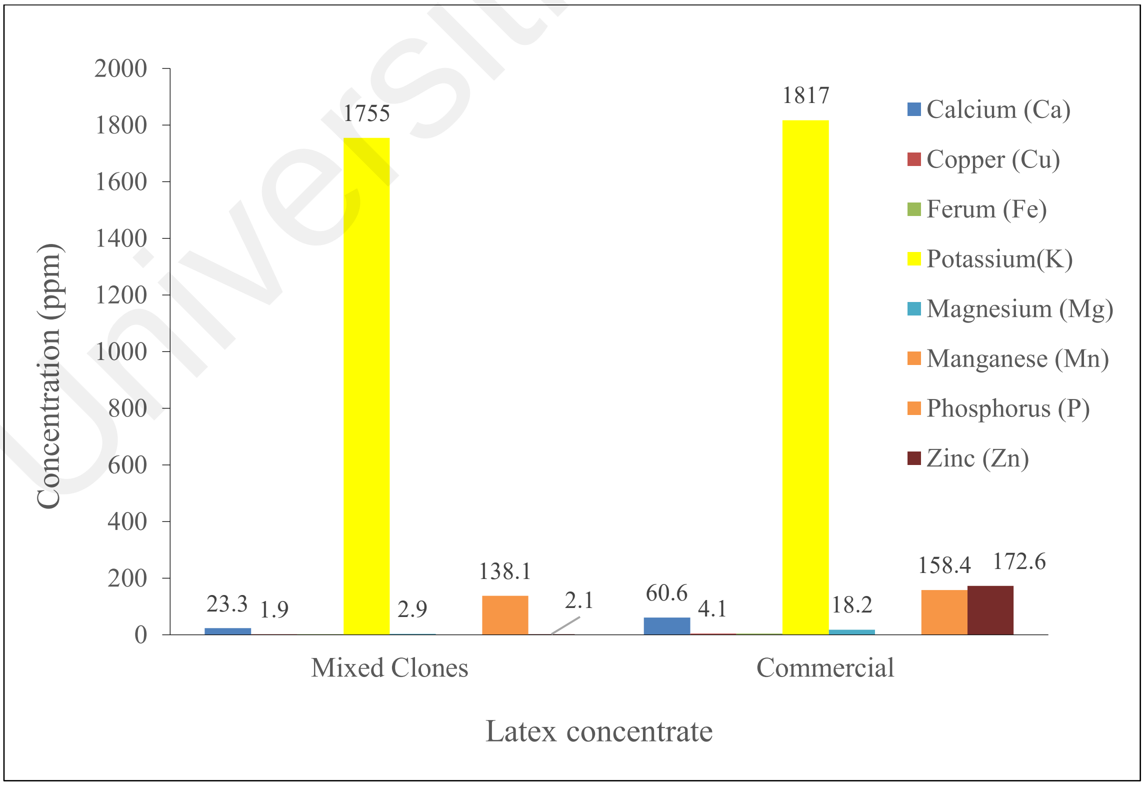


Figure 4.1: Metallical contents in MCLC and CLC.

4.1.2.2 The metallical content in MCLC films

Table 4.4 shows the metallical contents that are present in MCLC films treated with different filler loadings of calcium carbonate. It was observed that nine metallics Al, Ca, Fe, K, Mg, Mn, Na, P and Zn, are commonly present in the MCLC. However, in this study, the focus was mainly on the 6 metallics Fe, Mg, Mn, Zn, P, and Ca as these metallics have the ability to give an impact on the properties of the rubber. It is known that the content of the metallics such as magnesium (Mg), manganese (Mn), and iron (Fe) in rubber products alleviate oxidation of the products (Bien & Robert, 1983). The two metallics, Cu and Mn are known to enhance the oxidation of the rubber. Both metallics were not detected in MCLC and CLC. According to ISO 2004 specification, the concentration of Cu and Mn should not be more than 8 ppm and 2 ppm, respectively. Otherwise, the rubber products have the potential to degrade, and the shelf life of rubber products could be reduced. In addition, Cu contamination can cause discolouration of rubber products during ageing (Duangthong et al., 2017; Yip, 1990).

Based on the results obtained in Table 4.4 and Figure 4.2, Ca and Mg contents increased as the filler loadings increased. A similar trend was observed for Fe and Mn. However, for the metallic Zn, the amount decreased as the filler loading increased. Zn plays an important role in NR products as the Zn cations from ZnO or Zn compounds reacts with an organic accelerator to give active zinc-accelerator complexes, which is one of the main steps in the vulcanisation process (Chapman & Porter, 1988; Ducháček, 1972).

ZnO is a common additive for both dry and latex-based formulations. As an activator of the vulcanisation process, ZnO has the ability to enhance the crosslinking efficiency in latex products such as gloves, condoms, teats and etc. The important function of ZnO is to generate the accelerator as the vulcanisation reaction proceed. By the addition of ZnO in latex compound reduces the latex stability and film clarity when used above 2.0 pphr.

However, ZnO is classified as a hazardous chemical and the excess release of which is highly toxic to aquatic species (Anand et al., 2015; Fernando et al., 2012; Junlapong et al., 2019). In Table 4.4, it was observed that the significant value of K at 35 pphr filler loading severely decreased from 600 ppm to 377 ppm. The sudden decrease might be due to instrument error. However, by looking at trends from control (0 pphr) until 40 pphr filler loadings, it appears that the values of potassium in MCLC film are consistent from 10 pphr until 40 pphr filler loadings as compared to the control sample.

Table 4.4: The metallical contents in MCLC films at different loadings of $CaCO_3$

***Metallic content (ppm)**	**Filler loadings (pphr)**						
	Control	**10**	**20**	**25**	**30**	**35**	**40**
Aluminium (Al)	15	23	34	44	29	37	39
Calcium (Ca)	6671	26428	60961	61112	59343	77572	93854
Copper (Cu)	ND	ND	ND	ND	ND	ND	ND
Ferum (Fe)	19	29	40	44	38	48	52
Potassium (K)	624	625	726	700	600	377	695
Magnesium (Mg)	16	446	880	980	914	1250	1503
Manganese (Mn)	ND	ND	0.1	0.5	ND	1.2	1.9
Natrium (Na)	198	278	332	344	320	339	391
phosphorus (P)	146	140	142	127	126	135	119
Zinc (Zn)	6671	6121	5821	5323	4086	4969	4712

*All the results obtained is the mean value of 3 samples
ND (non-detected): value obtained was below the limit of detection

4.1.2.3 The metallic content in MCLC films with SA-treated $CaCO_3$

Stearic acid treated $CaCO_3$ dispersion at 30 pphr filler loading was added into the MCLC films. The $CaCO_3$ dispersion was treated with stearic acid at various concentrations namely 0.5, 1.0, 3.0, 4.0, 5.0 and 7.0 by % *w/v*. The MCLC films were prepared at 30 pphr filler loading based on the interference study carried out earlier which indicated that chemical interference was more obvious at 30 pphr filler loading as discussed under sub-topic 4.3.3. A similar formulation and procedure were used in the sample preparation, as stated under sub-topic 3.3.3 (Tables 3.4 and 3.5). In this analysis, the metallics, namely Fe, Cu, Mg, Zn, phosphorus and Mn, were evaluated according to the MRB test method (UPB/P/058, 2015). Table 4.5 shows the metallic's present in MCLC films containing SA-treated $CaCO_3$ such as Fe, Mg, Zn, Cu, phosphorus and Mn. No clear trend was observed in the metallical contents of the films at various treatments. Inconsistent results in the metallic content may be due to the interaction between the SA-treated $CaCO_3$ and MCLC. The Mg content of the MCLC films was very much higher than Control 1 (untreated $CaCO_3$, at 6.6 ppm). In addition, the Ca content in all the MCLC films filled with SA-treated $CaCO_3$ was higher compared to Control 1 (5781 ppm), with the highest value of 59231 ppm Ca recorded in a film loaded with 0.5% SA treated $CaCO_3$. The high Mg and Ca contents are believed to originate from the stearic acid coated on the $CaCO_3$ particles (Mihajlović et al., 2009). It is well known that the presence of high Mg^{2+} ions causes destabilisation of the latex concentrate. The Zn contents in MCLC films loaded with $CaCO_3$ treated at various concentrations of SA (0.5% until 7.0%) were lower compared to Control 1 (untreated $CaCO_3$) as shown in Table 4.5. High Zn content present in untreated MCLC films (Control 1) might also come from the accelerator (ZBuD) and antioxidant (ZnO) that was added into NR latex compounds. However, lower Zn content in MCLC films filled SA-treated $CaCO_3$ because of less zinc chelation with sulphur bonds. Zinc chelation is responsible for stabilising the sulphur bonds and influences the position of the S-S bond. The SA-treated $CaCO_3$ has hindered the zinc chelation with sulphur bonds. Besides that, zinc oxide is a widely used compound in the rubber industry due to the excellent properties that it shows as an activator and, consequently, its role in the mechanism of accelerated sulphur vulcanization has been extensively studied (Guzmán et al., 2012; Zhao et al., 2018).

Table 4.5: The metallic's content that presence in MCLC film with SA- treated $CaCO_3$

SA concentration (%)	*Metallics content (ppm)						
	Fe	Mg	P	Zn	Mn	Cu	Ca
Control 1	8	7	70	2964	ND	ND	5781
0.5	43	560	63	1765	3	ND	59231
1.0	36	939	58	1600	3	0.3	40864
3.0	31	1083	77	2035	4	0.5	45939
4.0	24	485	89	1505	2	ND	37578
5.0	30	887	53	1810	3	0.6	38655
7.0	27	532	79	1715	1	ND	39741

*All the results obtained is the mean value of 3 samples
ND (non-detected). The value obtained was below the limit of detection

4.1.3 Thermal analysis and Identification of $CaCO_3$ contents in MCLC films filled with untreated and SA-treated $CaCO_3$

4.1.3.1 Thermogravimetric analysis (TGA) of MCLC films filled with untreated $CaCO_3$

The $CaCO_3$ contents in MCLC films were determined by using TGA following MRB in-house test method. Figure 4.2 shows the thermograms of unaged unmilled and milled MCLC films containing 0 pphr, 10 pphr, 30 pphr and 40 pphr $CaCO_3$ loadings. The rubber, $CaCO_3$, CaO and impurities contents were calculated based on the thermograms obtained. Milling was carried out by passing the film through a small-scale creeping machine at least 6 times. All the samples were analysed by TGA following the MRB test method (UPB/P/010, 2010).

Prior to the analysis of the MCLC films at various filler loadings by TGA, a sample containing only $CaCO_3$ dispersion was analysed. Figure 4.2 also shows the results of rubber, $CaCO_3$ and ash contents at 10 pphr, 30 pphr and 40 pphr $CaCO_3$ loadings. On heating, the $CaCO_3$ decomposed into carbon dioxide (CO_2) and calcium oxide, CaO. The

CaO in the residue was calculated based on the decomposition of $CaCO_3$. All the details of the calculation are as shown in Appendix C.

Tables 4.6 and 4.7 show the percentage of rubber, $CaCO_3$ and ash contents of unaged milled and unmilled MCLC films with and without rubber chemicals, respectively. The rubber chemicals added include KOH, potassium laurate, sulphur dispersion, ZBuD, ZnO and Wingstay L (Table 3.8). Figure 4.3 shows the $CaCO_3$ content present in unaged MCLC films with and without milling. While Table 4.6 shows the percentage of rubber, $CaCO_3$ and ash contents of aged MCLC film with rubber chemicals added into it. The purpose of carrying out this experiment was to evaluate the $CaCO_3$ content with and without the addition of rubber chemicals and to observe filler distribution in MCLC films. In Table 4.6, the values of percentage $CaCO_3$ and CaO contents without filler (0 pphr) might come from instrument error or any rubber chemicals left in the instrument and not entirely burned by oxygen gas. Based on Figure 4.3, there was no significant difference in the results of $CaCO_3$ between unmilled or milled MCLC films. Therefore, the milled aged MCLC films were carried out. Generally, the $CaCO_3$ content of unmilled samples was slightly higher as compared to milled samples. This can be attributed to the inhomogeneity of the sample. In Figure 4.3, it was observed that the $CaCO_3$ content in MCLC film with rubber chemicals added indicated that there was no significant difference from 0 pphr until 25 pphr. Above 25 pphr filler loading, the $CaCO_3$ content of milled samples were higher values compared to unmilled samples. The discrepancy in the small amount of filler (less than 1.0 %) detected for 0 pphr filler may originate from rubber chemicals such as ZBuD and ZnO that were added during latex compounding. The lower results in the unmilled samples compared to milled may be due to inhomogeneity arising from the uneven distribution of filler in the MCLC films.

Figure 4.3 shows the $CaCO_3$ content of unaged milled samples at different filler loadings after solvent extraction at 60 ºC for 16 hours. The $CaCO_3$ content of samples after solvent extraction was slightly lower as compared to samples that were not extracted. The scatter in the results of $CaCO_3$ and ash obtained at high filler loadings in samples that had undergone solvent extraction and ageing could have been due to the removal of some part of the non-rubbers, including filler from the samples. Ageing was also observed to reduce filler content, as shown in Table 4.8 and Figure 4.3. The higher content of CaO as compared to ash at filler loadings above 25 pphr indicated less $CaCO_3$ content in MCLC films being pick-up during the dipping process. The ash content comes from the latex since more NR latex at above 25 pphr filler loading.

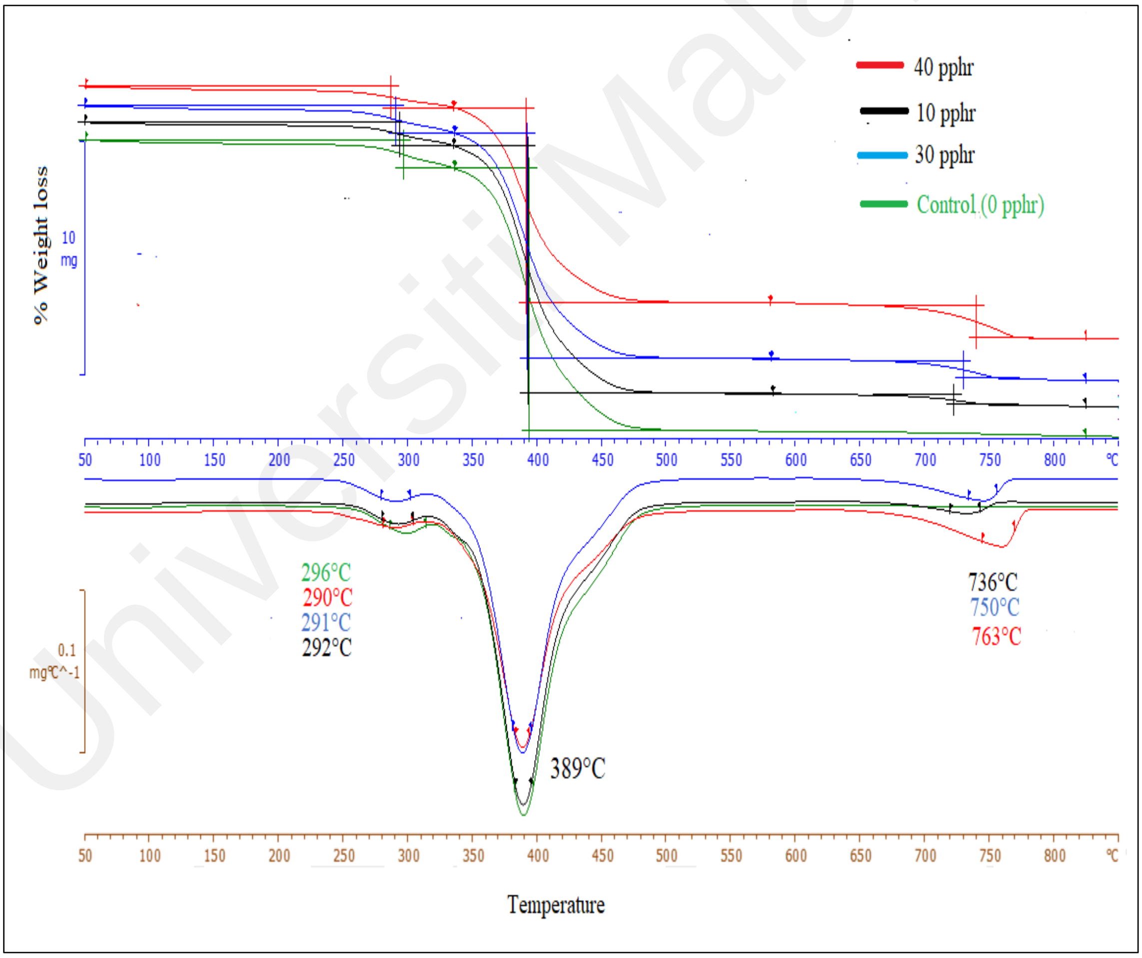


Figure 4.2: The thermogram of rubber, $CaCO_3$ and ash contents at Control, 10 pphr, 30 pphr and 40 pphr filler loadings.

Table 4.6: The rubber, $CaCO_3$ and ash contents of unaged MCLC films added with filler and rubber chemicals

	Filler content (pphr)	Rubber content (%)	$CaCO_3$ content (%)	CaO content (%)	Ash content (%)
Unmilled (Unaged)	0	96.6	1.0	0.6	2.3
	10	90.8	7.2	4.0	1.9
	20	79.3	13.8	7.7	1.8
	25	75.7	18.5	10.3	0.3
	30	70.0	20.2	11.3	3.1
	35	75.2	23.2	13.0	1.7
	40	69.8	24.4	13.6	0.5
Milled (Unaged)	0	94.4	0.7	0.4	1.7
	10	90.7	7.5	4.2	1.7
	20	75.9	14.8	8.2	1.0
	25	74.7	17.3	9.7	0.3
	30	69.2	22.5	12.6	1.6
	35	68.0	28.8	16.1	0
	40	68.5	24.7	13.9	0

Table 4.7: The rubber, $CaCO_3$ and ash contents of unaged MCLC latex films prepared with filler only

	Filler content (pphr)	Rubber content (%)	$CaCO_3$ content (%)	CaO content (%)	Ash content (%)
Unmilled (Unaged)	0	95.9	0	0	0.5
	10	88.6	8.9	5.0	0.8
	20	80.6	16.6	9.3	1.4
	25	77.0	18.2	10.1	0.5
	30	72.7	22.6	12.7	0.5
	35	71.2	24.6	13.8	0
	40	68.1	27.8	15.6	0
Milled (Unaged)	0	91.9	0	0	0.6
	10	88.2	9.1	5.1	0.1
	20	79.4	15.0	8.4	0.3
	25	80.0	17.8	10.0	0.6
	30	72.0	22.2	12.4	0.4
	35	71.8	22.2	12.4	0.9
	40	67.6	27.1	15.2	0.6

Table 4.8: The rubber, $CaCO_3$ and ash contents of milled, aged MCLC films

	Filler content (pphr)	Rubber content (%)	$CaCO_3$ content (%)	CaO content (%)	Ash content (%)
Milled (Aged)	0	98.3	0	0	1.2
	10	94.8	3.6	1.9	1.7
	20	90.6	8.1	4.6	1.2
	25	87.5	12.2	7.0	0.8
	30	78.2	20.8	11.7	0.8
	35	82.2	17.3	10.4	1.0
	40	80.8	18.9	11.1	0.8

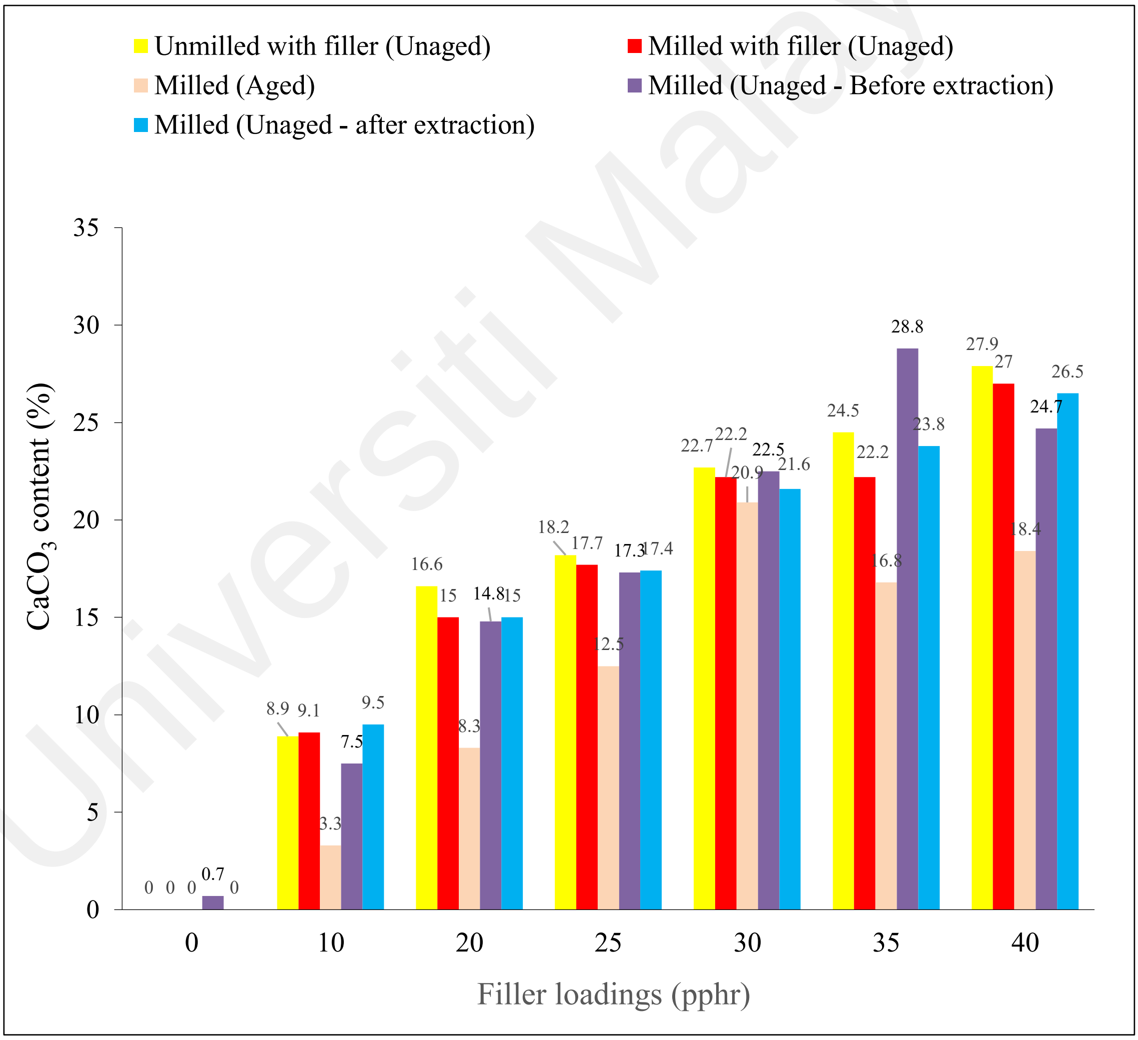


Figure 4.3: The $CaCO_3$ content of unaged (unmilled and milled), aged (milled) and unaged (milled) before and after extraction of MCLC films at different filler loadings.

4.1.3.2 Evaluation of dried SA-treated $CaCO_3$ content by thermogravimetric analysis (TGA)

The SA-treated $CaCO_3$ at various concentrations at 0.5, 1.0, 3.0, 4.0, 5.0 and 7.0 by % (*w/v)* was evaluated by using TGA as shown in Figure 4.4. The main purpose of the investigation was to identify the presence of SA that might be coated on the surface of $CaCO_3$ particles and to measure the amount of SA. Besides that, to find out the accuracy of the amount of filler ($CaCO_3$) being pick-up during the dipping process of MCLC films and also to investigate the $CaCO_3$ distribution in MCLC films uniform and homogenous. Fatty acids such as stearic acid is the most common surfactant used to precipitate calcium carbonate. In a study on dried $CaCO_3$ coated with stearic acid, stearic calcium stearate and magnesium stearate. Gilbert et al. (2001) reported that the maximum amount of organic material adsorbed on $CaCO_3$ was stearic acid. Figure 4.5 shows the thermogram of treated $CaCO_3$ at 1.0% and 3.0% concentrations of SA. At 1.0% SA-treated $CaCO_3$ the $CaCO_3$ and ash contents were 99.2% and 56.3%, respectively whilst at 3.0% the $CaCO_3$ and ash contents were 95.9% and 55% respectively. The content of SA that possible being detected on the $CaCO_3$ particles were at 3.0% to 7.0% were 2.8%, 5.0%, 4.0% and 6.9%, respectively, as shown in Figure 4.4. Detection and quantification of SA was based on the temperature of decomposition during thermal analysis, as shown in Figure 4.5. Unfortunately, the limit of detection of SA by the TGA used for the analysis is more 3.0% SA-treated $CaCO_3$. Stearic acid present on the surface of $CaCO_3$ particles (treated $CaCO_3$) was observed to be degraded at a higher temperature (806 ºC and 810 ºC) compared to pure $CaCO_3$ (802 ºC) (Devamani et al., 2016). The anomalous slightly higher amount of $CaCO_3$ determined for Control 1 (untreated $CaCO_3$) as compared to treated $CaCO_3$ at 0.5% and 1.0% concentrations may be due to some contamination in the analyser or sample. However, the treated $CaCO_3$ contents was observed to decrease with increasing concentration of SA viz from 3.0% to 7.0%. The amount of SA determined at 3.0% to 7.0% concentrations of the treated $CaCO_3$ may not originate completely from the coating on the $CaCO_3$ particles. Earlier studies have stated that the adsorption of stearic acid on the $CaCO_3$ may be partly chemical and partly physical and occur by the formation of multilayers, wherein the first layer is built based on strong chemical forces, with the following layers are physically adsorbed over the chemisorbed layer (Mihajlović et al., 2013; Shi et al., 2010).

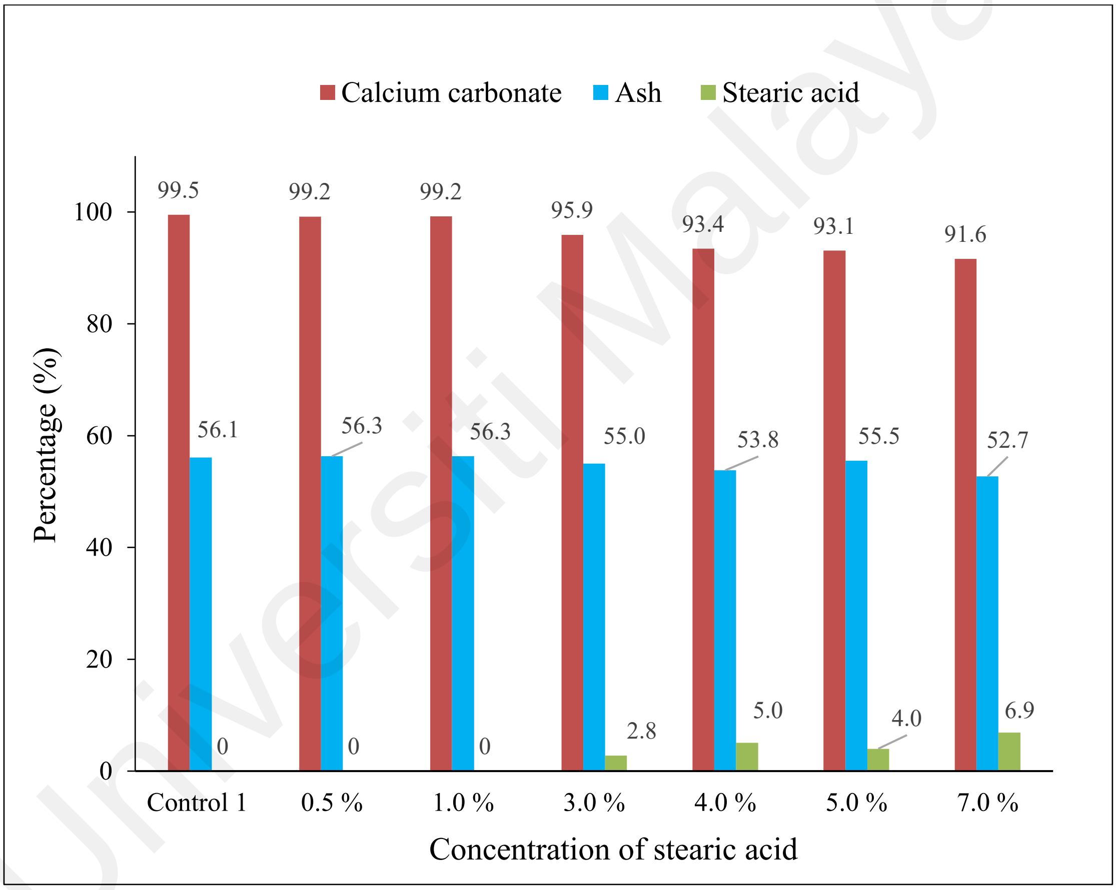


Figure 4.4: The contents of stearic acid, $CaCO_3$ and residue by TGA analysis.

