CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

The studies contained in this thesis were carried out in two segments; the first one deals with the extraction of oil, optimization, kinetics and characterization of oil. The second was on production and characterization of biodiesel. These studies were made over three different biodiesel feed stock *S. foetida, C. pentandra* and *P. americana* seeds. The general materials and method involved were discussed in this chapter. This may slightly varied depending on the nature of the above sources, which were discussed in their respective chapters.

3.2 CHEMICALS AND REAGENTS USED

Solvents like methanol, n-hexane, petroleum ether (boiling range 30-60°C), tert-butyl methyl ester (MTBE), carbon tetra chloride, chloroform, tetra hydro furan (THF) were obtained from Merck, Mumbai, India. KOH (98% purity), NaOH (99.5%) and anhydrous sodium sulphate were purchased from Sisco research laboratories, Mumbai, India. H$_2$SO$_4$, HCl, acetic acid, diatoms earth were purchased from Rankem (Delhi, India). All solvents and chemicals obtained were used as received with out any further purification.

The standard reagents for GC analysis were high pure water free methanol, BF$_3$ (Boron trifluoride) reagent (125 g BF$_3$ L$^{-1}$ in methyl alcohol), methanolic NaOH (0.5 M) and n-Heptane were of chromatograph grade was purchased
from Fischer Scientific. The standard FAME mixture of 37 fatty acid components used for calibration was obtained from Supelco, USA.

3.3 TOTAL OIL ESTIMATION

The extraction methods present for most solid samples usually involve a substantial amount of wet chemistry. The only available alternative to the traditional wet method of solvent extraction is Soxhlet type of apparatus which allows nearly 100% oil recovery and user friendliness. The total oil content of the feedstock is determined by Soxhlet extraction. It is also used to find the effectiveness of different solvent for extraction.

3.3.1 Soxhlet Apparatus

The apparatus was first described in 1879. It is a versatile tool that can be used to separate a single to hundreds of grams of oil with nearly 100% recovery. The basic procedure comprises of a solid sample to be placed in a porous container and allowing condensed solvent to extract continuously.

3.3.1.1 Components and working of Soxhlet apparatus

Figure 3.1 shows the instrumental setup of Soxhlet apparatus. The function of the condenser is to cool the solvent vapor and cause it to condense back into the distilling pot. A porous container is to hold the solid sample and allow for the condensed solvent to saturate and pass through thereby extracting the oil. Distilling pot holds the solvent pool and serves as a reservoir for the concentrated oil. During operation the solvent vapor, generated by gently heating the reservoir, condenses and is allowed to drip back onto the porous sample cup. The liquid condensate that drips onto the sample performs the extraction which then passes through the container and as long as needed. As it progresses the oil is concentrated in the reservoir. The oil yield obtained was expressed in terms of mass percentage of the samples and calculated using Equation (3.1).
\[ \text{Oil yield (wt\%)} = \frac{\text{Mass of oil extracted (g)}}{\text{Mass of seed (g)}} \times 100 \] (3.1)

Figure 3.1  Experimental setup for Soxhlet extraction
3.4 BATCH EXTRACTION

Laboratory scale extraction was carried out in a batch process. 10 g of seed and 100 mL solvents were taken by weight ratio in a 250 mL screw cap conical flask and kept inside temperature-controlled shaker. The oil distributes between the two phases (seed and solvent) depending on its partition coefficient. The rate at which the transfer of solute takes place from the seed to the extracting solvent depends on the process parameters. The mixing rate was kept constant at 250 rpm. Once the required time was attained, the mixing was stopped, cooled and the liquid-solids phases were separated. The mass of oil extracted at each time interval was determined gravimetrically after removing the solvent by vacuum distillation.

3.5 EXTRACTION KINETICS

Extraction kinetic study is the investigation on effect of experimental conditions influencing the speed of extraction mechanism.

