CHAPTER 1

INTRODUCTION

Natural rubber (NR) and palm oil are the major primary commodities of Malaysia. In this regard, there are many economic and technological incentives to produce a new polymer from existing commodity polymer than to develop polymer from a new monomer. The idea of utilizing palm oil-based products to react with NR was mooted. Hence, the aim of this project was to study the reactions of palm oil-based alkyds and polyhydroxyalkanoates (PHA). A background of epoxidized natural rubber (ENR), alkyd and PHA is thus presented in general. The research methodology including background and scopes of study are then briefly discussed at the end of this chapter.

1.1 Epoxidized natural rubber

Natural rubber (NR) is commonly obtained from the latex of the Hevea brasiliensis tree, which is originally grown in South America. NR is a polyolefin with a precise *cis*-1,4-isoprene molecule and the weight-average molecular weight ($\overline{M_w}$)[1,2] is generally between the limits of 1.6 and 2.3×10^6.

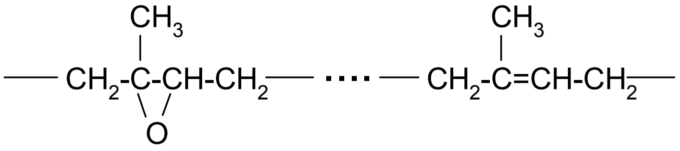
The epoxidized natural rubber (ENR) is a derivative from the chemical modification of NR, where a portion of the carbon–carbon double bonds of the NR molecular chains are chemically converted to epoxy groups. ENR can be prepared by partial epoxidation of NR latex either with peroxyacetic acid[3] or peroxyformic acid which forms ‘in-situ’ from hydrogen peroxide and formic acid[4].

When the NR is epoxidized, its chemical and physical properties change according to the extent to which the mole percentage of modification is introduced. This leads to the improvements of NR properties such as increase in oil resistance, glass transition temperature, polymer viscosity, polarity as well as low gas permeability, good wet grip, high damping characteristics[5], where some of these properties are similar to those of synthetic rubbers[6,7].

ENR-50 is produced when NR is epoxidized to 50 mole %. Its air permeability has become similar to butyl rubber (IIR) and its oil resistance is close to acrylonitrile-butadiene rubber (NBR). The tensile strength and fatigue properties of ENR-50 are improved tremendously. Since ENR-50 was still able to undergo strain crystallization like NR[8], it has a significantly higher tensile strength than NBR with much improved fatigue performance. However, ENR-50 has been found to appear as a highly damping rubber with very low room-temperature resilience, even less than that of NR or NBR[5].

1.1.1 Chemical modification of ENR

ENR can be represented by the following structure, where each molecule has one epoxy and unsaturated sites.



$$-CH_2-\overset{CH_3}{\underset{\setminus\, O\, /}{C-CH}}-CH_2- \cdots -CH_2-\overset{CH_3}{C}=CH-CH_2-$$

These two reactive functional sites in ENR could be utilized for chemical modifications to change the properties of the base polymer and diversify the applications. While the double bonds can be involved in addition reactions such as vulcanization by sulfur and peroxide, the epoxy groups provide alternative sites for interaction with compounds having other functional groups[9-12] such as carboxylic and amino groups.

1.1.1.1 Vulcanization of ENR

The first commercial method for vulcanization has been attributed to Charles Goodyear. His process (heating natural rubber with sulfur) was first used in Springfield, Massachusettes in 1841[13]. Ever since, there has been continued progress towards the improvement of the process, which was then being referred to as vulcanization.

Vulcanization converts rubber from a linear polymer to a three-dimentional network by crosslinking the polymer chains. It can be defined as a process which increases the retractile force and reduces the amount of permanent deformation remaining after removal of the deformation force[13]. Thus, vulcanization increases elasticity while decreases plasticity.

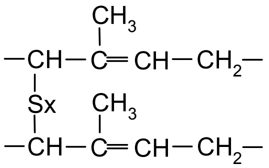
According to the theory of rubber elasticity, the retractile force to resist a deformation is proportional to the number of network-supporting polymer chains per unit volume of elastomer[14]. A supporting polymer chain is a linear polymer segment between network junctures. An increase in the number of junctures or crosslinks gives an increase in the number of supporting chains. In an unvulcanized linear high polymer (above its melting point), only molecular chain entanglements constitute junctures.

Vulcanization, thus, is a process of chemically producing junctures by the insertion of crosslinks between polymer chains. A crosslink may be a group of sulfur atoms in a short chain, a carbon to carbon bond, a polyvalent organic radical, an ionic cluster, or a polyvalent metal ion. The process is usually carried out by heating the rubber, mixed with vulcanizing agents in a mould under pressure.

1.1.1.1.1 Sulfur vulcanization

Natural rubber is usually vulcanized under sulfur vulcanization systems, together with activators and organic accelerators. The activators, usually zinc oxide and stearic acid, increase the number of crosslinks and stiffness, but amount used in sulfur vulcanization are not critical provided that certain minimum levels are exceeded. The accelerator increases the rate of cure and the efficiency of sulfur crosslinking.

In a normal sulfur vulcanization system, the temperatures required are in the range of 120-160°C when the sulfur-sulfur bonds in the elemental S_8 sulfur rings start to break[15]. In any case, the primary sites of attack are hydrogens on the α carbon on the double bond, producing the structure as shown below.



where, x = 2, 4 ,6, 8.

As a result, the physical properties of the crosslink networks, such as resilience, stiffness and resistance to creep, are generally increased.

In fact, the physical properties obtained depend on the types of crosslink formed and the extent of main-chain modification by side reactions. This is usually being largely determined by the vulcanization system, although cure time and temperature also have an important effect[16]. Generally, there are three types of typical sulfur vulcanization systems, namely, conventional vulcanization (CV), efficient vulcanization (EV) and semi-EV.

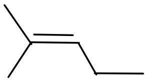
A CV system contains high sulfur level, 2-3.5 phr and low level of accelerator, typically 0.3-1 phr. At optimum cure, the vulcanizates contain mostly polysulfidic crosslinks with a relatively high level of chain modification. These polysulfide networks generally have good tensile, tear and fatigue properties and excellent resistance to low-temperature crystallization. However, they are susceptible to reversion, i.e. loss of properties on overcure and to oxidative ageing. Other drawbacks include high compression set and high rates of secondary creep and stress relaxation at elevated temperatures, due to exchange reactions between polysulfidic crosslinks.

Low sulfur levels, typically 0.25-0.7 phr, with high accelerator levels, typically 2.5-5 phr will give mainly monosulfidic crosslinks with lower level of chain modification. They are therefore known as efficient vulcanization (EV) systems. These systems give vulcanizates that are more resistant to reversion, oxidative ageing and high temperature compression set due to the higher thermal stability of monosulfidic crosslinks. However, compared to CV vulcanizates, EV vulcanizates have a lesser extent of resistance to tearing, fatigue and wear and lower tensile strength. Besides, their resistance to low temperature crystallization is poor and their rubber-to-metal bonding may be difficult. Therefore, EV systems are used for the vulcanization of thick articles and those used at elevated temperatures.

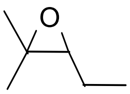
For semi-EV systems, intermediate sulfur level of 1-2 phr and 2.5-1 phr of accelerator are often used. The vulcanizates have physical properties intermediate between those of CV and EV vulcanizates. In fact, they give some improvements in reversion, ageing resistance and compression set compared with CV vulcanizates, but resistance to fatigue and low temperature crystallization is impaired. However, they have higher scorch safety, particularly when sulfenamide accelerators are used in the system.

1.1.1.1.2 ENR vulcanizates

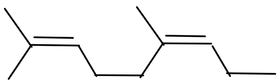
Gelling and Morrison had shown that vulcanization by sulfur alone is faster and more efficient for ENR than NR and indicated that isolated double bonds react more rapidly than continuous double bonds[17]. From their experiments with model olefin and epoxy under the conditions required for the sulfur vulcanization of ENR, it appeared that sulfur reacted with olefin (structure 1) rather than epoxy (structure 2) to produce crosslinks. Further investigation revealed that sulfur had greater extent of reaction with structure (4) and structure (5) than a mixture of structure (1) and structure (2) or structure (3). Since sulfur reacts with olefins by a chain mechanism, the slower rate of reaction with structure (3) is ascribed to cyclization of a chain-propagating species, where the cyclization required a neighbouring double bond which is present in structure (3) and in NR. However, ENR-50 with olefin groups on either side of an adjacent epoxy group, has provided effective blocking towards such cyclization reactions. Therefore, the higher the concentration of epoxy groups in the rubber chain, the stronger is the effect of epoxy-activated double bonds in sulfur vulcanization[18]. Recent studies on ENR vulcanization by Ismail *et al.* indicated that these epoxy-activated double bonds in ENR had resulted shorter scorch time and cure time as well as increased torque values and these changes enhanced sulfur vulcanization by producing higher crosslink density in ENR[19-25].



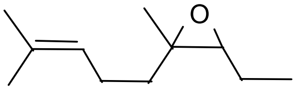
Structure (1)



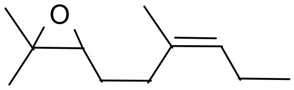
Structure (2)



Structure (3)



Structure (4)



Structure (5)

On the other hand, Gelling and Morrison have reported that the sulfur vulcanizates of ENR showed poor aging properties. This was attributed to the acid-catalyzed ring-opening reactions of the epoxy groups where these acids were produced by the thermal decomposition of oxidized sulfides[17]. Besides, these poor ageing properties of ENR were also believed to be due to the residual acidity that was inherited from the NR modification (epoxidation) with peroxide.

In terms of sulfur vulcanization systems, ENR cured with the CV system was found to exhibit poor ageing properties as compared to the unmodified NR[26-29] and this was due to different aging mechanism, in which sulfur acids from the oxidation of sulfides ring-opened the epoxy groups to give crosslinks[17]. These resulted in a substantial increase in modulus and consequently reduction in tensile strength. Therefore, conventional sulfur vulcanization of ENR is only applied when special formulations with a high level of base to counteract this acid problem are used. However, there may be a scorch problem arising from the CV system, unless the pH of the rubber is suitably adjusted, in order to avoid acid catalyzed ring opening occurring at low pH, and to accelerate the vulcanization process by base at high pH[26].

Therefore, it has been reported that the use of a semi-EV system was more suitable for ENR vulcanizates in which the sulfur to accelerator ratio are about the same[27-29] and semi-EV cures give better fatigue but inferior aging properties compared to EV systems[26]. Besides, Amu *et al.* has suggested that a low sulfur curing system (EV or semi-EV systems) was the preferred choice for satisfactory processing safety as well as imparting the ageing performance of ENR vulcanizates during mastification (mill-breakdown process)[30]. However, Sadequl *et al.* has reported that the influence of accelerator/sulfur ratio of different vulcanization systems on the scorch time of ENR became less significant as vulcanization temperature increased[19].

In general, vulcanization increases elasticity of rubber but decreases plasticity[13]. The plasticity of ENR vulcanizates can be modified through blending with polyolefins under dynamic vulcanization to produce thermoplastic vulcanizate (TPV). During dynamic vulcanization, only the rubber phase is vulcanized upon molten-stage mixing with a thermoplastic at high temperature[31-33].