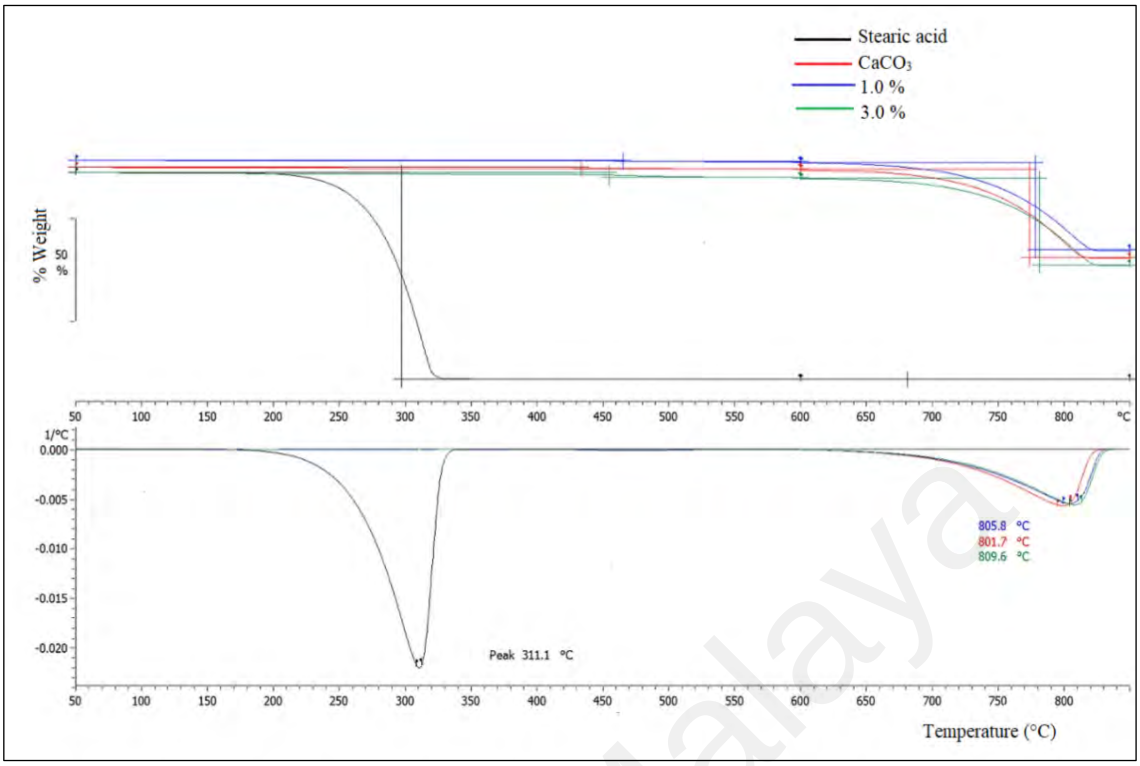


Figure 4.5: The thermogram of stearic acid, $CaCO_3$ and SA-treated $CaCO_3$ at 1.0% and 3.0% concentrations.

4.1.3.3 Thermogravimetric analysis (TGA) of MCLC films filled with SA-treated $CaCO_3$

The MCLC films filled with treated $CaCO_3$ was analysed by TGA in order to determine the amount of SA-treated $CaCO_3$ present in it. Previous works have shown that it was possible to deduce the composition and distribution of filler in NR latex films by thermal analysis. The researchers suggested that the filler was able to disperse evenly in the NR latex compound before dipping was carried out and that the addition of filler helped to increase the interaction between rubber and filler particles during latex compounding (Amir Hashim et al., 2004; Roslim & Amir Hashim, 2010).

Figure 4.6 shows the amount of SA-treated $CaCO_3$ in unaged and aged MCLC films compared to Control 1 (untreated $CaCO_3$). The results of aged films filled with SA-treated $CaCO_3$ were slightly higher compared to unaged films except for the sample filled with 7.0% SA-treated $CaCO_3$ and Control 1, whereby the amount obtained was approximately the same. The aged sample filled with 0.5%, 1.0% and 5.0% treated $CaCO_3$ also obtained higher results of $CaCO_3$ content as compared to unaged samples. However, there was no significant difference in the results obtained at 3.0% and 4.0% SA-treated $CaCO_3$ for both aged and unaged films. The slight decrease in results of unaged films filled with SA-treated $CaCO_3$ as compared to Control 1 indicated that the pickup of SA-treated $CaCO_3$ during latex dipping process was less from 2.4% until 16.2% in the MCLC films. While in aged samples, the increase in treated $CaCO_3$ contents indicated that the amount of SA-treated $CaCO_3$ in samples also increased as compared to Control 1 at 21.3% until 38.8%. It is assumed that heating during the ageing process had improved the cross-link interaction between the SA-treated $CaCO_3$ and natural rubber particles. The inconsistent result obtained may be due to insufficient amount of SA available to attach or cover all the surface of $CaCO_3$ particles as well as the uneven distribution of SA-treated $CaCO_3$ in the latex during compounding stage. This was based on the results of 7.0% SA-treated $CaCO_3$ and Control 1 (untreated) samples. The result of unaged samples was slightly lower as compared to Control 1, whilst the result of aged samples was higher as compared to Control 1. These observations indicated that the SA-treated $CaCO_3$ in MCLC films were more strongly bonded to the rubber particles compared to untreated $CaCO_3$ under both conditions.

Figures 4.7 and 4.8 shows the thermograms of SA-treated $CaCO_3$ at various concentrations as compared to pure SA and $CaCO_3$. The thermograms of pure SA and $CaCO_3$ will help in identifying the thermal curves of SA-treated $CaCO_3$ in the MCLC films. Figure 4.7 shows SA decomposed at 311 °C. At 1.0% and 3.0% SA treated $CaCO_3$ in MCLC films, the oxidation of the organic substances occurred at 390 °C and 389 °C, while the decomposition of the $CaCO_3$ component occurred at 762 °C and 759 °C as shown in Figure 4.8. Control 1 in Figure 4.8 also shows the transition of the SA degradation region, and this might be due to the MCLC film that contained rubber and

$CaCO_3$ that was untreated. The Control and MCLC samples indicate a transition of SA degradation regions due to the degradation of additives that are present in MCLC films. According to a study by Mihajlović et al., generally for calcite modified with fatty acids, two main decomposition stages were visible between the temperature range of 25 °C to 600 °C. The first stage, between 25 °C and 200 °C which, corresponds to a loss of water or elimination of physisorbed acid that was not removed by washing procedure. The main decomposition step between 200 °C and 400 °C corresponds to the oxidation of organic substances that is chemisorbed at the calcite surface. The third decomposition step starts at about 580 °C, which corresponds to the decomposition of calcite (Mihajlović et al., 2009).

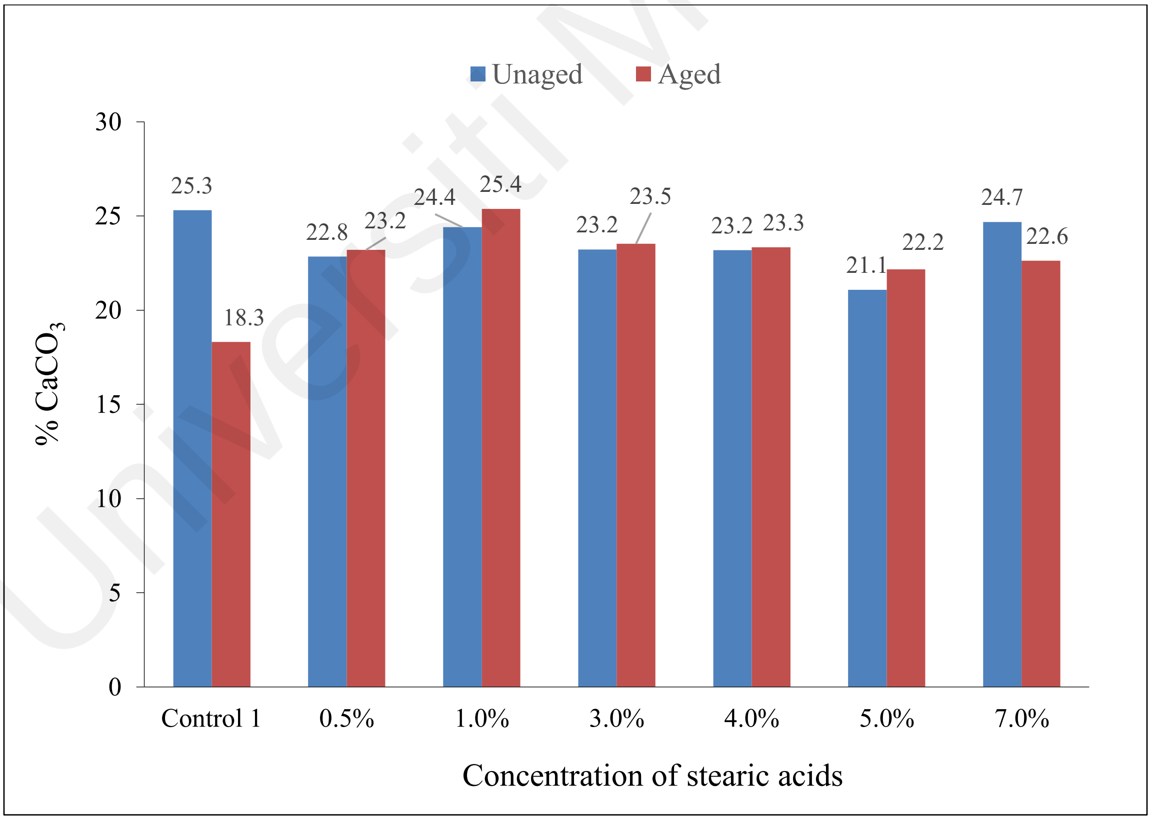


Figure 4.6: The $CaCO_3$ content in MCLC films with Control 1 (untreated) and SA-treated $CaCO_3$.

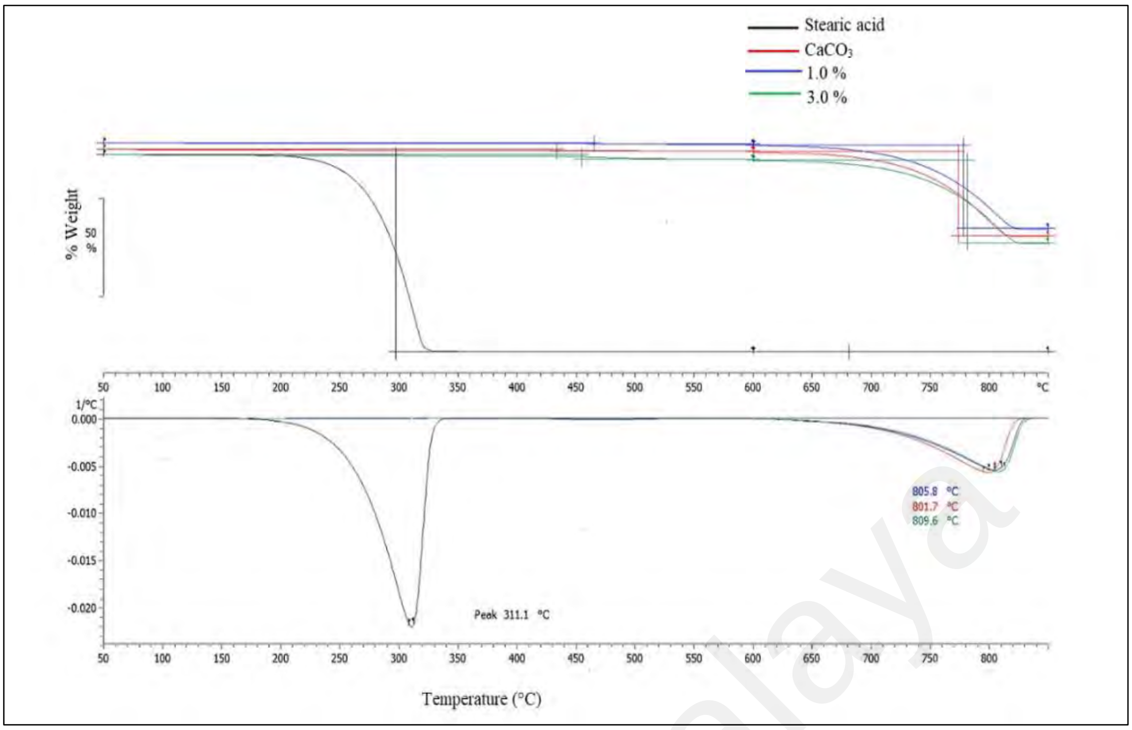


Figure 4.7: The thermogram for MCLC films filled with $CaCO_3$ treated with 1.0% and 3.0% SA, pure SA and $CaCO_3$.

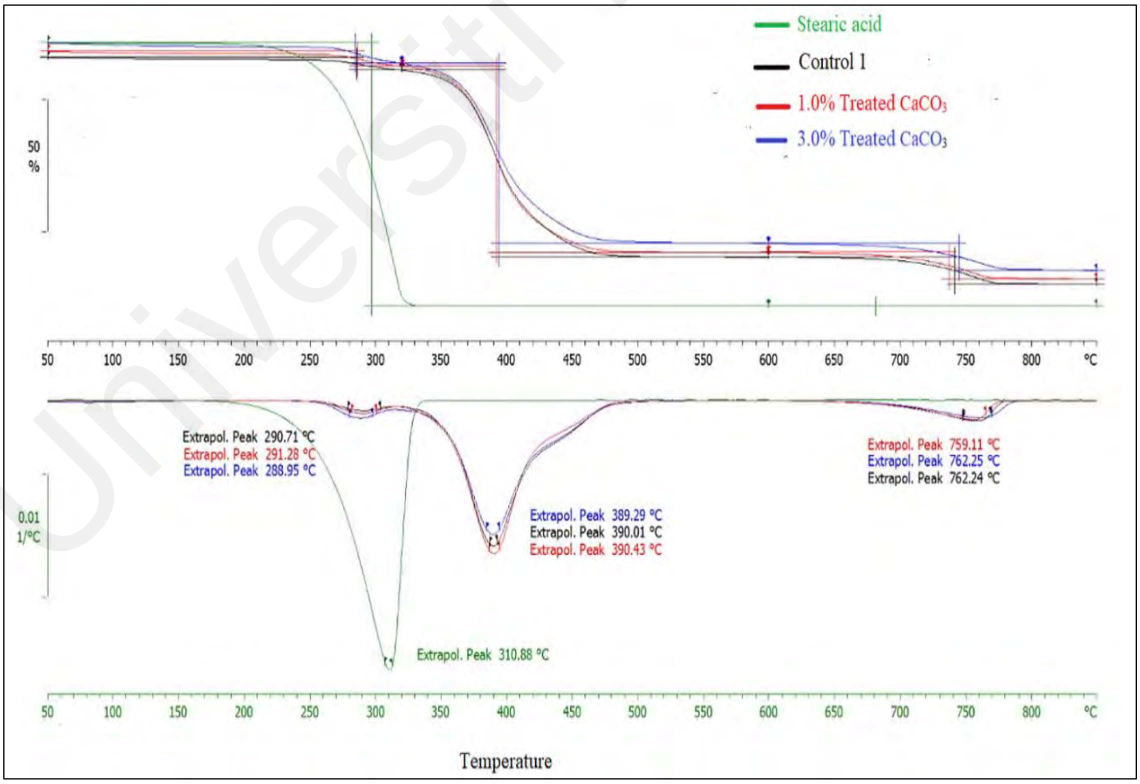


Figure 4.8: The thermogram of MCLC films filled with $CaCO_3$ treated with 1.0% and 3.0% SA as compared to pure SA and Control 1 (untreated $CaCO_3$).

4.1.3.4 The glass transition temperature (T_g) of MCLC films filled with untreated $CaCO_3$

The glass transition temperature (T_g) is a fundamental polymer characteristic that influences the properties of materials. It is an analytical technique used to study the thermal oxidation of elastomers and in the NR industry, it is widely applied to study thermal oxidation of NR or its stabilisation by certain antioxidants (Bonfils et al., 2001; Boonsomwong et al., 2020; Loadman, 1985). It is known that the natural rubber has long and regular macromolecule chains without large substituent, with the spatially oriented structural unit. Thus, their segments are moveable and at low temperatures, they can freely rotate around simple chemical bonds. With the increasing of the content of irregularities in the rubber chain or under the presence of large substituents, the T_g is increasing (Matador, 2007). In this study, the main reason to carry out this experiment was to observe the change in T_g of MCLC films filled with filler. Figure 4.9 shows the value of T_g in MCLC films. From the observation, the T_g results of MCLC films at different filler loadings were quite consistent. The T_g values seem to show a slight increase from Control (0 pphr) until 20 pphr filler loadings and then begin to decrease after 20 pphr filler loadings. The values of T_g begin to be consistent from 20 pphr until 40 pphr filler loadings. The slight changes in T_g of MCLC film from Control (0 pphr) to 20 pphr filler loadings may be due to the contribution of polysulphidic cross-linkages restricting molecular mobility. Polysulphidic cross-linkages is the bridging of two polymer chains are linked by three or more sulphur atoms (Chapman & Porter, 1988; Kim et al., 2020). The T_g of MCLC films from 25 pphr until 40 pphr was of similar magnitude as the Control. It seems that no effect on the T_g by fillers is present in MCLC films. The T_g of MCLC films increased by 1.2% when filler loadings increased from 0 pphr to 20 pphr filler loadings and then decreased by about 1.1% after 20 pphr until 40 pphr filler loadings. It seems that the presence of crystalline region in rubber matrix (MCLC) also might be led to a small elevation of the T_g by reducing the mobility of the amorphous region besides the chemical cross-link of the MCLC films (Hiranobe et al., 2021; Nicholson, 2006). Besides, a gradient of chain mobility proceeding from the polymer in direct contact with the filler surface to bulk behaviour was reported as well as bridges of glassy polymer between filler aggregates where confinement of the polymeric macromolecules was particularly extreme. Conversely, there are also a large number of studies reported there were no significant effect of the presence of filler on the local segmental dynamics of the polymer and the bulk glass transition (Huang et al., 2015).

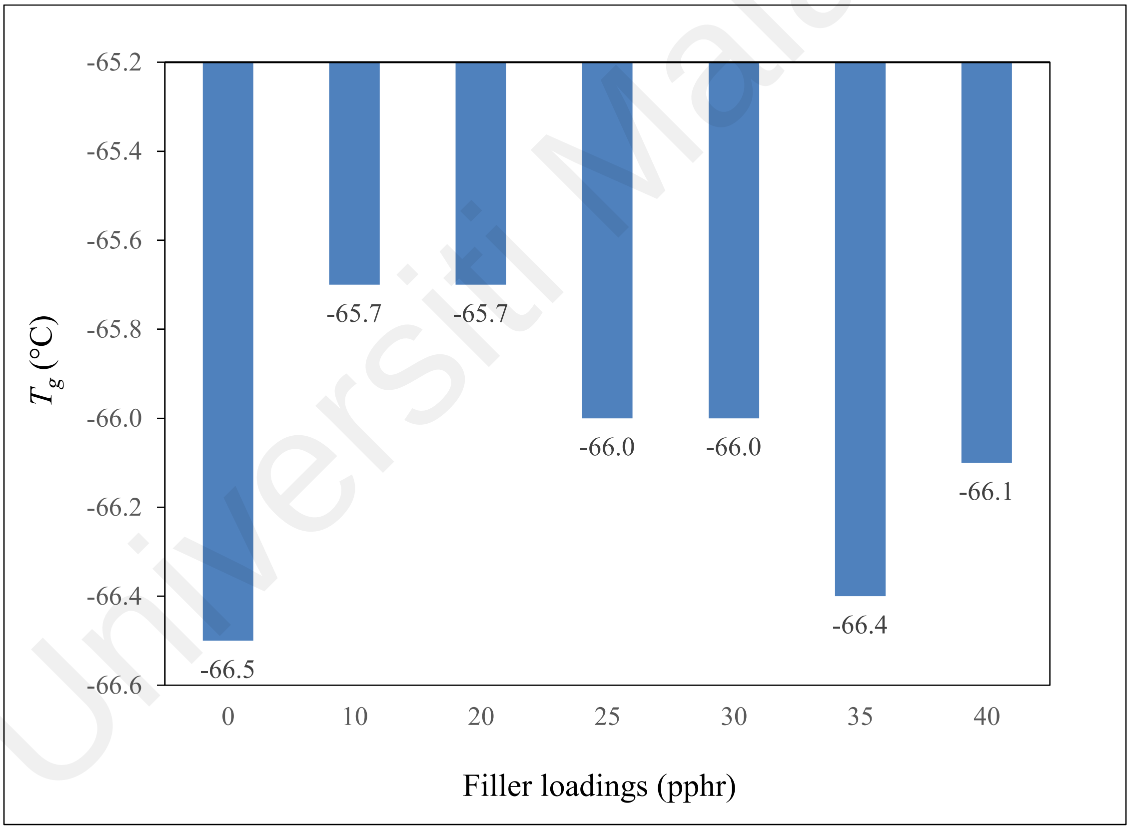


Figure 4.9: The midpoint of glass transition temperature (T_g) of MCLC films with untreated $CaCO_3$.

4.1.3.5 The glass transition temperature *(T_g)* of MCLC films filled with SA-treated $CaCO_3$

The glass transition temperature is a temperature range where the polymer substrate changes from a rigid glassy material to a soft (not melted) material and is usually measured in terms of the stiffness or modulus (Asiah et al., 1986; Loadman, 1985). In this study, the T_g of MCLC films with untreated and treated $CaCO_3$ with SA in $CHCl_3$ medium at various concentrations was carried out to measure the T_g of samples by DSC. Figure 4.10 shows the midpoint of the glass transition temperature of the SA-treated filler in MCLC films. From the results obtained, the T_g of MCLC film filled with $CaCO_3$ treated with 0.5% to 3.0% SA decreases. However, the T_g increased in a film filled with 4.0% SA-treated filler. Then, it begins to decrease again at 5.0% and increase at 7.0% concentration. The reduction in T_g of the MCLC films indicated that the sample was stiffer while the high T_g of SA-treated $CaCO_3$ at 0.5%, 4.0% and 7.0% was more elastic as the values obtained were more towards the T_g of NR, which is at -72 ºC. The T_g of a polymer is influenced by the flexibility and stiffness of the main backbone chain and the nature of the side group (Loadman, 1985).

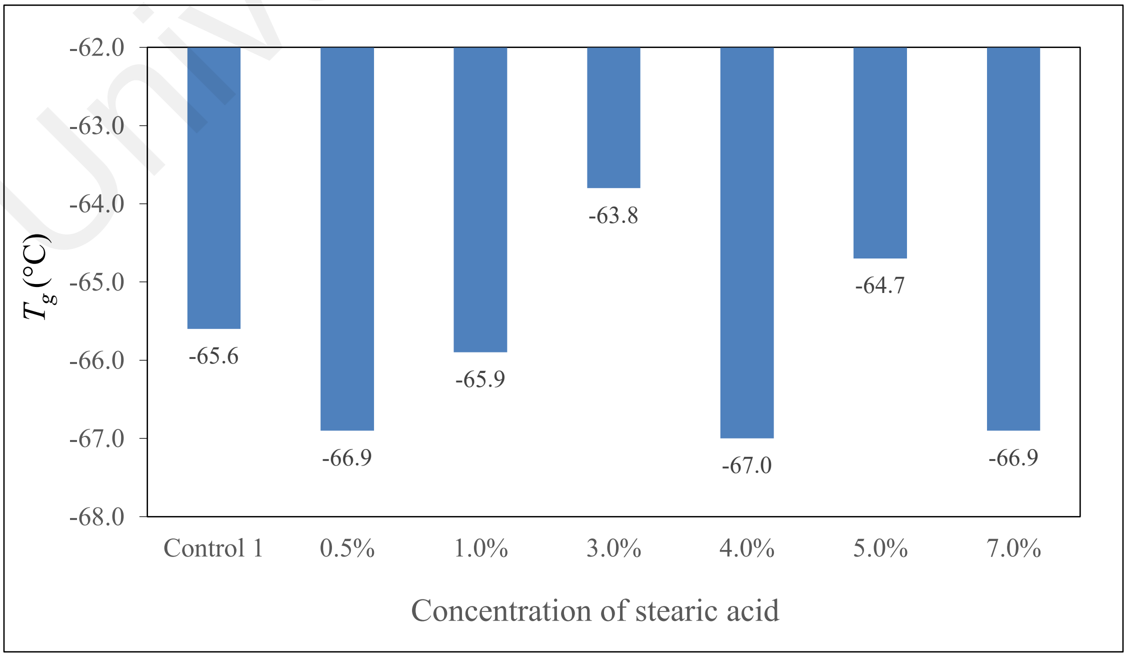


Figure 4.10: The midpoint (°C) of T_g in MCLC films with SA-treated $CaCO_3$.

4.1.3.6 Identification of untreated $CaCO_3$ in MCLC films by Fourier transform infrared spectroscopy (FTIR)

FTIR is one of the essential tools used to identify the functional groups that are present in the rubber materials. In the IR region, each functional group has several patterns of vibration (stretching, bending, etc.) which lead to several absorption bands. The absorption bands are unique for a particular functional group and can be used as a tool for characterisation and identification purposes. The absorbance of these bands is proportional to their amount present based on Lambert-Beer law (Zhang, P. et al., 2008). In this study, the presence of $CaCO_3$ in MCLC films at different filler loadings was investigated by FTIR. According to the literature, three carbonate peaks are commonly observed when carbonate is present in a rubber matrix. The position of the peaks are at 1437 cm^{-1}, 873 cm^{-1} and 712 cm^{-1}. In this study, a strong and broad peak was noted at 1450 cm^{-1}, a second strong but small peak was observed at 1375 cm$^{-1,}$ and a third weak peak at 850 cm^{-1}. It was observed that the samples containing 30 pphr filler depicted higher absorbance as compared to samples containing10 pphr filler. This indicated that as more filler was added to the rubber, a higher absorbance was shown in Figure 4.11. The higher absorbance was due to the filler that intercalates between rubber particles which is organic material identified by FTIR. At 1450 cm^{-1} it was observed that the $CaCO_3$ peaks became strong and broad as the filler loadings increased.

