CHAPTER 2

MATERIALS AND METHODS

2.1. MATERIALS

*Adenanthera pavonina* seeds were collected from Anna University Campus, Chennai. Methanol (99.9 % purity), n-hexane (99 % purity) and Calcium nitrate (98 % Purity) were purchased from Merck India Ltd. Chloroform (> 99.9% purity) and anhydrous strontium nitrate were purchased from SRL Chemicals India Ltd. Tungstophoric acid and silica gel were purchased from Sigma Aldrich (Chennai, India). Potassium hydroxide, oxalic acid, potassium bromide, Isopropanol, KBr and CDCl₃ were obtained from Merck India Ltd for testing and analysis.

2.2. METHODS

2.2.1. Preliminary operation

*Adenanthera pavonina* (Saga) seed was initially weighed and allowed for moisture removal operation in a hot air oven at 40 °C until it reached zero weight loss. 2.8 wt% of moisture was removed from saga seed (moisture content of the fresh saga seed was analysed by gravimetric method). Moisture removed saga seed was crushed and it was separated based on the size of the granular particle by sieving as per the ASTM standard.
2.2.2. Lipid extraction by Soxhlet

Extraction set up generally consists of a three necked round bottom flask (250 ml). The middle neck of the round bottom flask was connected to a sample jar with a reflux condenser, a thermometer was positioned in one of the two side necks and the third neck was used for taking samples. The flask was submerged in a heat controlled water bath (Suganya & Renganathan 2012). Crushed seed of 25 gm was taken in a jar and 300 mL of total solvents were used per batch for 8 hr.

2.2.3. Lipid extraction by superheated solvent extractor and quantification

Superheated extraction followed by separation setup with schematic diagram is shown in Fig. 2.1. Each extraction operation with 25 gm of crushed saga seed was taken in to the extraction vessel. Height, diameter and thickness of the extraction vessel are 200 mm, 38.1 mm and 4 mm respectively. All lines (6.35 mm) and vessels were fabricated by using stainless steel. 0.2 μm pore size filter cloth was used for both side of the vessel to allow the solvents freely. Appropriate temperature and flow rate of mixed solvent from storage vessel in to the extractor through pre-heater was maintained by controlling temperature using electrical heating coil and hot air oven and by extraction vessel inlet needle valve adjusted manually (Mohammad et al 2012). The pressure was maintained by adjusting the extraction vessel outlet needle valve manually (Pressure control valve). The solvents were pumped (Milton Roy- Electromagnetic dosing pump with flow of up to 2.14 lph and pressure up to 50 bar) through the pre-heater into the extraction vessel continuously maintained at constant temperature, pressure and flow rate. Afterwards the extract was collected in a vessel equipped with flash column for the purpose of mixed
solvent separation. These separated solvents were reused for next cyclic extraction operation. Furthermore, traces of solvent present in oil were completely removed by simple distillation and reused.

Extract yield was quantified by the following expression.

\[ Y_{oil} = \frac{W_f - W_r}{W_i} \times 100 \]  

.........(2.1)

Where, ‘\( Y_{oil} \)’ is the extracted yield or oil yield in terms of percentage, ‘\( W_i \)’ is initial weight of saga seed taken in extraction vessel and ‘\( W_r \)’ is the weight of raffinate. All units were represented in weight basis.

**Fig. 2.1.** Superheated mixed Solvent extraction schematic diagram - set up. (1-Solvent storage vessel, 2-High pressure pump, 3-Inlet valve, 4-Bypass valve, 5-Extractor, 6-Hot air oven, 7-Outlet valve, 8-Flash column, 9-Condensor, 10-Heating coil, TI-Temperature indicator, PI-Pressure indicator, CW-Cooling water)
2.2.4. Lipid Characterization