Kinetics deal with the experimental determination of reaction data from which rate laws and rate constants are obtained. Relatively simple rate laws exist for zero-order (for which reaction rates are independent of concentration), first-order and second-order. The other orders can be derived from the above order. In consecutive reactions, the rate-determining step often determines the kinetics. In consecutive first-order reactions, a steady state approximation can simplify the rate law.

The activation energy for extraction is experimentally determined through the Arrhenius equation and the Eyring equation. The main factors that influence the extraction rate include; the physical state of the
substance, the concentrations of the substance, the temperature at which the extraction occurs and whether or not any catalysts are present in the extraction.

The rate law or rate equation for an extraction process is an equation that links the reaction rate with concentrations or pressures and constant parameters (normally rate coefficients and orders). To determine the rate equation for a particular system one combines the extraction rate with a mass balance for the system.

Solid liquid extraction is a common and efficient technique in producing oil for biodiesel production. Solid liquid extraction, sometimes called leaching, involves the transfer of a soluble fraction (the solute or leachant) from a solid material to a liquid solvent. Solvent extraction was developed because it allows more complete extraction at lower temperatures. The kinetics of oil extraction from oil seeds dependent on a number of factors. These include the composition and morphology of the raw material and the structural and mechanical properties of the flakes after hydrothermal treatment and milling before preparation of the material for extraction. During the extraction itself, the most important conditions are the temperature and duration of the extraction, as well as the polarity of the solvent used for extraction.

Solvent extraction process can be considered in terms of the usual rate Equation (3.2)

\[
\text{Rate of Extraction} = \frac{\text{Driving force}}{\text{Resistance}} \quad (3.2)
\]

In this case, the driving force is the difference between the concentration of the component being transferred, the solute, at the solid interface and in the bulk
of the solvent stream. Therefore a general reaction rate equation (Equation 3.3) for oil extraction from seeds can be written as

\[
\frac{dy}{dt} = kY^n
\]  

(3.3)

Taking ln on both sides

\[
\ln \frac{dy}{dt} = \ln k + n \ln Y
\]  

(3.4)

This is of linear form $y= mx+c$; where $Y$ is the percent oil yield; $t$ is the time of extraction (min); $k$ is the extraction constant; and $n$ is the reaction order. Since the percent oil yield increased in the course of time, the terms $dy/dt$ have a positive sign (Topallar and Gecgel 2000). Using the experimental values and applying the Differential Method, $\ln dy/dt$ versus $\ln Y$ was plotted. From the plot the following can be calculated;

1. An order of extraction kinetics can be found from the values of $n$ obtained, from the slopes of the straight lines.

2. The reaction rate constants were calculated from the intercepts.

3.5.1 Calculation of Activation Energy

Activation energy may also be defined, as the minimum energy required for starting a chemical reaction. The activation energy of a reaction is usually denoted by $E_a$ and given in units of kJ mole$^{-1}$. At a more advanced level, the Arrhenius activation energy term from the Arrhenius equation is the best regarded as an experimentally determined parameter that indicates the sensitivity of the extraction rate to temperature.
The Arrhenius equation gives the quantitative basis of the relationship between the activation energy and the rate at which a reaction proceeds. From the Arrhenius equation, the activation energy can be expressed in the Equation (3.5) (Sepidar et al 2009, Topallar and Gecgel 2000, Levenspiel 2003).

\[ k = A e^{(-E_a / RT)} \]  \hspace{1cm} (3.5)

where \( A \) is the frequency factor for the reaction, \( R \) is the universal gas constant, \( T \) is the temperature in K and \( k \) is the reaction rate coefficient. While this equation suggests that the activation energy is dependent on temperature, in regimes in which the Arrhenius equation is valid this is cancelled by the temperature dependence of \( k \). Thus, \( E_a \) can be evaluated from the reaction rate coefficient at any temperature (within the validity of the Arrhenius equation).

A plot of \( \ln k \) versus \( 1/T \) gives a straight line whose slope represents the activation energy of extraction, \(-E_a/R\), and whose intercept is the Arrhenius constant, \( \ln A \). Thus, the activation energy and the Arrhenius constant were calculated.

