



CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Biodiesel

Sheehan et al., (1998) defined biodiesel in the final report of Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus as a substitution of renewable diesel fuel. There are several advantages of biodiesel towards all of economical, environmental and industrial aspects; for example, reducing dependence on foreign petroleum, leveraging limited supplies of fossil fuels, mitigating greenhouse gas emissions, reducing air pollution and related public health risks, benefiting the domestic economy. In addition, biodiesel is an eco-friendly, alternative diesel fuel prepared from domestic renewable resources i.e, vegetable oils (edible or non-edible) and animal fats, that's runs in engines-cars, buses, trucks, construction equipments, boats, generators, and oil home heating units. Various edible and non-edible vegetable oils, like rice barn oil, coconut oil, Jatropha oil, castor oil, cottonseed oil, which are either surplus and are non-edible type, can be used for the preparation of biodiesel. It is an alternative fuel derived from the conservation of agricultural lipids and a simple alcohol into fatty acid alkyl ester and glycerol and is defined as "mono alkyl esters of fatty acids derived from vegetable oil or animal fats". Methanol has been the most commonly used alcohol in the commercial production of biodiesel. These natural oils and fats are made up main of triglycerides. These triglycerides have striking similarity to petroleum derived diesel so that it is known as "biodiesel". Biodiesel functions in current diesel engines, and is a possible candidate to replace fossil fuels as the world's primary transport energy source (Bajpai and Tyagi, 2006).

The biodiesel has many positive attributes and they are being summarized as:

- It is plant-derived, not petroleum-derived, and as such its combustion eliminates life cycle carbon dioxide emissions, a "greenhouse" gas since carbon dioxide

emitted during combustion is recycled in the photosynthesis process occurring in the plants used as raw materials for biodiesel production.

- It can be domestically produced, offering the possibility of reducing petroleum imports.
- The higher cetane number of biodiesel compared to fossil diesel indicates the potential for higher engine performance.
- It is biodegradable.
- Relative to conventional diesel fuel, biodiesel reduces emission of particulate matter by 40%, unburned hydrocarbons by 68%, carbon monoxide by 44%, sulphates by 100%, PAHs (polycyclic aromatic hydrocarbons) by 80%, and carcinogenic nitrated PAHs by 90%, on average. The use of biodiesel significantly reducing the environmental pollution.
- The superior lubricating property of biodiesel increases the engine efficiency.
- The higher flash point of biodiesel makes it safe to store.
- The biodiesel molecules are simple hydrocarbon chains, containing no sulphur which is concern in crude oil derived petroleum products.
- Biodiesel contains higher amount of oxygen (up to 10%), which ensures complete combustion of hydrocarbons.
- Use of biodiesel will lead to increased energy independence as well as increased economic activity from fuel production and utilization.
- Generation of new employment opportunities in cultivation, processing and production of biodiesel.
- Greening of wastelands and regeneration of degraded forest-lands, thereby helping in eco-restoration and preventing further land degradation.
- Empowerment of village community through enhanced livelihood opportunities.

2.2 *Jatropha curcas* L. (Physic nut)

According to Chhetri et al. (2007), Physic nut or *Jatropha curcas* L. is a bush/small tree belonging to Euphorbiaceae family. This is a non-edible oil-bearing plant widespread in arid, semi-arid and tropical regions of the world. *Jatropha* is a

drought resistant perennial tree that grows in marginal lands and can live over 50 years (Bosswell, 2003). *Jatropha* grows almost anywhere, even on gravelly, sandy and saline soils. It can thrive on the poorest stony soil. It can grow even in the crevices of rocks. The leaves shed during the winter months form mulch around the base of the plant. The organic matter from shed leaves enhance earth-worm activity in the soil around the root-zone of the plants, which improves the fertility of the soil. Regarding climate, *Jatropha* is found in the tropics and subtropics and likes heat, although it does well even in lower temperatures and can withstand a light frost. Its water requirement is extremely low and it can stand long periods of drought by shedding most of its leaves to reduce transpiration loss. *Jatropha* is also suitable for preventing soil erosion and shifting of sand dunes.

The *Jatropha* tree has several beneficial properties such as its stem is being used as a natural tooth paste and brush, latex from stem is being used as natural pesticides and wound healing, its leaf as feed for silkworms among other uses (Chhetri et al., 2007). The uses of *Jatropha* plant are concluded in Table 2.1.

Table 2.1 The uses of *Jatropha* plant

Parts of <i>Jatropha</i> plant	Beneficial uses
Whole plant	<ul style="list-style-type: none"> - Water erosion prevention and for conservation - Promising live fence - Useful as green manure - Useful in controlling sand drift
Roots	<ul style="list-style-type: none"> - Used as ethnomedicine
Leaves	<ul style="list-style-type: none"> - Used as ethnomedicine - Used as a dye given tan and brown colour - Useful as botanical
Latex	<ul style="list-style-type: none"> - Used as ethnomedicine - Resembles shellac - Use for making ink
Seeds	<ul style="list-style-type: none"> - Source of oil - Useful as illuminant, lubricant, in soap and

Parts of Jatropha plant	Beneficial uses
	candle making - Used as medicine both internally and externally
Bark	- Yields tannin
Twig	- Use as medicine - Use as herbal tooth brush