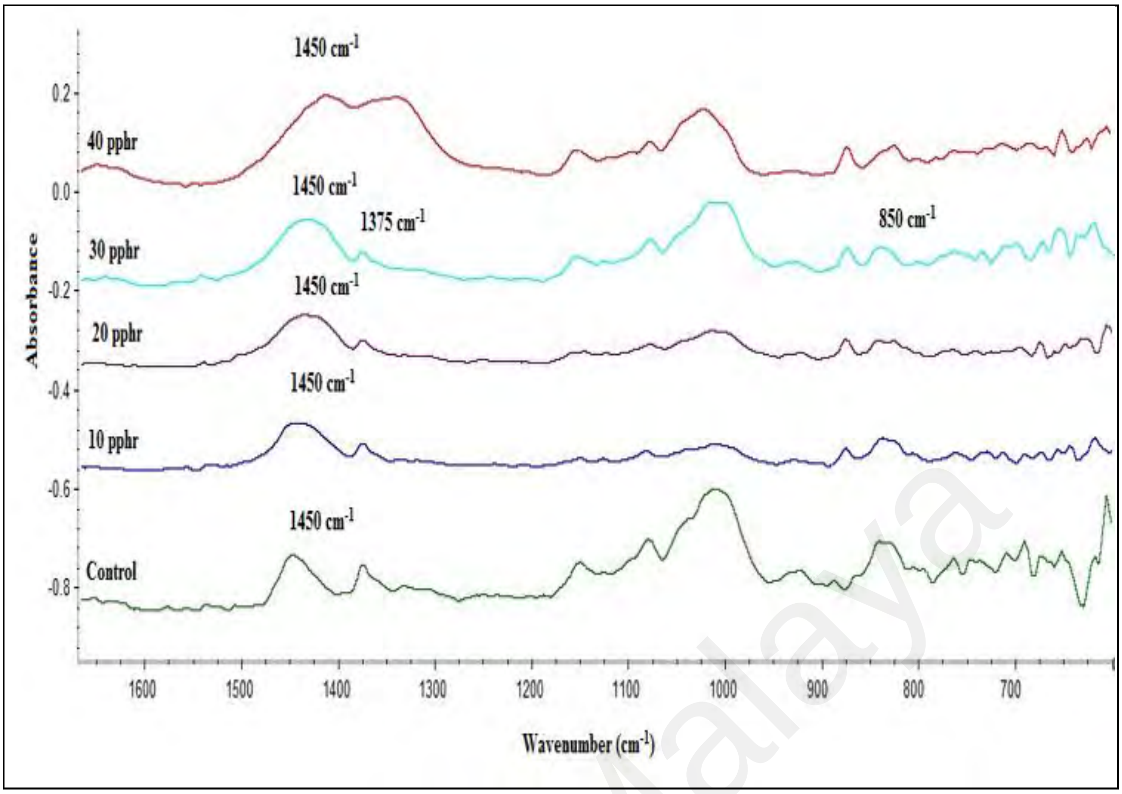


Figure 4.11: FTIR spectra of MCLC films with the presence of $CaCO_3$ peaks at range 1350 cm^{-1}– 1500 cm^{-1} and about 850 cm^{-1}.

Natural rubber shows N-H stretching band at 3375 cm^{-1}, amide and amide bonding at 1650 cm^{-1} and the out of plane bending vibration from =C-H bond at 840 cm^{-1} (Figure 4.12). The absorption bands at 1650 cm^{-1} and 1540 cm^{-1} are indicative of amide and amine groups, thus confirming the presence of nitrogenous materials in natural rubber. From the shift in the absorption peaks at 1450 cm^{-1} to 1425 cm^{-1} and that of 840 cm^{-1} to 875 cm^{-1} and the appearance of a broad peak at 1650 cm^{-1}, it was deduced that there was an interaction between $CaCO_3$ and MCLC film. The broad peak at 1650 cm^{-1} can be attributed to the protein that attached to NR latex (Chaikumpollert et al., 2011).

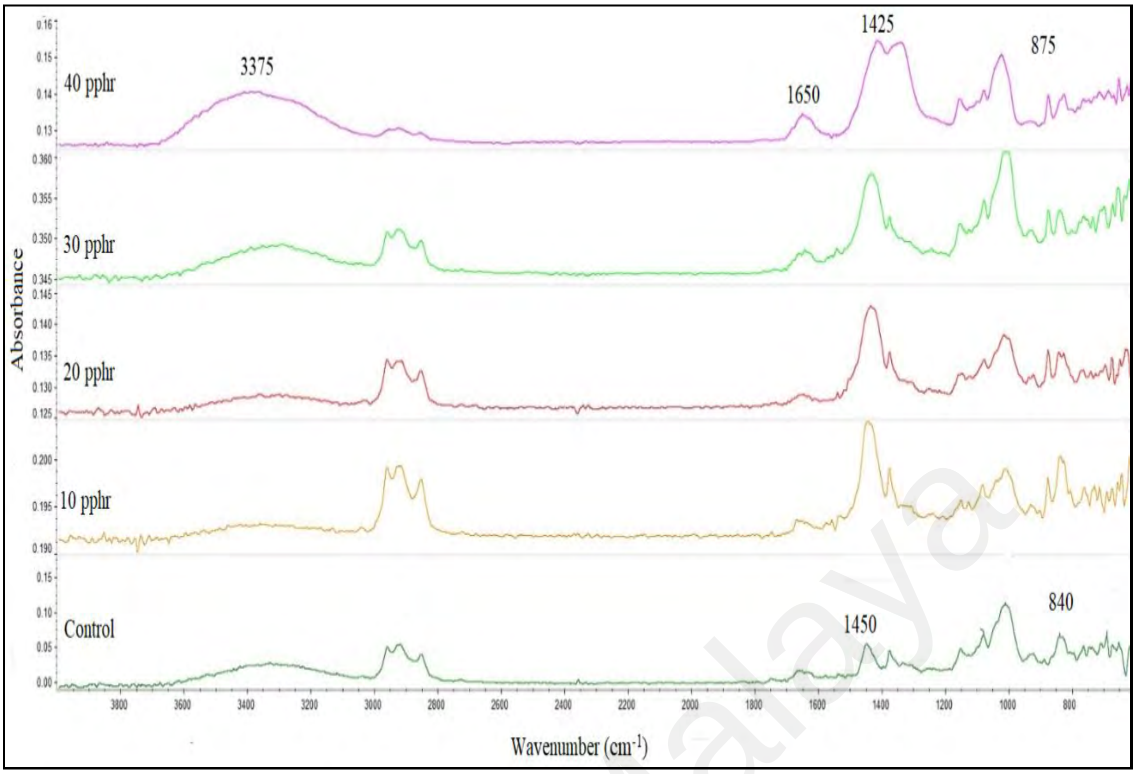


Figure 4.12: FTIR spectra of MCLC films unfilled (Control) and filled with $CaCO_3$.

4.1.3.7 Evaluation of dried dispersion of SA-treated $CaCO_3$ by Fourier transform infrared spectroscopy (FTIR)

The dried dispersions were evaluated by FTIR to identify and determine the amount of SA on the surface of $CaCO_3$ particles. The dried treated $CaCO_3$ was ground into a powder form with KBr before analysing it by FTIR. Figures 4.13 and 4.14 show the FTIR spectra of the treated $CaCO_3$ dispersions as compared to pure SA. Figure 4.13 shows a strong and broad peak between 1200 cm^{-1} to 1700 cm^{-1}. The presence of a strong and sharp peak at 875 cm^{-1} and a strong small peak at 710 cm^{-1} (Figure 4.14) was also observed. Figure 4.14 shows the FTIR spectra of treated $CaCO_3$ dispersions at 0.5%, 1.0%, 3.0% and 7% SA concentrations as compared to the spectra of pure stearic acid. The characteristic peaks for stearic acid at 2925 cm^{-1}, 2850 $cm^{-1,}$ and 710 cm^{-1} as observed in Figure 4.13 were present in the dispersions.

The absorbance of the characteristic peaks of stearic acid at 2925 cm^{-1}, 2850 cm^{-1} and 710 cm^{-1} were observed in the $CaCO_3$ treated with SA at higher concentrations, namely

3.0%, 4.0%, 5.0% and 7.0% concentration. The peaks were hardly distinguishable at 0.5% and 1.0% treated $CaCO_3$ especially at 2925 cm^{-1} and 2850 cm^{-1}. This may be due to insufficient amount of SA present to coat or cover the $CaCO_3$ particles. The significant peak at 875 cm^{-1} and 710 cm^{-1} was also observed for all SA treated $CaCO_3$. According to the peaks in the spectra obtained at 2925 cm^{-1}, 2850 cm^{-1} and 710 cm^{-1}, it can be confirmed that the $CaCO_3$ was attached to stearic acid.

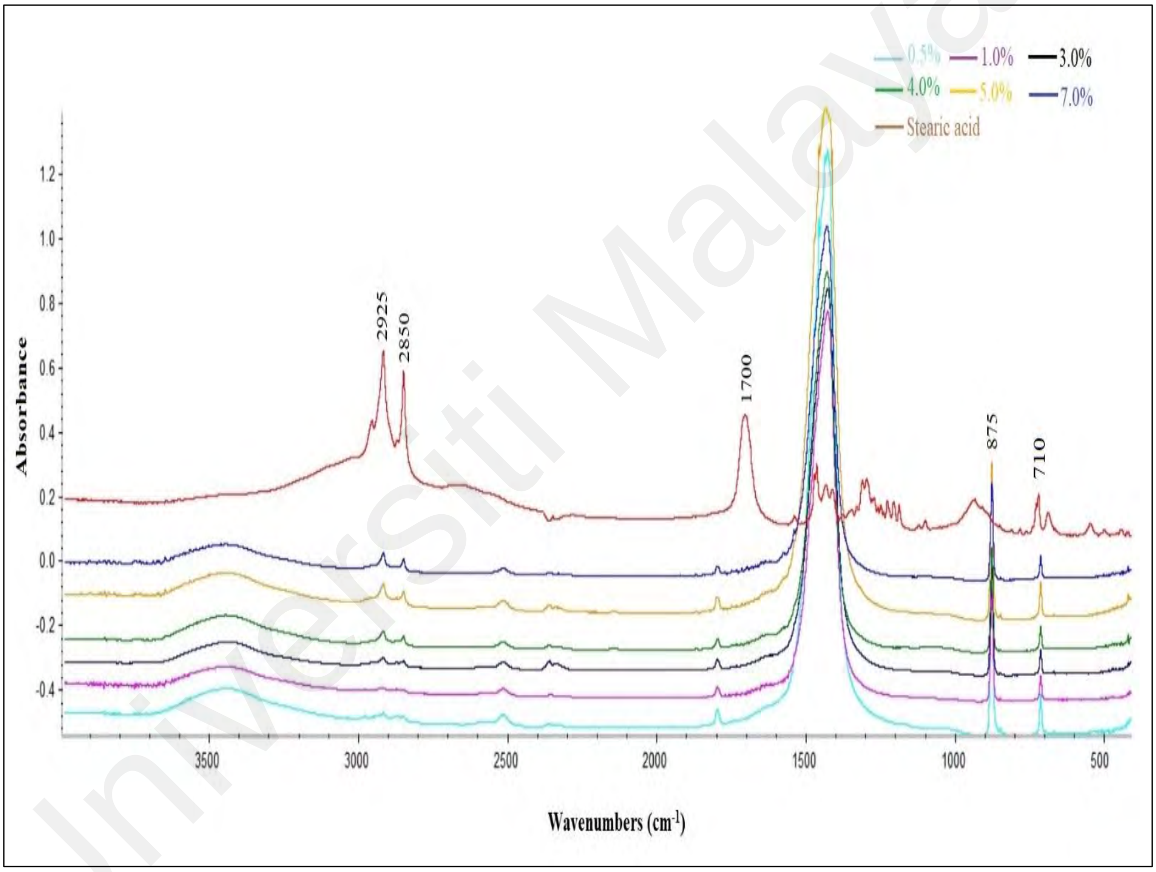


Figure 4.13: The FTIR spectra indicate the dispersion of treated $CaCO_3$ as filler from 0.5 % until 7% SA concentrations as compared to a pure stearic acid.

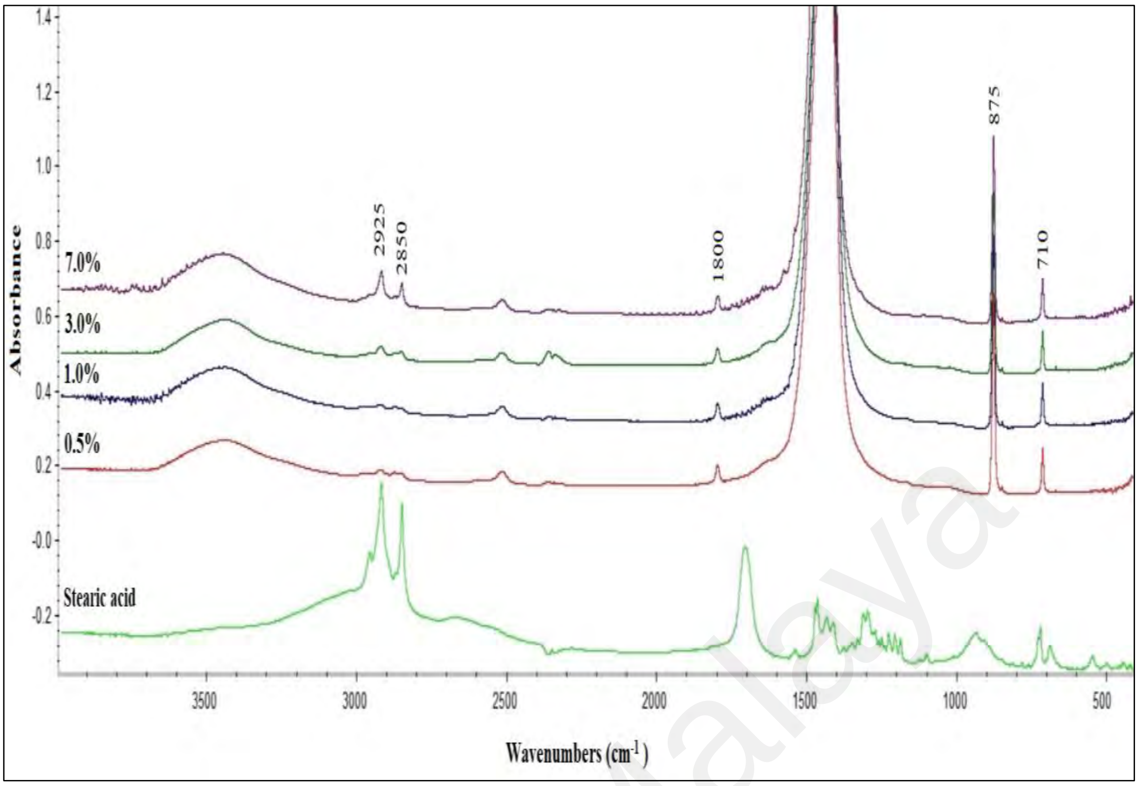


Figure 4.14: The FTIR spectra of treated $CaCO_3$ at 0.5% , 1.0%, 3.0% and 7.0% concentrations as compared to pure stearic acid.

4.1.3.8 Identification of SA-treated $CaCO_3$ in MCLC films by Fourier transform infrared spectroscopy (FTIR)

The MCLC films filled with SA-treated $CaCO_3$ were analysed by using FTIR to identify the presence of stearic acid (SA) in the sample. Figure 4.15 shows the spectrum of the MCLC films filled with treated $CaCO_3$ from 0.5% to 7.0% stearic acid concentrations, Control (without $CaCO_3$) and Control 1 (untreated $CaCO_3$). Based on the spectra obtained as shown in Figure 4.15, it was observed that a strong and sharp peak at 1450 cm^{-1} was present in Control 1 (untreated $CaCO_3$), Control (without $CaCO_3$) and all SA-treated $CaCO_3$ excluding at 4.0% and 5.0% concentrations. In Figure 4.16, a strong small peak was present at 875 cm^{-1} for MCLC films loaded with 1.0%, 3.0% and 7.0% SA-treated $CaCO_3$. This indicates the presence of stearic acid. When lowering the scale of spectra at range 600 cm^{-1} to 1650 cm^{-1} (Figure 4.17), it observed that at 3.0% and 7.0% SA-treated $CaCO_3$ the peaks have slightly shifted from 1430 cm^{-1} to 1445 cm^{-1} which indicate that the amount of SA on the surface of $CaCO_3$ increased as compared to Control 1 (untreated $CaCO_3$) and low concentration of SA at 0.5% SA-treated $CaCO_3$. Therefore, it can be assumed that the peaks at 875 cm^{-1} might be attached to stearic acid, while the peak at 834 cm^{-1} indicates the presence of $CaCO_3$ that was not attached to stearic acid (Croitoru et al., 2017).

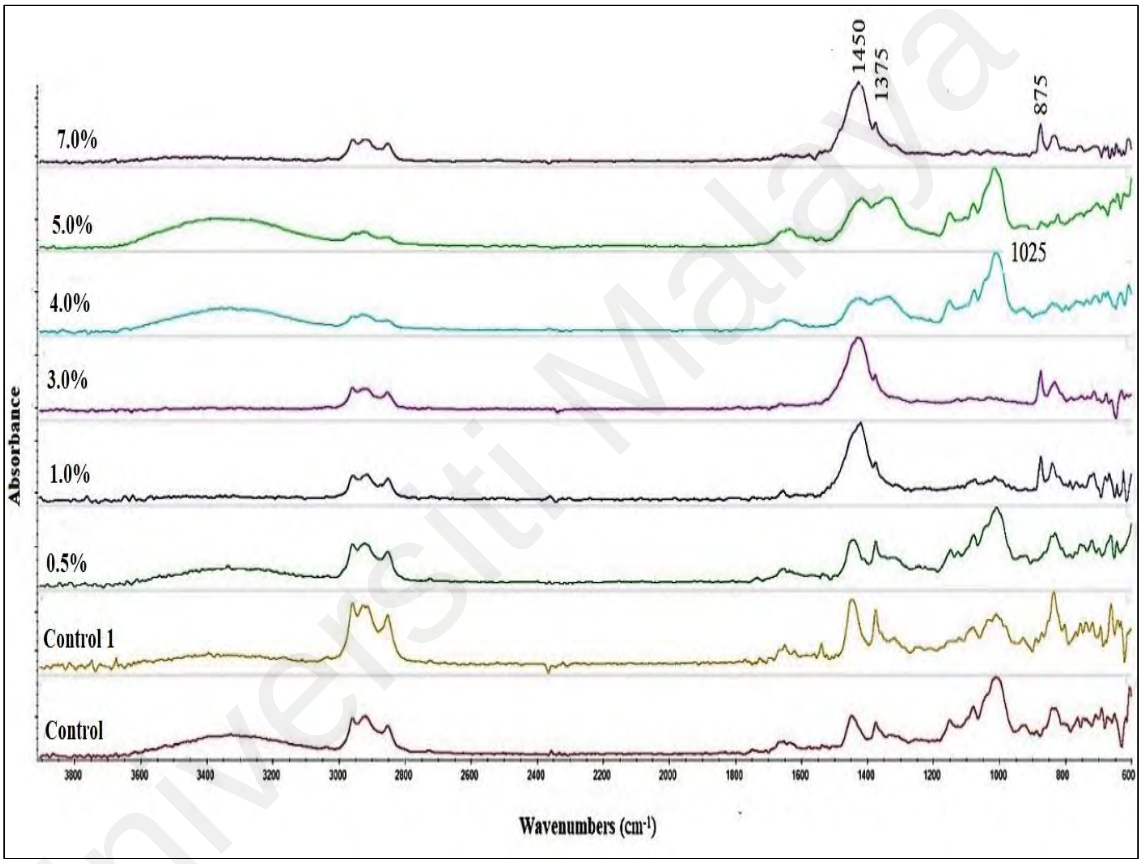


Figure 4.15: The spectrum of MCLC film for Control (without $CaCO_3$), Control 1 (untreated $CaCO_3$) and MCLC filled with the SA-treated $CaCO_3$ at various concentrations.

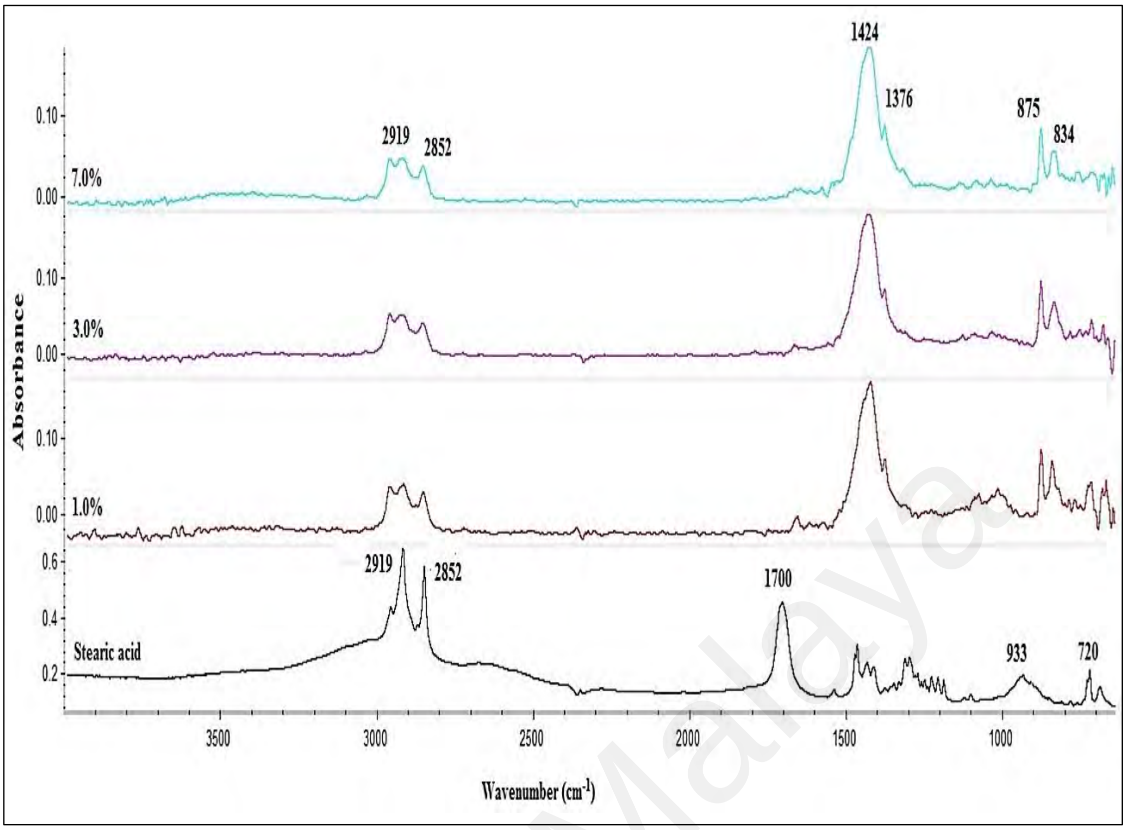


Figure 4.16: The FTIR spectra of MCLC films with SA- treated $CaCO_3$ at 1.0%, 3.0% and 7.0% concentration of SA as compared to pure stearic acid.

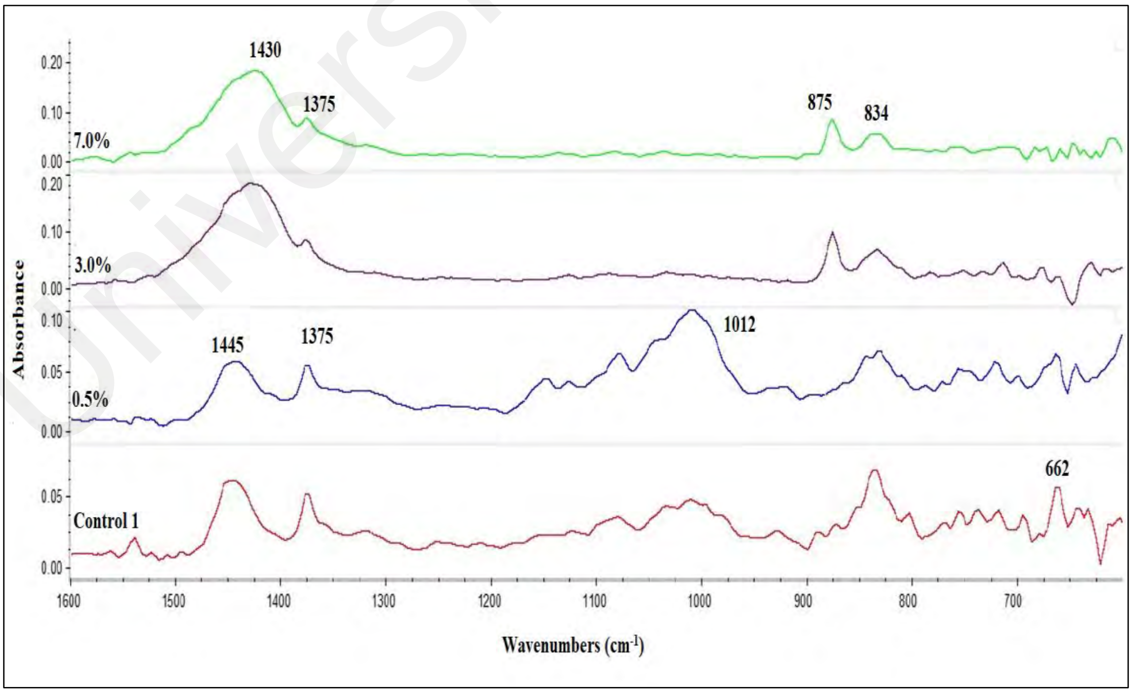


Figure 4.17: The FTIR spectra of MCLC films filled with 0.5%, 3.0% 7.0% SA-treated $CaCO_3$ as compared to Control 1 (Untreated $CaCO_3$) at lower wavenumber (range 600 cm^{-1} to 1600 cm^{-1}).

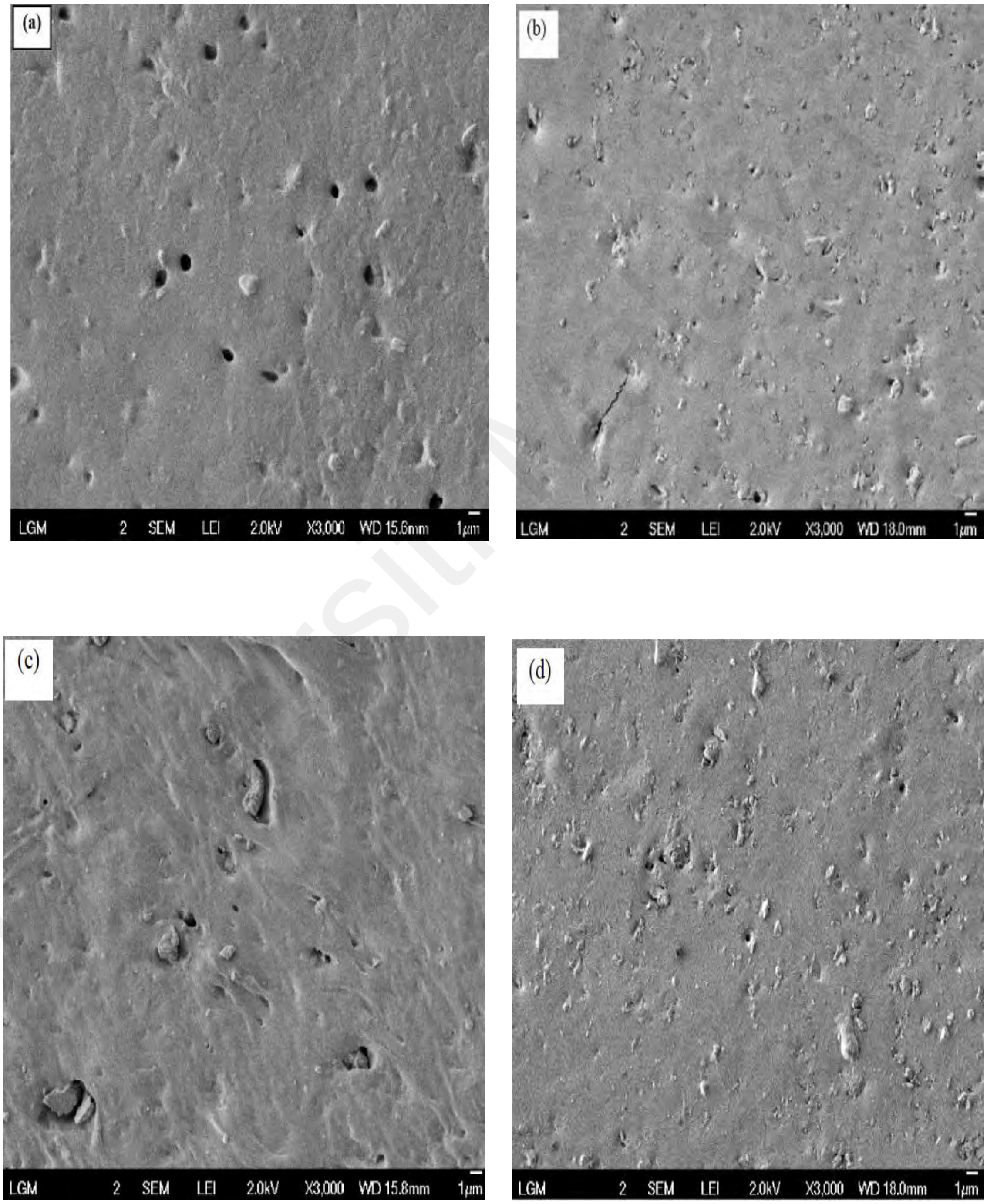
4.2 Surface morphology of MCLC films

The surface morphology of MCLC films at different loadings and after exposure to ozone have been observed to investigate the effect of $CaCO_3$ in MCLC films. Then, the surface morphology of calcium carbonate ($CaCO_3$) was treated with SA in a chloroform medium at various concentrations in powder form to ensure SA coated the $CaCO_3$ particle. The surface morphology of MCLC films filled with SA-treated $CaCO_3$ was also being observed.