### 3.5.2 Calculation of Activation Thermodynamic Parameters

Gibbs energy is a thermodynamic potential that measures the process-initiating work obtainable from a thermodynamic system at a constant temperature and pressure (isothermal and isobaric). The activation thermodynamic parameters were calculated from the following Equation (3.5), (3.6) and (3.7) according to the transition state theory (Sepidar et al 2009, Topallar and Gecgel 2009, Levenspiel 2003).

\[ A = \frac{RT}{Nh} \frac{n!}{\pi^m} \]  \hspace{1cm} (3.6)
\[ \Delta H^* = E_\alpha - RT \] (3.7)

\[ \Delta G^* = \Delta H^* - \Delta S^* \] (3.8)

where \( N \) is the Avogadro's constant, \( h \) is the Planck's constant, \( \Delta S^* \) is the activation entropy, \( \Delta H^* \) is the activation enthalpy and \( \Delta G^* \) is the activation free energy or Gibb's energy.

### 3.5.3 Calculation of Thermodynamic Parameters

The activation thermodynamic parameters were calculated in the following Equations (3.9) and (3.10) according to the transition state theory (Sepidar et al 2009, Topallar and Gecgel 2009, Levenspiel 2003).

\[ K = \frac{Y_T}{Y_u} \] (3.9)

\[ \ln K = -\frac{\Delta G}{RT} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \] (3.10)

where \( K \) is the equilibrium constant, \( Y_T \) is the percent oil yield at temperature \( T \), \( Y_u \) is the percent unextracted oil, \( \Delta H \) is the enthalpy change, \( \Delta S \) is the entropy change and \( \Delta G \) is the free energy or Gibb's energy.

A plot of \( \ln Y_T \) versus \( 1/T \) at time \( t \), gives as straight line whose slope represents the enthalpy change of extraction, \( \Delta H.R \). Thus, the enthalpy change was calculated for oil extraction. The \( \Delta H \) value obtained was indicating the physical and chemical nature of the oil extraction process (Liauw et al 2008, Topallar and Gecgel 2000, Khraisha 2000).
3.6 PHYSICAL AND CHEMICAL ANALYSIS OF OIL

3.6.1 Viscosity (Brookfield Viscometer)

The viscosity or resistance to flow, of a material can be determined by a rotational viscometer. The viscometer can also be used to approximate other flow characteristics by relating viscosity and flow for a known composition. The specific rotational speed and the spindle used as well as the temperature of the test sample can have a significant impact on the value determined. So it is best to use the same conditions throughout a test series.

3.6.1.1 Procedure

Brookfield model LVF viscometer, spindle set stopwatch and thermometer were used to determine the viscosity of oil. The viscometer is leveled by adjusting the feet or by rotating the viscometer on the mounting shaft until the bubble is centered before each use. The speed control was set to 60 rpm on the upper surface of the knob was found on the left side of the housing. The sample and container were placed under viscometer. The temperature of the sample was measured and recorded.

An appropriate spindle was selected for viscosity measurement. The number of the spindle was engraved at the top of each spindle. Using the adjustment knob on the viscometer stand the spindle was carefully lowered into the sample to the immersion mark etched into the spindle shaft. With one hand depress and hold the brake, found on the back of the viscometer head, firmly down while turning on the viscometer. The brake was released once the viscometer starts rotating smoothly for 60 seconds. The brake was depressed firmly and the viscometer was turned off, while continuing to hold the brake down. This keeps the dial indicator in place until the value can be read. If the dial indicator cannot be seen in the gauge window, turn the viscometer on and off
quickly until the needle comes into view, maintaining firm pressure on the brake. The value on the viscometer gauge and number of the spindle were recorded. The brake was released. The correct multiplier for the spindle used was referred from Table 3.1. The viscosity was calculated in centipoises by multiplying the meter reading with the multiplier corresponding to the particular spindle used. This was then converted into \( \text{mm}^2 \text{S}^{-1} \).