Source: http://www.jatrophabiodiesel.org/jatrophaPlantation.php?_divid=menu2

Jatropha usually grows below 1400 meters of elevation from sea level and requires a minimum rainfall of 250 mm, with an optimum rainfall between 900-1200 mm. Jatropha grows on well-drained soils with good aeration and is well adapted to marginal soils with low nutrient content. On heavy soils, root formation is reduced. Jatropha is a highly adaptable species, but its strength as a crop comes from its ability to grow on very poor and dry sites (Bosswell, 2003). This plant is not even browsed by animals for its leaves. Recently *J. curcas* is being considered as one of the most promising potential oil source to produce biodiesel in Asia, Europe and Africa. Chhetri et al. (2007) discussed the multiple use of Jatropha for different purposes. Among other parts of the Jatropha tree, the seed has so far been found appropriate for numerous uses. Several studies have shown that there exists an immense potential for the production of plant based oil to produce biodiesel. Azam et al. (2005), studied the prospects of fatty acid methyl esters (FAME) of some 26 non-traditional plant seed oils including Jatropha to use as potential biodiesel in India. Among them, *Azadirachta indica*, *Calophyllum inophyllum*, *J. curcas* and *Pongamia pinnata* were found most suitable for use as biodiesel and they meet the major specification of biodiesel for use in diesel engine. Moreover, they reported that 75 oil bearing plants contain 30% or more oil in their seed, fruit or nut. Subramanian et al. (2005) reported that there are over 300 different species of trees which produce oil bearing seeds. Thus, there is a significant potential for non-edible oil source from different plants for biodiesel production as an alternative to petrodiesel. Pramanik (2003) reported the oil content in Jatropha seeds and kernel to be in ranges from 26-50% by weight of the

seed and ranges from 21-74% by weight of the kernel. It is a rapidly growing tree and easily propagated. The *Jatropha* seeds and kernels are shown in Figure 2.1.



Figure 2.1 *Jatropha* seeds and kernels

Source: www.jatropha.org.nz

2.2.1 *Jatropha* oil extraction

For extraction of the *Jatropha* oil, two main methods have been identified: mechanical extraction and chemical extraction (Aderibigbe et al., 1997 & Forson et al., 2004). Prior to oil extraction the *Jatropha* seeds have to be dried (Henning, 2000 & Tobin and Fulford, 2005). Seed can be dried in the oven (105 °C) or sun dried (3 weeks). Mechanical expellers or presses can be fed with either whole seeds or kernels or a mix of both, but common practice is to use whole seeds. For chemical extraction only ground *Jatropha* kernels are used as feed.

1. Mechanical expellers

For mechanical extraction of the oil from the seed, either a manual ram press or an engine driven screw press can be used (*Jatropha Handbook*, Forson et al., 2004 & Henning, 1998). Henning (2000), stated that engine driven screw presses extract 75–80% of the available oil, while the manual ram presses only achieved 60–65%. The efficiency range of engine driven screw presses can be broadened corresponds to the fact that seeds can be subjected to a different number of extractions through the expeller. Up to three passes is common practice. Pretreatment of the seeds, like cooking, also can increase the oil yield (Beerens, 2007).

2. Chemical extraction

Table 2.2 summarizes the reaction temperature, reaction pH, time consumption and oil yield of different chemical extraction methods tested on *Jatropha*. The n-hexane method is the most common and results in the highest oil yield, but also takes most time. In aqueous enzymatic oil extraction the use of alkaline protease gave the best results for both available studies (Winkler et al., 1997 and Shah et al., 2005). Furthermore, it is shown that ultrasonication pretreatment is a useful step in aqueous oil extraction (Shah et al., 2005). Adriaans (2006) concluded that solvent extraction is only economical at a large-scale production of more than 50 tons biodiesel per day. However, he did not recommend the conventional n-hexane solvent extraction because of environmental impacts (generation of waste water, higher specific energy consumption and higher emissions of volatile organic compounds) and human health impacts (working with hazardous and inflammable chemicals). Using aqueous enzymatic oil extractions greatly reduces these problems (Adriaans, 2006) as do the use of supercritical solvents (mainly supercritical CO₂) or biorenewable solvents as bio-ethanol and isopropyl alcohol. Although the new generation n-hexane extraction units are very efficient and produce far less environmental burdens than the older units, further research on these alternative solvents is recommended as on their commercial viability. Moreover, Foidl and Mayorga (2004) presented the use of supercritical isopropanol or CO₂ in a continuous mechanical oil extraction system only leaving 0.3% by weight of oil in the cake.

Table 2.2 *Jatropha* oil extraction efficiency from chemical extraction methods

Extraction method	Reaction temperature (°C)	Reaction pH	Time consumption (hr)	Oil yield (%)
Soxhlet extraction				95-99
1st acetone	-	-	24	
2nd n-hexane	-	-	48	
AOE ^(a)				
Winkler et al., 1997	-	-	2	38
Shah et al., 2005	50	9	6	38
Ultrasonication & AOE	50	9	6	67

Extraction method	Reaction temperature (°C)	Reaction pH	Time consumption (hr)	Oil yield (%)
AEOE ^(b)				
Hemicellulase or cellulose Alkaline protease	60	4.5	2	73
Winkler et al., 1997	60	7	2	86
Shah et al., 2005	50	9	6	64
Alkaline protease with 5 min of ultrasonication as pretreatment	50	9	6	74
Three-phase partitioning	25	9	2	97

Note:(a) means aqueous oil extraction. (b) means aqueous enzymatic oil extraction.