Studies of the dynamically vulcanized poly(vinyl chloride)/epoxidized natural rubber (PVC/ENR) blends have been carried out by some researchers[34-37]. They indicated that the blends showed a pseudoplastic behaviour and were processable as thermoplastic elastomers.

ENR can be dynamically vulcanized with polypropylene to prepare TPV based on the ENR/polypropylene (ENR/PP) blends. Nakason *et al.* demonstrated that the physical properties of the blends were influenced by various curing systems, i.e., sulfur, peroxide and a mixture of sulfur and peroxide-cured systems[38]. They found that the ENR/PP blends using the mixed-cure system exhibited higher values in mixing torque, shear stress, shear viscosity, tensile strength and elongation at break than those of the sulfur and peroxide-cured systems, respectively. They attributed these increments to a formation of S-S, C-S combination with C-C linkages in the ENR phase.

1.1.1.2 ENR blends

There are several methods to modify polymer properties. Although it is possible to synthesize a copolymer, it is much easier to blend two existing polymers to obtain the required properties of the two. In the thermodynamics of mixtures, the exothermic specific interactions between polymers have a profound influence on the phase behaviour of binary polymer blends. To form a miscible blend, the free energy of mixing must be negative, $\Delta G_m \leq 0$ and the second derivative with respect to composition $\partial^2 \Delta G_m / \partial \phi^2 > 0$, where

$$\Delta G_m = \Delta H_m - T\Delta S_m \qquad [1.1]$$

where, ΔH_m and ΔS_m are the enthalpy and entropy of mixing, respectively.

ϕ refers to the volume fraction of the polymer in the solution.

For high molecular weight polymers, ΔS_m is negligibly small and the sign of ΔG_m is dominated by ΔH_m. In general, ΔH_m is negative only if there are specific associative interactions between the two polymers. Therefore, the formation of miscible polymer blends depends on the occurrence of specific associative interactions between the constituents in a blend and this may be categorized as shown in Table 1.1.

For ENR, the epoxy groups are found to be effective in causing specific interactions in ENR/polymer blends[41,48,49]. The presence of epoxy groups in ENR introduces polarity and made the rubber more versatile for rubber-rubber or rubber-plastic blending[28].

Mohanty *et al.* has carried out a series of investigations to study the chemical reaction in the blends of ENR and poly(ethylene-*co*-acrylic acid) (PEA)[50-53]. They reported that the blends were miscible as the PEA proportion exceeded 50 wt %. This miscibility has been attributed to the esterification reaction between the *in-situ* generated –OH groups of ENR and the free –COOH groups of PEA during melt blending. In their further research, Mohanty *et al.* have developed ionomers from the blend of PEA and ENR[52]. Zinc ionomers were formed by neutralizing the blends with zinc oxide in the presence of zinc stearate.

Table 1.1

The specific interactions between the constituents in a miscible polymer blend

Specific interaction	Example of miscible blend	References
Hydrogen bonding	Blend of poly(vinyl chloride) (PVC) and epoxidized styrene-butadiene copolymer.	Margaritis *et al*. (1989)[39]
	Blend of polyurethane (PU) and the ethylene-methyl acrylate copolymer.	Santra *et al*. (1993)[40]
Dipole – dipole interaction	Blend of epoxidized natural rubber (ENR) and PVC.	Varughese *et al*. (1988)[41]
Ion – dipole interaction	Blend of polystyrene ionomers and poly(alkylene oxides).	Eisenberg and Hara (1984)[42]
Ion-ion interaction	Blend of polytetrafluoroethlene (PTFE) and poly(ethylene acrylate)	Murali and Eisenberg (1982)[43]
Intramolecular repulsive interaction	Blend of poly(methyl methacrylate) (PMMA) with the copolymers of styrene and acrylonitrile within a restricted range of copolymer composition.	Stein *et al*. (1974)[44]
Co-crystallization	Although it is a rare phenomenon, isomorphic polymer blends are miscible in both molten and crystalline states.	
	Blend of poly(vinyl fluoride) and poly(vinylidene fluoride).	Natta *et al*. (1965)[45]
	Blend of poly(isopropyl vinyl ether) and poly(sec-butyl vinyl ether)	Natta *et al*. (1969)[46]
	Blend of ultrahigh molecular weight polyethylene (UHMWPE) with high density polyethylene (HDPE) or low density polyethylene (LDPE)	Ree *et al*. (1987)[47]
Chemical reaction	It is either via an esterification reaction or by the formation of direct covalent bonds.	
	Blends of ENR (50 mol %) and poly(ethylene-co-acrylic acid) (PEA)(6 wt %) are partially miscible up to 50 % by weight of PEA and completely miscible beyond this proportion.	Mohanty *et al*. (1995)[48]

A number of studies have established that poly(vinyl chloride)/ENR, (PVC/ENR) blends are miscible[41,48,54-57]. A recent work by Ratnam and Zaman[58-62] indicated that the electron beam irradiation had enhanced the PVC/ENR blend homogeneity as well as the mechanical properties of the blend. They attributed this enhancement to the occurrence of irradiation-induced crosslinking in ENR, which was associated with the irradiation-induced ring opening side chain reaction of the epoxy group and irradiation-induced oxidation at the cis double bond. These irradiation-induced reactions were believed to be responsible for the elevated H-bonding interactions between ENR and PVC in the blends.

On the other hand, crosslinking of ENR with monobasic acid[11] or dibasic acid[10] via the formation of ester linkages has been reported elsewhere. This interaction was attributed to the ring opening of the epoxy groups by the acid groups.

Manna *et al.* has indicated that ENR can be chemically interacted with the precipitated silica upon blending at high temperature. They showed that a higher rate of interaction could occur in the rubber-filler blends with the presence of silane coupling agent, namely *N*-3-(*N*-vinyl benzyl amino) ethyl-γ-aminopropyl trimethoxy silane monohydrochloride[63]. This interaction was claimed due to the greater extent of polymer-filler coupling bond formation, where the epoxy groups of ENR were ring-opened by the silane coupling agent through its $-NH_2^+-$ groups, forming the C-N bonds between the coupling agent and ENR.

The recent work published by Yew *et al.* has shown that the addition of ENR improved the tensile properties of poly(lactic acid)/rice starch (PLA/RS) composites[64]. They also indicated that ENR played an important role in enhancing the degradability of the PLA/RS which was attributed to its polarity (the presence of epoxy groups). They showed that ENR could form favourable interaction with PLA and RS, presumably via hydrogen bonding. The hydrogen bonding was believed to occur between the epoxy group in ENR with the carbonyl group in PLA and the hydroxyl groups of RS.

1.1.1.3 Application

1.1.1.3.1 Tyres

Both ENR-50 and ENR-25 exhibit good wet grip characteristics and have been examined as tyre tread materials. In particular, ENR-25 compounds containing silica or silica/black have been found to give lower rolling resistance than NR and better wet grip than oil-extended styrene-butadiene rubber (OESBR), providing an ideal combination of these properties for tyre treads. On the other hand, the low air permeability properties of ENR-50 have also been utilized in the application in tyre inner liners and inner tubes.

1.1.1.3.2 Biodegradable thermoplastic elastomer

Recently, the addition of ENR to some biodegradable thermoplastic polymer has been studied by several researchers[64,65]. It has been proved that ENR can also play a crucial role in producing the biodegradable thermoplastic elastomer.

Mishra *et al.* has developed a green thermoplastic elastomer based on polycaprolactone/ENR (PCL) blend[65]. This PCL/ENR blend was biodegradable and could be employed as a heat shrinkable material. This material was prepared by crosslinking PCL/ENR blend by dicumyl peroxide during melt mixing process. Such heat shrinkable polymer has a potential application in the packaging industry, cable industry and heat shrinkable tube production[66].

Another biodegradable thermoplastic elastomer has been developed by Yew *et al.* recently[64]. They have demonstrated that the addition of ENR in poly(lactic acid)/rice starch composites (PLS/RS) led to some improvements in tensile properties as well as enhanced the biodegradability of the PLA/RS composites when exposed to water and α-amylase enzymatic treatments[64]. They attributed the enhancement in biodegradability to the polarity of ENR with the presence of epoxy groups.

1.1.1.3.3 General rubber goods

ENR with combination of improved properties has a wide range of application for general rubber goods[67]. The oil resistance of ENR is being utilized for an application in oil resistant cut thread. Besides, the fatigue lives of ENR compounds have been applied in an oil resistant flexible tubular conveyor or in combination with its higher damping properties for the application in vibration isolation mounts. The high wet grip properties of ENR have also being applied to produce non-slip flooring materials and sports shoe soling compounds. Furthermore, ENR has a promising application in adhesive. Due to its high adhesion to PVC, ENR-50 is being utilized as a cover compound for PVC core conveyor belting.

1.2 Alkyd

Alkyd resins are polyfunctional oil-modified polyesters, synthesized by reacting polybasic acids with polyhydric alcohols together with a vegetable oil, animal oil or its derivatives, via a step-wise polymerization process[68].

Kienle was the first person to introduce the term “Alkyd” in 1927[69], and is derived from “al” of alcohol and “cid” of acid, where “cid” was later changed to “kyd” for the sake of euphony[70]. Alkyd was the first synthetic polymer being applied in surface coatings industry[71]. During 1912 to 1915, the General Electric Co. demonstrated the film-forming capabilities and properties of the condensation products of polyhydric alcohols and polybasic acids, where the coatings binders were composed of glyptals, i.e.

reaction products of glycerol and phthalic anhydride. However, these glyptals have limited application due to their brittleness and they were later modified with fatty acids and oils, which were then termed as alkyds by Kienle.

Ever since alkyds were first introduced some 80 years ago, they have enjoyed a consistent annual growth[68]. Alkyds are one of the widely used synthetic resins in the paint and surface coating industry. Alkyd-based coating is well known for its fast dryness, good corrosion protection, high gloss and ease of application even over poorly treated surface. Besides, alkyds tend to be lower in cost yet give coatings that exhibit fewer film defects during application[72]. Since the alkyd reaction is one of the most versatile resin-forming reactions, the properties of alkyds can be modified through physical or chemical blending with other polymers[73]. Examples of polymers that commonly used to do physical blending with alkyds are nitrocellulose, urea-formadehyde, melamine-formadehyde, chlorinated rubber and chlorinated paraffin; whereas, polymers commonly used to modify alkyds chemically are styrene, phenolics, silicones, epoxies, isocyanates, formaldehyde. Whether the modification is physical or chemical, the properties and performance of the blended system will be enhanced than that of any individual resin, especially in flexibility, toughness, adhesion and durability of the coatings.

1.2.1 Alkyd raw materials

1.2.1.1 Polyhydric alcohol

A polyhydric alcohol is an alcohol with more than one hydroxyl group (-OH). Table 1.2 lists some examples of common polyhydric alcohols used for preparation of alkyd.