4.2.1 Surface morphology of MCLC films filled with untreated $CaCO_3$

The surface morphology of MCLC films was analysed to observe the effect of filler on the surface of MCLC film. Figures 4.18 (a) to (g) show the surface morphology of MCLC films from Control (0 pphr) to 40 pphr filler loadings as analysed by FESEM. From figure 4.18 (a), it can be seen that the Control, MCLC with no filler added, had a smoother surface as compared to the films treated with filler. An increasing amount of filler rendered the surface of the film to be less smooth, as seen in the micrographs of films with 20 pphr, 25 pphr and 30 pphr of filler. At filler loading of 20 pphr and above, it was observed that agglomerates presumably containing the filler started to appear, as shown in Figures 4.18 (c), (d) and (e). Nevertheless, the surface of the film appeared smooth even at 35 pphr filler loading as shown in Figure 4.18 (f). A significant crack was observed on the surface of the film containing 40 pphr filler as shown in Figure 4.18 (g). It was also noted that the film surface became stiffer as filler loadings increased, which could be related to interfacial adhesion between filler and the rubber phase.

According to literature, the smooth surface of the rubber film on the addition of filler could be related to the particle size of the fillers. It is postulated that the smaller the particle diameter, the higher is the specific surface area, thus increasing the contact area between filler and rubber particles (Lay et al., 2019; Mohamad Akmal et al., 2013; On et al., 2012). When dried, the rubber particles in the film coalesced to form a relatively stable latex film, where the surface material of each rubber particle formed boundaries around the coalesced particles (Baker & Fulton, 2012; Roslim & Amir Hashim, 2010).



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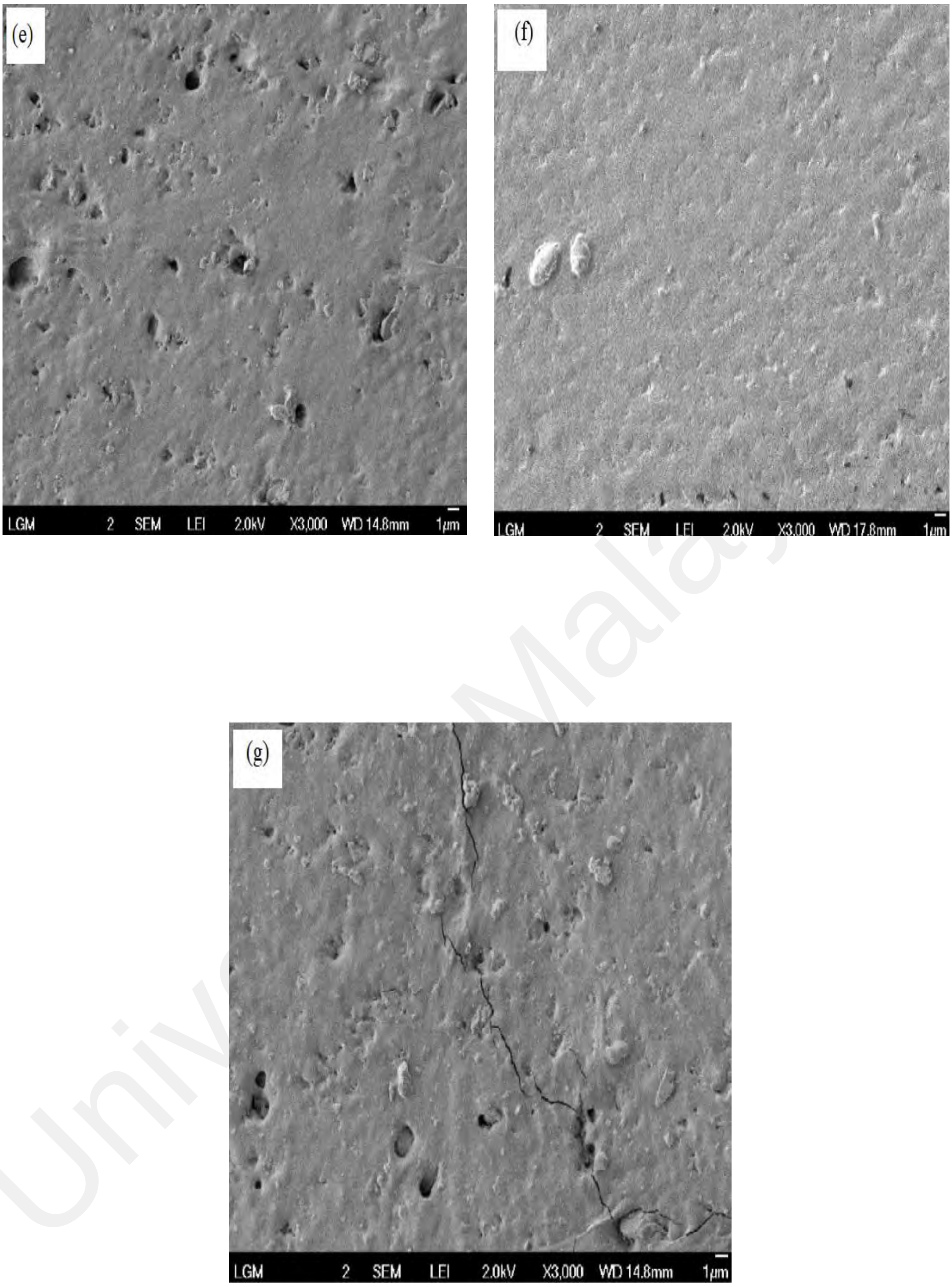
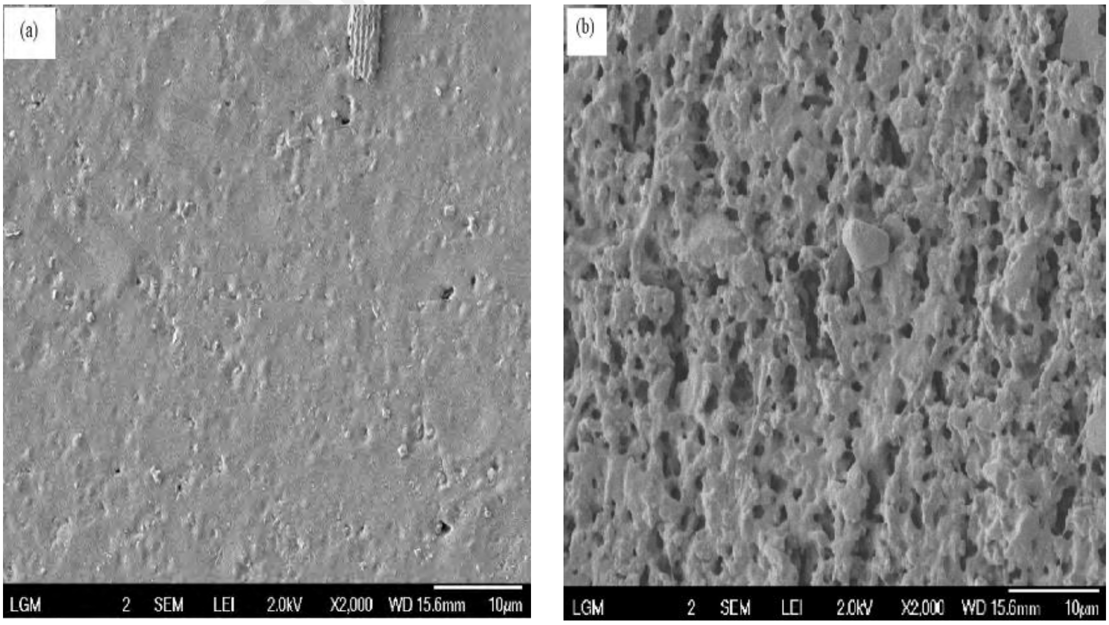


Figure 4.18: The surface morphology of MCLC films (a) Control (0 pphr), (b) 10 pphr (c) 20 pphr (d) 25 pphr (e) 30 pphr (f) 35 pphr (g) 40 pphr filler loadings at 3000x magnification.

4.2.2 Surface morphology of MCLC films after exposure to ozone concentration

The surface morphology of MCLC films was monitored after exposure to ozone at a concentration of 10 pphm from 3 to 24 hours by using a 7x magnifying glass before analysis by FESEM. It was observed that no cracks occurred in all the films at the different (0 pphr until 40 pphr) filler loadings after exposure to ozone under 10% strain and at a temperature of 40ºC for 3, 6 and 9 hours at 260 ml/min. Figures 4.19, 4.20 and 4.21 show the surface morphology of MCLC latex films at 10, 30 and 40 pphr filler loadings before and after exposure to ozone at different exposure times by using FESEM. The surface morphology of films without any filler (0 pphr) at all exposure times to ozone was observed to be smooth with no visible rough surface. However, at 10 pphr filler loadings, the roughness of the film surface started to be visible. Increasing roughness was noted with increasing exposure time. A similar observation was noted for films at 30 pphr and 40 pphr filler loadings, with the roughness of the surface appearing after 9 hours exposure at 10 pphm ozone concentration.



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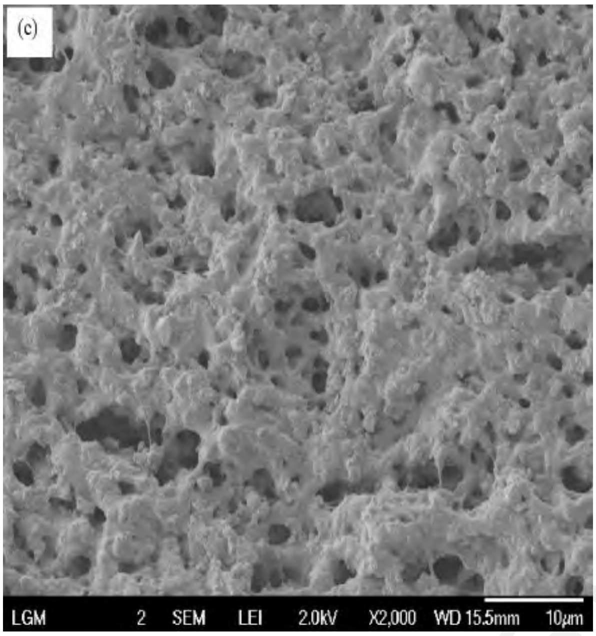


Figure 4.19: The surface morphology of MCLC films at 10 pphr filler loading before and after exposure ozone at various times. (a) Control, (b) 6 hours and (c) 24 hours exposure at 2000x magnification.

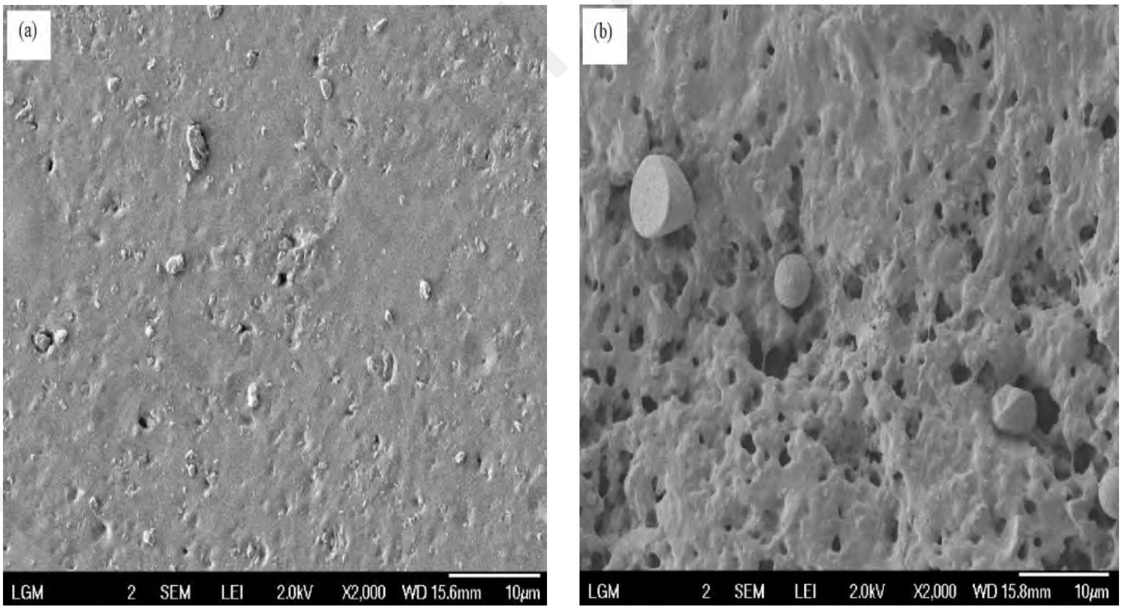


Figure 4.20: The surface morphology of MCLC films at 30 pphr filler loading before and after exposure to ozone after various period of exposure. (a) Control, and (b) 24 hours exposure at 2000x magnification.

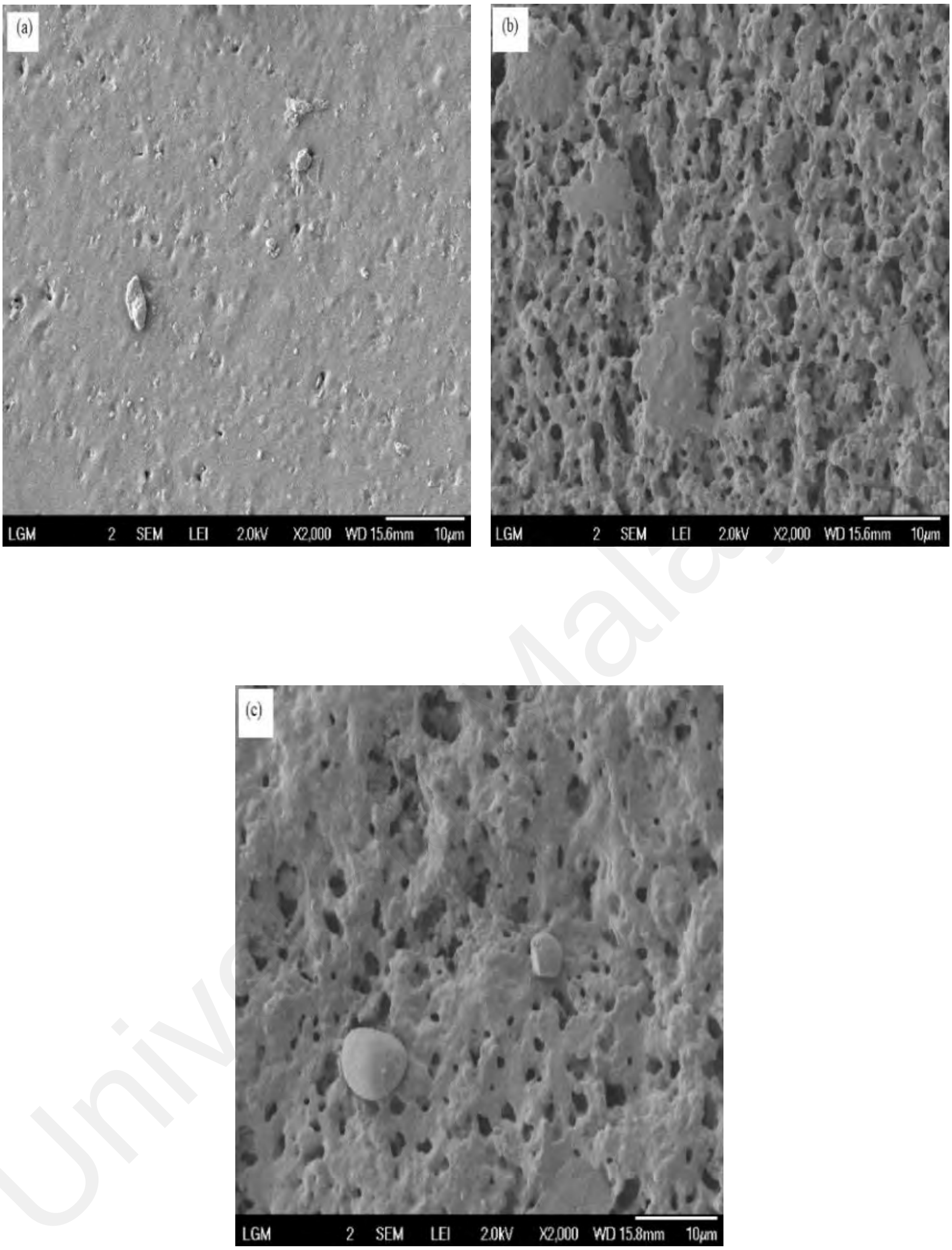
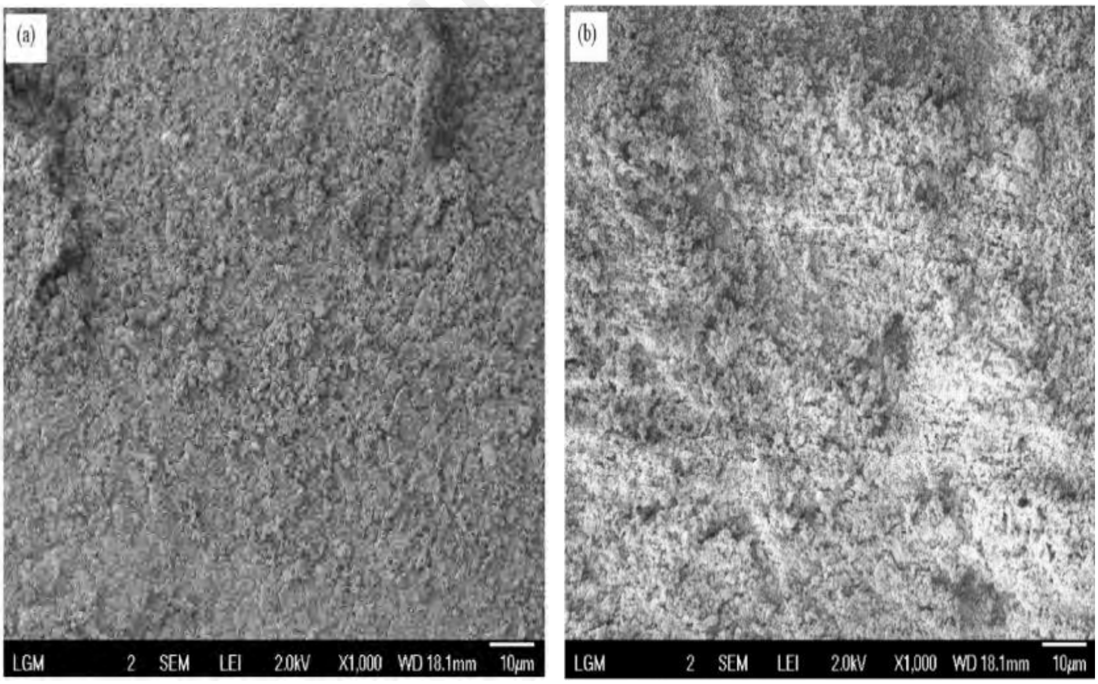


Figure 4.21: The surface morphology of MCLC films at 40 pphr filler loading before and after exposure to ozone for various periods of exposure. (a) Control, (b) 6 hours and (c) 24 hours exposure at 2000x magnification.

4.2.3 Surface morphology of SA-treated $CaCO_3$ in dispersion and solid form

Figure 4.22 shows the surface morphology of $CaCO_3$ treated at various SA concentrations as observed by FESEM. Based on the observation, it was noted that the surface morphology of SA-treated $CaCO_3$ was slightly different from the Control 1 sample. The surfaces of treated $CaCO_3$ samples were finely granular like and denser, particularly samples treated with a high concentration of SA. Initially, the distribution of the fine granules for $CaCO_3$ treated at 0.5% SA was uneven. However, the distribution became more even at increasing SA concentration up to 3.0% above which the treated $CaCO_3$ started to agglomerate. The agglomeration of treated $CaCO_3$ was more obvious at 5.0% SA. At 7.0% SA, the surface morphology of the treated $CaCO_3$ resembled that of the Control sample. Its surface was not effectively coated with SA, and this explained the reduction of SA-treated $CaCO_3$, as shown in Figure 4.4 sub-topic 4.1.3.2.



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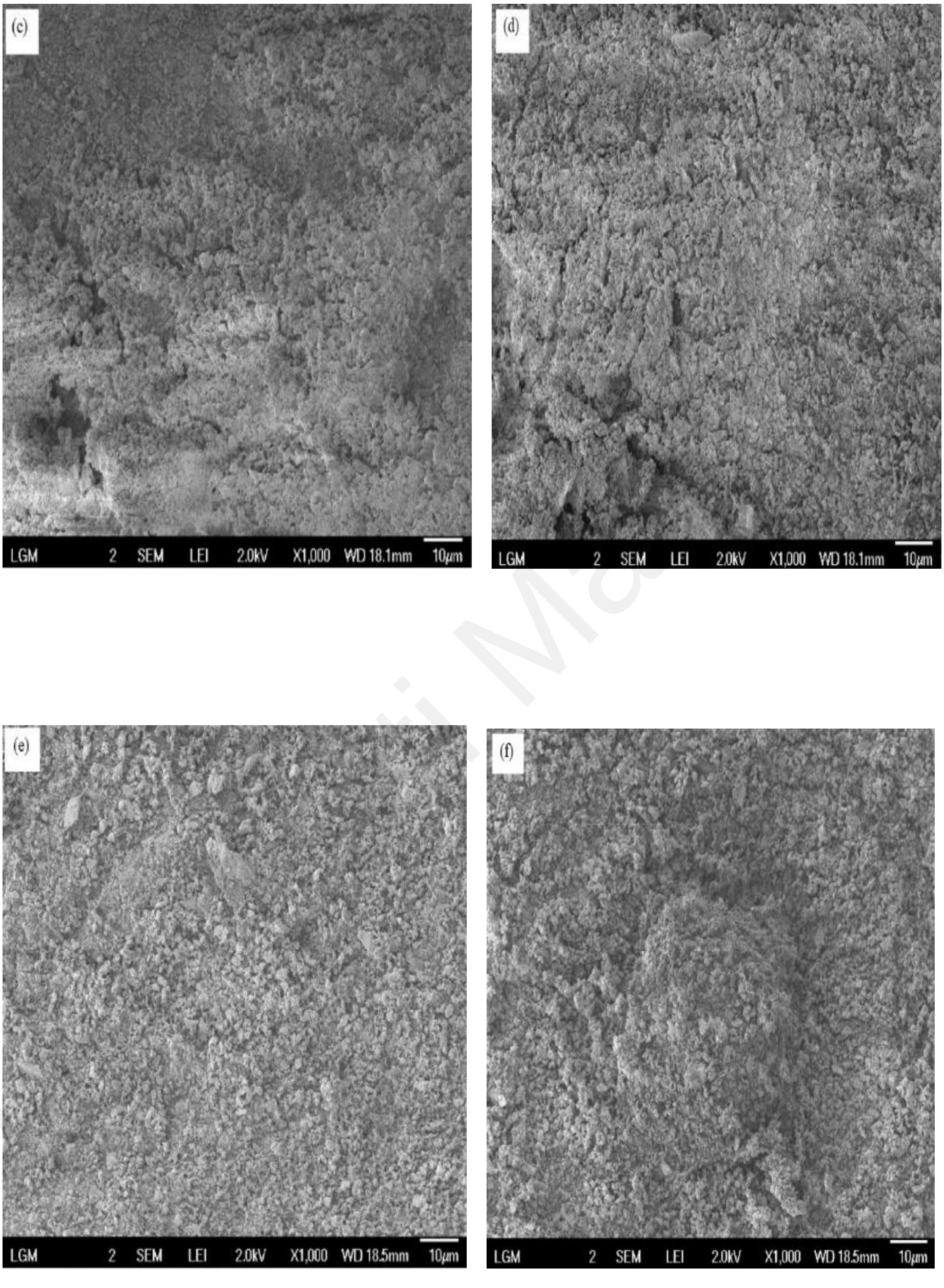

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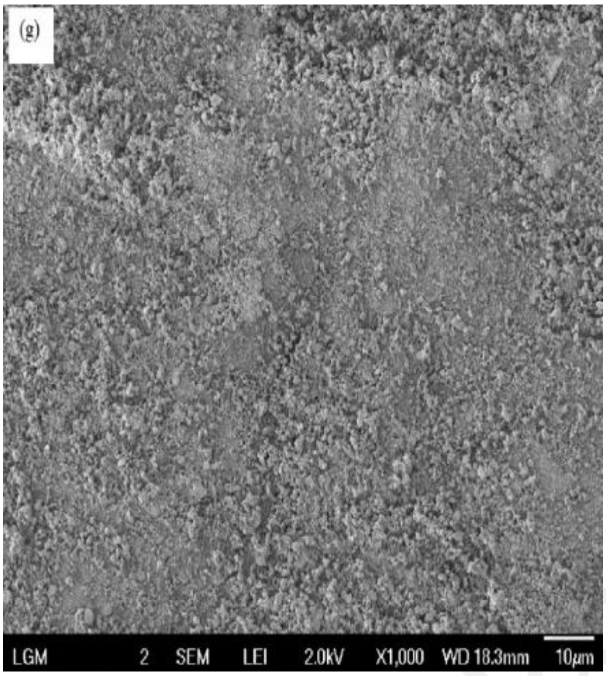
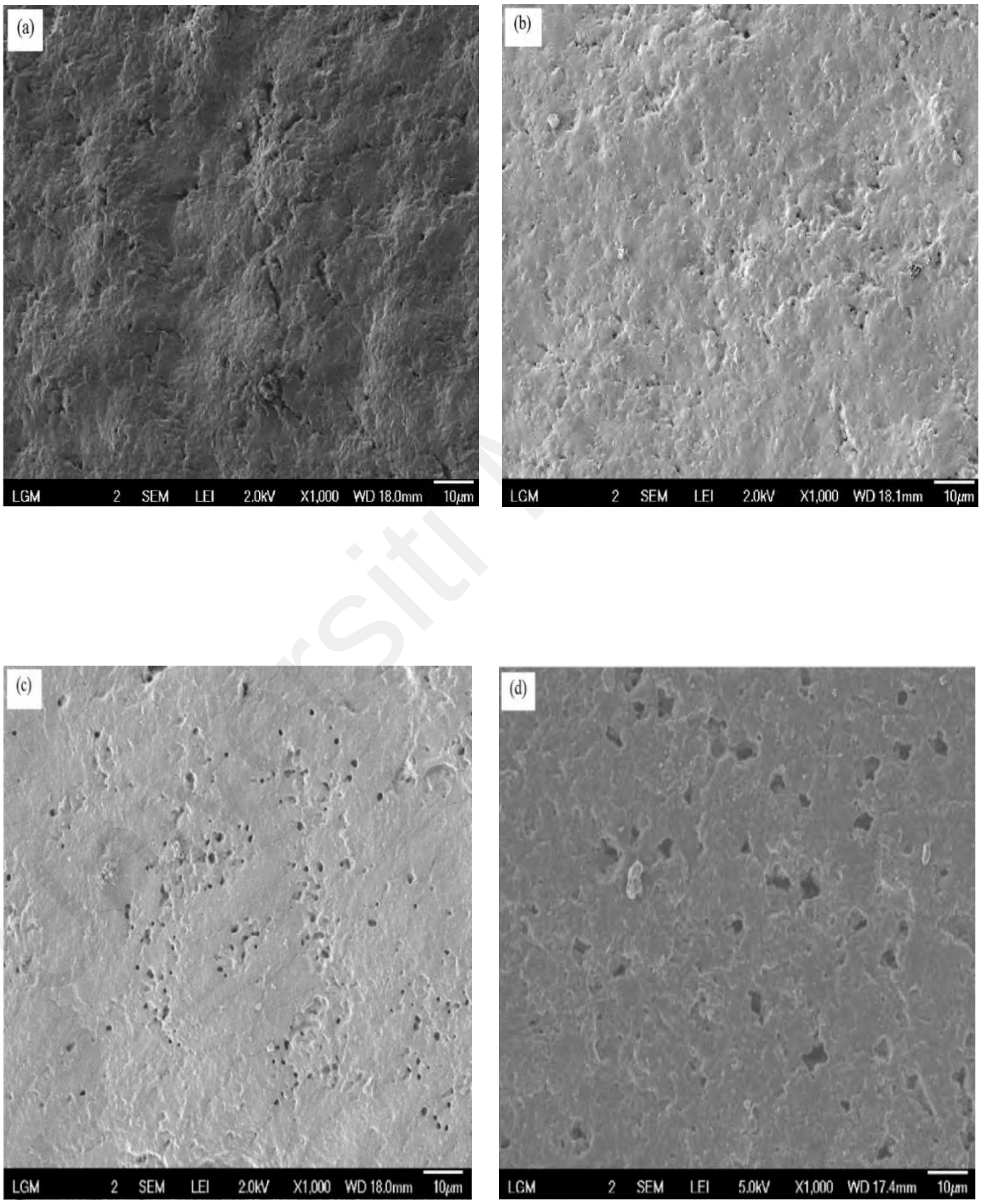


Figure 4.22: The surface morphology of untreated and treated $CaCO_3$ (a) Control 1 (b) 0.5% (c) 1.0% (d) 3.0% (e) 4.0% (f) 5.0% and (g) 7.0% SA concentrations at 1000x magnification.