Source: Gübitz et al., 1999; Heller, 1996; Forson, 2004; Shah et al., 2005; Winkler et al., 1997 and Shah et al., 2004.

2.2.2 Jatropha oil characteristics

Jatropha oil can be used as fuel in diesel engines directly and by blending it with methanol (Gübitz et al., 1999). Engine tests with Jatropha oil were done in Thailand, showing satisfactory engine performance (Takeda, 1982). The chemical structure of Jatropha oil is shown in Figure 2.2

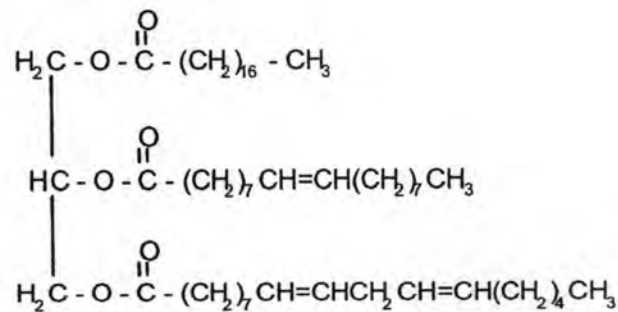


Figure 2.2 Chemical structure of Jatropha oil

Source: Jongschaap et al., 2007

The role of Jatropha as a substitution for diesel is very remarkable. Many researchers have studied its potential for use as neat oil, as transesterified oil (biodiesel), or as a blend with diesel (Narayana and Ramesh, 2006; Pramanik, 2003;

Augustus et al., 2002). The fatty acid methyl ester of the oil of *Jatropha* was found most suitable for use as biodiesel and it meets most of the specifications of biodiesel standards of USA, Germany and European Standard Organization (Azam et al., 2005). The composition and characteristics of the crude *Jatropha* oil are given in Table 2.3. It is important to note that the oil quality is dependent on the interaction of environment and genetics; therefore, the values of each characteristic show a very wide range. As for the seed size, seed weight and oil content, it is believed that also for the oil quality the environmental conditions have a larger impact than the genetics (Kaushik et al., 2007). More research is necessary to study toward the improvement of seed quality and seed yield in order to obtain large amount of oil. The *Jatropha* oil contains more than 75% unsaturated fatty acid, which is reflected in the pour and cloud point of the oil. The fatty acids composition of *Jatropha* oil is shown in Table 2.4 which obviously seen that the oil is dominated by oleic acid (C18:1) followed by linoleic acid (C18:2).

Table 2.3 Crude *Jatropha* oil composition and characteristics

Characteristics	Values	References
Saponification number (mg/g)	202, 192.4 ^[a] , 190.2 ^[b] , 112	Winayanuwattikun et al., 2008; Foidl et al., 1996; Ajiwe et al., 1996
Iodine value (mg iodine/g)	108.40, 95.2 ^[a] , 106.6 ^[b] , 121.60	Winayanuwattikun et al., 2008; Foidl et al., 1996; Ajiwe et al., 1996
Cetane number	48.91, 51, 45	Winayanuwattikun et al., 2008; Kandpal and Madan, 1995; Reddy and Ramesh, 2005
Viscosity at 30 °C (cSt)	38.8 ^[a] , 37.0 ^[b] , 49.9	Foidl et al., 1996; Reddy and Ramesh, 2005
Specific gravity at room temperature (g/cm ³)	0.9180, 0.939	Kandpal and Madan, 1995; Ajiwe et al., 1996
Sulphur content % (kg/kg) × 100	0.13	Kandpal and Madan, 1995
Calorific value (kJ/kg)	9470, 39774	Kandpal and Madan, 1995; Reddy and Ramesh, 2005
Flash point (°C)	240	Foidl et al., 1996; Kandpal and

Characteristics	Values	References
		Madan, 1995; Reddy and Ramesh, 2005
Cloud point (°C)	2	Achten et al., 2008
Acid value (mg KOH/g)	5.18	Ajiwe et al., 1996
Density (kg/m ³)	918	Reddy and Ramesh, 2005
Carbon residue % (kg/kg) × 100	0.44	Reddy and Ramesh, 2005