Table 1.2

Some of the common polyhydric alcohols used for preparation of alkyd

Bivalent alcohol	Formula
Ethylene glycol	$HO(CH_2)_2OH$
Propylene glycol	$CH_3CH(OH)CH_2OH$
1.3-butylene glycol	$CH_3CH(OH)CH_2CH_2OH$
Pentanediol	$HO(CH_2)_5OH$
Neopentyl glycol	$HOCH_2C(CH_3)_2CH_2OH$
Diethylene glycol	$HO(CH_2)_2O(CH_2)_2OH$
Triethylene glycol	$HO(CH_2)_2O(CH_2)_2O(CH_2)_2OH$
Dipropylene glycol	$CH_3CH(OH)CH_2OCH_2CH(OH)CH_3$
Trivalent alcohol	**Formula**
Glycerol	$HOCH_2CH(OH)CH_2OH$
Trimethylolethane	$CH_3C(CH_2OH)_3$
Trimethylolpropane	$CH_3CH_2C(CH_2OH)_3$
Polyvalent alcohol	**Formula**
Pentaerythritol	$C(CH_2OH)_4$
Dipentaerythritol	$(CH_2OH)_3CCH_2)_2O$
Sorbitol	$HOCH_2(CHOH)_4CH_2OH$

From Table 1.2, the most common polyhydric alcohols used in alkyd formulation are ethylene glycols, glycerol and pentaerythritol. As ethylene glycols are only bifunctional, they are mainly used to formulate unmodified resins which are thermoplastics that are linear and cannot be crosslinked except in the case of unsaturated polyesters. Longer chain glycols, such as propylene glycol and diethylene glycol will only provide the finished resins with even greater flexibility. Therefore, they are usually used together with glycerol in an alkyd formulation. If ethylene glycols are used with a drying oil via alcoholysis process, then the glycerol present in the oil becomes the determining factor in formation of resin.

Among these polyhydric alcohols, a trivalent alcohol, especially glycerol is preferable in common alkyd formulation due to its trifunctionality. With this trifunctionality, glycerol is able to add more branching in the alkyd macromolecule, providing a three-dimensional polymeric network and thus results in a high molecular weight resin. However, there is a difference in reactivity of the hydroxyl groups in glycerol, where the terminal hydroxyl groups ($-CH_2OH$) called primary or α-hydroxyl groups are alike the central group (-CHOH) contains a secondary or β-hydroxyl group. All the three hydroxyl groups react at about the same rate with fatty acids at 180-280°C, but the α-hydroxyl groups react more rapidly than the β-hydroxyl groups with dibasic acids, such as phthalic[74].

Pentaerythritol, $C(CH_2OH)_4$, is made by condensing formaldehyde and acetaldehyde in the presence of alkali and water. It is a solid material of high melting point (253°C), which has four primary reactive hydroxyl groups. It then forms a much more complex resin with phthalic anhydride than with glycerol. Hence, only medium-long and extra long oil length alkyds, with the content of fatty acids about 65-70 %, are commercially available in order to avoid rapid gelation during manufacturing[74]. However, its reactivity can be reduced, either by partial replacement with glycol (functionality 4 plus functionality 2 is equivalent to average functionality 3), or more easily by the use of larger proportions of modifying fatty acids. Because the four hydroxyl groups are equivalent in reactivity, pentaerythritol behaves more uniformly than glycerol when esterified, and the course of the esterification can be more readily predicted. Besides, the product molecules have a more symmetrical structure. However, commercial pentaerythritols are not pure, containing up to 15-20 % of dipentaerythritols which may affect the properties of the final alkyds as predicted. Owing to the excessive reactivity of pentaerythritol compared to glycerol, the manufacture of short and medium drying oil alkyds is almost impossible.

1.2.1.2 Polybasic acid

A polybasic acid is an acid containing at least two hydrogen atoms capable of substituting metal in a molecule or neutralizing a base, namely, an acid having a basicity of at least two. Examples of polybasic acids are saturated dibasic acids such as succinic acid, adipic acid, azelaic acid and sebacic acid; unsaturated dibasic acids are such as terephthalic acid, maleic acid, fumaric acid and isothalic acid; whereas, anhydrides of saturated dibasic acids or unsaturated dibasic acids are phthalic anhydride, maleic anhydride, glutaric anhydride and succinic anhydride. Figure 1.1 shows the chemical structures of the mentioned polybasic acids.

The saturated dibasic acids are usually for the manufacture of plasticizing alkyds. For example, the alkyds synthesized using adipic or sebacic acids are predominantly linear and less rigid.

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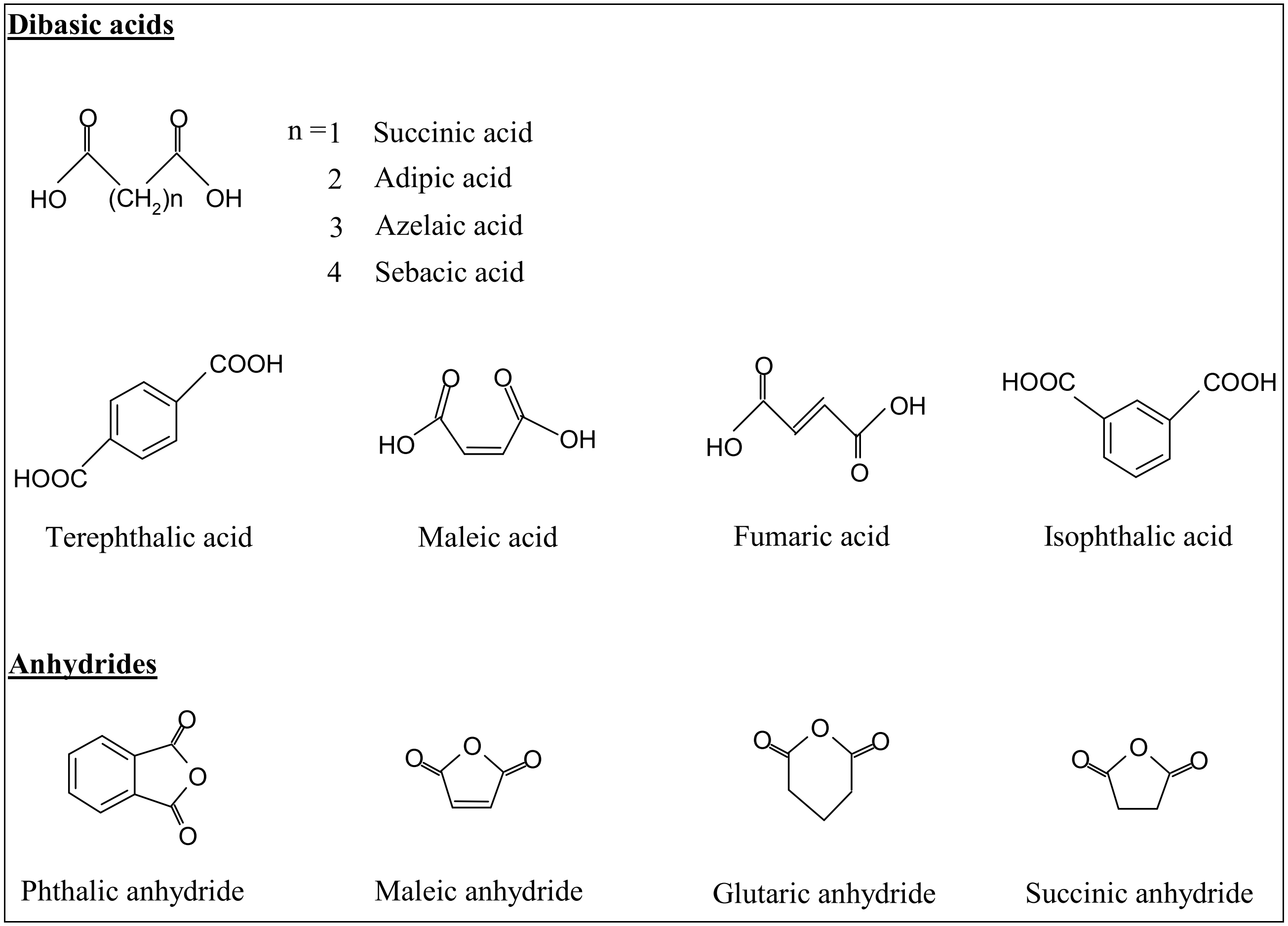


Figure 1.1: Some of the common polybasic acids used for preparation of alkyd

Among the polybasic acids, unsaturated polybasic acids or their anhydrides are preferable, from the reason that they are obtainable at a low cost and could form hydrogen bond among molecules and thus providing higher tackiness to the final alkyd. By far, the most widely used unsaturated polybasic acid is phthalic anhydride (PA). It has the advantage that the first esterification reaction proceeds rapidly by opening the anhydride ring and thus reduces the reaction time. PA also has a relatively low melting point, where the crystals can melt, dissolve and react readily in the reaction mixture at 131°C. The shorter time at lower reaction temperature can thus reduces the extent of side reactions of the polyol components.

Maleic acid, or more commonly maleic anhydride (MA), is usually used together with PA in the formulation of drying oil alkyds. Besides its difunctionality, MA also provides additional functionality arising from its double bond, which enables it to form the polyunsaturated alkyds. In normal alkyd manufacture, the use of MA is usually restricted to 1-10 % of the PA content[74]. Such replacement is sufficient to produce improvements in colour, processing time and water resistance when compared with maleic-free alkyds. However, the replacement of PA by MA in excessive quantities may cause gelation during synthesis.

Fumaric acid (FA) is treated similarly to MA. It is less toxic than MA, exhibits less loss by sublimation and is claimed to give a faster reaction leading to the formation of faster drying alkyd of better colour. However, compare to MA, FA requires a higher processing temperature for solubility and liberates an additional mode of water during processing.

Terephthalic acid (TPA) is made by oxidizing *p*-xylene or di-isopropyl benzene. It is used almost entirely in glycol-terephthalic ester resins for the production of synthetic fibres and films, with great strength, elasticity and relatively good chemical resistance.

Isophthalic acid (IPA) has a much higher melting point (354°C) than PA and thus is more difficult to use since it does not easily melt into the reaction mixture. High temperatures are required for longer times than with PA and hence more dimerization of fatty acids occurs with IPA, resulting in the final resin of higher viscosity. The longer reaction time at higher temperature also leads to greater extent of side reactions of the polyol components. However, polyesters of IPA are more resistant to hydrolysis than those of PA in the pH range of 4 to 8, the most important range for exterior durability of coating.

1.2.1.3 Vegetable or animal oils

Vegetable or animal oils are the modifier in an alkyd formulation. Examples of vegetable oils are palm oil, palm kernel oil, soybean oil, olive oil, colza oil, sesame oil, wood oil, castor oil and linseed oil; whereas, animal oils are such as fish oil, whale oil, beef oil, mutton oil and hoof oil. Natural resins such as rosin, amber and shellac; and synthetic resins such as ester gum, phenol resin, carbon resin and melamine resin, also have been reported as a modifier in the alkyd formulation.

From the recent trend of emphasizing environmental friendly, it is preferable to employ an alkyd resin derived from resources other than petroleum. Hence, naturally occurring oils, either vegetable or animal, should be utilized in alkyd formulation.