Properties such as density, average molecular weight, iodine value, acid value, FFA content and Saponification value of extracted *Adenanthera pavonina* oil were analyzed using the standard procedures (AOCS 1998; Vicente et al 2004). 5 g of saga seed oil was transferred in to individual 100 mL conical flasks. About 0.05 g of Potassium Hydroxide (1% by weight of the oil) was dissolved in 1.44 g anhydrous methanol. Completely dissolve the KOH in methanol, apply heat if required. Transfer the resulting solution slowly to the sample in a conical flask with stirring. After complete transfer of KOH solution in flask continue the stirring for 120 minutes to complete the reaction. After completion of the reaction time pipette out and transfer approximately 2-3 mL of reaction mixture to 5mL test tube and keep it for phase separation. Separated FAME was used for the fatty acid composition present in saga seed oil were analysed by gas chromatography (GC) analysis. One gram of oil was taken and fatty acid composition analysis was carried out by using the gas chromatography which consists of CHEMIT GC 8610 flame ionization detector in the column BPX-70. Nitrogen and hydrogen was used as carrier gas and oxygen was used for detonation purpose. The data was composed with Winchrom software. Thin layer chromatography (TLC) was used to separate and determine the concentration of different types of lipid groups present in the saga seed oil (Sam et al 2008).

2.2.5. Catalyst preparation

The heterogeneous catalysts were synthesised by two step impregnation followed by calcination techniques as follows.
2.2.5.1. **Synthesis of CaHPW\textsubscript{12}O\textsubscript{40}/SiO\textsubscript{2}**

The silica supported calcium salt of Tungstophoric acid catalyst was synthesized by two step impregnation technique according to reference (Ming Huang et al 2011). The two step impregnation method is shown in scheme 2.1. In this technique, initially ten mole of silica support was impregnated in to 20 mL solution contains one mole of calcium nitrate tetra hydrate at constant stirring condition. Then the mixture solution was evaporated at 80 °C for 1 hr and dried overnight at 110 °C. Obtained powder was calcined at 400 °C for 3 hr. Second step, the calcined silica supported calcium nitrate was added into a 20 mL solution contains one mole of Tungstophoric acid with continuous stirring, after completion of addition the mixture was dried at 110 °C and calcined at 400 °C for 3 hr to get Ca(HPW\textsubscript{12}O\textsubscript{40})/SiO\textsubscript{2}. Finally 10.7 gm of the white fine crystal powder was obtained.

**Scheme 2.1.** Ca(HPW\textsubscript{12}O\textsubscript{40})/SiO\textsubscript{2} preparation by two step impregnation method.
2.2.5.2. Synthesis of SnHPW$_{12}$O$_{40}$/SiO$_2$

The strontium salts of heteropolyacids catalysts were prepared via two step impregnation according to reference (Ming Huang et al 2011). In a typical synthesis, 1.8 g silica support was impregnated with a 20 mL solution containing 0.635 g of Sr(NO$_3$)$_2$ with constant stirring, then the mixture solution was evaporated to dryness at 80 °C. The powder was dried at 110 °C overnight and calcined at 400 °C for 2 h. The calcined sample was added into a 20 mL solution containing 8.64 g of H$_3$PW$_{12}$O$_{40}$·xH$_2$O with continuous stirring, then dried at 110 °C and finally calcined at 300–400 °C for 3 h to get CaHPW$_{12}$O$_{40}$/SiO$_2$ catalysts.

**Scheme 2.2.** Sr(HPW$_{12}$O$_{40}$)/SiO$_2$ preparation by two step impregnation method.
2.2.6. Characterization of catalyst

In heterogeneous catalysis, the reaction is believed to take place at active sites on the catalyst surface. Hence, most important physical properties of a solid catalyst are those related to the surface. The characterization of catalysts can be broadly divided into two categories for convenience: structural and textural characterization. The structural characterization means properties associated with the geometry and the long-range order of a solid catalyst. The properties associated with a porous catalyst are influenced by the porous nature of a support material which are termed as the texture of a catalyst (Ramaswamy 2003). The characterization of the surface area, pore volume and pore size distribution will be discussed in detail in this section.

- Surface area of catalysts

The Brunauer-Emmett-Teller (BET) surface area is mostly used to characterize a solid catalyst’s surface property. Brunauer, Emmett and Teller derived the equation (BET equation) for physical adsorption of gases on solid surfaces leading to multilayer adsorptions (Brunauer et al 1938). The BET equation (Eq. 2.2) was based on some assumptions: (1) the surface is energetically uniform; (2) the condensation of a layer of gas can proceed to multilayers; and (3) the adsorbed molecules do not interact laterally. The simple form of Brunauer-Emmett-Teller (BET) equation can be written as:

\[
p/(V_a (p_0-p)) = 1/(V_m c) + p(c-1)/(V_m p_0 c)
\]  \hspace{1cm} \text{(2.2)}

\[p\] is the pressure of gas, \[V_a\] is the volume of gas adsorbed, \[V_m\] is the BET monolayer volume of gas, and \[c\] is the constant term.
Where,

- \( p \) is the equilibrium pressure, mmHg
- \( p_o \) is the saturated vapor pressure of the adsorbate at liquid nitrogen temperature
- \( V_a \) is the volume of gas adsorbed at equilibrium;
- \( c \) is an isothermal constant;
- \( V_m \) is adsorbate monolayer volume.