4.2.4 Surface morphology of MCLC films filled with SA-treated $CaCO_3$

Figure 4.23 shows the surface morphology of MCLC films with treated $CaCO_3$ at 0.5, 1.0, 3.0, 4.0, 5.0 and 7.0 by % (*w/v*) of stearic acid concentrations as well as Control (without $CaCO_3$) and Control 1(untreated $CaCO_3$) by FESEM. Morphology of MCLC films surfaces reflects the dispersibility of filler in samples. Surface morphology of MCLC films observed via FESEM showed that the Control sample (without $CaCO_3$) had a smooth surface as compared to Control 1 (untreated $CaCO_3$) and all treated samples. From the FESEM micrographs, the surface morphology of MCLC films with 0.5% and 1.0% SA-treated $CaCO_3$ have a smoother surface as compared to 3.0%, 4.0%, 5.0% and 7.0% SA-treated fillers. The surface morphology of the later was rougher and coarser. This indicated that at lower concentrations the SA- treated $CaCO_3$ (0.5% and 1.0%) was well dispersed in the MCLC films. The rougher and coarser surface at the higher SA concentrations might be due to undulating voids that occurred between latex and SA-treated $CaCO_3$ particles (Roslim & Amir Hashim, 2010). It was also observed that no agglomeration occurred on the surfaces of all the MCLC films containing the fillers treated with various SA concentrations.



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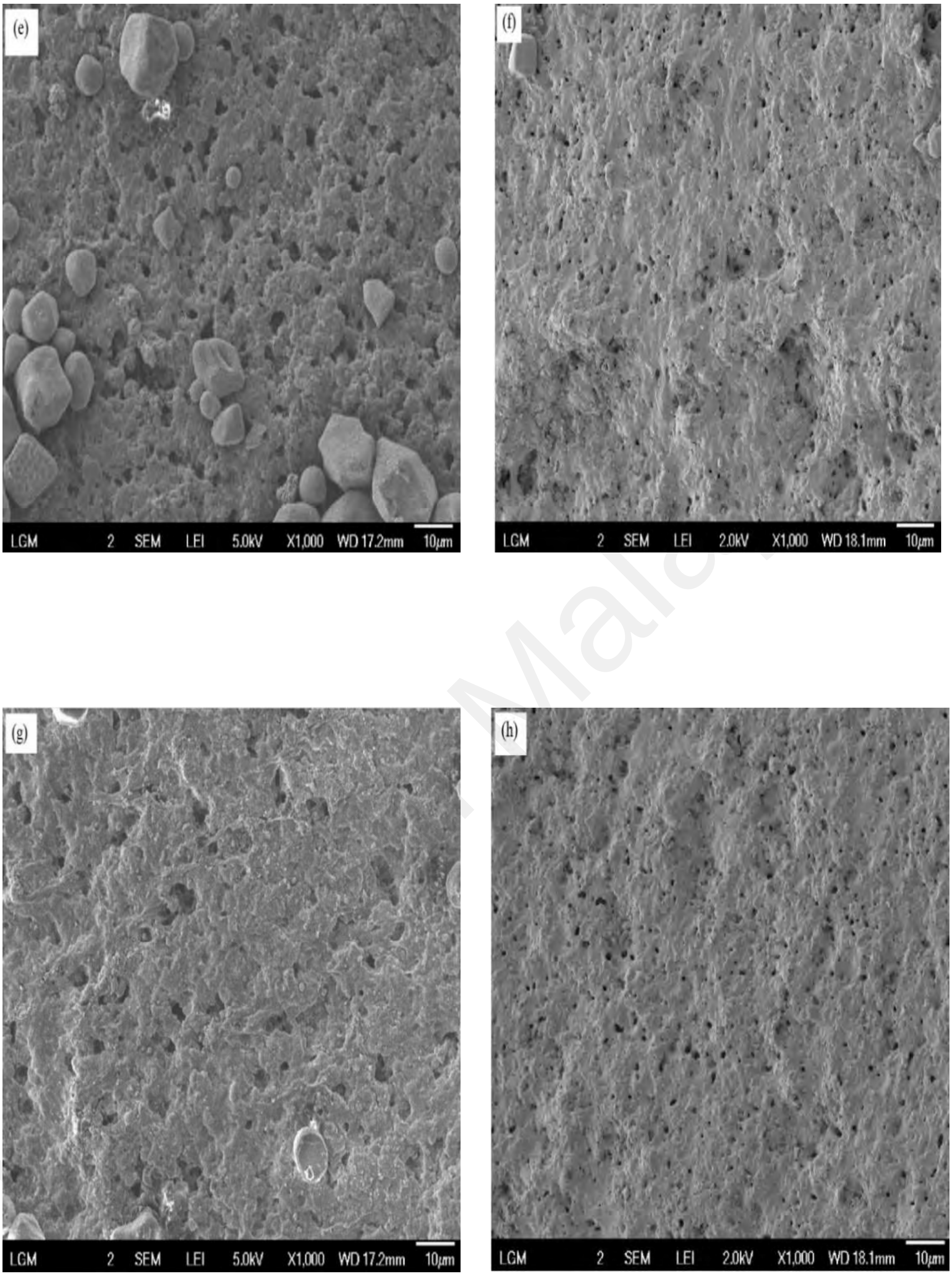

Figure 4.23: The surface morphology of MCLC films (a) Control (without $CaCO_3$) (b) Control 1 (untreated $CaCO_3$) (c) 0.5% (d) 1.0%, (e) 3.0% (f) 4.0% (g) 5.0% and (h) 7.0% SA-treated $CaCO_3$ at 1000x magnification.

4.3 Total protein content of MCLC films filled with untreated $CaCO_3$ and SA-treated $CaCO_3$

The effect of filler on the total and extractable protein content of aged and unaged MCLC films at different filler loadings was investigated. The total protein content was calculated from the percentage of nitrogen (N), whilst the extractable protein (EP) content was determined by ASTM 5712 (2015) test method. The effect of filler on total protein content under all the conditions as mentioned above and after exposure to ozone at 10 pphm concentration for 3, 6, 9 and 24 hours was also investigated.

The total protein content of MCLC films filled with $CaCO_3$ treated at various concentrations of stearic acid was evaluated based on the nitrogen (N) and extractable protein (EP) contents based on the modified Lowry assay. The total nitrogen (N) content was determined by the combustion technique using the Automatic analyser. The total nitrogen content was multiplied by a factor of 6.25 to convert it to estimate total protein content (Faridah Hanim et al., 2018). The EP content was determined from a buffered phosphate extract and the protein assay was analysed using a Microplate Reader. The tests were performed for both the unaged and aged MCLC films untreated and treated SA-treated $CaCO_3$. In addition, the EP was determined with non-corrected and corrected of interfering substances. In the corrected method, the copper reagent was replaced by water in an alkaline tartrate solution in the colour development stage during the protein assay (Mok et al., 2005).

4.3.1 Nitrogen content of MCLC films filled with untreated $CaCO_3$

The MCLC films at different filler loadings were aged by heating in an oven at 70 °C for 7 days before solvent extraction to remove the non-rubbers constituents from the films. Figure 4.24 shows the result of the percentage of N and total protein in MCLC films added with the filler at various filler loadings. The total protein content for unaged and aged MCLC films at the various filler loadings was estimated by multiplying the percentage of N by a factor of 6.25 (Faridah Hanim et al., 2018; Mahendra et al., 2021; Nun-anan et al., 2018; Promsung et al., 2021; Nurul Hayati et al., 2015).

The percentage of N and total protein contents in MCLC films values increased with increasing the $CaCO_3$ as a filler loading. The highest percentage of N and total protein contents were obtained at 35 pphr filler loading with values of 1.95% and 12.2%, respectively. However, the percentage of N and total protein content decreased slightly between 30 pphr to 40 pphr filler loadings. The increase in the percentage of N and total protein contents with increasing $CaCO_3$ loading can only be from the $CaCO_3$ as no other chemicals were added in the MCLC during latex compounding. However, the increase in total protein content in the end products may originate from non-rubber substances such as protein and other rubber chemicals that contain amine groups (Hasma, 1992). The decrease in the percentage of N between 30 pphr and 40 pphr may be due to lower filler uptake on the MCLC films during the dipping process as no other chemicals were added in the MCLC compound. As shown in Figure 4.3, the filler content in MCLC film increased with increasing filler loadings up to 35 pphr, after which a slight decrease in filler content was observed in films that were loaded with 40 pphr filler (Ruhida & Aziz, 2017).

The effect of $CaCO_3$ on the nitrogen content in MCLC films at different filler loadings before and after ageing at 70 °C for 7 days was investigated. The MCLC was compounded with $CaCO_3$ at different loadings and chemicals according to the formulation shown in Table 3.6 (Chapter 3). As observed in Figure 4.25, the percentage nitrogen (N) for the unaged samples was higher compared to the aged samples. There was a slight increase in the values of percentage N for unaged samples from 0.71% to 0.78% as compared to Control (without filler) at 0.65%. This can be attributed to the homogenous unaged samples during the milling process.

Ageing resulted in a slight decrease in percentage N in samples loaded with 10 pphr to 25 pphr filler, after which the percentage N increased with increasing filler loadings up to 40 pphr filler. This can be attributed to the loosening of the filler particles that are attached to the rubber cross-link or are trapped in between the rubber particles (Hasma et al., 2006; Yip et al., 1997). As more filler was added into the MCLC compound, more of the non-rubbers would be inside the MCLC film, including other chemical ingredients that were added into the MCLC compound. The increasing N content may also originate from the chemical ingredients such as accelerator (ZBuD), protective agent (ZnO), and coagulant ingredient $Ca(NO_3)_2$ added during preparation of dipped MCLC films. The chemicals that have a tendency to increase the N content in samples rubber chemicals such as accelerator (ZBuD, $C_{18}H_{36}N_2S_4Zn$), protective agent (Wingstay L, Butylated reaction product of ρ-cresol and dicyclopentadiene), and coagulant ingredient $Ca(NO_3)_2$ added during preparation of dipped MCLC films. The ZBuD and Wingstay L have a content of N. The percentage of $CaCO_3$ was lower in aged samples compared to unaged samples. Prolonged heating and ageing could have decomposed some of the $CaCO_3$. In addition, some of the chemical ingredients and part of the non-rubbers were removed during the extraction process.

As more filler was added into the MCLC compound, more of the non-rubbers will be incorporated into the MCLC film. This was substantiated by the results of filler content obtained through the TGA analysis as shown in Figure 4.3 under sub-topic 4.1.3.1. The lower percentage of $CaCO_3$ in aged MCLC films was attributed to prolonged heating and ageing that may have resulted in decomposition of the $CaCO_3$.

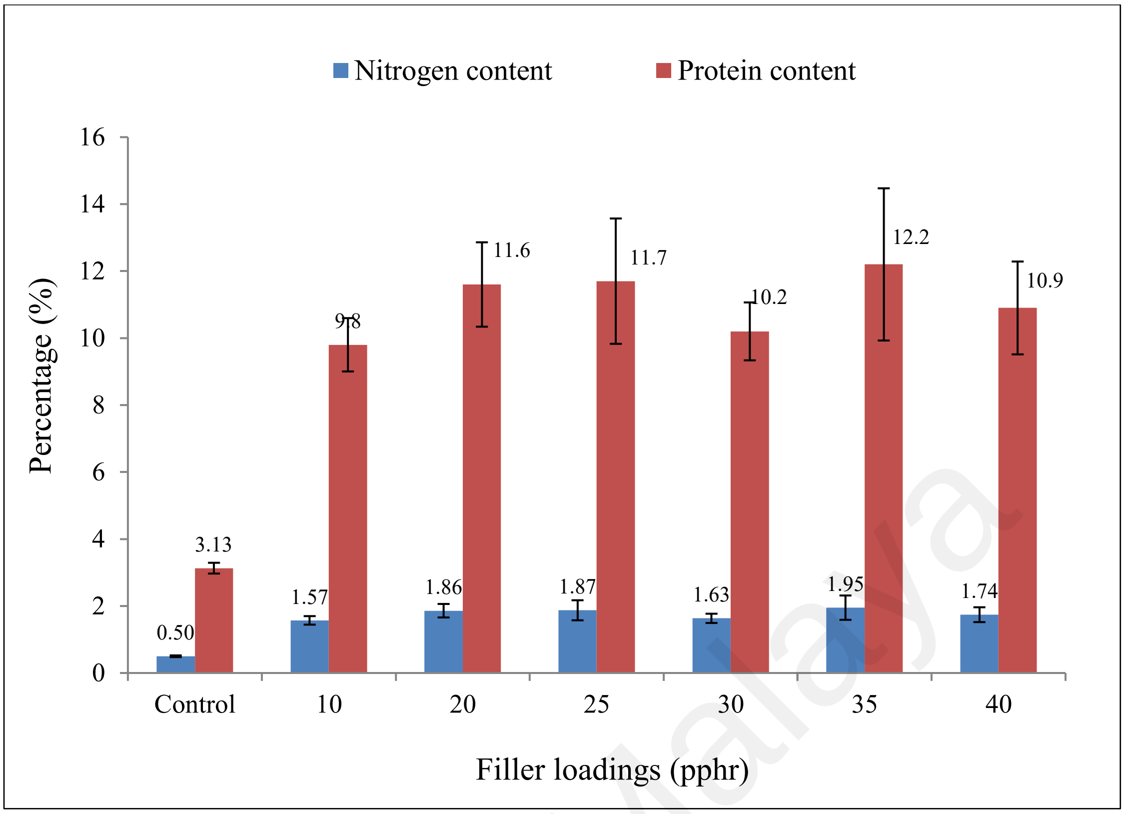

Figure 4.24: Nitrogen and protein contents in MCLC films filled with untreated $CaCO_3$ and without rubber chemicals.

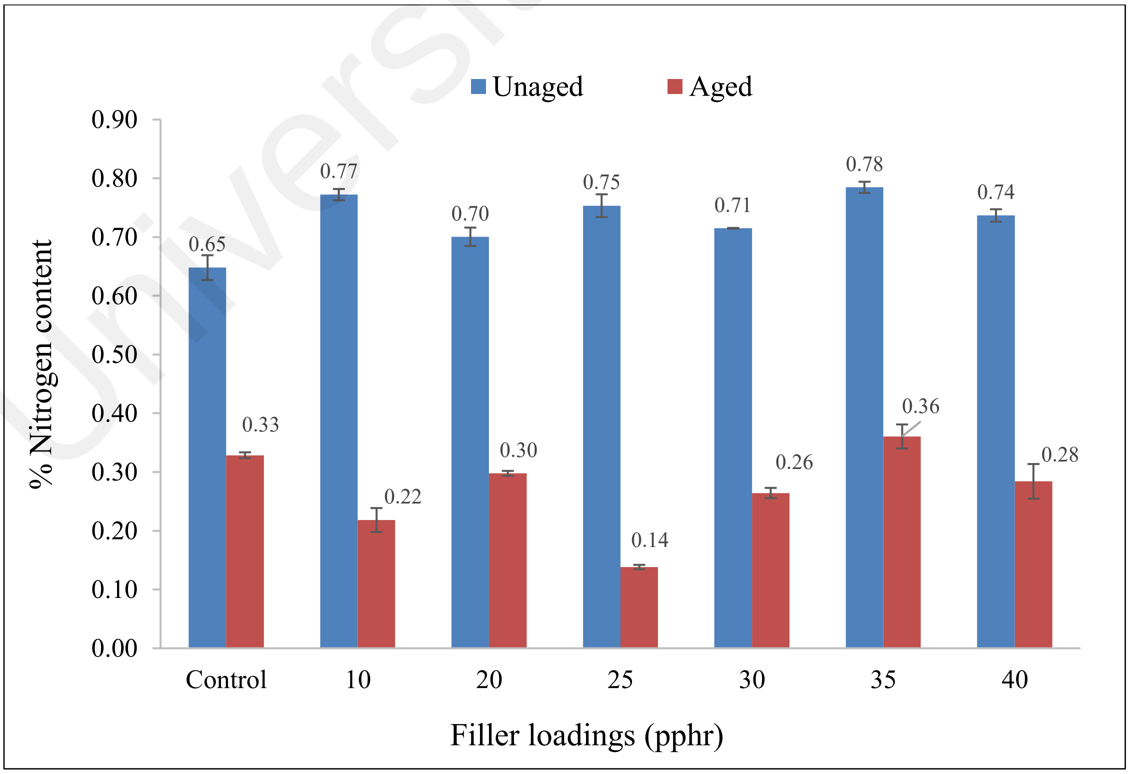

Figure 4.25: Nitrogen content of unextracted, unaged and aged MCLC films filled with untreated $CaCO_3$.

Figure 4.26 shows the percentage of N for the unaged MCLC films after extraction with acetone for 16 hours. It was observed that percentage N before extraction was higher as compared after extraction samples. It is postulated that during the extraction process, acetone, being polar dissolved the non-rubbers that were not cross-linked to the rubber (Xu et al., 2017). Percentage nitrogen of the extracted aged Control sample was of similar magnitude to the unextracted, aged Control sample as shown in Figure 4.26. The percentage of N content for aged samples after the extraction was higher as compared to samples that were not extracted from 10 pphr until 25 pphr and 40 pphr filler loadings. However, at 30 pphr and 35 pphr filler loadings, the percentage of N obtained was lower. From 10 pphr up to 25 pphr filler, the latex content was higher compared to filler. Removal of non-rubber in the MCLC films was therefore easier. As more filler was added in the MCLC compound (40 pphr) a similar trend was obtained from 10 pphr until 25 pphr, and this might be due to the ability of filler to pick up during the dipping process.

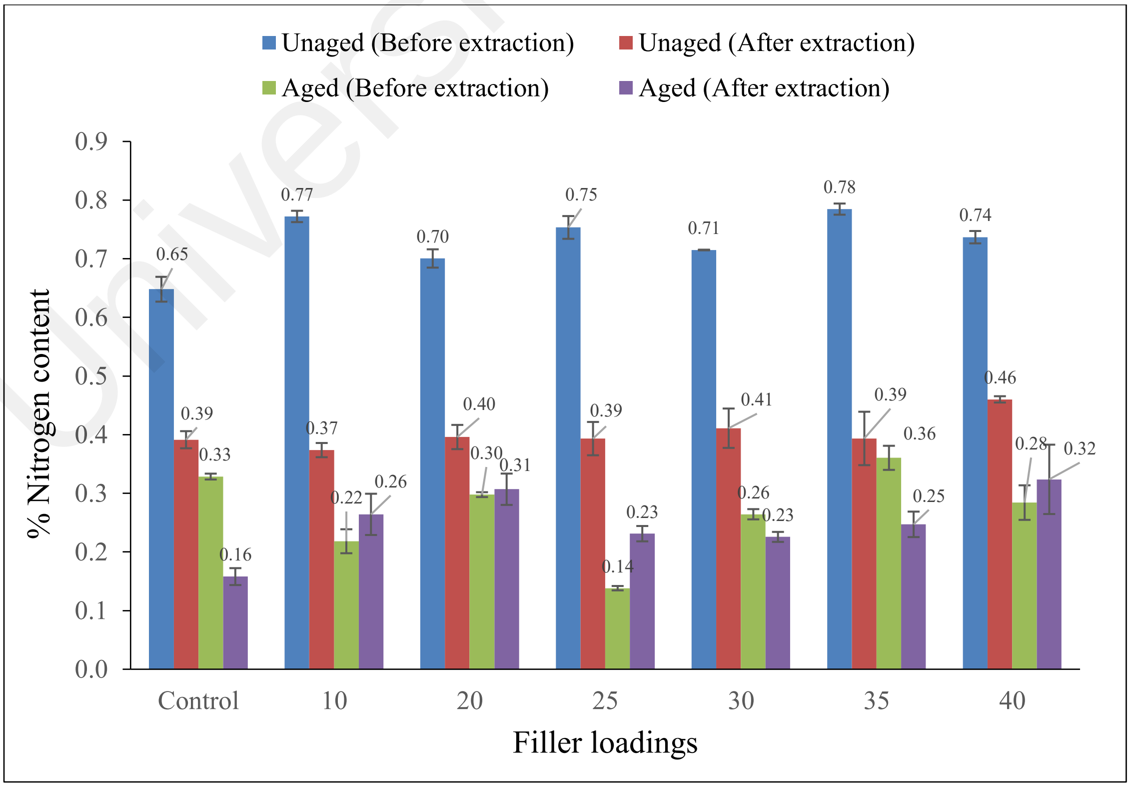

Figure 4.26: The content of nitrogen for unaged and aged MCLC films filled with untreated $CaCO_3$ before and after extraction.

4.3.2 Nitrogen content in MCLC films filled with SA-treated $CaCO_3$

Figure 4.27 shows the percentage of nitrogen (N) content of unaged and aged MCLC films with $CaCO_3$ treated at various concentrations of stearic acid in a chloroform medium. Overall, the N content of the film for Control 1 (untreated $CaCO_3$) of the unaged samples was higher compared to unaged treated samples. It is difficult to identify the trend of the N content due to the inconsistency in the results obtained. It was observed that unaged $CaCO_3$ treated with SA at 0.5%, 4.0% and 7.0% had higher nitrogen content compared to aged SA-treated $CaCO_3$. However, at 1.0% and 5.0%, treated $CaCO_3$ in MCLC films exhibited the opposite results. Besides, at 3.0% of a treated $CaCO_3$, the percentage of N of unaged and aged samples has produced similar values at 0.71% and 0.72%, respectively. By comparing results obtained in Figure 4.24 sub-topic 4.3.1 at 30 pphr filler loadings, the nitrogen content was obtained at 1.63%, which is higher as compared to the unaged sample in Figure 4.27, which was obtained at 0.71%. The N content was presumed to be related to the presence of a protein in the rubber and indicates the presence of some residual nitrogenous components, probably amino-acids bond directly to polyisoprene molecules (Marinho & Tanaka, 2000). According to the study by Dalrymple & Audley (1992), they have found that total nitrogen content of NR glove that prepared from standard latices produced twice that of the film 'free' of non-protein nitrogen.

By comparing the value of percentage of N content between samples with untreated $CaCO_3$ and without $CaCO_3$, it was observed that the percentage of N content for Control 1 (Untreated $CaCO_3$) at 30 pphr for unaged and aged was obtained at 0.93% and 0.63%, respectively. At 3.0% SA-treated $CaCO_3$ for unaged and aged was obtained at 0.71% and 0.72%, respectively. When compared to Control (without filler) sample, the percentage of N content for unaged and aged (0.65% and 0.33%) respectively. The sample at 30 pphr loading for unaged and aged, the percentage of N content was obtained at 0.71% and

0.26%, respectively. Therefore, for the untreated $CaCO_3$ sample at 30 pphr loading as compared to Control, there was an increase of percentage of N content by 9.0% for unaged and reduction at 21.0% for ageing samples. In the case of SA-treated $CaCO_3$, as compared to Control 1, there was a reduction of percentage of N content for 24.0% for the unaged sample and an increase at 14.0% for the ageing sample. However, by comparing between unaged and aged samples for untreated and SA-treated samples, the difference of percentage of N content was at 63.0% (decrease) and 1.0% (slight increase). This also indicates that the unaged SA-treated $CaCO_3$ has reduced the percentage of N content, but the ageing process has increased the percentage of N content. However, there was no significant result between unaged and aged samples that were filled with 3.0% SA-treated $CaCO_3$.

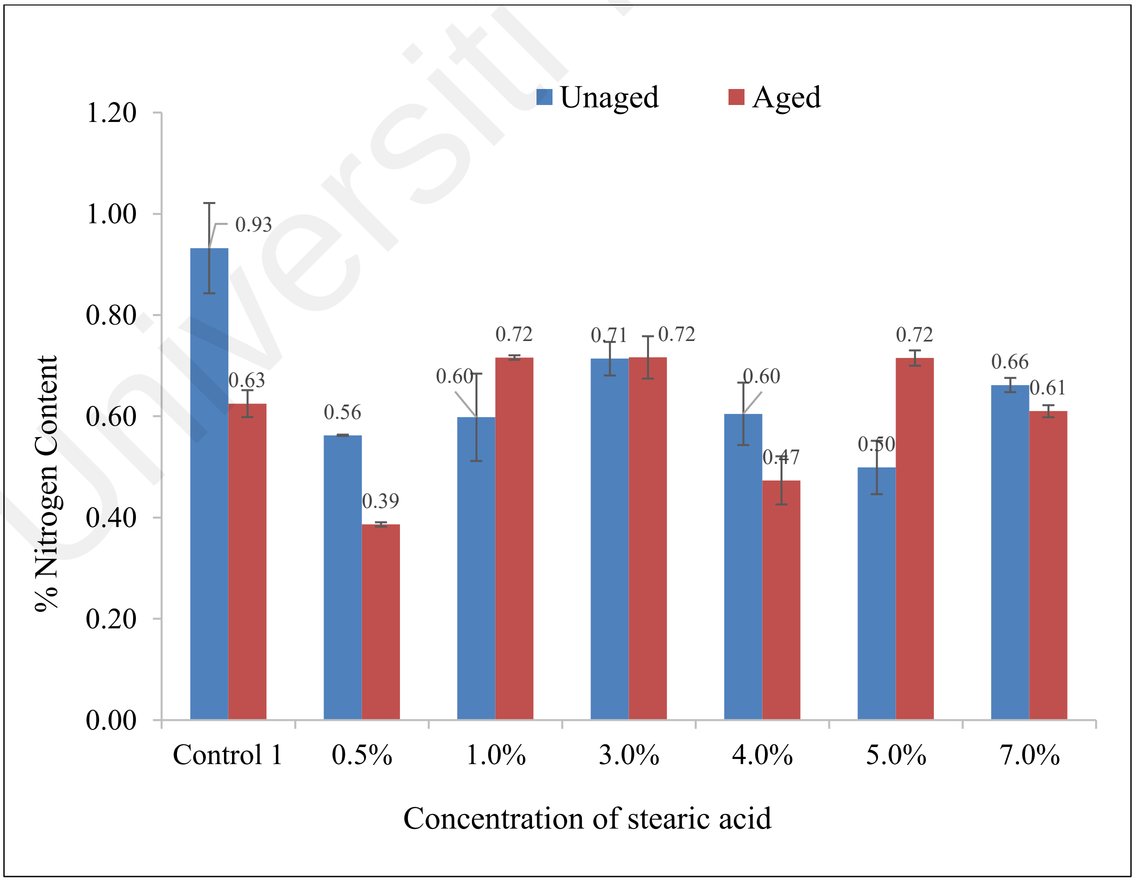

Figure 4.27: Nitrogen content of unaged and aged MCLC films filled with SA-treated $CaCO_3$.

4.3.3 Extractable protein content in MCLC films filled with untreated $CaCO_3$

The main idea of measuring total protein content and N content is to determine the N content that was not insoluble with water since the EP content is the amount of EP that is soluble to PBS (water-based). Figure 4.28 shows the results of non-corrected and corrected EP content of unaged and aged MCLC films at different filler loadings. In general, it was observed that the EP contents of the unaged MCLC film decreased with increasing filler loadings of up to 25 pphr filler, after which the effect tends to plateau off. A similar trend was observed for the aged MCLC films. The higher corrected EP content of the aged MCLC films at 35 pphr and 40 pphr filler loadings compared to the corrected EP content of the unaged samples could be due to inferences from the $CaCO_3$ filler when added in excess into MCLC. Clonal variation, denaturation of protein and insolubilisation of some EP in the samples are possible contributory factors to the trend observed (Ruhida & Aziz, 2017; Yeang & Faridah, 1993; Yip et al., 1997). As recommended by the test protocol in ASTM D5712, an optional correction was used to resolve the chemical interference attributed to $CaCO_3$ in the Phosphate Buffer Saline extracts of MCLC films during the colour development in the protein assay. A similar assay was carried out in the absence of the copper (Cu^{+2}) reagent according to ASTM D5712 (2015). This step prevents the formation of protein-Cu complex, thus ensuring the reduction of the Folin-Ciocalteu's phenol reagent primarily to the reaction of the amino acids present in the protein complex. Based on a previous study of NR latex gloves, the optional correction resulted in lower EP values (Mok et al., 2005).