In fact, the naturally occurring oils consist of triglycerides, tri-esters of glycerol and fatty acids. Some triglycerides are drying oils, but many are not. Alkyds formulated from drying oils are able to air-dry at ambient temperature, when the oils react with oxygen, crosslink and form solid films. The reactivity of drying oils with oxygen results from the presence of diallylic groups, (i.e., two double bonds are separated by some methylene groups, $-CH=CHCH_2CH=CH-$) or conjugated double bonds. Some authors classify oils as drying, semidrying and non-drying based on their iodine value[75]. Although iodine values can serve as satisfactory quality control specifications, they are sometime not useful and can be misleading in defining a drying oil or for predicting reactivity. A useful empirical relationship is that non-conjugated oils that have a drying index greater than 70 are considered as drying oils[76]. The drying index is calculated as follows:

$$\text{Drying index} = (\%\ \text{linoleic acid}) + 2(\%\ \text{linolenic acid}) \qquad [1.2]$$

1.2.2 Classification of alkyd

There are many ways of classifying alkyds. Generally, alkyd resin is classified into different oil length (OL) based on the amount of oil (or fatty acid) in its formulation[77,78] and can be expressed in equations [1.3] and [1.4], respectively.

$$\text{Oil length} = \frac{W_{Oil}}{W_{Alk} - W_{H_2O}} \times 100\% \qquad [1.3]$$

$$\text{Oil length} = \frac{1.04 \times W_{FA}}{W_{Alk} - W_{H_2O}} \times 100\% \qquad [1.4]$$

where, W_{Oil} is the weight of "oil", W_{Alk} is the weight of the finished alkyd, W_{H_2O} is the weight of water evolved during esterification and W_{FA} is the weight of fatty acids. The 1.04 factor in equation [1.3] converts the weight of fatty acids to the corresponding weight of triglyceride oil.

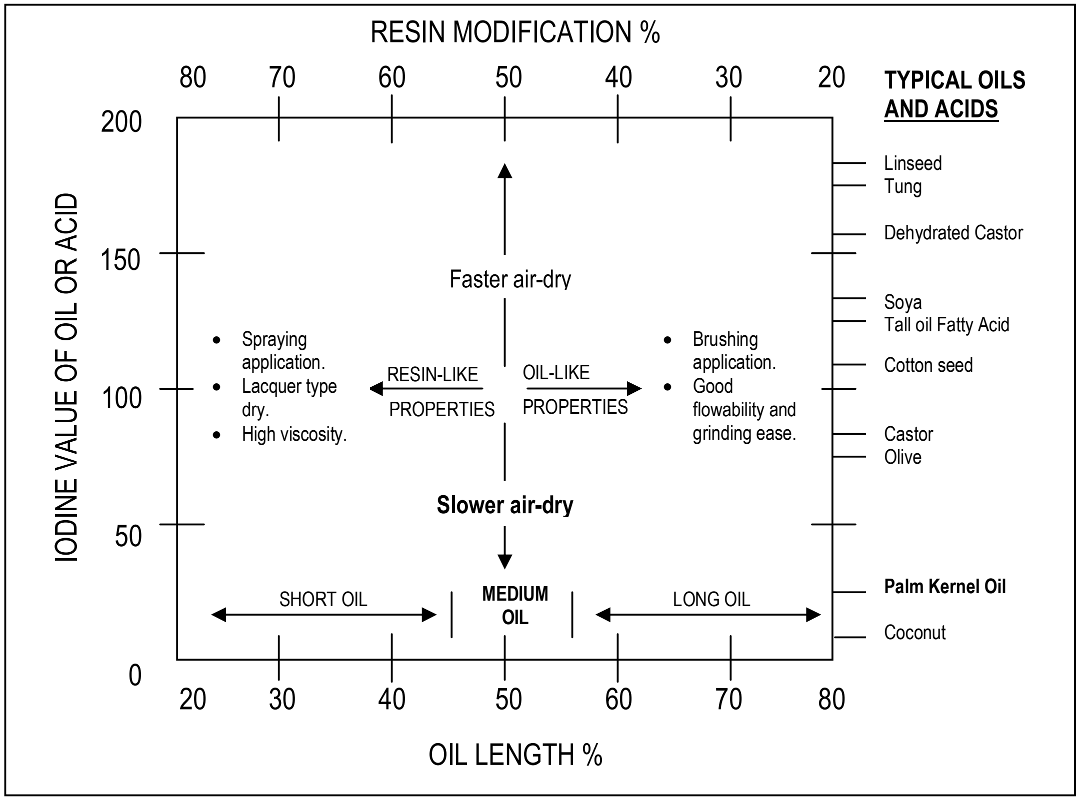


Figure 1.2: The properties to be expected from an alkyd of different oil length and iodine number

(Adapted from Alkyd Resin Technology[79] , page 175)

Figure 1.2 shows the properties to be expected from the alkyd of different oil length and iodine number[79]. From this figure, short oil length alkyds show resin-like properties which have higher viscosity, tackiness and impart the hardness of a coating; whereas, the long oil length alkyds show oil-like properties, where they are softer, having higher flowability and form coating with less tackiness. The medium oil length alkyd corresponds to an even weight mixture of resin-like and oil-like properties.

Another classification is to classify alkyds into oxidizing and non-oxidizing types[80]. An oxidizing alkyd contains drying (unsaturated) or semi-drying oils or fatty acids and often have an oil length in excess of 45 %. A non-oxidizing alkyd contains non-drying (saturated) oils or fatty acids and therefore the alkyd is not capable of forming coherent film by air oxidation. Hence, the non-oxidizing alkyd is used as polymeric plasticizer or as hydroxyl-functional resin, which is crosslinked by melamine-formadehyde or urea-formadehyde resins or by isocyanate crosslinkers. The oil length for non-oxidizing alkyds is usually formulated below than 45 %[80].

The other classification is by distinguishing the unmodified with modified alkyds. Modified alkyds contain other monomers in addition to polyhydric alcohols, polybasic acids and fatty acids. Examples are styrenated alkyds[81-83] and silicone alkyds[84-86].

1.2.3 Alkyd preparation process

1.2.3.1 Synthesis from oils or fatty acids

Alkyds that are formulated from an identical chemical composition will exhibit different properties and performance depending on their preparation processes. There are various procedures in preparing alkyd resins[87-89]. In general, alkyds can be prepared from two processes, i.e. fatty acid and alcoholysis (or monoglyceride).

In the fatty acid process, alkyds are prepared from fatty acids. Such alkyds can be produced in various methods and the three simplest and common methods are shown as follows.

The first process can be performed in a single step where all the raw materials, polyhydric alcohol, fatty acid and polybasic acid, are added together at the start of the reaction and the esterification is carried out simultaneously in range of 220-250°C[80]. Thus, there is a free competition among the –COOH groups with the –OH groups. An alkyd with more branching is synthesized.

The second process can be conducted in two steps. The polyhydric alcohol (glycerol) and polybasic acid (PA) are first reacted together at 180°C until a soft clear viscous resin is obtained. Then, warm fatty acids are added and the reaction temperature is raised to 180-220°C[80]. In this case, the –COOH groups of PA are deliberately reacted with the primary –OH groups of glycerol to form glyceryl phthalate before fatty acid is added. Therefore, the –COOH groups of fatty acid are forced to esterify with the leftover –OH groups of glyceryl phthalate. Hence, the alkyd's backbone is linear.

The third process was carried out under a step-by-step esterification of the fatty acids, which involves a stepwise addition of fatty acids, from 40-90 %, at 180-250°C[90]. By withholding a part of this chain-terminating ingredient, the polymer is predominantly linear and of high molecular weight.

On the other hand, in the alcoholysis (monoglyceride) process, alkyds are prepared from glyceride oil in a two-stage procedure. The first stage in the process is to convert the polyhydric alcohol and glyceride oil into a single homogeneous monoglyceride phase at 230-250°C in the presence of a catalyst. At the second stage, this monoglyceride in turn provides a solvent for the polybasic acid added next to react under esterification in order to complete the alkyd reaction at 180-250°C. This process is usually applied when PA is used as the polybasic acid since PA is not soluble in the oil but is soluble in the glycerol. Hence, transesterification of oil with glycerol must be carried out as a separate step before PA is added or otherwise glyceryl phthalate gel particles would form early in the first stage of the process. The comparisons of the characteristics of the two processes are summarized in Table 1.3.

Table 1.3

Characteristics of the alkyd preparation process

Preparation process	Characteristics
Alcoholysis (monoglyceride)	a) The rate of esterification slows down at higher acid value. b) Boding and gelation occur at slightly higher acid value. c) The alkyd tends to be marginally softer and tackier. d) The alkyd tolerates more aliphatic hydrocarbon thinner.
Fatty acid	a) Low acid value is more rapidly attained. b) Bodying and gelation occur at lower acid value. c) The alkyd tends to be harder and less tacky. d) The alkyd tolerates less aliphatic hydrocarbon thinner.

Goldsmith has proposed a plausible explanation for the difference of the alkyds' characteristics prepared by different processes[91]. He indicated that the differences were due to the differential rates of reaction between –OH and –COOH groups of raw materials, depending on their specific location on the parent molecules. Figure 1.3 illustrates the chemical structures proposed for alkyds formed by fatty acid procedure and Figure 1.4 illustrates the chemical structures proposed for alkyds formed by alcoholysis (monoglyceride) procedure. Comparisons of the proposed chemical structures can be made by considering these alkyds are synthesized from the same raw materials and formulations of fatty acid, PA and glycerol.

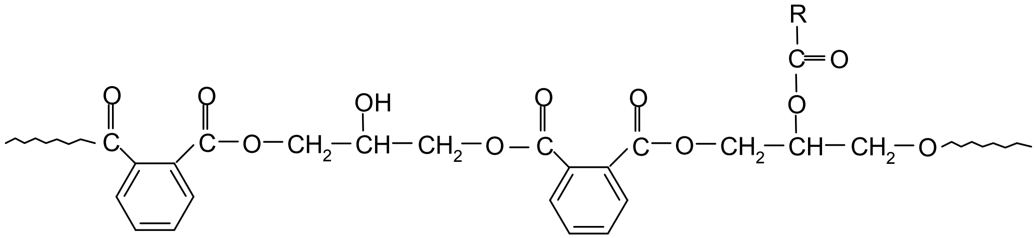
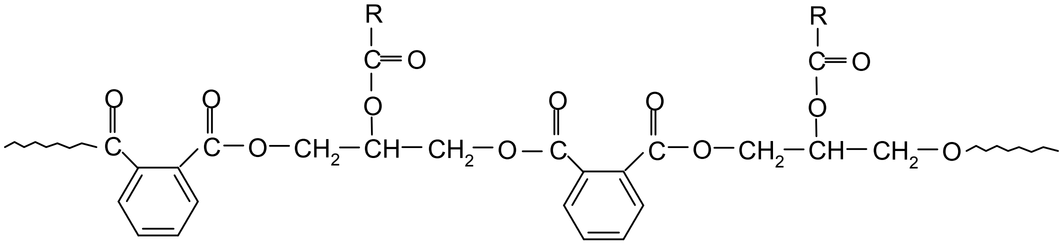
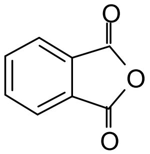
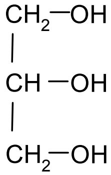
Alkyd	Fatty acid procedure
Short oil length 1 mole FA 2 moles PA 2 moles Glycerol	
Long oil length 2 moles FA 2 moles PA 2 moles Glycerol	
Where, R-COOH Fatty acid	 Phthalic anhydride  Glycerol