**Table 3.1 Multiplying factor corresponding to the particular spindle**

<table>
<thead>
<tr>
<th>Spindle</th>
<th>Multiplier for readings at 60 rpm</th>
<th>Viscosity range (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1 to 100</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>50 to 500</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>400 to 2000</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>1000 to 10000</td>
</tr>
</tbody>
</table>

### 3.6.2 FFA and Acid Value Determination

The acid value is the number of milligrams of potassium hydroxide necessary to neutralize fatty acids in 1 gram of sample. The sample was weighed into an Erlenmeyer flask, diluted with neutral alcohol and titrated with 0.1 N methanolic KOH or 0.5 N aqueous NaOH, depending on the expected acid value.

#### 3.6.2.1 Procedure

An appropriate amount of sample was weighed into a tared Erlenmeyer flask. The weight was recorded. 100 mL of an appropriate neutral alcohol and a few drops of the phenolphthalein indicator solution were added. A stir bar was placed in the flask and mixed thoroughly to dissolve sample, using heat if necessary. Table 3.2 was used as a guide and titrated with the appropriate solution until a faint, pink endpoint appears and persists for 30 seconds. The volume of
titrant was noted when endpoint reached. The acid value was calculated by using the Equation (3.11) and the FFA content can be calculated using Equation (3.12). The acidity in terms of meq g\(^{-1}\) can be calculated using Equation (3.13).

\[
\text{Acid value, mg KOH g}^{-1} = \frac{(\text{mL of titrant}) \times (N \text{ of titrant}) \times (56.1)}{(\text{Sample wt.)}}
\]  

\[
\text{FFA\%} = \frac{(\text{mL of titrant}) \times (N \text{ of titrant}) \times (\text{Avg. Mwt. of fatty acid})}{(\text{Sample wt.}) \times (10)}
\]  

\[
\text{Acidity, meq g}^{-1} = \frac{(\text{mL of titrant}) \times N}{(\text{Sample wt.})}
\]  

Table 3.2 Sample weight needed to obtain a titration volume under 7 mL

<table>
<thead>
<tr>
<th>Expected Acid Value</th>
<th>Weight of sample (g)</th>
<th>Weighing accuracy (±g)</th>
<th>Titrating solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 1</td>
<td>20</td>
<td>0.05</td>
<td>0.1N KOH</td>
</tr>
<tr>
<td>1 to 4</td>
<td>10</td>
<td>0.02</td>
<td>0.1N KOH</td>
</tr>
<tr>
<td>4 to 15</td>
<td>2.5</td>
<td>0.01</td>
<td>0.1N KOH</td>
</tr>
<tr>
<td>15 to 75</td>
<td>0.5</td>
<td>0.001</td>
<td>0.1N KOH</td>
</tr>
<tr>
<td>75 to 375</td>
<td>0.5</td>
<td>0.001</td>
<td>0.5N NaOH</td>
</tr>
<tr>
<td>375 to 1875</td>
<td>0.1</td>
<td>0.0002</td>
<td>0.5N NaOH</td>
</tr>
</tbody>
</table>

3.6.3 Saponification Value

The saponification value is the amount of alkali necessary to saponify a definite quantity of the sample. It is expressed as the number of milligrams of KOH required to saponify one gram of the sample. A sample is refluxed in 0.5 N methanolic KOH for 1.5 h and titrated using 0.5 N HCl.

3.6.3.1 Procedure

The sample was melted and mixed thoroughly to ensure homogeneity. The appropriate amount of sample was weighed into an Erlenmeyer flask using Table
3.3. The weight was recorded. 50 mL of 0.5 N KOH solution was pipetted into the flask. Further some boiling stones added and refluxed for 1.5 h. It was made sure that cold water flowing through the condensers, so as to aid in the condensing of the sample back into the Erlenmeyer flasks.