Figure 4.28 also show the results of non-corrected and corrected EP content of unaged and aged MCLC films, respectively. In the corrected method, the copper reagent was replaced by water in an alkaline tartrate solution in the colour development stage during the protein assay. It was observed that the correction step resulted in lowering the EP values of both the aged and unaged MCLC film at the different filler loadings up to

30 pphr, corresponding to filler loading of about 20% (Table 4.8). The unexpected higher non-corrected EP values for the aged samples at filler loadings above 30 pphr corresponding to about 20% filler might be due to chemical interference from $CaCO_3$ that was present in excess. Ageing may have also caused loosening of the filler particles, thus rendering interference in the protein assay. The EP content was the lowest for MCLC films as compared to the Control sample. At 30 pphr filler loading, the non-corrected EP content was markedly reduced by about 42% and 64% for unaged and aged films, respectively, whereas the corrected EP content was reduced by about 40% and 59%, respectively.

Chemical interference from the rubber chemicals present in the formulation may also be an additional factor in the protein assay. To elucidate this, MCLC film mixed with the filler at different filler loadings were prepared as mentioned earlier in Table 3.7 sub-topic 3.3.5.1. Figure 4.29 shows the amount of EP content in MCLC mixed with the filler at different filler loadings. It was observed that the EP content increased more than 100% for MCLC film containing only 30 pphr filler. In contrast, the EP was remarkably reduced by about 32% for latex film containing 30 pphr filler and rubber chemicals. The decrease in EP may be due to the presence of rubber chemicals that were bound to the proteins and consequently impeded the proteins from being leached out during EP determination. This explained the reduction in EP content when rubber chemicals and filler are present together in MCLC films.

It is established that the Type 1 latex allergy is associated with the protein extractable from NR latex products whilst the Type IV latex allergy is mainly due to the chemical additives in rubber such as accelerators and antioxidants (Hasma et al., 2003; Hasma et al., 2004; Yip, 1998; Yip et al., 1997). The proteins in latex dipped products elutable from samples during the extraction process is about 1.0% by weight of latex, and some parts of it are on the surface of the rubber particles. Although the total protein in products is expected to be present in a relatively constant amount, its composition is highly variable. The interfering substances could also originate during the extraction of the samples, as stated by Yeang and Faridah (1993). According to the author, the extraction solution was first precipitated after the addition of trichloroacetic acid (TCA) or phosphotungstic acid (PTA) prior to centrifugation of the precipitate into a pellet of protein. Then, the protein pellet was solubilised with 0.1 M sodium hydroxide before adding alkaline copper tartrate (non-corrected) to the solution. A protein assay was carried out with Folin's reagent at the colour development stage. It is at this stage that interference is believed to occur due to the interaction of the calcium ions in solution with the alkaline copper tartrate during the protein assay. As more $CaCO_3$ are added into the MCLC films, the degree of interferences is expected to increase, thus increasing the colour development and resulting in higher EP values for the 30 pphr and 40 pphr filler loading the unaged samples was higher than with the correction. Besides, both $CaCO_3$ and protein are non-rubbers, but ion Ca^{2+} can interfere with the protein assay when measuring EP content absorbance by a microplate reader. In the case of aged samples, some of the $CaCO_3$ may have been decomposed into calcium oxide (CaO) and gaseous carbon dioxide (CO_2) on heating. The CaO reacts with the phosphate-buffered saline (PBS) buffer to form soluble calcium hydroxide. This soluble calcium hydroxide subsequently interferes with the EP measurement reflecting on the high EP content (Song et al., 2014). This is a possible reason the EP values of the corrected samples were slightly lower compared to the non-corrected EP content. However, inconsistent trends of EP results of aged samples were observed, and further study needs to be carryout.

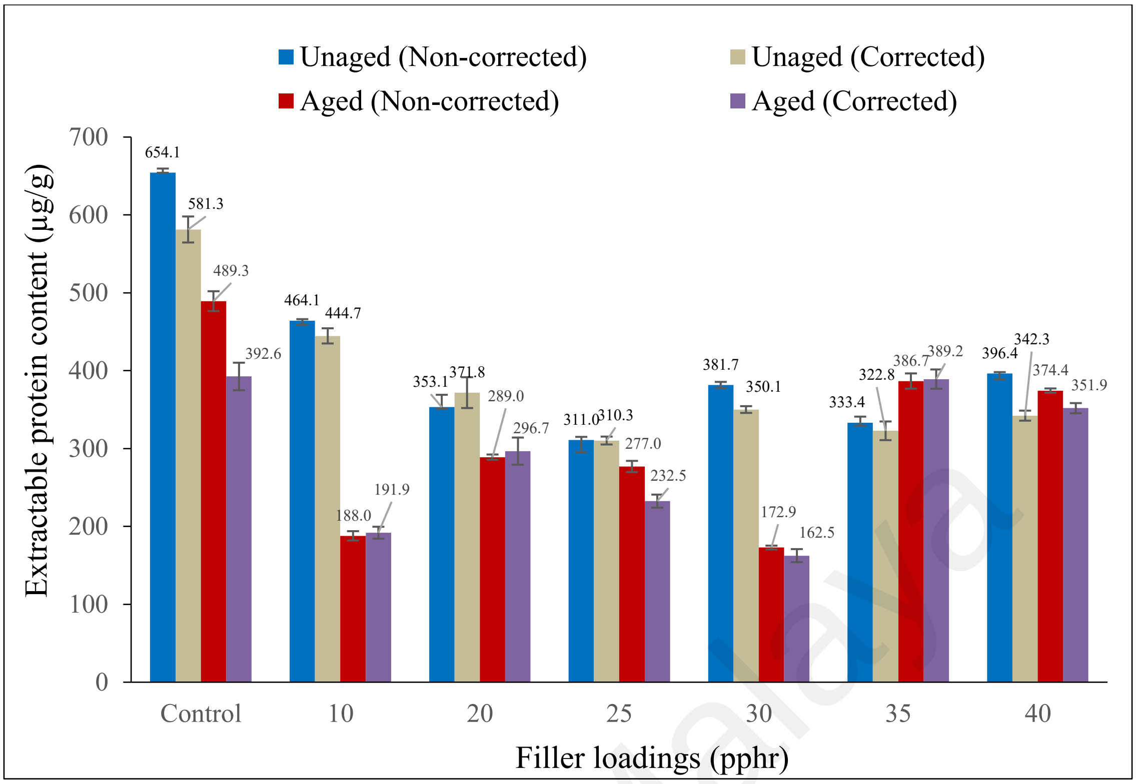

Figure 4.28: The non-corrected and corrected EP content on unaged and aged MCLC films filled with untreated $CaCO_3$.

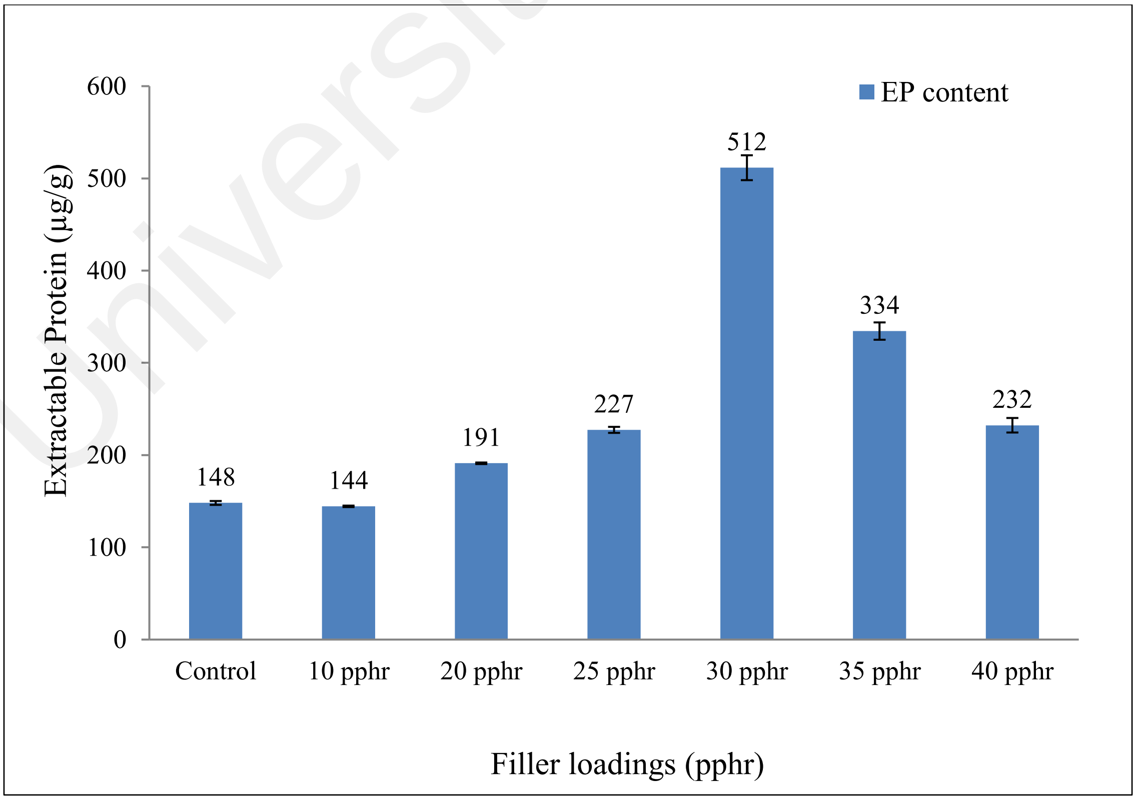

Figure 4.29: The EP content of MCLC films at various filler loadings.

4.3.4 Extractable protein content in MCLC films after ozone exposure

It is well known that degradation of natural rubber products is related to cracking of the rubber surfaces on exposure to ozone and a major type is thermo-oxidative ageing. The chemical reactions have occurred between the rubber chain and oxygen. This could lead to the composed of rubber chains due to chain scission and finally affect the properties (Andrews & Braden, 1961; Payungwong et al., 2021). The double bonds located mainly on the surfaces of the rubber are easily attacked by ozone molecules. Degradation was observed to be severe when the surfaces were exposed to ozone.

An experiment was conducted to investigate the effect of ozone treatment on EP content of MCLC film at various filler loadings. The MCLC film at various filler loadings was exposed to ozone at a concentration of 10 pphm with 10% strain applied to the samples at intervals. The EP content was measured according to ASTM D5712 test method. Figure 4.30 show the values of EP content before and after exposure to the ozone at 10 pphm concentration for 3, 6, 9 and 24 hours. It was observed that the addition of fillers up to 30 pphr tend to decrease the EP contents in the MCLC films. On the other hand, exposure to ozone generally increased the EP contents, with increasing exposure to ozone resulting in a further increase in the EP content. The reduction in EP contents may be due to the loosening of the double bond during ozonation, as illustrated in Figure 4.31 (Roberts, 1988). According to Andrew and Braden (1961), the ozone molecule attacks NR in both the strained and unstrained state, but that in the latter case, the extent of penetration is so small as to resist typical methods of detection. The surface strain appears to allow much deeper penetration of ozone and the formation of microscopic cracks. However, at a filler level of 40 pphr, the EP contents was not significantly affected by ozone exposure.

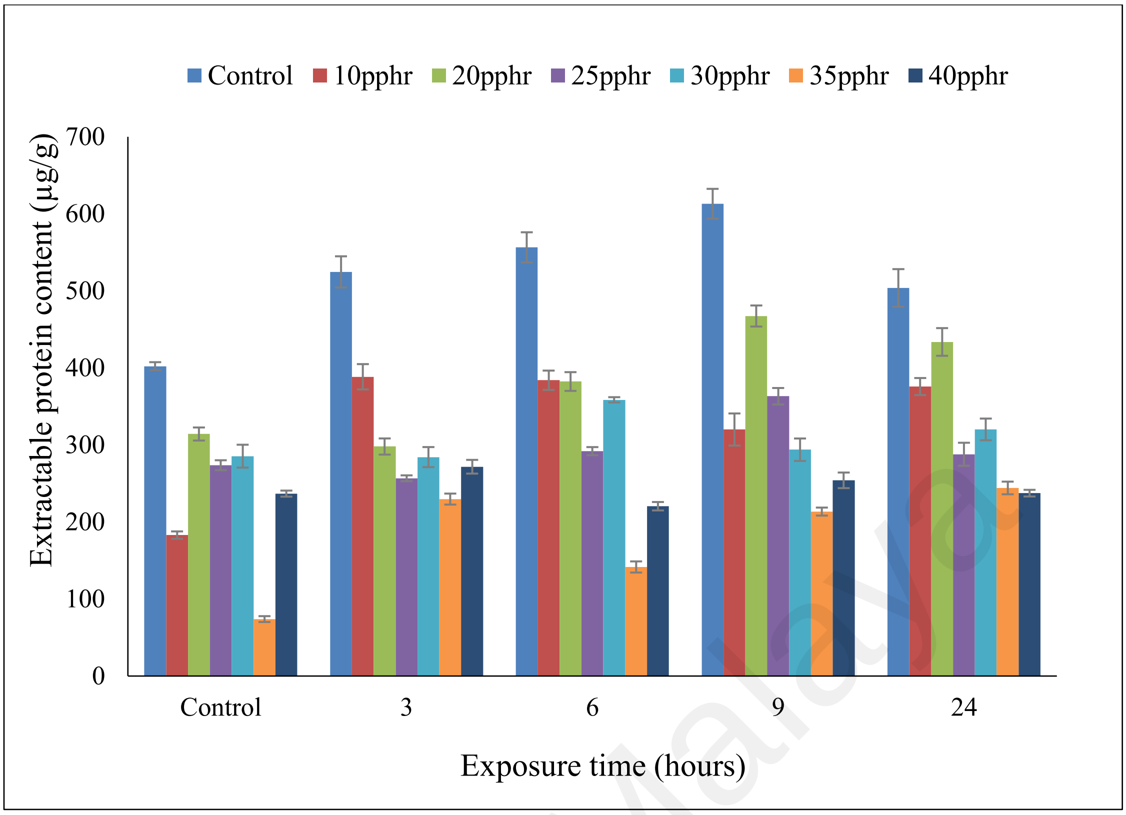

Figure 4.30: The EP content in MCLC films filled with untreated CaCO₃ after exposure to ozone at 10 pphm concentration.

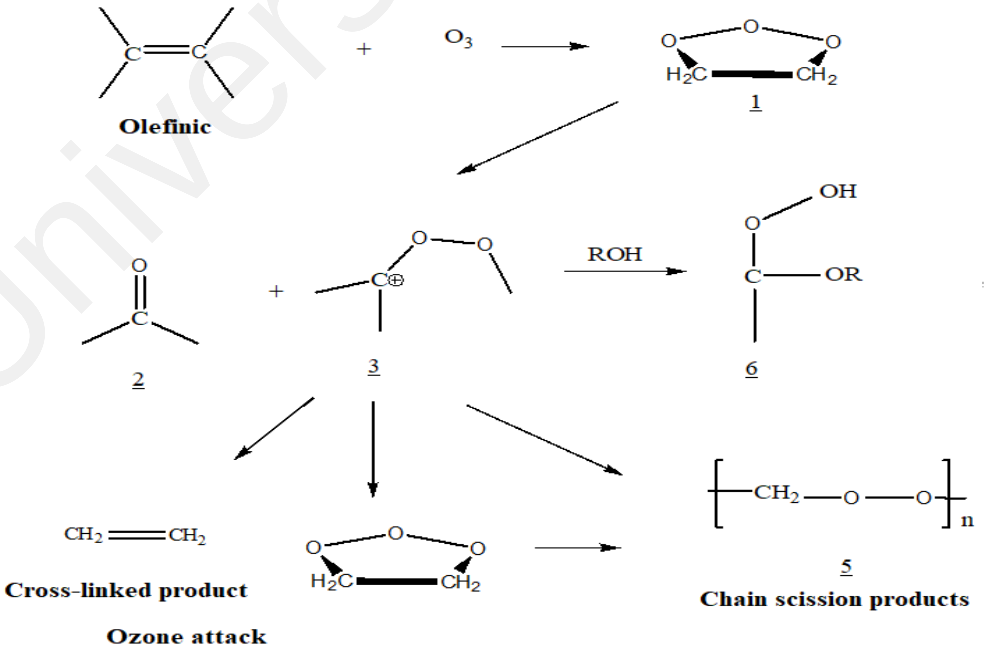

Figure 4.31: The mechanism of the ozonation process (Roberts, 1988).

4.3.5 Extractable protein content in MCLC films filled with SA-treated $CaCO_3$

Figures 4.32 and 4.33 show the non-corrected and corrected EP contents of unaged and aged NR latex films. Figure 4.32 shows that non-corrected EP content for the unaged samples was higher compared to aged samples except at the 4.0% SA concentration. However, no significant difference was observed in the values of unaged and aged samples for the non-corrected EP content at 3.0% concentration. It was also observed that there was a reduction in EP content from Control 1 until 3.0% concentrations and a slight reduction from 3.0% to 7.0% concentrations. This indicated that the stearic acid was bonded to the $CaCO_3$. In Figure 4.33, it was observed that the results of corrected EP contents for the unaged and aged films were inconsistent. The corrected EP content of unaged films at 0.5%, 4.0% and 7.0% obtained higher results compared to aged samples. However, the corrected EP content of aged samples at 1.0%, 3.0% and 5.0% obtained higher results compared to unaged samples with the highest value at 417.4 μg/g (3.0% treated). This observation was probably due to the contribution of chemical interferences from the rubber chemicals in the latex compounds (Hasma et al., 2004). The test method provides an optional method to correct absorbance values for samples whose values were suspected to be affected by interferences from non-proteinaceous substances present in the samples. The correction is based on the reaction of the Folin reagent in distilled water in place of the copper (Cu^{+2}) reagent (non-corrected), thus preventing the formation of the protein- copper complex. Under normal conditions (non-corrected) the Cu^{2+} ion binds with protein to form complexes that subsequently reduce Folin reagent, resulting in the development of a blue coloured solution that is absorbed in 750 nm (Mok et al., 2005).

The SA-treated $CaCO_3$ was predicted to reduce the EP content. The non-corrected and corrected for both unaged and aged NR latex films were analysed, as shown in Figures 4.34 and 4.35, respectively. From Figure 4.34, as expected, the corrected EP content of unaged NR latex films was lower compared to non-corrected EP content due to the removal of chemical interferences during the complexion protein with distilled water. It also found that all the non-corrected and corrected EP contents of treated samples were lower as compared to the control 1 (untreated filler). For aged samples in Figure 4.35, it was found that the results of non-corrected EP content at Control 1, 0.5%, 4.0% and 7.0% were higher as compared to the corrected EP content. Theoretically, it is expected that the corrected EP values should be lower than the non-corrected EP values as the interferences have been taken into account when water was used to substitute the Cu^{2+} ions according to ASTM D5712. However, at 1.0%, 3.0% and 5.0% concentrations, the opposite results were obtained, which the non-corrected were lower as compared to the corrected EP content. The reduction of EP values might be due to stearic acid presence on the treated $CaCO_3$ and the contribution of heating during the ageing process. This might be due to the chemical interference that happened from MCLC films filled with SA-treated $CaCO_3$ (Ruhida et al., 2020). Therefore, further research should be carried out to rectify the interference that might be occurred from the ion Ca^{2+} in $CaCO_3$ dispersions.

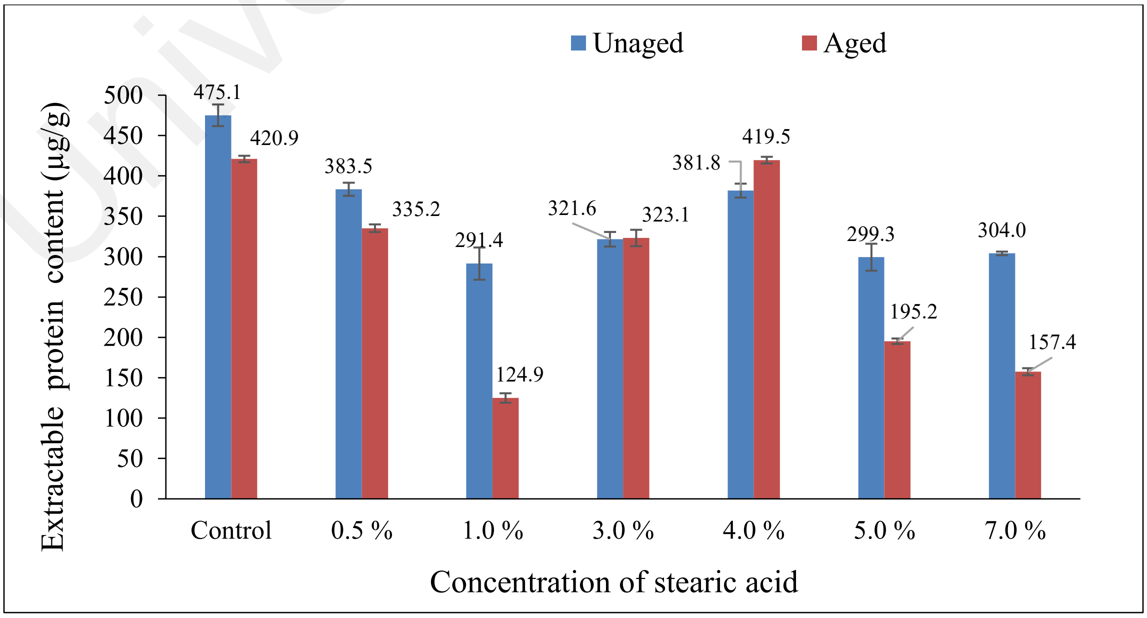

Figure 4.32: The non-corrected EP content of unaged and aged MCLC films filled with SA-treated $CaCO_3$.

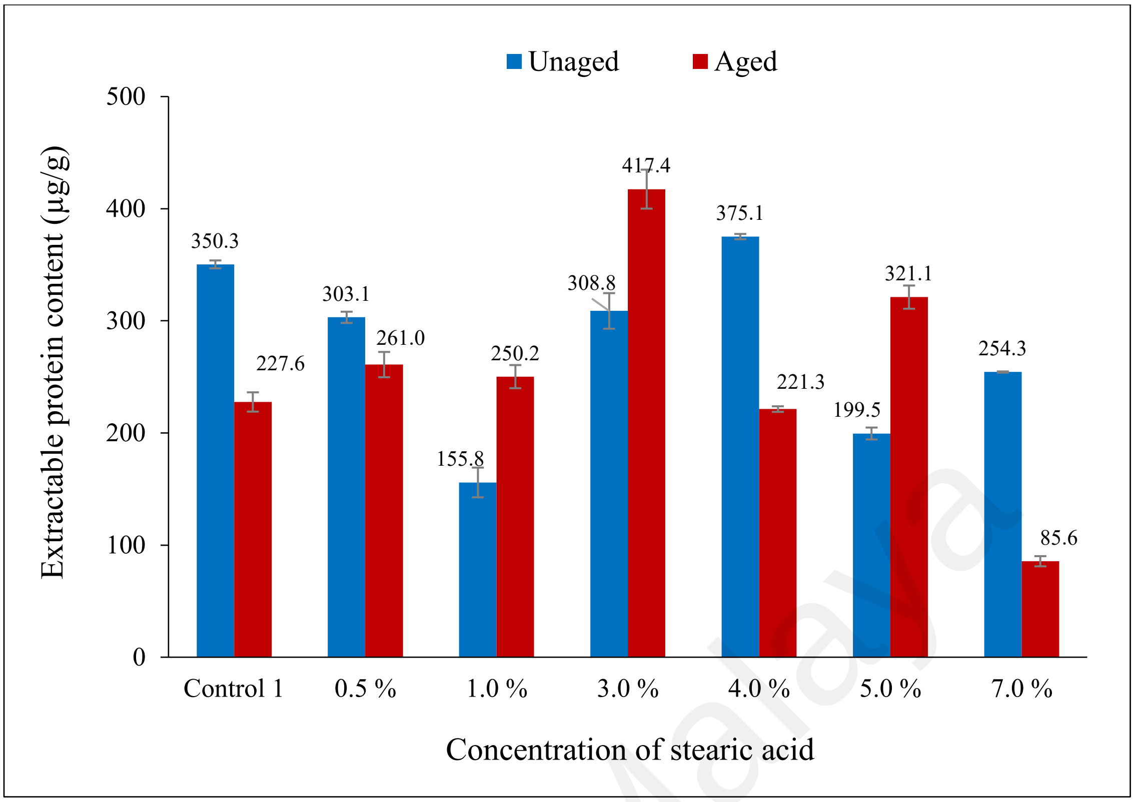

Figure 4.33: The corrected EP content of unaged and aged MCLC films filled with SA-treated $CaCO_3$.

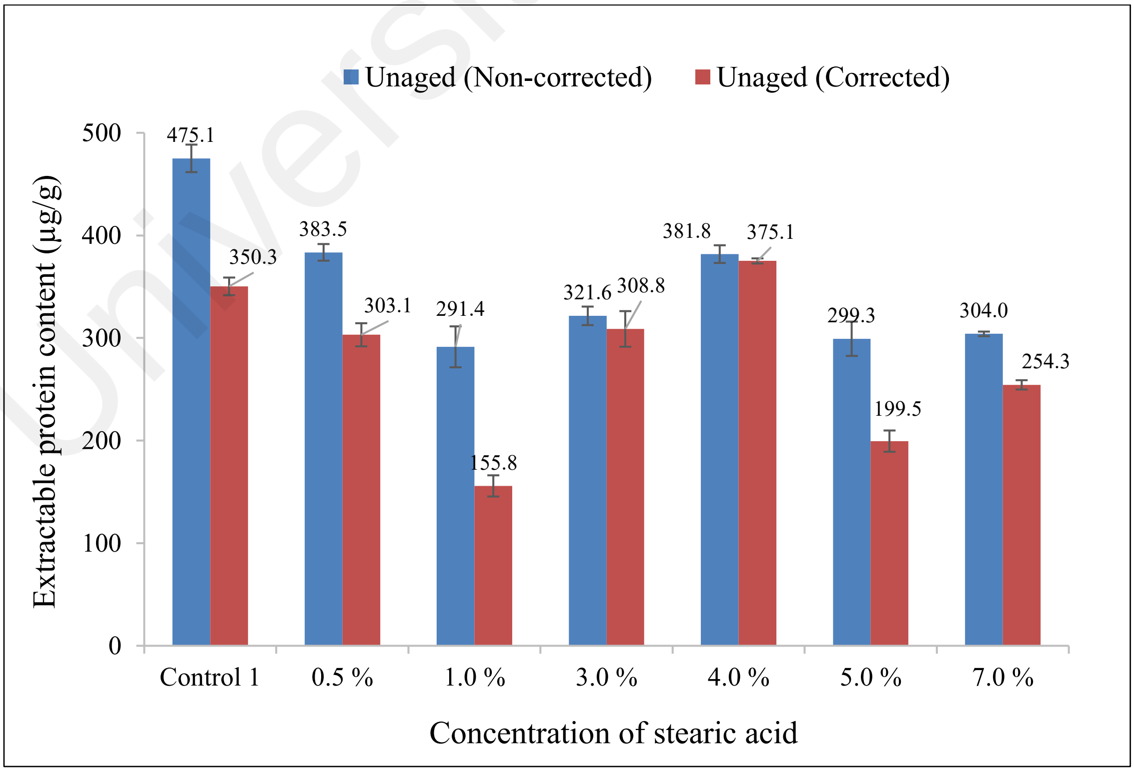

Figure 4.34: The corrected and non-corrected EP content of unaged MCLC films filled with SA – treated $CaCO_3$.