Note: [a] means Variety Caboverde. [b] means Variety Nicaragua

Table 2.4 Fatty acid composition

Fatty Acid	Structure	% by weight
Lauric	12:0	0.14, 0.31
Myristic	14:0	0.17, 0-0.1, 0.1 ^[a] , 0.1 ^[b]
Palmitic	16:0	14.82, 14.1-15.3, 12.8, 13.38, 15.1 ^[a] , 13.6 ^[b]
Palmitoleic	16:1	0.81, 0-1.3, 0.88, 0.9 ^[a] , 0.8 ^[b]
Stearic	18:0	4.15, 3.7-9.8, 7.3, 5.44, 7.1 ^[a] , 7.4 ^[b]
Oleic	18:1	40.98, 34.3-45.8, 44.8, 45.79, 44.7 ^[a] , 34.3 ^[b]
Linoleic	18:2	38.61, 29.0-44.2, 34.0, 32.27, 31.4 ^[a] , 34.3 ^[b]
Linolenic	18:3	0.27, 0-0.3, 0.2 ^{[a],[b]}
Arachidic	20:0	0.06, 0-0.3, 0.2 ^[a] , 0.3 ^[b]
Behenic	22:0	0-0.2, 0.2 ^[a]
Total Saturated		20.8-26.3
Total Unsaturated		72.7-78.7

Note: [a] means Variety Caboverde. [b] means Variety Nicaragua

Source: Winayanuwattikun et al., 2008; Gübitz et al., 1999; Foidl et al., 1996; Kandpal and Madan, 1995; Ajiwe et al., 1996; Chhetri et al., 2007; Reddy and Ramesh, 2005

2.2.3 Use of the Jatropha oil

As biodiesel: The oil from Jatropha can be directly used in older diesel engines or new big motors running at constant speed (e.g. pumps, generator). Blending with fossil diesel and/or other fossil fuels belongs to this option as well. The oil can also be transesterified into Jatropha (m)ethyl esters that can be used in conventional diesel engines or diesel engines with adapted parameters. The calorific value and cetane number of Jatropha oil are comparable to diesel, but the density is high. The high viscosity of the Jatropha oil, which is considered as a potential alternative fuel for the compression ignition (C.I.) engine, is decreased by blending with diesel. Pramanik (2003) reported that significant improvement in engine performance is observed compared to vegetable oil. Acceptable thermal efficiencies of the engine were obtained with blends containing up to 50% volume of Jatropha oil. From the properties and engine test results, it has been established that 40–50% of Jatropha oil can be substituted for diesel without any engine modification and preheating of the blends.

As pesticide: Apart from its use as biodiesel, the oil has been used to produce biocides (insecticide, molluscicide, fungicide and nematocide) (Shanker and Dhyani, 2006). There are several publications indicate that the Jatropha oil and aqueous extract from oil has highly potential as an insecticide. For instance, it has been used in the control of insect pests of cotton including cotton bollworm and on pests of pulses, potato and corn.

As soap: The glycerine that is a by-product from biodiesel transesterified can be used to produce soap as well as from crude Jatropha oil itself. In either case the process produces a soft durable soap and is a simple one, well adapted to household or small-scale industrial activity.

As medicinal use: The Jatropha seed oil also can be applied to treat eczema and skin diseases and to sooth rheumatic pain (Heller, 1996). The linoleic acid (C 18:2) content in Jatropha kernel oil is of possible interest for skincare. Furthermore, Goonasekera et al., (1995) showed that various solvent extracts of Jatropha have an abortive effect. The oil has a strong purgative action and is also widely used for skin

diseases and to soothe pain such as that caused by rheumatism. The oil is used as a cathartic purgative and for the treatment of skin ailments (Duke, 1988).

2.2.4 Jatropha seed and its toxicity

The seeds of *Jatropha* are a good source of oil, which can be used as a diesel substitute. However, the seeds of *Jatropha* are, in general, toxic to humans and animals. The high concentration of phorbol esters present in *Jatropha* seed has been identified as the main toxic agent responsible for *Jatropha* toxicity (Adolf et al., 1984; Makkar and Becker, 1997). Several cases of *Jatropha* poisoning in humans after accidental consumption of the seeds have been reported with symptoms of giddiness, vomiting and diarrhoea and in the extreme condition even death has been recorded (Becker and Makkar, 1998). In general, ingestion of 3-5 seeds causes marked nausea, gastro-intestinal irritation, abdominal pain, vomiting and sometimes diarrhoea. In severe cases patients were clinically dehydrated, but made a rapid recovery after intravenous fluid replacement (Joubert et al., 1984; Abdu-Aguye, 1986; Mampane et al., 1987). Ionizing radiation treatment could serve as a possible additional processing method for inactivation or removal of certain antinutritional factors such as phorbol esters, phytates, saponins and lectins (Siddhuraju et al., 2002). It is not possible to destroy phorbol esters by heat treatment because they are heat stable and can withstand roasting temperature as high as 160 °C for 30 minutes. However, it is possible to reduce its concentration in the meal by chemical treatments. Complete removal of the toxins is therefore necessary before *Jatropha* oil can be used in industrial applications or in human medicine, the oil must be shown to be completely innocuous before it is used commercially. The negligible concentration of phorbol esters that could be existed in the oil should be 0.27 mg/g as Goel et al., (2007) reported it is the concentration of phorbol esters in nontoxic Mexican varieties.