Figure 1.3: Chemical structure proposed for alkyds formed by fatty acid procedure

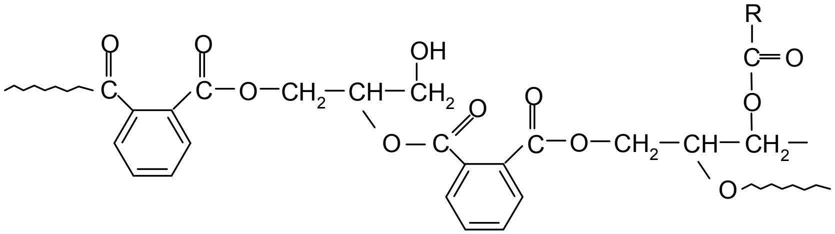
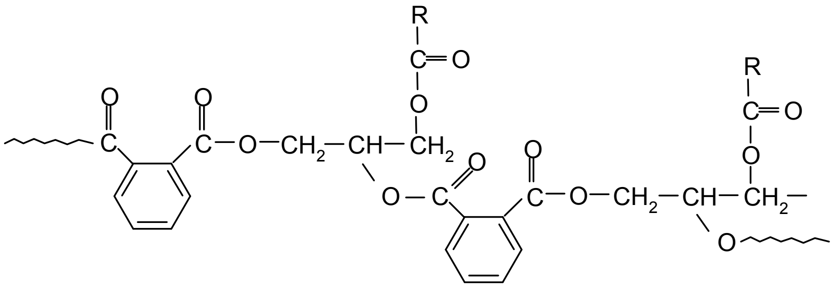
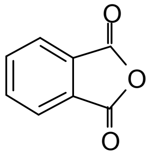
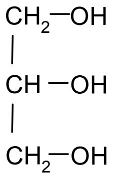
Alkyd	Alcoholysis procedure
Short oil length 1 mole FA 2 moles PA 2 moles Glycerol	
Long oil length 2 moles FA 2 moles PA 2 moles Glycerol	
Where, R-COOH Fatty acid	 Phthalic anhydride  CH_2-OH, $CH-OH$, CH_2-OH Glycerol

Figure 1.4: Chemical structure proposed for alkyds formed by alcoholysis (monoglyceride) procedure

From Figure 1.3, in the fatty acid preparation process, there is a free competition between the –COOH groups of fatty acids and PA, with the –OH groups of glycerol, assumed that all added at the beginning. Compare to PA, the long hydrocarbon chain in fatty acid creates steric hindrance and acts as a structural block in order to esterify with the nearby hydroxyl groups. Therefore, the –COOH groups of fatty acid that lag behind in esterification with the primary –OH groups in glycerol have to settle for connections with secondary –OH groups.

As shown in Figure 1.4, in the alcoholysis (monoglyceride) process, the competition is more rigged, where the –COOH groups of fatty acid are deliberately reacted with the primary groups of glycerol before any PA is added. Thus, the –COOH groups of the diacid PA are placed at a competitive disadvantage and are forced to react with the leftover –OH groups.

1.2.3.2 Process variation

Esterification is a reversible reaction; therefore, the rate of removal of water from the reactor is an important factor affecting the rate of esterification. Alkyds can be formed by esterification[70], where the reaction occurs between the –COOH and the –OH groups either belong to the same molecule or in two different compounds according to the reaction as follows:

$$\text{R-COOH} + \text{R'-OH} \longleftrightarrow \text{RCOOR'} + \text{H}_2\text{O}$$

For the alkyds synthesized from anhydrides, the reaction involves two distinct steps, as shown in Figure 1.5. In the first step, esterification proceeds rapidly by opening the anhydride ring, which happens at a lower temperature and caused the formation of half-esters. There is no water of reaction evolved. On further heating, the second step continues and long chain molecules are produced which contain free hydroxyl groups in excess. This step happens at a slower rate and water of reaction is released[70].

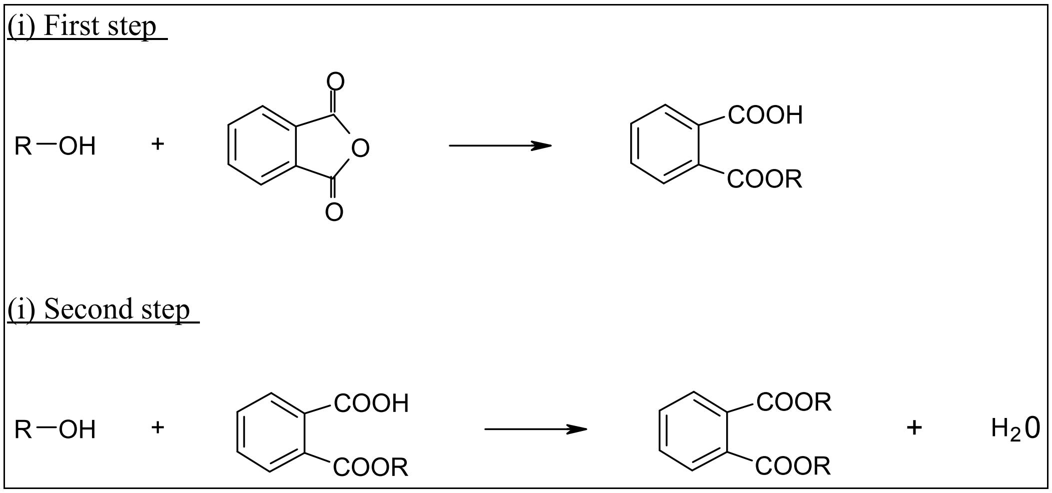


Figure 1.5: The esterification reaction for the alkyds synthesized from anhydrides

Esterification can be conducted either in the absence of solvent (fusion cook) or with the presence of solvent (solvent cook).

In a fusion cook, a flow of inert gas is usually maintained through the reactor, to prevent ingress of air and to remove water of reaction. There is a considerable loss of volatile reactants and PA sublimation (in the reflux condenser) during a fusion cook, particularly at higher temperature. Therefore, fusion cook is generally applied in the formulation of long oil length alkyds in order to reduce the effect of unanticipated losses on the predicted functionality.

In a solvent cook, the presence of solvent facilitates the removal of the water of reaction, allowing a shorter processing time being achieved without heating to excessively high temperature, where a typical temperature ranges for solvent process is around 200-240°C. The solvent vapour blanket serves as an inert atmosphere, preventing ingress of air, thus enabling light colour products to be polymerized with a minimum of inert gas usage. Furthermore, the accumulation of sublimed solid monomers, mainly PA, in the reflux condenser, can be returned into the reactor together with solvent reflux.

1.2.3.3 Progress of reaction

Both the acidity and viscosity of an alkyd during esterification are affected by the reaction temperature and reaction time. Figure 1.6 and Figure 1.7 show the relationship of the reaction temperature, time, viscosity and acid number during the preparation of a typical medium oil linseed alkyd[89]. Hence, the choice of a reaction temperature is important in an alkyd synthesis, where the temperature must allow the reaction to be carried out within a reasonable time period to reduce the operation cost, yet not so elevated to cause destructive decomposition, discolouration and an excessive loss of volatile material during the reaction.

From Figure 1.6 and Figure 1.7, it is no doubt that the progress of reaction in an alkyd preparation can be followed and controlled by periodically checking the acidity and viscosity, where the disappearance of carboxylic acid is followed by titration and increase in molecular weight is followed by viscosity, commonly using Gardner bubble tubes or rheometer. However, determination of acid number and viscosity takes some time in sampling as the reaction is continuing in the reactor. While time limit is required for viscosity determination, viscosity also depends strongly on solution concentration and temperature. Hence, acid number titration is generally chosen as a more convenient method to monitor the progress of reaction.

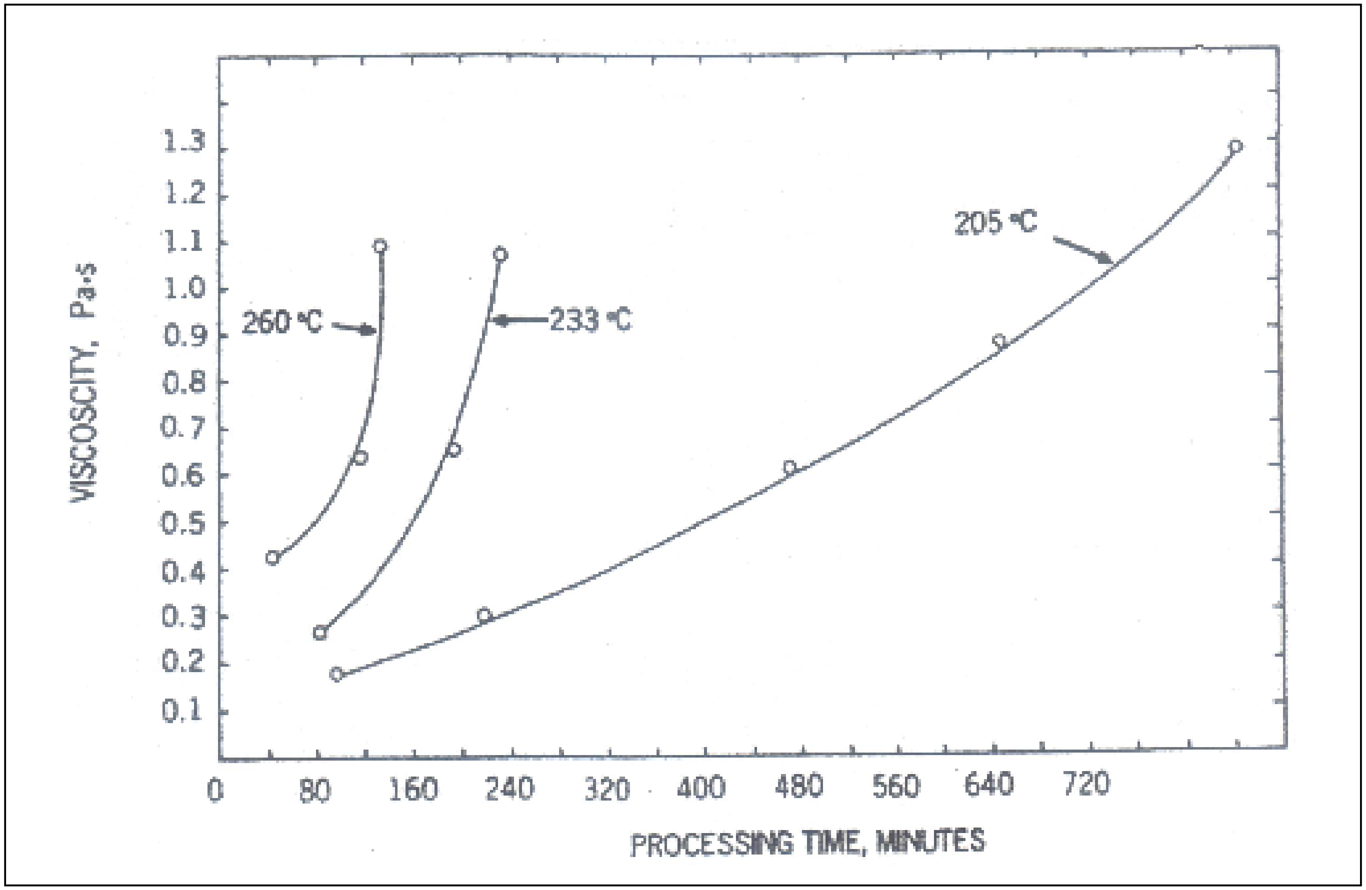


Figure 1.6: Effect of esterification temperature and reaction time on viscosity of a typical medium oil linseed alkyd