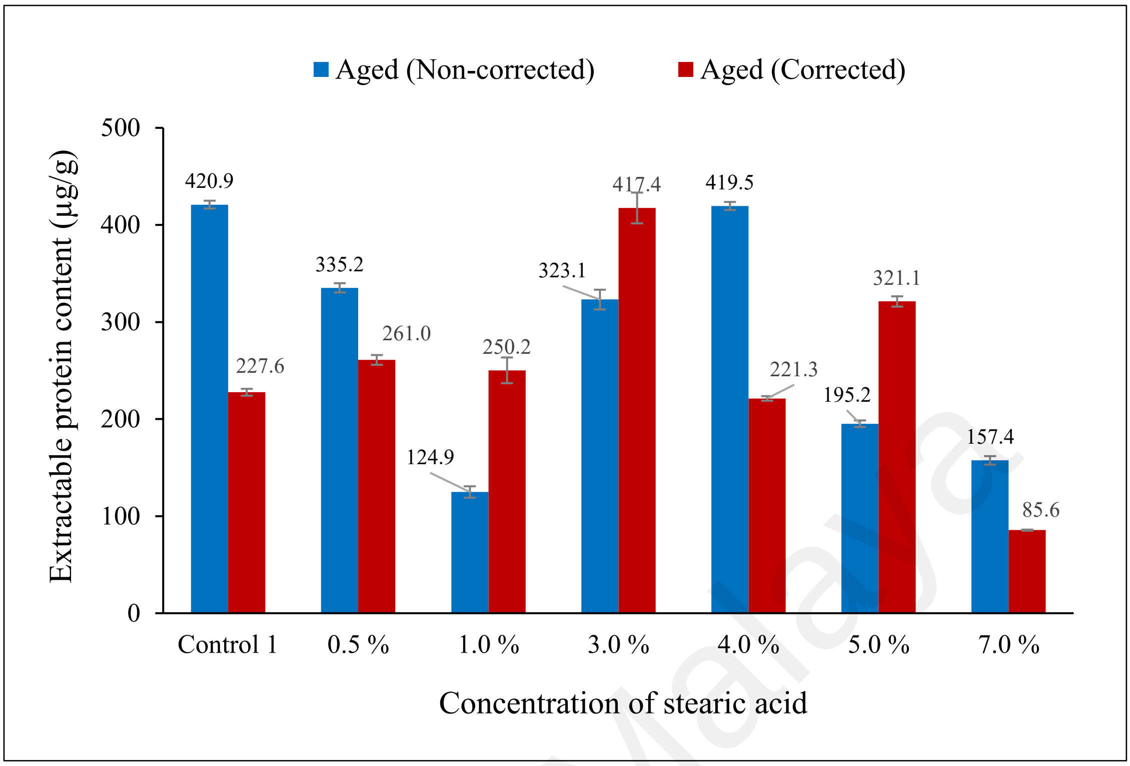

Figure 4.35: The corrected and non-corrected EP content of aged MCLC films filled with SA-treated $CaCO_3$.

4.4 Mechanical properties of MCLC films

4.4.1 Tensile strength of MCLC films filled with untreated $CaCO_3$

Figures 4.36, 4.37 and 4.38 show the results of tensile strength, tensile strain and tensile modulus at 300 and 500 MPa of unaged and aged MCLC films samples at different filler loadings, respectively. It was observed that there was a slight decrease in tensile strength for both unaged and aged films filled with fillers as compared to the Control sample, Figure 4.36. Tensile strength appeared to decrease with increasing filler loadings from 10 pphr to 40 pphr. As filler is introduced into the latex compound, the elasticity of the natural rubber is reduced, thus resulting in lower tensile strength at higher content of filler. The results of tensile strength for unaged film at 25 pphr and 30 pphr was slightly higher as compared to aged samples. This may be due to fillers that are dispersed in latex and not bonded to the rubber particles (Khamsook & Rattanawan, 2006). The similar tensile strength between unaged and aged film without filler maybe due to insufficient vulcanisation process for the unaged sample. Although, during the leaching process of unaged films, it could increase the tensile strength and modulus but had little effect on the crosslink concentration. Therefore, due to insufficient vulcanisation of the unaged sample might increase the tensile strength during the ageing process (Amir Hashim et al., 1997).

Heating during ageing increased the vulcanisation process of the MCLC films resulting in enhanced stiffening. This is known to arise from the interaction of the curatives present such as sulphur, that were dispersed with the filler particles and the rubber molecules. The increase in mechanical properties arising from the interaction between the curatives, filler and rubber particles on heating have also been reported by other researchers. The reaction enhanced the stiffening of latex film (Jarkasi et al., 2015; Manroshan & Baharin, 2005). Likewise, the decreasing tensile strain of MCLC films with increasing filler loading for both unaged and aged samples can be attributed to such interaction. As more filler was added into the latex compound, the properties attributed to natural rubber, such as crystallinity exhibited upon stretching was also reduced.

Subsequently, the increase in tensile modulus at 300 and 500 MPa, as shown in Figure 4.37, with increasing filler loadings, was ascribed to the stiffening effect that occurred from the interaction between filler and rubber particles. As mentioned earlier, heating during the ageing process resulted in interaction between the curing agent and rubber molecules, but the addition of filler in between rubber molecules led to reduction of cross-link concentration of the MCLC films. This explains the decrease in mechanical properties, particularly in tensile strength of vulcanised filled NR film with the addition of fillers. However, further investigation is required in order to understand the interaction fully. The modulus at M300 for aged films at 30 pphr filler loading indicate a slightly higher as compared to unaged films might be due to the insufficient time of curing during the vulcanisation process of latex films as shown in Figure 4.38.

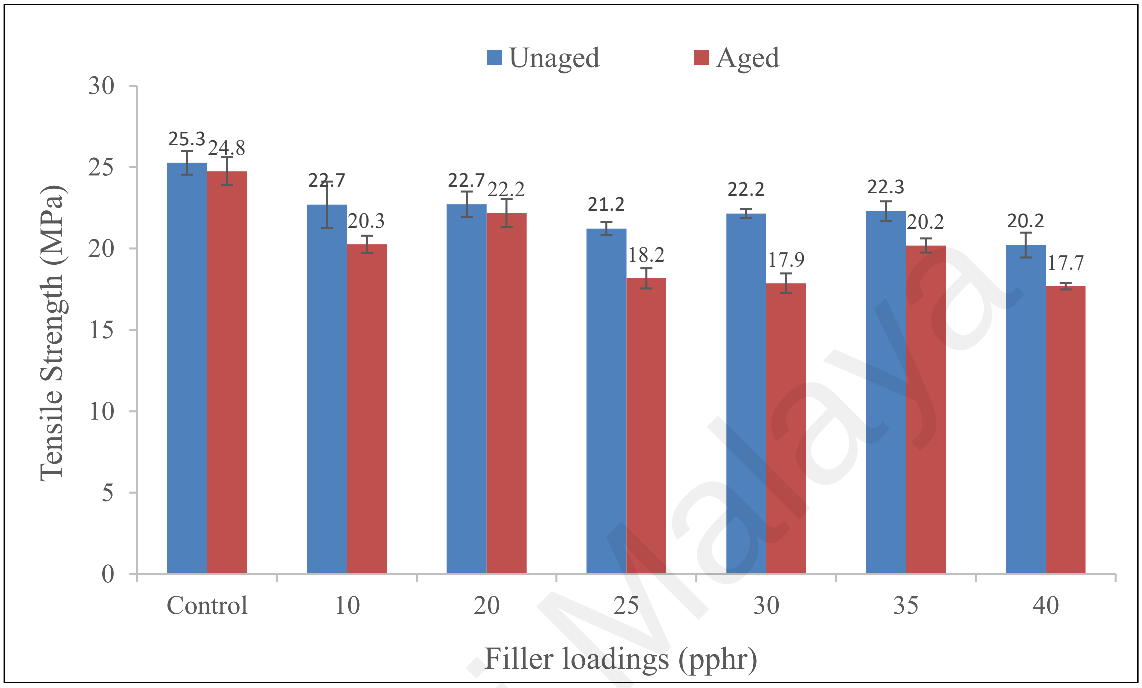

Figure 4.36: Tensile strength of unaged and aged MCLC films filled with untreated $CaCO_3$.

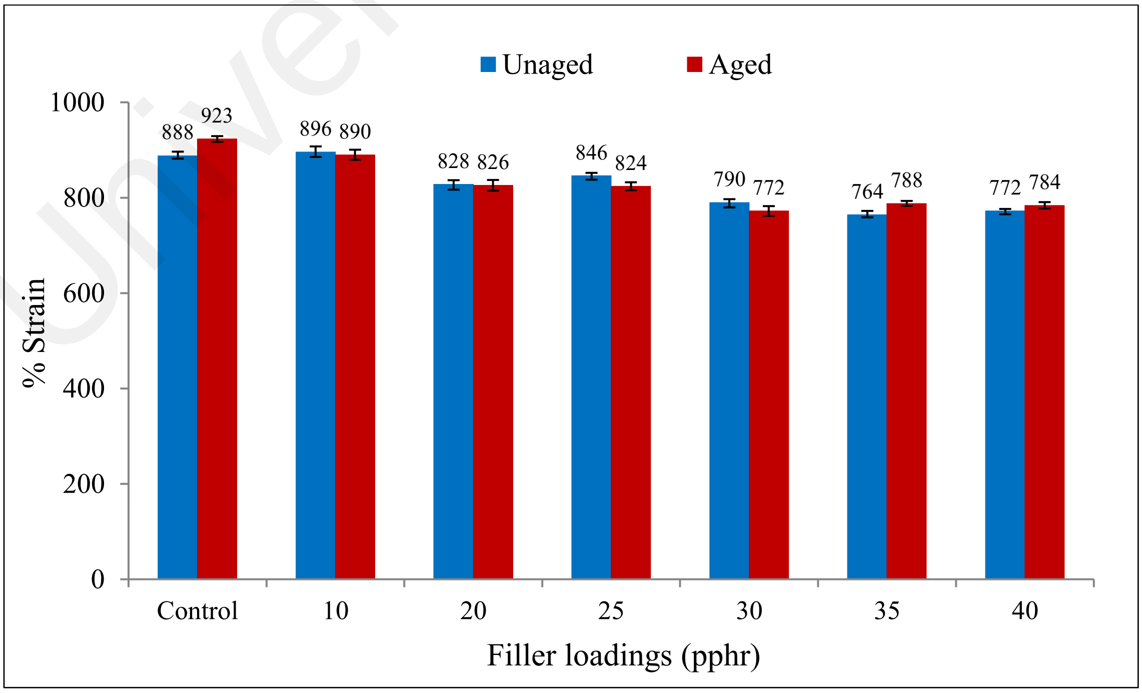

Figure 4.37: The percentage of strain for unaged and aged MCLC films filled with untreated $CaCO_3$.

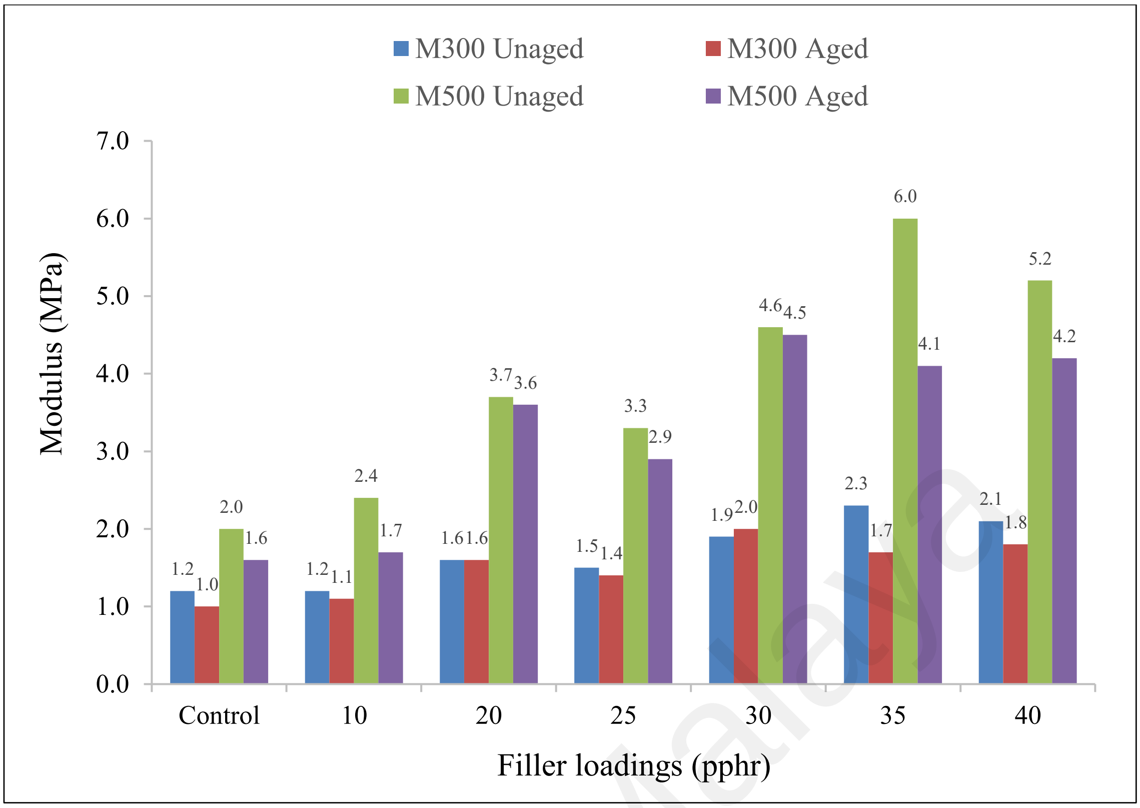

Figure 4.38: The modulus at 300% and 500% for unaged and aged MCLC films filled with untreated $CaCO_3$.

4.4.2 Tensile strength MCLC films filled with SA-treated $CaCO_3$

The correlation between tensile strength and cross-link concentration was studied in MCLC films filled with SA-treated $CaCO_3$. Based on the findings of the previous study, it was stated that higher cross-link concentration resulted in higher tensile strength. Another factor that may influence the tensile strength is the amount of orientated crystallinity in the vulcanised and stretched rubber. Cai et al. (2003) reported that the addition of filler in NR latex caused some physical reinforcing effect from the filler-rubber interphase interaction (Cai et al., 2003; Roslim & Amir Hashim, 2010). Figure 4.39 shows the results of tensile strength for unaged and aged MCLC films with SA-treated $CaCO_3$. It was observed that the tensile strength of unaged samples was higher compared to aged samples. Initially, the tensile strength of unaged samples progressively increased from Control 1 (untreated $CaCO_3$) to MCLC films filled 0.5% SA-treated film before reduction of tensile strength MCLC films filled from 1.0% until 7.0% SA-treated $CaCO_3$. It also observed at 7.0% SA-treated $CaCO_3$, the tensile strength of unaged films was slightly lower as compared to aged films. The reduction in tensile strength of the unaged film may be due to the lesser amount of SA being attached to the surfaces of $CaCO_3$ particles and has a tendency to be like untreated $CaCO_3$. Untreated $CaCO_3$ in MCLC compounds were intercalated in between rubber particles and has the ability to lower the strength of polysulphidic cross-linkages between rubber particles.

Figures 4.40 and 4.41 show the tensile modulus at 100% (M100), 300% (M300) and 500% (M500) strain of unaged and aged MCLC films, respectively. From Figure 4.41, it was observed that the tensile modulus at M500 was higher compared to tensile modulus at M300 and M100 in MCLC films with Control 1 (untreated $CaCO_3$) sample and $CaCO_3$ treated with 0.5% to 7.0% SA. The stiffness of the samples was observed to increase with the increasing amount of treated fillers added. However, for unaged samples, there was no significant difference in the tensile modulus at M300 and M100 for all samples. The tensile modulus at 500% strain decreased from Control 1 until 3.0% treated $CaCO_3$ and decreased severely at 4.0% and 5.0% SA-treated $CaCO_3$. The reduction in tensile modulus on ageing is mainly due to increasing stiffness. The heating process during ageing has played a role in increasing the tensile modulus and stiffness of MCLC film. The increased of the tensile modulus during ageing might be due to insufficient vulcanisation process during preparation of samples. A previous study by Ho & Khew (1999) has stated that the increase of tensile strength after the ageing process happens when the vulcanisation is much faster than diffusion, cross-linking take place on the surface of the latex particle before the reactants can diffuse into the core. This has led to an unvulcanised core surrounded by cross-linking shell of rubber molecules. Therefore, complete vulcanisation could not achieved (Ho & Khew, 1999; On et al., 2012). Therefore, it was believed that ageing and contribution of the heating process could enhance and complete the vulcanisation. This could increase the tensile strength of the sample. Meanwhile, the stiffness of the sample increased due to more SA-treated $CaCO_3$ being added into the MCLC films.

Figure 4.42 shows the percentage of strain for unaged and aged MCLC film with SA-treated $CaCO_3$. A higher %strain was observed for unaged MCLC films as compared to aged films. For unaged samples, increasing the concentration of SA to treat the $CaCO_3$ filler resulted in increasing % strain in the MCLC films. However, for aged films, there was a slight initial decrease in % strain in the 3.0% SA treated $CaCO_3$ before the % strain became consistent in the 4.0% to 7.0% SA- treated $CaCO_3$. It is postulated that the SA may be bonded less tightly to the rubber particles in these MCLC films, thus properties manifested are more akin to natural rubber.

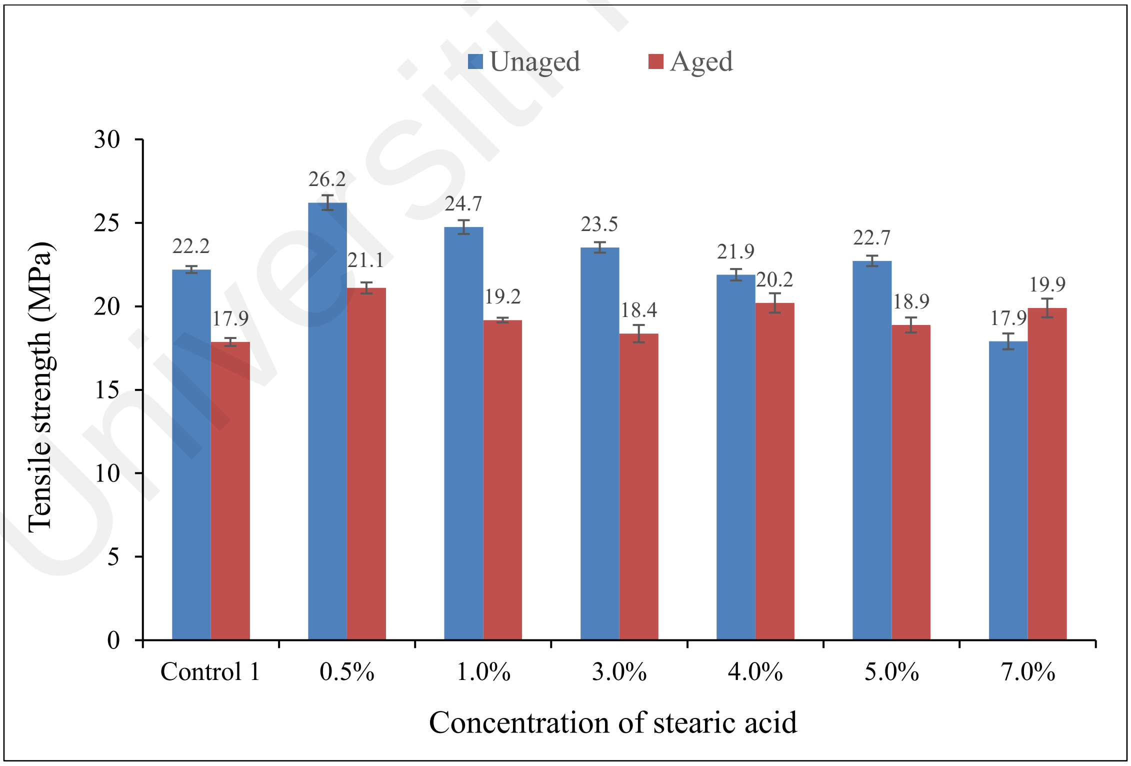

Figure 4.39: The tensile strength of unaged and aged MCLC films filled with SA- treated $CaCO_3$ at various concentrations.

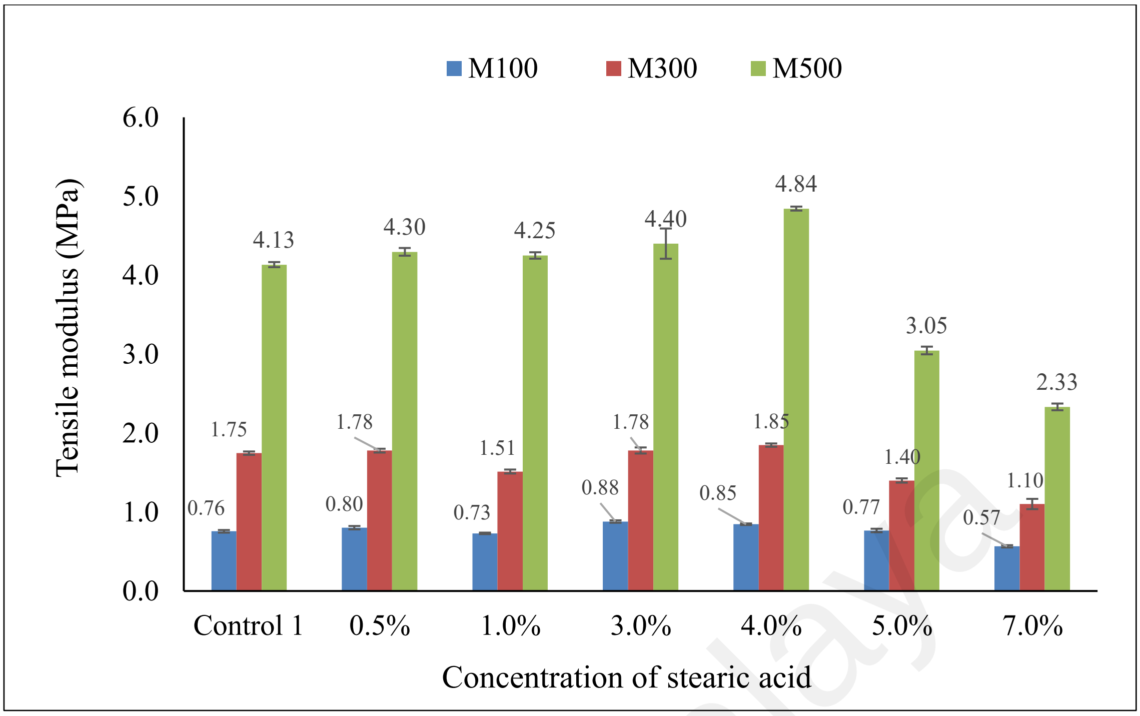

Figure 4.40: The tensile modulus of unaged MCLC films filled with SA-treated $CaCO_3$ at ε = 100%, 300% and 500%.

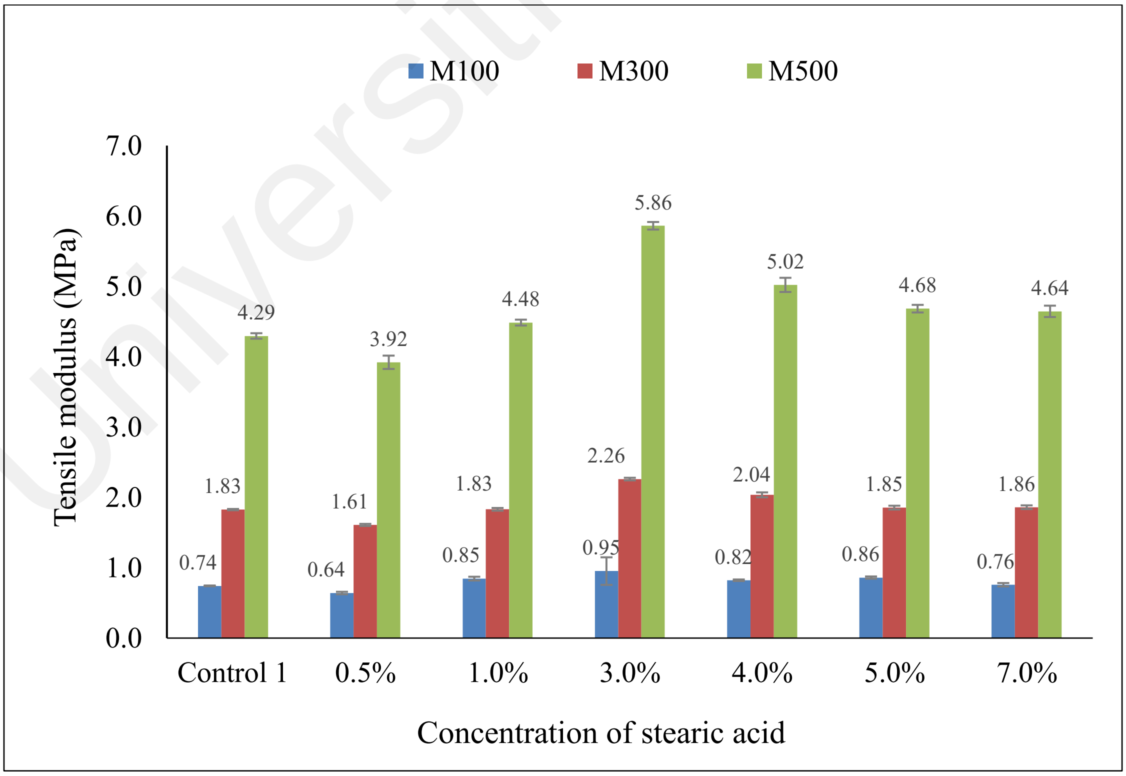

Figure 4.41 : The tensile modulus of aged MCLC films filled with SA-treated $CaCO_3$ at ε = 100%, 300% and 500%.

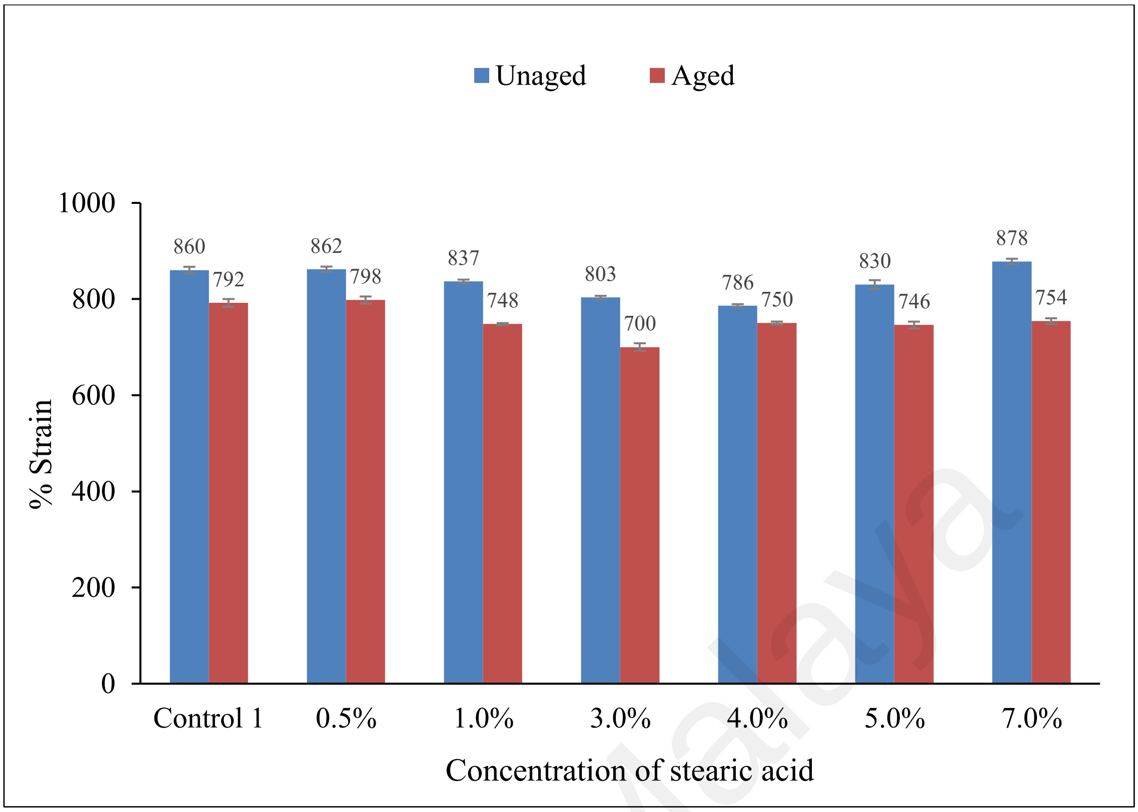

Figure 4.42: Percentage strain of unaged and aged MCLC film filled with $CaCO_3$ treated with stearic acid at various concentrations.