By plotting \( \frac{p}{V_a(p_o-p)} \) vs. \( \frac{p}{p_o} \) and determining \( V_m \) from the slope of the resultant straight line in the partial pressure range of 0.05 to 0.35, the surface area can be calculated. Nitrogen is commonly used as the adsorbate at liquid nitrogen temperature. The applicability and limitation of the BET theory rests on the type of materials used which are micro, meso, macro or even non-porous and types of adsorption isotherms for nitrogen. BET surface area values can be determined without any limitations with good accuracy from isotherms which are of strong adsorbent-adsorbate interactions (Gregg & Sing 1967). With isotherms which are typically of very weak adsorbent-adsorbate interactions, more concern is needed to determine the BET surface area values with a sufficient accuracy (Sing 1970).

**Pore volume and pore size distribution**

The Barrett-Joyner-Halenda (BJH) method is a typical method for calculating pore size distributions (Barrett et al 1951). The BJH method is based on a model of the adsorbent as a collection of cylindrical pores. The theory accounts for capillary condensation in pores using the classical Kelvin equation, which in turn assumes a hemispherical liquid-vapor meniscus and a well defined surface tension. The BJH method uses the Kelvin equation to correlate the partial pressure of nitrogen in equilibrium with the porous solid to
the size of the pores where capillary condensation takes place. The Kelvin equation is as followed as:

\[ RT \ln \left( \frac{p}{p_0} \right) = -2\sigma V_m/r_k \]  

Where,

- \( r_k \) is the Kelvin radius,
- \( \sigma \) is the nitrogen surface tension at temperature \( T \),
- \( T \) is the temperature,
- \( R \) is Universal gas constant,

The pore size distribution is obtained by analysis of the desorption isotherm.

The BET and BJH analysis is carried out with \( N_2 \) (77 K) (BELSORP-mini II, BEL INC. Japan). All samples are subjected to a preliminary treatment in a vacuum at 200°C for 5 h. To determine the sorption isotherms, the amount of molecules adsorbed on the samples are measured as a function of the relative pressure \( p/p_0 \). The specific surfaces are calculated from the adsorption parts of the isotherms at 77 K in the relative pressure varying from 0.01 to 0.35 \( p/p_0 \) with \( N_2 \).

**SEM and EDAX analysis**

The surface morphology of the prepared calcium salt of Tungstophoric acid and Ca(HPW\(_{12}O_{40}\))/SiO\(_2\) were characterized by scanning electron microscopic (SEM). The elements of the prepared acid catalyst were examined by energy dispersive X-ray (EDX). The electron microscopic investigations are carried out with a DSM 982 Gemini, Zeiss corp., Germany, equipped with a 4-quadrant solid-state BSE detector, a high brightness in-lens
SE detector and a lateral SE detector. The DSM 982 GEMINI is applied with a thermal (Schottky-type) field-emission electron source (SFE). Element-specific quantification is performed by the dedicated EDX unit, equipped with a 30 mm² Si(Li) detector INCA PentaFet™, FWHM 129 eV @ MnKα (Oxford corp., England).

- **FTIR, XRD and TGA**

HPW presence and structural reliability at molecular intensity were characterized by FTIR spectra and XRD. Stability of the catalyst was examined by thermo gravimetric analysis.