(Adapted from The Chemistry and Processing of Alkyd Resins[89])

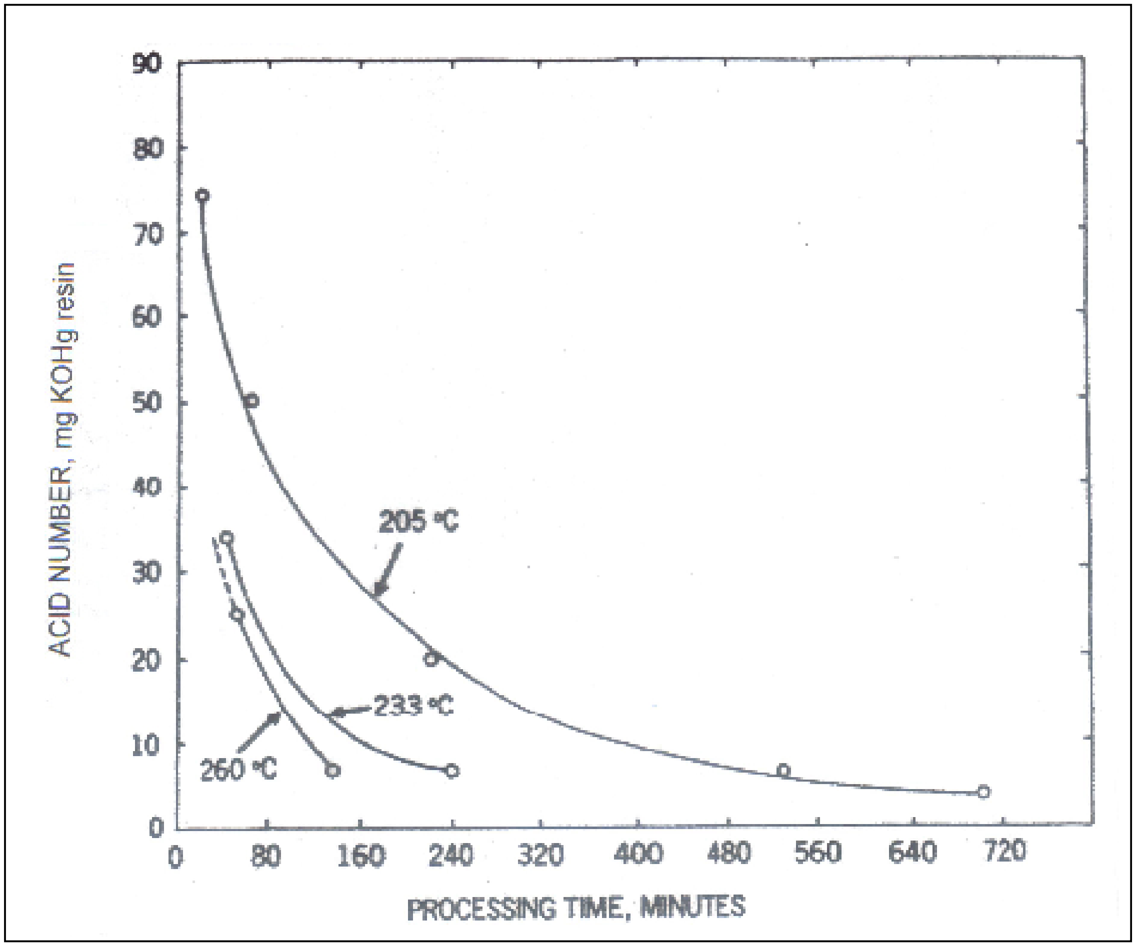
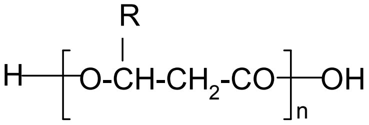


Figure 1.7: Effect of esterification temperature and reaction time on acid value of a typical medium oil linseed alkyd

(Adapted from The Chemistry and Processing of Alkyd Resins[89])

1.3 Polyhydroxyalkanoates

Polyesters of the microbial polyhydroxyalkanoates (PHA) family are thermoplastic in nature and they have biodegradable and biocompatible properties[92-96]. They are polyesters of various HAs which are synthesized by a variety of microorganisms as carbon or energy reserve materials. They are synthesized and intracellularly accumulated as distinct granules, usually when an essential nutrient such as nitrogen or phosphorus is available only in limiting concentrations in the presence of excess carbon source[94,97]. PHAs can be completely degraded to water and carbon dioxide under aerobic conditions and to methane under anaerobic conditions by microorganisms. Generally, PHA can be represented by the following structure, where each molecule of the PHA has one hydroxyl and one carboxylic terminal group.



$$H-\left[O-\overset{R}{\overset{|}{C}}H-CH_2-CO\right]_n-OH$$

where, R stands for an aliphatic group with 3,5,7,9, and 11 carbons.

Poly(3-hydroxybutyrate) [P(3HB)] was the first PHA to be discovered in 1925 by Lemoigne[98], who isolated the polymer from *Bacillus megaterium* by chloroform extraction and demonstrated that it was a polyester of 3-hydroxybutyric acid[99,100]. The general knowledge of microbial P(3HB) was then summarized in a comprehensive review by Dawes and Senior in 1973[101].

In fact, PHA possess properties similar to various synthetic thermoplastics like polypropylene and hence can be extruded, moulded, spun into fibres, made into films and used to make heteropolymers with other synthetic polymers. In spite of its numerous advantages, PHA has not yet been able to replace conventional plastics on a large scale due to its high cost.

1.3.1 Classification of PHA

PHAs can be divided into two groups depending on the number of carbon atoms in the chain, i.e. short-chain length (scl) and medium-chain length (mcl). The difference is mainly due to the substrate specificity of the PHA synthases that can accept 3HAs of a certain range of carbon length[102]. The PHA synthase of *Alcaligenes eutrophus* can polymerise 3HAs consisting of 3-5 carbon atoms to produce scl-PHAs. Whereas, the PHA synthase that is present in *Pseudomonas oleovorans* can only accept 3HAs of 6-14 carbon atoms and thus produces mcl-PHAs consisting of 6-14 carbon atoms.

Polymerization imparts the [R]-stereo-chemical configuration to the repeat units, resulting in fully isotactic polyesters[103-106]. This stereoregularity enhances crystallization so that the scl-PHAs with short alkyl side chains, e.g. poly-3-hydroxybutyrate, poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) are highly crystalline; whereas the mcl-PHAs with longer side chains, e.g. poly-3-hydroxyoctanoate, PHO tend to be more amorphous and elastomeric[107].

Many species of Pseudomonas are able to produce large quantities of mcl-PHA from different substrates such as alkanes, alkenes, free fatty acids, and triacyglycerols[108-114]. A mcl-PHA with a number of potential applications, including biodegradable elastomers and adhesives, can be produced when these bacteria are provided with a substrate high in unsaturated fatty acids, such as soybean oil or linseed oil. These olefinic groups can be further chemically modified to produce polymers with different properties.

However, the mcl-PHAs exhibit low melting temperatures (T_m) that vary between 40 and 60°C and glass transition temperature (T_g) values well below 0°C[115-117]. As a result the mcl-PHA polymers tend to soften at ambient temperatures, an effect that seriously limits potential application as biodegradable elastomers. This can be overcome by crosslinking the unsaturated side chains.

1.3.2 PHA biosynthesis

There are four different pathways for the synthesis of PHAs found to date[94,97] as shown in Figure 1.8[119].

In *R. eutropha*, β-ketothiolase carries out the condensation of two molecules of acetyl-CoA to acetoacetyl-CoA. An NADPH-dependent acetoacetyl-CoA reductase then carries out its conversion to 3-hydroxybutyryl-CoA. The third and the final step is the polymerisation reaction catalysed by PHA synthase[102].

In *R. rubrum*, the pathway differs after the second step where the acetoacetyl-CoA formed by β-ketothiolase is reduced by a NADH dependent reductase to L-(+)-3-hydroxybutyryl-CoA which is then converted to D-(–)-3-hydroxybutyryl-CoA by two enoyl-CoA hydratases.

A third type of PHA biosynthesis pathway is found in most *Pseudomonas* species belonging to rRNA homology group I. *P. oleovorans* and other *Pseudomonas* species accumulate PHA consisting of mcl 3-hydroxyalkanoic acid if cells are cultivated on alkanes, alkanols or alkanoic acids[108-110].

The fourth type of PHA biosynthetic pathway is present in almost all *Pseudomonas* species belonging to rRNA homology group II. This pathway involves the synthesis of copolyesters consisting of mcl-3HAs from acetyl-CoA. This pathway has not been studied in detail[118].