A blank was prepared and run simultaneously with the samples by pipetting 50 mL of 0.5 N KOH into an empty flask. Some boiling stones were added and refluxed along side the samples. After 1.5 h of refluxing, the condensers inner surface was rinsed with about 25 mL of deionized water. The rinsed water was collected in the Erlenmeyer flasks. The flasks were removed from the condenser and allow the sample solutions to cool to room temperature. About 3 to 5 drops of phenolphthalein indicator was added to each flask and mixed thoroughly with a stir bar. The samples were titrated with 0.5 N HCl, until the pink colour just disappears. The respective titration volumes used to reach each endpoint was recorded. The saponification value was calculated using Equation (3.14).

Table 3.3  Sample weight needed to obtain an expected saponification value

<table>
<thead>
<tr>
<th>Saponification Value Expected</th>
<th>Sample weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 59</td>
<td>10.0 to 12.0</td>
</tr>
<tr>
<td>50 to 79</td>
<td>9.0 to 11.0</td>
</tr>
<tr>
<td>80 to 99</td>
<td>7.0 to 8.6</td>
</tr>
<tr>
<td>100 to 119</td>
<td>5.7 to 7.0</td>
</tr>
<tr>
<td>120 to 139</td>
<td>4.9 to 5.9</td>
</tr>
<tr>
<td>140 to 159</td>
<td>4.2 to 5.1</td>
</tr>
<tr>
<td>160 to 179</td>
<td>3.9 to 4.8</td>
</tr>
<tr>
<td>180 to 199</td>
<td>3.3 to 4.1</td>
</tr>
<tr>
<td>200 to 219</td>
<td>3.0 to 3.7</td>
</tr>
<tr>
<td>220 to 239</td>
<td>2.7 to 3.4</td>
</tr>
<tr>
<td>240 to 259</td>
<td>2.5 to 3.1</td>
</tr>
<tr>
<td>260 to 279</td>
<td>2.2 to 2.7</td>
</tr>
<tr>
<td>280 to 300</td>
<td>2.2 to 2.7</td>
</tr>
</tbody>
</table>
3.6.4 Iodine Value (Wijs Method)

The Iodine value is a measure of the unsaturation of fatty acids and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample (percent iodine absorbed). A sample was dissolved in chloroform and then reacted, in the dark, with Wijs solution for a particular time. KI and deionized water were added to the flask and the solution was titrated with 0.1 N sodium thiosulfate.

3.6.4.1 Procedure

An appropriate amount of sample was weighed into a tared Erlenmeyer flask. The weight of the sample recorded. The flask was labeled accordingly. Adding 25 mL of chloroform to the flask and flask was swirled to dissolve the sample. If needed, the flask was heated on a steam bath to completely dissolve the sample. After the sample solution has been cooled to room temperature, 25 mL of Wijs solution was pipetted into the flask and swirled the contents till it was thoroughly mixed. A blank sample was prepared by pipetting 25 mL of chloroform and 25 mL of Wijs solution into an empty Erlenmeyer flask. The flasks were labeled accordingly. The flasks were stopped with ground glass stoppers. About 5 mL of the 15% KI solution was pipetted into the stopper well. The flasks were stored in dark place for 30 minutes (60 minutes for expected iodine values greater than 150) to allow the reaction to take place completely.

After the completion of reaction, all the flasks were removed from the dark at the same time. About 20 mL of the 15% KI solution and 75 mL of deionized water were added to each of the flasks. The flasks were stirred well with a stir bar. The blank sample was titrated using 0.1 N sodium thiosulfate to reach the pale yellow endpoint. Nearly 2 mL of the starch indicator solution was added to the
flask and continue titrating until the blue colour just disappears (usually a white endpoint). The titration of each sample was repeated. The iodine value was calculated by using Equation (3.15).

\[
\text{Iodine Value} = \frac{\text{(mL Blank} - \text{mL sample)} \times (\text{N of HCl}) \times (12.69)}{(\text{Sample wt.})}
\]  

(3.15)

3.6.5 Unsaponifiable Matter

Unsaponifiable matter determines the amount of matter soluble in fats and oils, which cannot be saponified by caustic alkali.