- **Acid–base titration**

Ca(HPW₁₂O₄₀)/SiO₂ was characterized by the determination of its H⁺ content using acid-base titration method. 0.1 N of KOH was prepared and standardized with oxalic acid. KOH solution was taken in a burette and titrated with 10 ml of neutralized solvent contains 1 gm of sample with phenolphthalein indicator. The end point of pale pink color is noted and acid value was calculated as follows:

\[
A_v = V \times N \times E/W \tag{2.4}
\]

Where,

- \(A_v\) is an Acid value in gmole/g of sample,
- \(V\) is volume of KOH consumed in ml,
- \(N\) is normality of KOH,
- \(E\) is equivalent weight of KOH,
- \(W\) is weight of sample in g,
2.2.7. **Transesterification Reaction Procedure**

The schematic diagram of the biodiesel process unit was shown in Fig. 2.2. The transesterification reactions were executed at realized temperature in a 250 ml three-neck round bottom flask equipped with a reflux condenser at one side neck and Ultrasonicator was immersed on to a reaction mixture through middle of the neck. In this research, *Adenanthera pavonina* seed oil was extracted as per the previous research report (Ramachandran Kasirajan et al 2014) and has been used as a feed stock. The molar ratio of alcohol such as methanol to *Adenanthera pavonina* seed oil required was calculated treating three mole of FFA as one mole of triglyceride.

*Adenanthera pavonina* seed oil and the known amount of prepared catalyst were charged into the round bottom flask. When the reaction required temperature was reached, methanol was added into the flask. Once methanol addition started, the reaction mixture was sonicated at 40 kHz frequency. After the completion of reaction, the catalyst was separated from the reaction mixture by filtration. Then the mixture was allowed to settle overnight for the separation of glycerol. Then methanol was removed from biodiesel using rotary evaporation.
2.2.8. Biodiesel characterization

The biodiesel produced from Adenanthera pavonina seed oil was characterized by $^1$H-NMR spectroscopy and Thermogravimetric analysis such as TGA/DTA.

$^1$H-NMR Spectroscopy

Methyl ester produced from saga seed oil by the transesterification process, was characterized by $^1$H-NMR. The $^1$H-NMR spectra were obtained using a BRUKER 500 MHz AVANCE III instrument with CDCl$_3$ as solvent and TMS as an internal standard. $^1$H spectra were recorded with pulse duration of 45 °C and 16 scans. A simple formula (Eq. 2.5) for conversion (%) is
\[ X_{BD} = \frac{2A_{CH3}}{3A_{CH2}} \times 100 \] 

Where, \( X_{BD} \) is the percentage conversion of saga seed oil to Methyl ester, \( A_{CH3} \) is an integration value of the methoxy protons of the Methyl ester (the strong singlet), \( A_{CH2} \) is an Integration value of the methylene protons. Factors 2 and 3 were derived from the fact, that the methylene carbon possesses two protons, while the alcohol (methanol derived) carbon has three attached protons.

### 2.2.9. Acetylation Reaction Procedure

Acetylation reaction mechanism is shown in Scheme 2.3. The reaction were carried out in a 250 ml three neck round bottom flask considered as a batch reactor equipped with heating mantle (Leonardo et al 2010). The sonicator was connected in a middle neck of the flask, water cooled reflux condenser and thermometer was linked with another two side necks, respectively. Initially, 20 gm of glycerol was taken in a reactor and desired quantity of acetic acid and Ca(HPW\(_{12}O_{40}\))/SiO\(_2\) catalyst were added into the reactor. The reaction was carried out at particular temperature up to the complete conversion and the sample was collected from the reaction mixture at specific time interval during the reaction time period. Final reaction mixture was containing mono, di and triacetin, glycerol, acetic acid and catalyst. The catalyst was separated by filtration and used directly without any further pre-treatment for next runs to identify the activity of the catalyst.
Scheme 2.3: Acetylation Reaction Mechanism of glycerol to mono, di and triacetin

2.2.10. Characterization of bio additive’s

Selectivity and conversion of biofuel additives were analyzed by gas chromatography – mass spectrometry (GC-MS-QP), (Shimadzu, 2010) equipped with VF-5 ms capillary column (Length – 30 mm, Diameter - 0.25 mm, Film Thickness - 0.25 μm). The column temperature of each run was started at 70 °C for 2 min, then raised to 300 °C and maintained at 300°C for 10 min. The GC conditions were mentioned as column oven temperature at 70 °C, injector temperature at 260 °C, Injection mode as split, split ratio of 10, flow control mode as linear velocity, column flow at 1.51 ml/min and carrier gas as helium with 99.9995% purity. MS conditions were maintained as ion source temp at 200 °C, interface temp at 240 °C, scan range as 40 – 1000 m/z, solvent cut time as 5 min, MS start time as 5 (min), MS end time as 35 (min) and Ionization with EI (- 70ev).