2.3 Phorbol esters

According to Vogg et al. (1999), phorbol esters (PEs) are naturally occurring tri- and tetracyclic diterpenes. They are classified according to their structures into three classes: tiglane, daphnane and ingenane esters. These compounds have been

isolated from only two plant families, *Euphorbiaceae* and *Thymeleaceae*. PEs are known to exert a plethora of biological effects including inflammation, tumor promotion, cell proliferation and differentiation. Most of their biological actions appear to be mediated through binding to the major phorbol ester receptor, protein kinase C, a family of kinases which have a central role in intracellular signal transduction and gene expression. It is also well known that differences in biological effects of different phorbol esters are strictly structurally controlled. These properties of PEs have been widely used in pharmacology and biochemistry to investigate different physiological and pathological processes involving PKC signalling mechanisms.

In addition, the Division of Occupational Health and Safety reported that phorbol esters are toxic and suspected carcinogens. The possible exposure routes could be through dermal and the ingestion in which will give the outcome of severe irritation of tissues (the skin, eyes, mucous membrane, and lungs) and induced sensitivity.

2.3.1 Phorbol esters and their chemical structure

Phorbol esters are defined as “polycyclic compounds in which two hydroxyl groups on neighboring carbon atoms are esterified to fatty acids”. The structure of the phorbol esters is dependent on the tetracyclic diterpene carbon skeleton known as tigliane. Tigliane is the fundamental alcohol moiety in the phorbol esters. Tigliane contains four rings designated as A, B, C, and D (Figure 2.3). Hydroxylation of this basic structure at different positions and then ester bonding to various acid moieties results in formation of large varieties of phorbol ester compounds. The phorbol, the parent diterpene of phorbol esters, contains five hydroxyl groups with different reactivity towards acylation (Hecker and Schmidt, 1974). Ring A is on the left and trans linked to the 7-member ring B. Ring C is 6-membered and cis linked to the cyclopentane D ring.

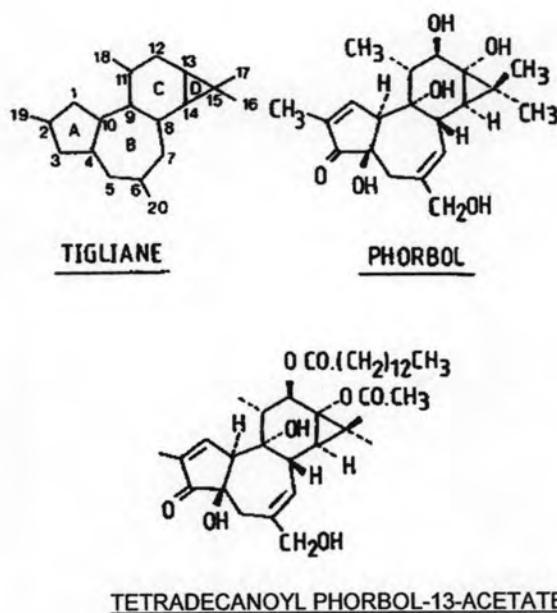


Figure 2.3 Structure of tetradecanoyl phorbol-13-acetate (TPA)

Source: Evans, 1986

2.3.2 Isolation and detection of phorbol esters

The naturally occurring phorbol esters are unstable and are susceptible to oxidation, hydrolysis, transesterification, and epimerization during isolation procedures (Haas et al., 2002). Due to their oxygen sensitivity, the isolation must be conducted in oxygen-free conditions: solvents must be degassed and extraction should be conducted under continuous flow of nitrogen or argon. The isolation protocols involved derivatization of the functional groups in phorbol ester, mainly acylation or esterification of hydroxyl groups by chemical agents. The derivatized phorbols are then separated using different high performance liquid chromatography (HPLC) protocols. Goel et al., (2007) mentioned in their experiment that the phorbol esters have been isolated from the methanol extracts of the seed kernels and quantified using HPLC.

2.3.3 Detoxification of phorbol ester-containing plant ingredients

There are various physical and chemical ways to destroy these phorbol esters in feeds. Heat treatment followed by solvent extraction to remove phorbol esters could result in elimination of most of the antinutrients and toxins from the toxic variety. The heat treatment in combination with the chemical treatment of sodium hydroxide and sodium hypochlorite has also been reported to decrease the phorbol ester level in *Jatropha* seed meal to 75%. A study conducted with *Jatropha* seed oil reported that deacidification with sodium hydroxide and potassium hydroxide and bleaching with different agents reduced the phorbol ester level to 55%. In addition, there is a report reveal that degumming and deodorization treatments able to decrease phorbol esters to a lower extent (Hass and Mittelbach, 2000). The degumming removed lecithin while the deodorization was used to remove the undesirable volatile and odoriferous materials. The hydrolysis products of TPA, 12-*O*-tetradecanoyl phorbol (TP) and phorbol acetate (PA), make TPA inactive as tumor promoters (Mentlein, 1986).

2.3.4 Beneficial effects of phorbol esters

Besides numbers of negative effects toward human health and livestock, as mentioned before, there are some positive effects posed in research publication of Goel et al., (2007) which are tumor inhibitors, inhibit human immunodeficiency virus (HIV) replication, and possess antileukemic activity.