4.4.3 Tear strength of MCLC films filled with untreated $CaCO_3$

Figure 4.43 shows the tear strength of MCLC films at different filler loadings for unaged and aged films. As expected, lower tear strength of the aged samples was observed compared to the unaged samples from Control until 40 pphr filler loadings. The lower tear strength is due to the breakdown of the adhesion between the rubber and filler particles with heat and ageing. It was observed that the tear strength in unaged films increased from Control to 35 pphr filler loading but reduced drastically at 40 pphr filler loading. Initially, increasing the addition of filler into MCLC films increased the tear strength of the film. However, as more filler was introduced into the MCLC film, the distribution of fillers present between the rubber particles created a weak zone between the rubber particles leading to a reduction in tear strength (Azemi, 2005).

Knotty tearing was observed from Control until 35 pphr filler loadings while a steady tear was observed at 40 pphr filler loading for unaged samples. Nevertheless, Azemi has stated that there was no clear scientific evidence on the reduction of tear strength at specific filler loadings. The author suggested that anisotropy in NR latex film through the occurrence of knotty tearing indicated the ability of NR latex to strain-crystallise with protein molecules acting as reinforcing filler and polysulphidic crosslink (Azemi, 2005). In another study, three alternative methods were reported that helped to increase the tear strength of latex films through the addition of certain fillers, resin and a small proportion of synthetic latices (Idrus et al., 2011).

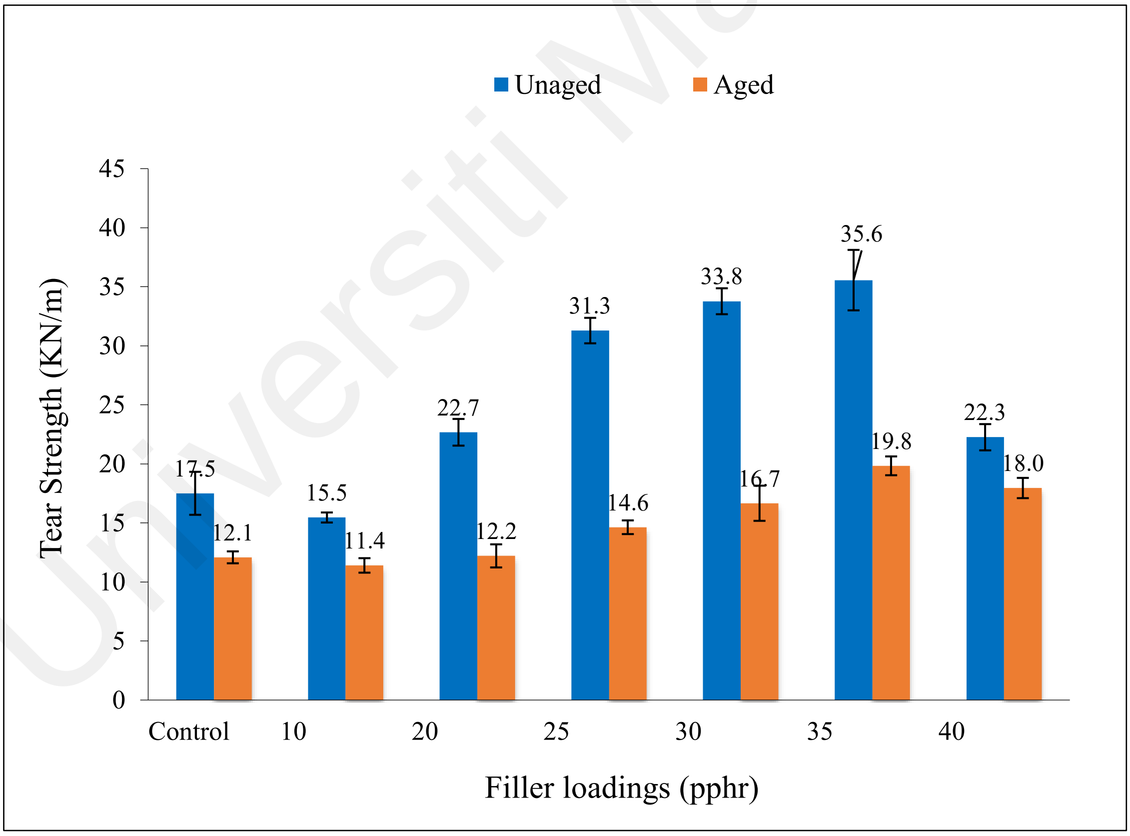

Figure 4.43: Tear strength of unaged and aged MCLC films filled with untreated $CaCO_3$.

4.4.4 Tear strength of MCLC films filled with SA-treated $CaCO_3$

Figure 4.44 shows the results of tear strength in MCLC films filled with SA-treated $CaCO_3$. The tear strength of unaged MCLC film was higher compared to aged films of the SA-treated $CaCO_3$ at all concentrations of SA used. No clear trend was observed for both unaged and aged films, and further investigation is needed to understand the phenomenon. However, the knotty tear was produced in MCLC fortified with SA-treated $CaCO_3$. The knotty tear is a phenomenon where the crack deviates at 90º from the intended tear path. This causes blunting of the tear tip and enhancement of the tear resistance. Knotty tearing is indicative of high tearing energy. The occurrence of knotty tear is associated with a strengthening structure that introduces anisotropy at the crack tip. In this case, the strengthening structure comes from strain-induced crystallisation produced by NR latex film, which has a regular microstructure (Azemi, 2005).

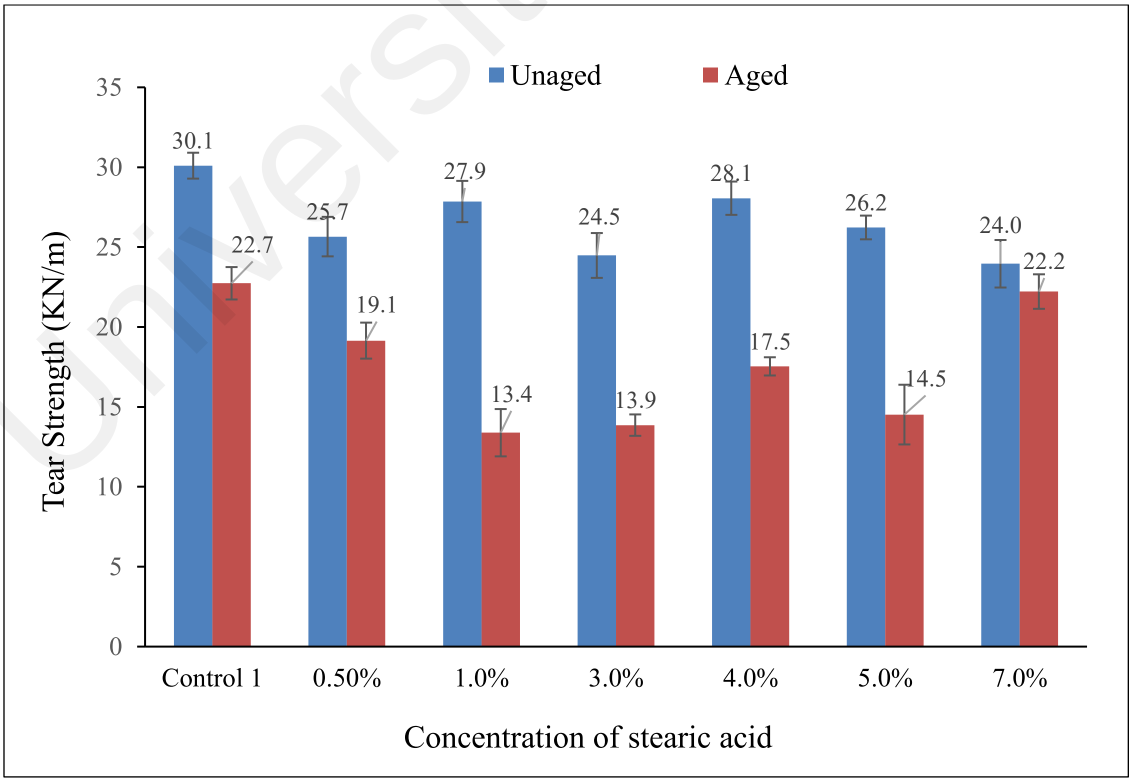

Figure 4.44: The tear strength of unaged and aged MCLC films filled with SA-treated $CaCO_3$ at various concentrations.

4.4.5 Relationship between $CaCO_3$ contents and cross-link concentrations of MCLC films filled with untreated $CaCO_3$

The effect of fillers on total protein content, surface morphology and mechanical properties (tensile and tear strength) of MCLC films were investigated. The relationship between $CaCO_3$ contents and cross-link concentrations of MCLC films was studied to ascertain whether a relationship exists between $CaCO_3$ content and cross-link concentration. The cross-link concentration of MCLC latex concentrate at different filler loadings was determined by using the solvent swelling test method and calculated according to the Flory Rehner equation (Flory & Rehner, 1943; Godbole et al., 2017; Neuburger & Eichinger, 1988). The presence of $CaCO_3$ was predicted to influence the cross-link concentration of rubber. Information on the changes in cross-link concentration will help in understanding the vulcanisation of rubber and predict the behaviour and performance properties of the rubber (Nasir & Teh, 1988). It is known that the cross-links concentration is also dependent on the quantity of sulphur present in the rubber vulcanisate (Hiranobe et al., 2021; Lewis, 1986).

The experiment was carried out to evaluate the effect of $CaCO_3$ addition into MCLC. Figure 4.45 shows the relationship between $CaCO_3$ contents and cross-link concentrations of MCLC films. Initially, the addition of the filler below 20 pphr affected the cross-link concentration. However, as more filler was added into the latex (more 20 pphr), the filler started to agglomerate between the rubber particles with not much effect on the cross-link concentration. The detailed calculation on the cross-link concentration at different filler loadings is as shown in Appendix F.

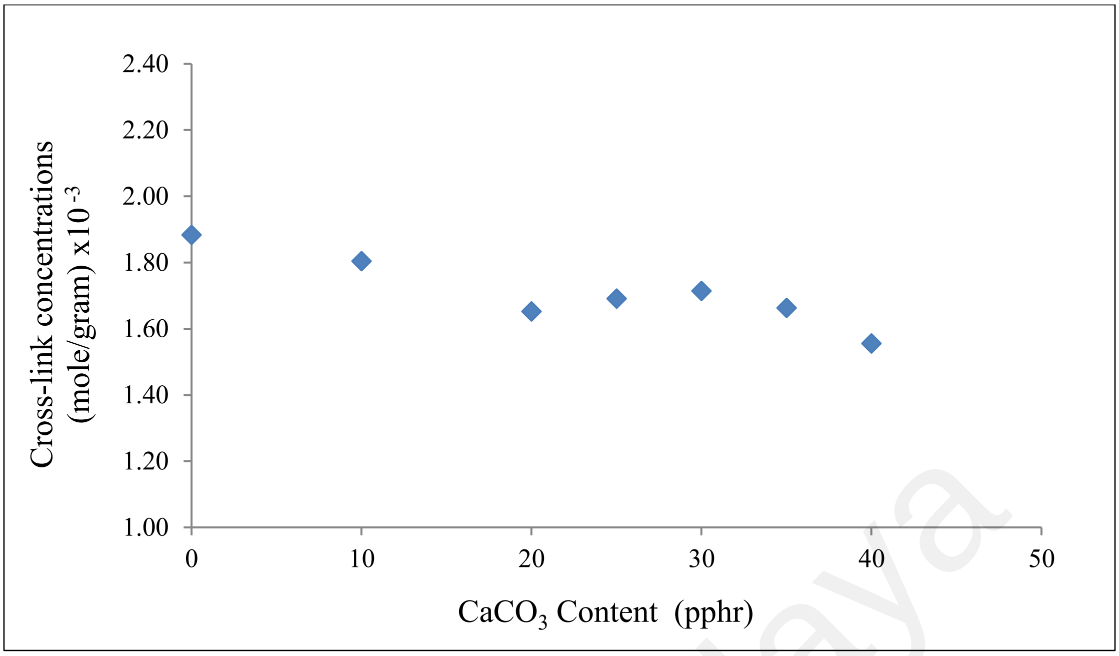

Figure 4.45: The relationship between filler content and cross-link concentrations in MCLC films filled with untreated $CaCO_3$.

4.4.6 Total sulphur, combined sulphur and free sulphur contents of MCLC films filled with untreated $CaCO_3$

According to the definition of the American Society for Testing and Materials (ASTM), vulcanisation is a chemical process in which the long chains of the rubber molecules become cross-linked by reactions with the vulcanising agent, usually sulphur, to form three dimensional structure (Joseph et al., 2015; Aprem et al., 2005). The three-dimensional network structure results in an improvement in elasticity and strength, conferring rubber with higher stability. A typical vulcanisation system in a compound consists of three components, namely activators, vulcanising agents, typically sulphur and accelerators. The terms cross-link and degree of cross-linking indicate the chemical interaction between sulphur and rubber and are affected by temperature, pressure and time. The cross-link concentration is used to measure the degree of crosslinking between rubber and sulphur (Adams & Johnson, 1953; Hiranobe et al., 2021). The degree of cross-linking determines the flexibility behaviours of the final rubber.

The results of total sulphur content, combined and free sulphur contents of unaged and aged MCLC films are as shown in Figure 4.46. It was found that there was no significant difference in the total sulphur content between unaged and aged MCLC films from Control (0 pphr) until 40 pphr filler loadings, as shown in Figure 4.46. However, the decrease in total sulphur content is significant from 0 to 25 pphr of filler loading, after which the total sulphur content was more or less the same at higher filler loading. The percentage of combined sulphur content of the aged samples was higher compared to the unaged samples. It also appears that the combined sulphur content decreased as the filler loadings increased, and the reduction was more significant up to 25 pphr filler loading. From Figure 4.46, it can be deduced that the presence of $CaCO_3$ and heat have an effect on the cross-link concentration of MCLC films and consequently on the strength of the MCLC films. The decrease in total and combined sulphur content with a concomitant decrease in cross-link density was seen to plateau off after 25 pphr filler loading. This suggests that at a filler loading of greater than 25 pphr, the presence of $CaCO_3$ added was too much in excess and no longer had any effect on the cross-linking. It is postulated that the excess calcium carbonate was present as agglomerates in the film.

Figure 4.46 also shows the percentage of free sulphur content on the unaged and aged MCLC films. It was observed that the free sulphur content of the unaged samples was higher compared to aged samples. According to Puacz et al. (1995), the results of free sulphur for unaged samples do not reflect the actual free sulphur concentration. In addition, the acetone used for extraction of the combined sulphur may be caused further vulcanisation of the rubber by the contribution of heating acetone at 70 °C might be a reason. In the aged samples, the amount of sulphur added as a cross-linking agent was entirely used for vulcanising the rubber. Therefore, extraction with acetone did not affect the concentration of free sulphur in the aged samples.

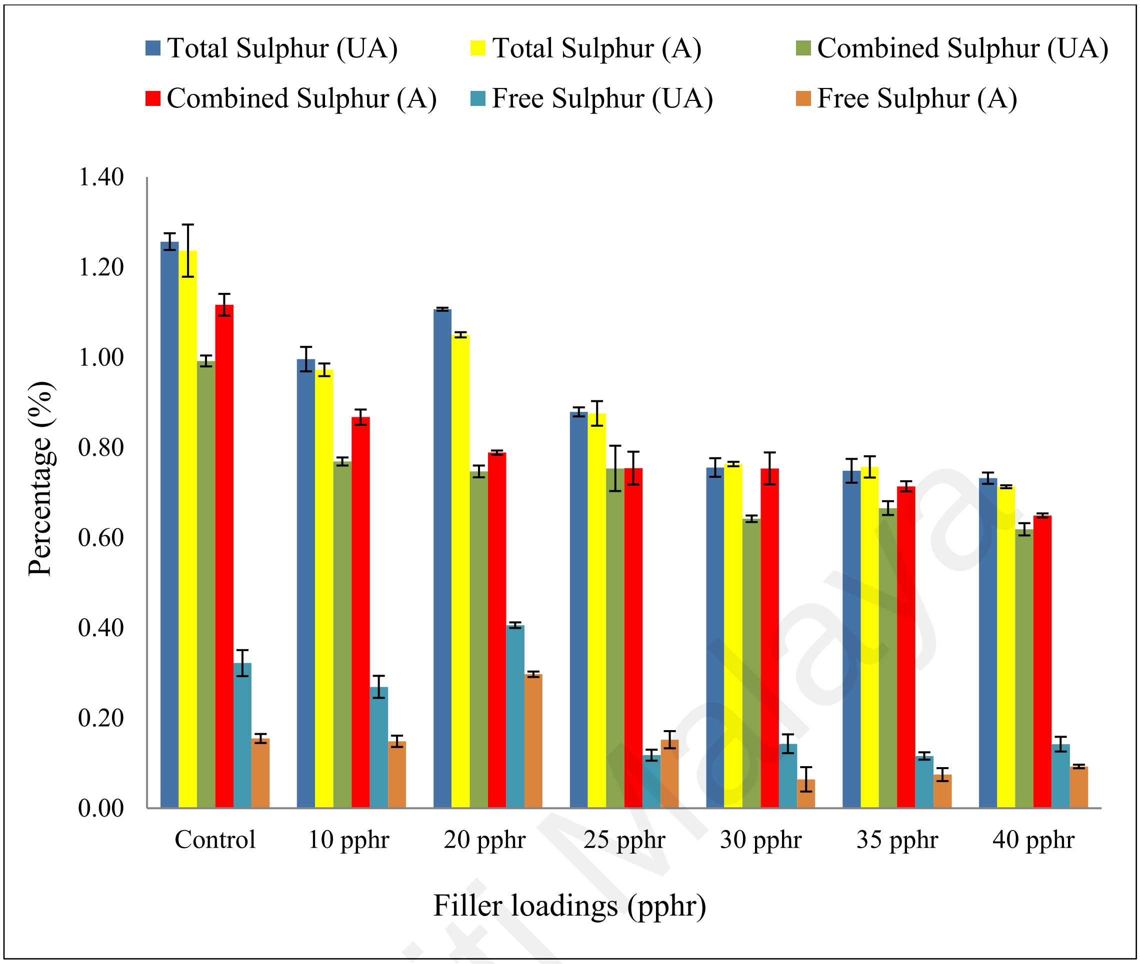

Figure 4.46: The total sulphur, combined sulphur and free sulphur contents of unaged (UA) and aged (A) MCLC films filled with untreated $CaCO_3$.

4.4.7 Total sulphur content of MCLC films filled with SA-treated $CaCO_3$

The total sulphur content in MCLC films filled with treated $CaCO_3$ was analysed to determine the percentage of sulphur present in the samples. The objective is to investigate the effect of the addition of filler on polysulphidic cross-link concentration, which in turn is related to the strength of the NR latex films. Figure 4.47 shows the percentage of total sulphur content for unaged and aged MCLC films added with SA- treated $CaCO_3$. It was observed that there was no significant difference in the results of total sulphur content between the unaged control sample and unaged samples containing SA-treated $CaCO_3$. In general, it was also observed that ageing did not affect the amount of total sulphur present in the MCLC films containing the different amounts of SA-treated $CaCO_3$. The slightly higher total sulphur content recorded for the aged Control 1 sample may be due to the inhomogeneity of the test sample.

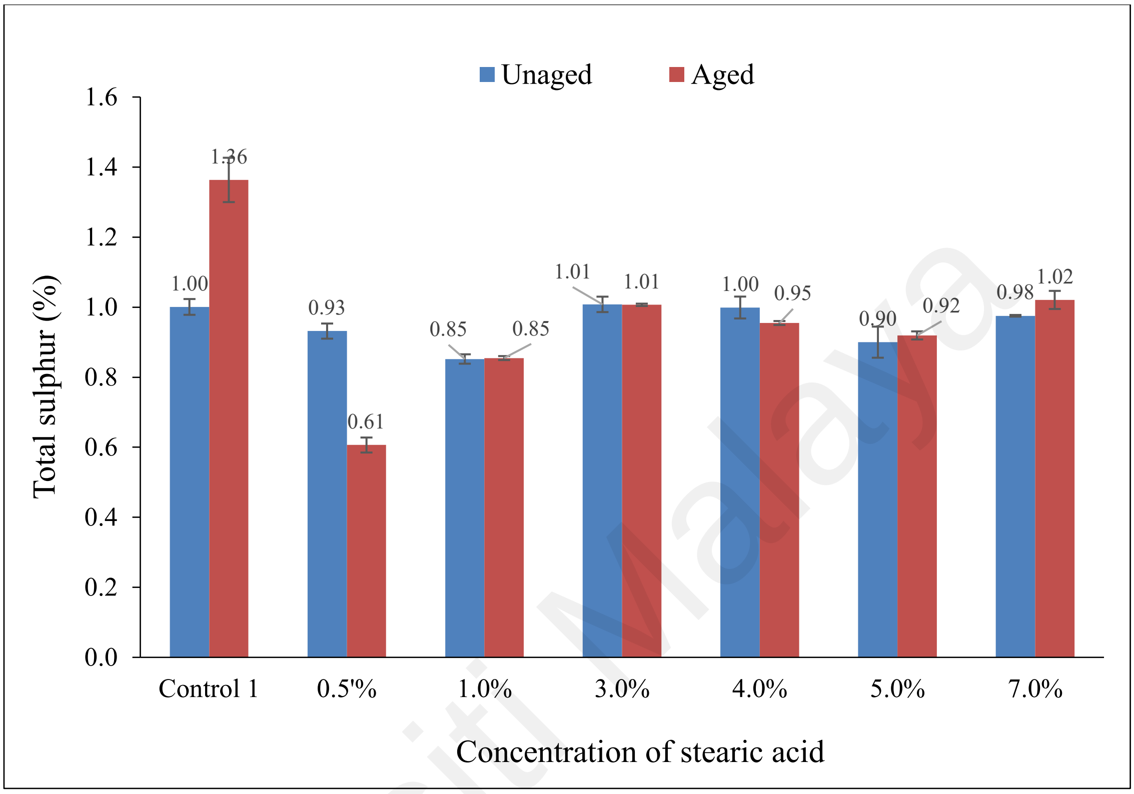

Figure 4.47: The content of total sulphur for unaged and aged MCLC films filled with SA-treated $CaCO_3$.

CHAPTER 5: CONCLUSION

The effect of fillers on surface morphology, mechanical properties and total protein contents in natural rubber latices are discussed in this study. The commercial filler, calcium carbonate, was chosen. Initially, $CaCO_3$ was added at different filler loadings. Based on the results of preliminary work carried out, a filler loading of 30 pphr was chosen because the values of $CaCO_3$, EP contents and tensile strength were obtained at optimum conditions. Therefore, the $CaCO_3$ was treated with SA at various concentrations in a chloroform medium. The addition of SA-treated calcium carbonate was expected to reduce the EP content of MCLC films without affecting adversely the mechanical properties. Findings from this research should benefit the Rubber Manufacturers in their effort to reduce the cost of production without compromising the quality of the NR product in terms of their chemical and mechanical properties. From the studies conducted in this thesis, several conclusions can be drawn.

i. The latex of MCFL and MCLC was clean, stable and free from foreign matters and non-polymeric impurities. The non-rubbers present in MCLC was much less than that of mixed clones field latex MCFL at 1.03% and 2.55%, respectively. The MST of the MCLC of 1314 seconds fulfilled and met the ISO 2004 specification.

ii. Results from TGA analysis showed that milling and ageing of MCLC films did not change the thermal properties of MCLC film significantly. However, the presence of other rubber chemicals such as ZBuD, Sulphur, ZnO and Wingstay L dispersions in the latex compound caused slight differences in the thermal properties of unmilled and milled MCLC films. The $CaCO_3$ content was slightly reduced, and this was attributed to heating and extraction that perhaps removed some of the non-rubber substances, including $CaCO_3$, from the MCLC films.

iii. By FTIR analysis, it was deduced that the peak at 873 cm^{-1} can be attributed to the presence of $CaCO_3$ in the MCLC film loaded with untreated fillers. For SA-treated $CaCO_3$, it was deduced that the peaks at 875 cm^{-1} might be attached to SA, while the peak at 837 cm^{-1} indicated the presence of $CaCO_3$ that was not attached to SA.

iv. From DSC analysis, there was no effect on the T_g by fillers present in MCLC films, although the T_g of MCLC film increased by approximately 1.2% from 0 pphr to 20 pphr filler loading and decreased by 1.1% from 25 pphr to 40 pphr filler loading. However, the reduction in T_g of MCLC films filled with SA-treated $CaCO_3$ indicated that the sample was stiffer and the high value of T_g at 0.5%, 4.0%, and 7.0% was more elastic since the values obtained were more towards the T_g of NR, which is at -72ºC.

v. FESEM micrographs revealed that the surface morphology of MCLC film from 20 pphr until 30 pphr loadings had a less smooth surface than the MCLC film without any filler. The $CaCO_3$ started to agglomerate at 35 pphr filler loading with a crack observed on the surface of MCLC film at 40 pphr filler loading. However, the cracks on the surface of MCLC films were observed after 24 hours of exposure to 10 pphm ozone concentration. No cracks were developed at 3, 6 and 9 hours exposure to ozone, with rough surface starting to appear after 9 hours of exposure. The MCLC films filled with SA-treated $CaCO_3$ at 0.5% and 1.0% had a smoother surface morphology compared to MCLC films containing $CaCO_3$ treated with SA at 3.0%, 4.0%, 5.0% and 7.0%. The surfaces were rougher and coarser.

vi. Nitrogen (N) and total protein increased with increasing filler loadings. The reduction in nitrogen content on ageing was due to the loosening of the $CaCO_3$ particles that were bonded to the rubber or $CaCO_3$ particles that stayed in between rubber particles on heating. In the case of SA-treated $CaCO_3$, it was observed that the unaged SA-treated $CaCO_3$ had reduced the percentage of N, but the ageing process has increased the percentage of N. However, there were no significant results of percentage N between unaged and aged samples that filled with 3.0% SA-treated $CaCO_3$ (0.71% and 0.72%).

vii. The EP content of unaged samples was higher than aged samples. The inconsistent trend between non-corrected and corrected EP content was believed to be due to hindered filler, chemicals interference and heating process. It was noted that the chemical interference occurred at 25 pphr filler loadings to 40 pphr filler loadings in the MCLC films. It was found that the EP contents in unaged MCLC films loaded with $CaCO_3$ treated with SA at all the concentrations studied were reduced compared to MCLC film containing untreated $CaCO_3$. The highest value of corrected EP content obtained was 417.4 µg/g with 3.0% SA treated $CaCO_3$. Generally, the unaged MCLC films had a lower EP content when correction was performed as recommended in the test method.

viii. The mechanical properties such as tensile and tear strength of MCLC films decreased as the filler loading increased. The tear strength of MCLC films increased as the filler loading increased from 10 pphr until 35 pphr filler loadings before decreasing at 40 pphr.

ix. In the case of MCLC films filled with SA-treated $CaCO_3$, it was observed that the reduction in tensile strength of the unaged film might be due to a lesser amount of SA being attached to the surfaces of $CaCO_3$ particles and has a tendency to be like untreated $CaCO_3$. Ageing also reduced the tear strength of the MCLC films. The tear strength of the unaged and aged MCLC films containing SA-treated $CaCO_3$ were lower as compared to MCLC film containing untreated $CaCO_3$.

x. The filler, $CaCO_3$, was observed to influence the cross-link concentration of rubber. Increasing the loading of filler resulted in decreasing cross-link concentration. Ageing did not affect the total sulphur content of MCLC films at different filler loadings. However, the combined sulphur content in aged samples decreased as the filler loadings increased. The effect of heat, solvent extraction and ageing may have influenced the decreasing content of combined sulphur.

From the above conclusions, the following recommendations are proposed:

I. To carry out an in-depth study on the effect of fillers on the chemical and mechanical properties of NR when exposed to ozone at different ozone concentrations and strain applications.

II. To delve further into the determination of extractable and allergenic content of NR latex films added with treated filler and rubber chemicals during compounding and the ensuing chemical interferences caused by the added chemicals. This will help in formulating steps to address the issues of Type IV latex allergy.

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