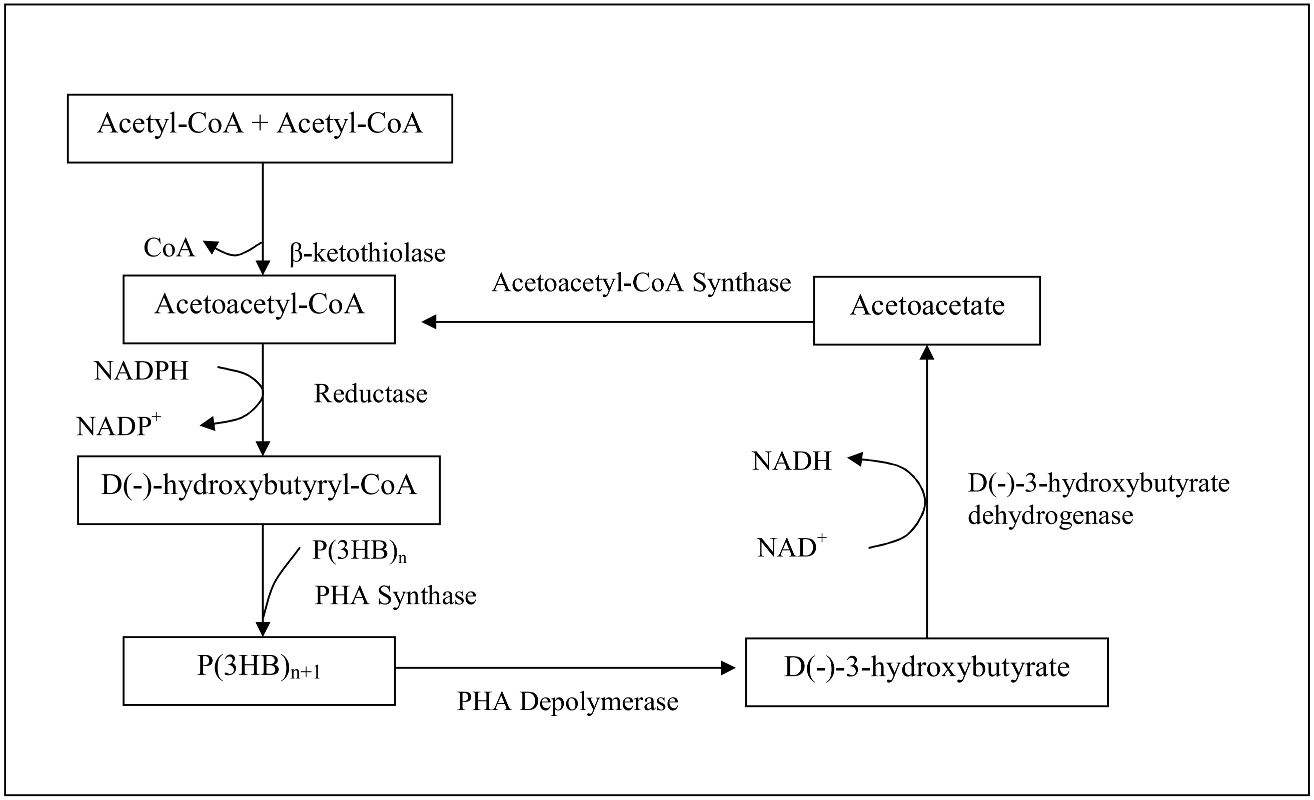


Figure 1.8: Metabolic pathway involved in the synthesis and breakdown of PHB in *R. eutropha*[119]

1.3.3 PHA production

Selection of microorganism for the production of PHA is based on several factors including the cell's ability to utilise an inexpensive carbon source, growth rate, polymer synthesis rate and the maximum extent of polymer accumulation. Preliminary calculation of PHA yields can be done by using equation for prediction of overall yields[120]. Besides, recovery of PHA is usually considered in PHA production as it significantly affects the overall economics. Table 1.4 shows the production of PHA by various bacteria[121].

In general, bacteria that are used for the production of PHAs can be divided into two main groups based on the culture conditions required for PHA synthesis. The first group of bacteria requires the limitation of an essential nutrient such as nitrogen, phosphorous, magnesium or sulfur for the synthesis of PHA from an excess carbon source. The bacteria included in this group are *R. eutropha* (previously known as *A. eutrophus*), *P. extorquens*, and *P. oleovorans*. The second group of bacteria do not require nutrient limitation for PHA synthesis and can accumulate polymer during growth. These bacteria include *Alcaligenes latus*, a mutant strain of *Azotobacter vinelandii*, and recombinant *E. coli*.

Table 1.4

Production of PHA by various bacteria[121]

Microorganism	Carbon source	PHA	PHA content (% w/v)	References
R. eutropha	Gluconate Propionate Octanoate	PHB PHB PHB	46-85 26-36 38-45	Liebergesell *et al*. (1994)[122]
Bacillus megaterium QMB1551	Glucose	PHB	20	Mirtha *et al*. (1995)[123]
Klebsiella aerogenes recombinants	Molasses	PHB	65	Zhang *et al*. (1994)[124]
Methylobacterium rhodesianum MB1267	Fructose/methanol	PHB	30	Ackermann and Babel (1997)[125]
M. extorquens (ATCC55366)	Methanol	PHB	40-46	Borque *et al*. (1995)[126]
Pseudomonas aeruginosa	Euphorbia and castor oil	PHA	20-30	Eggink *et al*. (1995)[111]
P. denitrificans	Methanol Pentanol	P(3HV) P(3HV)	0.02 55	Yamane *et al*. (1996)[127]
P. oleovorans	Glucanoate Octanoate	PHB PHB	1.1-5.0 50-68	Liebergesell *et al*. (1994)[122]
P. putida GPp104	Octanoate	PHB	14-22	Liebergesell *et al*. (1994)[122]
P. putida	Palm kernel oil	PHA	37	Tan *et al*. (1997)[128]
	Lauric acid Myristic acid Oleic acid	PHA PHA PHA	25 28 19	
P. putida BM01	11-Phenoxyun-decanoic acid	5POHV	15-35	Song and Yoon (1996)[129]
Sphaerotilus natans	Glucose	PHB	40	Takeda *et al*. (1995)[130]

where, PHB is polyhydroxybutyric acid, P(3HV) is polyhydroxyvaleric acid and 5POHV is poly(3 hydroxy-5-phenylvalerate).

The production of PHA can be normally done by batch or continuous fermentation using selected bacterial cultures in the presence of a variety of carbon substrates such as glucose, gluconates or methanol as the feed and a salt medium containing dissolved oxygen, ammonium, sulphate, phosphate, potassium or irons as nutrients[118].

For the fed-batch culture of bacteria belonging to the first group, a two-step cultivation method is often employed. In the first stage, a desired concentration of biomass is obtained without nutrient limitation and in the second stage essential nutrient is kept in limiting concentration to allow efficient PHA synthesis. For the cultivation of these bacteria, a limited mixture of carbon source and a nutrient should be fed at an optimal ratio to produce PHA with high productivity.

For the fed-batch culture of bacteria belonging to the second group, a nutrient feeding strategy is important to obtain a high yield of PHAs. Since PHA synthesis is not dependent on nutrient limitation in these bacteria, complex nitrogen sources such as steep liquor, yeast extract of fish peptone can be supplemented to enhance cell growth as well as polymer accumulation. Cell growth and PHA accumulation need to be balanced to avoid incomplete accumulation of PHA or premature termination of fermentation at low cell concentration.

On the other hand, a wide variety of PHA copolymers have also been reported to be isolated from different environmental resources, such as fresh water and marine cyanobacteria, marine sediments and sewage sludges[103,131,132].

1.3.4 PHA recovery

After the fermentation is over, the cells containing PHA are separated from fermentation broth by centrifugation. The harvested cells are then lysed for recovery of PHAs. A number of PHA recovery methods have been suggested[133]. Extraction using solvents such as chloroform[99], methylene chloride[134], propylene carbonate[135] and dichloroethane[136] can result in very pure PHA[137,138]. Usually, various lipids are co-extrated together with PHAs from the cells. To remove lipids, the PHAs are precipitated slowly by adding diethyl ether, hexane or lower alcohol as non-solvents. PHAs can be further purified by reprecipitation from chloroform solution. Alternatively, the bacterial cells are selectively destroyed and dissolved by treatment with dilute sodium hypochlorite solution for 30-60 minutes, where longer time may result in degradation of the PHAs as well. The isolated PHA granules are then purified and made free of lipids by washing with diethyl ether or methanol. Poly(3- hydroxybutyrate), P(3HB) granules can also be isolated by treating bacterial cells with enzymes that solubilize all of the cell components except P(3HB).

Hahn *et al.* has reported that PHA recovery using a dispersion of chloroform and sodium hypochlorite solution has the advantage of less polymer degradation, but this dispersion process also requires a large amount of solvent[139]. However, this extraction method requires large quantities of toxic and volatile solvent, which not only increases the total production cost but also has adverse environmental consequences[140].

Steinbüchel and Valentin indicated that the PHA granules themselves do not contain much contaminant[141]. Highly pure PHA can be produced by processes that break the cell and solubilize cellular material other than PHA. Therefore, a simple digestion method by inexpensive chemicals seems to be the most efficient and economical recovery process. Recently, Choi and Lee had developed a simple alkaline digestion method for the recovery of P(3HB) from recombinant *E. coli* cells[133].

On the other hand, ICI has developed a simple and economic process used to isolate P(3HB) granules from cells with an enzyme[142,143]. In general, PHA isolation by enzyme treatment involves the sonic oscillation treatment of an aqueous suspension of lysozyme-treatment cells to liberate the polymer, as reported by Griebel *et al.* in isolation of P(3HB) granules[144]. This procedure can solubilize all of the cell components except for P(3HB).

1.3.5 Quantification of PHA

PHA exists as discrete inclusions that are typically 0.2 ± 0.5 mm in diameter localized in the cell cytoplasm and can be visualized with a phase contrast light microscope due to their high refractivity[101]. When thin sections of PHA-containing bacteria are observed under transmission electron microscopy, the PHA inclusions appear as electron-dense bodies. Native PHA inclusions can be stained with Sudan Black[145] indicating that they are of a lipid nature[146,147]. Besides Sudan Black B, PHA is more specifically stained by the oxazine dye Nile Blue A, exhibiting a strong orange fluorescence at an excitation wavelength of 460nm[148]. While staining methods can be used to identify the presence of PHA, chemical analysis is often required to determine their monomeric compositions.

Several methods allow for the quantification of PHA compositions have been reported. The use of gas chromatography (GC) for the identification of PHA components was proposed. This method involves simultaneous extraction and methanolysis of PHA, in mild acid or alkaline conditions, to form hydroxyalkanoate methylesters which are then analysed by GC[149]. Another GC method for increasing PHA recovery was proposed by carrying out propanolysis in HCl[150] rather than acidic methanolysis in sulfuric acid.

Other recent methods for PHA quantification include HPLC[151], ionic chromatography, and enzymic determination[152]. HPLC measures only PHB and is based on conversion of PHB to crotonic acid followed by UV detection at 210 nm. PHA detection by ionic chromatography is based on the conversion of monomers to alkanoic acids. The determination involves acid propanolysis followed by alkaline hydrolysis with $Ca(OH)_2$ or acidic hydrolysis with concentrated H_2SO_4. After centrifugation, the sample is injected into an anionic column with a conductivity detector.

An enzymic estimation method was developed by Roche Molecular Biochemicals, USA (No. 127833)[152]. The 3HB is enzymically oxidized and the NADH produced from NAD^+ was reoxidised in the presence of iodonitro-tetrazoliumchloride to produce formazan, which was spectrophotometerically measured at 492 nm.

Recently, the determination of PHA inside intact cells by two-dimensional fluorescence spectroscopy and flow cytometry had also been proposed[153,154]. Cells stained with Nile Blue, show a clear fluorescence maximum between 570 nm and 605 nm when excited between 540 nm and 560 nm. A good correlation between fluorescence intensity and PHB concentration was obtained. However, differentiation of PHA composition was not possible with this method[154].

1.3.6 PHA properties

The physical properties of PHAs can be regulated by varying molecular structure, copolymer composition and chain length. Copolyesters ranging from hard crystalline thermoplastics to soft elastic rubbers can thus be made.

PHA products such as films, fibres, moulded objects and extruded profiles are environmentally biodegradable in soil, sludge and sea water, where the degradation can occurs very fast under optimal conditions. It has been found that the rate of biodegradation was influenced by a number of factors such as microbial population in a given environment, temperature, moisture level, pH, nutrient supply as well as composition, crystallinity, additives and surface area of PHA itself. Some microorganisms such as bacteria and fungi, *Pseudomonas lemoiguei* and *Alcaligenes faecalis* excrete extracellular P(3HA) that depolymerises enzymes to degrade the PHAs and utilize the intermediates as nutrients. This enzymatic degradation commonly occurs at the surface of the PHA item and the rate of surface erosion is strongly dependent upon both the molecular structure and copolymer composition of the PHAs. Table 1.5 shows the examples of some PHA degrading micro-organisms isolated from various environments[118,155-162].