2.4 Surfactant

Tadros T. F. (2005) explained the definition of surfactant or surface active agent that they are amphiphatic molecules. Surfactant molecule consists of a non-polar portion which has hydrophobic property (lyophilic), usually a straight or branched hydrocarbon or fluorocarbon chain containing 8-18 carbon atoms, and the chain is attached with the other portion of surfactant molecule defined as polar or

ionic portion with hydrophilic (lyophobic) property. A single surfactant molecule can be called as monomer at particular concentration in solution; surfactant molecules will associate to form larger units. These associated units are called micelles (self-assembled structures) and the first formed aggregates are generally approximately spherical. The concentration of which this phenomenon occur is known as critical micelle concentration (cmc). Each surfactant molecules has a specific characteristics cmc. at given temperature and electrolyte concentration. There are two types of micelle formations known as normal and reverse micelles. In aqueous solution, normal micelles are formed with the hydrophobic tail being in the internal and the hydrophilic head contact with aqueous phase. On the other hand, reverse micelles are formed in non-polar solvent or in oil with the hydrophilic head group being internal and the hydrophobic tail contact with non-polar phase. The formation of normal micelle and reverse micelle are illustrated in Figure 2.4.

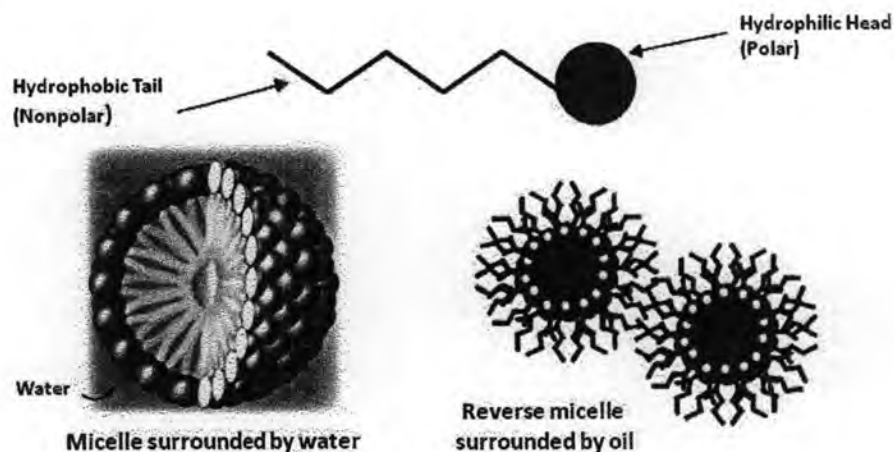


Figure 2.4 Normal micelle and reverse micelle

Source : www.enriquegortiz.com

2.4.1 General classification of surfactant

A classification is simply based on the nature of the hydrophilic group. Four main classes can be distinguished and called nonionic, anionic, cationic and zwitterionic.

Nonionic surfactant: The surfactant that the surface-active portion contains no charge and be able to adsorb onto either hydrophilic and hydrophobic surface and oriented toward the surface depending on the nature of surface. The most common nonionic surfactant is based on ethylene oxide or could be referred as ethoxylated surfactants.

Anionic surfactant: The surfactant that the surface-active portion contains negative charge and tends to adsorb onto the positive charge hydrophilic surface. This type of surfactant is the most widely used in industrial application because of their low cost of manufacturer. Several of commercial available anionic surfactant are sulphosuccinates, isothionates and taurates.

Cationic surfactant: The surfactant that the surface-active portion contains positive charge and tends to adsorb onto the negative charge hydrophilic surface. There are several researches that utilized this surfactant to do site remediation because of its unique property to be strongly absorbed onto most surfaces. The most common cationic surfactant is the quaternary ammonium compounds.

Zwitterionic surfactant: The surfactant that the surface active portion contains both positive and negative apparent without changing the charge of surface significantly.

2.4.2 Microemulsion

The term "microemulsion" refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules. A microemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with a surfactant. The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm, and has very low oil/water interfacial tension. Because the droplet size is less than 25% of the wavelength of visible light, microemulsions are transparent. The microemulsion is formed readily and sometimes spontaneously, generally without high-energy input. In

many cases a cosurfactant or cosolvent is used in addition to the surfactant, the oil phase and the water phase.

Windsor (1954) characterized four types of microemulsions that are most likely to be formed depending on the composition:

A Type I microemulsion is an oil-in-water microemulsion, where a portion of the oil has been solubilized by the surfactant. Conversely, a Type II microemulsion is a water-in-oil microemulsions, where a portion of water has solubilized by the surfactant. A Type III microemulsion is an emulsion with the oil and water solubilized by the surfactant that is often assumed to be bicontinuous, and may be in equilibrium with excess oil and water phases. This bicontinuous microemulsion can have varying proportions of oil and water, and is assumed to contain essentially all of the surfactant originally in the aqueous phase. All microemulsions by definition are thermodynamically stable, and in theory will never separate out into their oil and water constituents. Lastly, A Windsor Type IV microemulsion is a single-phase microemulsion system containing roughly the same amount of oil and water co-solubilized in the system.

In all four types of microemulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants.

Though it has been know that several factors determine whether a w/o or o/w system will be formed but in general it could be summarised that the most likely microemulsion would be that in which the phase with the smaller volume fraction forms the droplets i.e. internal phase.