Table 1.5

PHA degrading micro-organisms isolated from various environments[118]

Source from which isolated	Micro-organisms	Reference
Soil	*Aspergillus fumigatus* *Acidovorax faecalis* *Comamonas sp.* *Pseudomomas lemoignei* *Variovorax paradoxus*	Mergaert *et al*. (1993)[155] Mergaert *et al*. (1993)[155] Jendrossek *et al*. (1993)[156] Delafield *et al*. (1965)[157] Mergaert *et al*. (1993)[155]
Activated sludge	*A. faecalis* *Pseudomonas fluorescens*	Tanio *et al*. (1982)[158] Schirmer *et al*. (1993)[159]
Sea water	*Comamomas testosterone*	Kasuya *et al*. (1994)[160]
Lake water	*P. stutzeri*	Mukai *et al*. (1994)[161]
Anaerobic sludge	*Ilyobacter delafieldii*	Jansen *et al*. (1990)[162]

1.3.7 PHA application

Plastics are an essential part in our daily life and of almost all industries. Due to their versatile qualities of strength, lightness, durability and resistance to degradation, plastics have replaced glass, paper and metal in packaging, construction, health care and sanitation, agriculture and furniture. However, accumulation of recalcitrant plastics in the environment has become a serious problem. About a million marine animals face death every year by choking on floating plastic waste items that are mistaken by them as food items or by otherwise getting entangled in non-degradable plastic debris. Thus, the concept of biodegradable plastics is the key to solve the waste disposal problem. Biodegradable plastics can be divided into three categories as shown in Table 1.6[118]. Table 1.7 lists some of the manufacturers which are intensely involved in the manufacture and marketing of biodegradable plastics[121].

Table 1.6

Characteristics of the biodegradable plastics[118]

Biodegradable plastics	Characteristics
Chemically synthesized polymer	They are susceptible to enzymic or microbial attack. Since they do not match all the properties of plastics, they are not commercially viable as substitute for plastics. Examples are polyglycollic acid, polylactic acid, poly(ε-caprolactone), polyvinyl alcohol, poly(ethylene oxide).
Starch-based biodegradable plastics	Starch is usually added as filler and crosslinking agent to produce a blend of starch and plastic, for example, starch-polyethylene. This polymer matrix can be break down easily by soil micro-organisms in shorter degradation time. However, such plastics are only partially degradable. The fragments left after starch removal are recalcitrant and remain in the environment for a long time.
PHAs	They are the only 100 % biodegradable polymers. They are completely degraded to water and carbon dioxide under aerobic conditions and to methane under anaerobic conditions by microorganism in soil, sea, lake water and sewage. They possess properties similar to various synthetic thermoplastics like polypropylene and hence can be a good source of replacement of plastics.

Table 1.7

Manufacturers and the microorganisms, raw materials used for the production of biodegradable plastics[121]

Microorganism/raw material	Manufacturer
Alcaligenes eutrophus (H16)	ZENECA bio-products, UK (formerly ICI Ltd)
A. latus	Biotechnolgische Forschungs gesellschaft mbH (Austria)
Transgenic plants	Metabolix Inc (USA) Monsanto (USA) ZENECA Seeds (UK)
Recombinant *Escherichia coli*	Bio Ventures Alberta Inc. (Canada)
Starch	Warners Lambert (USA) Fertec, Italy (Ferruzi e Technologia) Biotec (Melitta) Emmerich (Germany) BASF Ludwigshafen (Germany) Bayer/Wolf Walsrode Leverkusen (Germany) Novamont Novara (Italy)
Cheap substrates	Polyferm Inc (Canada)
Bacteria	Biocorp (USA) Asahi Chemicals and Institute of Physical and Chemical Research (Japan)

PHAs are non-toxic, biocompatible, biodegradable thermoplastics that can be produced from renewable resources. Biodegradation of PHA is dependent on a number of factors such as microbial activity of the environment, and the exposed surface area, moisture, temperature, pH, molecular weight[163]. PHAs are degraded over a wide range of temperatures, even at a maximum of around 60°C with moisture levels at 55 %. Studies have shown that 85 % of PHAs were degraded in seven weeks[164,165]. PHAs have been reported to degrade in aquatic environments (Lake Lugano, Switzerland) within 254 days even at temperatures not exceeding 6°C[164].

PHAs have a high degree of polymerization, are highly crystalline, optically active and isotactic (stereochemical regularity in repeating units), piezoelectric and insoluble in water. PHAs have gained major importance due to their structural diversity and close analogy to plastics. Besides, having melting temperatures of 55-180°C, PHAs can be processed by common extruder and injection moulding equipments. The biodegradability and processibility of PHAs make them extremely desirable substitutes for synthetic plastics. Therefore, PHAs have attracted much research and development as well as commercial interests.

Depending on the property requirement by different applications, PHA can be either blended, surface modified or composited with other polymers, enzymes or even inorganic materials to further adjust their mechanical properties of biocompatibility. Blends of biodegradable PHAs or with other natural polymers such as starch, gelatine, dextrin and alginates with many polymers such as the nylons, poly(ethylene oxide), poly(vinyl chloride), poly(vinyl acetate) etc also hold high prospect of making biodegradable plastic items of diverse ranges[166,167]. Recently published work by Pramanik *et al.* indicated that some synthetic polyesters/copolyesters having biodegradable cross links and backbone links retain promising application as encapsulants for control release of drugs, fertilizers and pesticides[168-170]. Such links are reported to be based on the esterification of trimellitic acid or citric acid and alcoholic systems as glycerol, castor oil, and 1,2,6-hexane triol.

PHA is also useful as stereo regular compounds which can serve as chiral precursors for the chemical synthesis of optically active compounds[171,172,173]. Such compounds are particularly used as biodegradable carriers for long-term dosage of drugs, medicines, hormones, insecticides and herbicides. They are also used as osteosynthetic material in the stimulation of bone growth owing to their piezoelectric properties, in bone plates, surgical sutures and blood vessel replacements. Furthermore, PHAs also exhibit negligible oral toxicity and its ultimate biodegradation product, D(-)-3-hydroxybutyric acid, is a normal metabolite in human blood. Therefore, there are many possibilities to tailor-made PHA for medical implant applications or tissue engineering materials[174]. However, the medical and pharmaceutical applications of PHAs are limited due to the slow biodegradation and high hydraulic stability in sterile tissues[175].

Another important application of biodegradable PHAs is in agriculture field as a matrix for controlled or sustained release plant-growth regulators, such as fertilizers and other nutrients or pesticides. The chemicals are gradually released as the encapsulant polyester is degraded in soil.

Besides, PHAs are also considered as a source for the synthesis of chiral compounds (enantiometrically pure chemicals) and are raw materials for the production of paints. PHAs can be easily depolymerised to a rich source of optically active, pure, bi-functional hydroxyl acids.

1.4 Research methodology

1.4.1 Background

The work of Loo on the vulcanisation of ENR with dibasic acids has reported that crosslinking of ENR with dibasic acids could occur via the ring opening of the epoxy groups by the dicarboxylic acids and subsequent formation of diester linkages[10] with progressive increase in T_g with the extent of crosslinking. This reaction was examined at an elevated temperature of 160°C between 6-8 hours. Besides, work published by Gan and Burfield showed that ring opening of epoxy groups in ENR by monofunctional carboxylic acid, benzoic acid, leading to ester formation was the dominant reaction at elevated temperatures (125-160°C)[11]. They have found that the increase in T_g was due to the modified main chain structure, without involving any crosslinking.

Recent work by Chan *et al.* indicated that the carbonyl chain ends of poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) may trigger reaction with ENR at elevated temperatures of 220-234°C[176]. However, the blends were found to be immiscible and any reaction between the constituents was restricted to the interfacial region between the components. Up to date, there is little study on the reaction of ENR with the microbial thermoplastic polyesters, especially PHA, with good miscibility with ENR at elevated temperature.

After referring to the interesting findings reported by the above researchers, the ideas of investigating the reaction of ENR with palm oil-based alkyds and ENR with palm oil-based mcl-PHA were mooted. Furthermore, the ideas of synthesizing these palm oil based polymers to initiate the chemical reactions with ENR are mainly due to the fact that both natural rubber and palm oil are the major primary commodities of Malaysia[177,178]. Hence, any research that could produce new added values as well as enhancing the economic and technological incentives of rubber and palm oil should be encouraged among Malaysian researchers.

1.4.2 Scopes of study

Overall, the present work consists of two objectives. The first objective is to investigate the reactions of ENR with palm oil-based alkyds; while the second objective is to study the reactions of ENR and palm oil-based mcl-PHA. These two objectives will be achieved through the following work.

(a) To synthesize a series of palm oil-based alkyds that has both hydroxyl and carboxylic groups in different ratios of –OH to –COOH. This can be done by varying their acid and hydroxyl numbers in the alkyd formulations.

(b) To find evidence of alkyd that has been chemically incorporated into the ENR molecules from the FTIR and NMR studies.

(c) To measure the percentage of swelling, crosslink density, average molecular weight between crosslink and modulus of swollen crosslinked ENR in toluene.

(d) To establish the predominant reaction of these two functional groups (–OH and –COOH) in alkyds with epoxy groups in ENR at ambient temperature.

(e) To study the thermal stability of ENR at elevated temperature by measuring the intrinsic viscosity and calculating the viscosity average molecular weight (M_v).

(f) To study the thermal stability of mcl-PHA at elevated temperature by determining the acid number and then calculating the number average molecular weight (M_n).

(g) To find evidence that mcl-PHA has been chemically incorporated into the ENR molecules from the FTIR and NMR studies.

(h) To verify the miscibility of ENR and mcl-PHA from the DSC study.

(i) To calculate the epoxy contents of the reacted ENR from the ^{1}H-NMR spectra.

Therefore, this thesis consists of five chapters. Chapter 1 presents an introduction and background of the work. General information about ENR, alkyd and mcl-PHA are described, including their basic chemistry. The research methodology was also outlined. Chapter 2 provides a comprehensive description of the experimental techniques and raw materials used throughout the study. The procedures used to prepare the polymers for the reaction is described, together with the methods used to study the reaction between ENR and alkyd or ENR and mcl-PHA. In chapter 3, the reactions between ENR and alkyd were investigated. The progress of reaction in the alkyd preparation and its properties are studied. A plausible mechanism in the preparation of each alkyd is described. The FTIR, NMR and toluene swelling tests are used to study the reaction. This chapter is of interest because the predominant crosslinking reaction between the functional groups in ENR and alkyd is determined. For chapter 4, the reactions between ENR and mcl-PHA were studied. The thermal degradations of both ENR and mcl-PHA at higher temperature range are discussed. FTIR and NMR tests are used to study the reaction. A plausible mechanism in the reaction is depicted. Finally, chapter 5 summarizes the conclusions and provides suggestions for future research.