The key difference between emulsions and microemulsions are that the former, whilst they may exhibit excellent kinetic stability, are fundamentally thermodynamically unstable and will eventually phase separate. Another important difference concerns their appearance; emulsions are cloudy while microemulsions are clear or translucent. In addition, there are distinct differences in their method of preparation, since emulsions require a large input of energy while microemulsions do not. The latter point has obvious implications when considering the relative cost of commercial production of the two types of system.

2.4.3 Characteristic of microemulsion

Microemulsions are transparent (or translucent) dispersions containing two immiscible liquids with particles diameter in ranging of 5-200 nm that are generally obtained upon mixing gently the oil and water in the present of surfactant solution in the system. Normally, microemulsions are containing of at least 3 components, water oil and surfactant which may be one type or more than one or mixture of surfactant in some system. Generally, a second surfactant called cosurfactant, is added into the microemulsions system. The role of cosurfactant is to prevent formation of rigid structures such as gel, liquid crystal, participation etc. A cosurfactant can make the system lower viscosity, reduce the interfacial tension, increase the fluidity of the interface and thereby increasing the entropy of the system, increase the mobility of the hydrocarbon tail and allow greater phase due to its partition between the phases. Furthermore, other components such as additive, cosolvent, and electrolyte may be added into some systems. As a consequence of many potential area and ease of preparation interest has rapidly grown in the use of microemulsions in so many application such as oil recovery (Bidyut and Satya,2001).

In addition, Flaaten (2007) revealed toward the aspect of microemulsion characterization that when aqueous oil phase interaction under some conditions produces a microemulsion phase, which can transition from a Type I (oil in water) to Type III to Type II (water in oil) phase (or vice versa) with the systematic variation of several different parameters in the aqueous solution. Some parameters that cause a lower-to upper-phase transition for anionic surfactants such as sulfonates include increases in electrolyte strength (i.e. salinity), oil-soluble co-solvent concentration, alkyl chain length of the surfactant, oil aromaticity and propylene oxide number (PON), and some parameters that cause a upper- to lower-phase transition include decreases equivalent alkane carbon number in the oil, ethylene oxide number and water-soluble co-solvent concentration. A temperature increase generally causes an upper- to lower-phase transition for anionic surfactants; however, the reverse has been shown to occur for anionic sulfates with a high PON. The most notable phase behavior transition for anionic surfactant applications is from Type I to Type III to Type II as salinity increases.

2.4.4 Uses of microemulsion

Microemulsions have many commercially important uses:

- Water-in-oil microemulsions for some dry cleaning processes
- Floor polishers and cleaners
- Personal care products
- Pesticide formulations
- Cutting oils. Much of the work done on these systems have been motivated by their possible use to mobilize petroleum trapped in porous sandstone for enhanced oil recovery

A fundamental reason for the uses of these systems is that a microemulsion phase sometimes has an ultralow interfacial tension with a separate oil or aqueous phase, which may release or mobilize them from solid phases even in conditions of slow flow or low pressure gradients.

Microemulsions also have industrial applications, one of them being the synthesis of polymers. Microemulsion polymerization is a complex heterogeneous process where transport of monomers, free radicals and other species (such as chain transfer agent, co-surfactant and inhibitors) between the aqueous and organic phases, takes place. Compared with other heterogeneous polymerization processes (suspension or emulsion) microemulsion polymerization is a more complicated system. Polymerization rate is controlled by monomer partitioning between the phases, particle nucleation, and adsorption and desorption of radicals. Particle stability is affected by the amount and type of surfactant and pH of dispersing medium.

The kinetics of microemulsion polymerization has much in common with emulsion polymerization kinetics, the most characteristic feature of which is the compartmentalization, where the radicals growing inside the particles are separated from each other, thus suppressing termination to a high extent and, as a consequence, providing high rates of polymerization. In addition, microemulsion is also a method for the synthesis of magnetic nanoparticles.

2.4.5 Microemulsion as fuels

One of the direct advantages of microemulsion-based fuels is the presence of water in a stable microemulsion and they are successfully used to reduce soot formation. When the water is vapourized during the combustion, this will lower the heat released and the combustion temperature. As a direct consequence, the emission rate of gases like nitrogen oxides (NO_x) and carbon monoxide (CO) will decrease. The presence of water is also supposed to cause improved fuel atomization, minimization of particulate emission and sooting, and improved fuel economy in terms of price and miles/volume of the fuel. Another interesting feature of microemulsion-based fuel is their capacity to increase the octane number of gasoline and the corresponding octane number for diesel oils. Octane number improvers include formamide, glycols, urea, etc. In diesel fuels, many problems are overcome due to the high combustion temperatures (160–325°C). It is normal that diesel microemulsions contain watersoluble cetane number improvers. Microemulsions in fuels are also found to improve air–fuel contact and increase the flash point of fuel. A variety of microemulsions designed as fuel systems have been reported along with detergentless systems consisting of 1-butanol, diesel and water (Bidyut and Satya, 2001).

2.5 Related studies

The oil extraction technologies for biodiesel and oleochemical products purposes have been explored and innovated. Shah et al. (2004) studied the *Jatropha* oil extraction technique by using enzyme assisted three phase partitioning. The process consisted of simultaneous addition of t-butanol (1:1, v/v) and 30% (w/v) ammonium sulphate to the *Jatropha* seed kernels slurry. They discovered that combination of sonication and enzyme treatment with a commercial preparation of fungal proteases at pH 9, could obtain 97% oil yield within 2 hr which is faster than hexane soxhlet extraction. A year later, Shah et al. (2005) also studied the extraction of oil from *Jatropha* by combination of ultrasonication and aqueous enzymatic oil

extraction. They reported that use of ultrasonication as a pretreatment before aqueous oil extraction and aqueous enzymatic oil extraction was found to be useful. The results were shown that the maximum oil yield of 74% was obtained by processes of ultrasonication for 5 min followed by aqueous enzymatic oil extraction using an alkaline protease at pH 9.0. Other beneficial was found that use of ultrasonication also resulted in reducing the process time from 18 to 6 hr. Then in the year 2006, Naksuk studied the oil extraction from the palm kernels and the soybeans by using the surfactant aqueous-based solution. She conducted the mixed surfactants aqueous-based solution consisted of 3% Comperlan KD served as nonionic surfactant, 0.1% Alfoterra 145-5PO and Alfoterra 5-8PO served as anionic surfactants, and with neutral electrolyte, served as sodium chloride, scan. The results showed that this method gives the high efficiency extraction up to 85% of the method using hexane extraction. The quality of both palm and soybean oils obtained from hexane and surfactant aqueous-based solution were similar. Therefore, she concluded surfactant-based solution extraction technique seems to be promising as an alternative oil extraction method. Moreover, Berchmans and Hirata (2007) developed the technique of biodiesel production from reduction of free fatty acid in *Jatropha* oil from 15% FFA to be less than 1%. The reduction procedures were done by a two-step pretreatment process. The first step was carried out with 0.60 % w/w methanol-to-oil ratio in the presence of 1% w/w H_2SO_4 as an acid catalyst in 1-hr reaction at 50 °C. After the reaction, the mixture was allowed to settle for 2 hr and the methanol–water mixture separated at the top layer was removed. The second step was transesterified using 0.24 w/w methanol to oil and 1.4% w/w NaOH to oil as alkaline catalyst to produce biodiesel at 65 °C. The final yield for methyl esters of fatty acids was achieved 90% in 2 hr.

After the oil extraction investigation, to utilize *Jatropha* oil in the real industrializes aspect requires the detoxification process. In 1997, Aderibigbe et al., used heat to destroy the toxic substance from *Jatropha*. The results showed that curcin and antinutritional compounds were destroyed by heat except phorbol esters, although the boiling point of oil was heated. It could be implied that phorbol esters is heat resistance compound. Later in the year 2000, Hass et al., experimented on edible oil processing steps on phorbol esters detoxification. They found that deacidification step

and bleaching step were efficient for phorbol esters removal by 55% whereas degumming step and deodorization were not effective on phorbol esters removing. Three years later, Aregheore et al., studied the detoxification of lectin and phorbol esters in *Jatropha* using heat and chemical treatments. The experimental procedures were processing of seeds from a toxic variety of *Jatropha*, defatted and ground to obtain the meal. The meal was brought to heat and 14 different chemical treatments to detoxify the meal of lectin and phorbol esters. The results revealed that the untreated meal and the fully defatted meal had crude protein (CP) values of 25.6% and 52.8%, respectively. The methanol treated meal had 68.0% CP, while the mixing of NaOH and NaOCl treated meal had 55% CP. The increase in CP in methanol treated meal could be due to proportionate higher loss of non-protein components. Furthermore, they discovered the heat treatment did not decrease the concentration of phorbol esters and this confirmed the earlier reports of Makkar & Becker (1997) that heat alone cannot inactivate it. However, with the additional chemical treatments, the concentration of phorbol esters was reduced and the most effective among all the treatments carried out were treatment of 4 times washing with 92% methanol and treatment by addition of 4.0% NaOH (w/w) and 10% NaOCl (v/w). These treatment methods decreased the concentrations of phorbol esters to be 0.09 mg/g and 0.13 mg/g respectively. They also discussed that *Jatropha* meal as plant protein source can substitute for conventional meals such as the expensive soya bean meal. This treatment is promising, but in economic terms it is expensive to produce *Jatropha* meal from it. However, it can be exploited by small-scale industry. The price can be reduced if the methanol is recovered. Recently in the year 2008, Nokkaew et al., investigated the elimination of phorbol esters in *Jatropha* seed oil using 5 adsorbents: bentonite 200, bentonite 150, activated carbon, chitin and chitosan. They illustrated the results that bentonite 200 is the best adsorbent and the optimum conditions in adsorption were 15 in stirring time, 3.2% (w/v) for the amount of bentonite 200, room temperature and 100 rpm of stirring rate. The mentioned condition could remove 96-98% of phorbol esters in oil. In addition, they also eliminate phorbol esters in press cake by washing with 2-3% (w/w) of potassium hydroxide at room temperature for 45 min followed impregnation of press cake in 95% ethanol for one night give the untoxic press cake which suitable for animal feed.

In conclusion, this study mainly focuses on the oil extraction technology and elimination of phorbol esters using mixed surfactants aqueous-based solution base on the property of surfactant that could form microemulsion. The two types of surfactants that apply in this study are nonionic and anionic surfactants.