3.6.5.1 Procedure

Five grams of sample was weighed into a 250 mL Erlenmeyer flask with ground glass joint. The weight was recorded up to three decimal places. Added 50 mL of saponification reagent and pipetted into the flask. The flask was placed on a hot plate and a condenser was connected to it with cold water flowing through it. The solution was allowed to reflux for one hour. After one hour, the inner surface of the condenser was quantitatively rinsed with approximately 20 mL of deionized water.

The flask from the hot plate was removed and quantitatively transferred the solution to a stokes flask using deionized water. Enough water was added to the stokes flask to bring the fluid level to the neck of the flask (just below the bulb of the flask). About 50 mL of petroleum ether was added to the stokes flask. The flask was stoppered and mixed gently for one minute. The petroleum ether layer was transferred into a 500 mL separating funnel using the stopper and glass tubing assembly. The experiments were repeated until a total of five extractions are performed. The petroleum ether layers were continuously added to the same separating funnel.
Approximately 25 mL of the 10% reagent alcohol solution was added to the separating funnel. The separating funnel was stoppered and mixed gently for one minute. The 10% reagent alcohol layer was disposed. The experiments were repeated until a total of three washes were performed of the petroleum ether layer. The washed petroleum ether layer was transferred to a tared 250 mL beaker. The beaker was placed on a steam bath and the petroleum ether was evaporated to dryness. The beaker was placed inside a hot air oven at 105°C for approximately 10 minutes (or until a constant weight was achieved). The weight of the residue was measured. Using Equation (3.16), the percent unsaponifiable matter in the sample was calculated.

\[
\text{Unsaponification Matter \%} = \left( \frac{\text{Wt. of residue}}{\text{Sample wt.}} \right) \times 100
\]

3.6.6 Density and Specific Gravity (Pycnometer)

This test method is used to measure the specific gravity of all oils and fats. The specific gravity is one parameter that can be used to monitor the quality of these products. This test method covers the determination of the specific gravity of oils and liquid fats by calculating the ratio of the weight of a unit volume of the sample to the weight of a unit volume of water at 30°C.

3.6.6.1 Preparation of sample

Melted sample (use liquid oil directly) was filter through a filter paper to remove any impurities and the last traces of moisture was removed by keeping in hot air oven at 105°C. The sample was checked for complete dryness, which was then cooled to ambient temperature (30°C).
3.6.6.2 Standardization of pycnometer

Pycnometer was cleaned properly by filling with Chromic acid cleaning solution and letting it to stand for several hours. Pycnometer was emptied and rinse thoroughly with distilled water. It was filled with recently boiled water previously cooled to 20°C and placed in constant temperature water bath held at 30°C. After 30 min the water was adjusted level to proper point on pycnometer and stopper. It was removed from bath, wiped dry with clean cloth or towel and weighed.

3.6.6.3 Procedure

The dry pycnometer was filled with prepared sample in such a manner to prevent entrapment of air bubbles after removing the cap of the side arm. The stopper was inserted and immersed in water bath at 30°C and hold for 30 min. Oil coming out of the capillary opening was carefully wiped off. The bottle was removed from the bath and was cleaned, dried thoroughly. The cap was removed from the side arm and weighed quickly ensuring that the temperature does not fall below 30°C. Using Equation (3.17) and Equation (3.18), the specific gravity and density of the sample were determined.

\[
\text{Specific Gravity at 30 } ^\circ \text{C} = \frac{A-B}{C-A} \tag{3.17}
\]

\[
\text{Density at 30 } ^\circ \text{C, gml}^{-1} = \frac{A-B}{\text{Volume of pycnometer}} \tag{3.18}
\]

where, A is the weight in g of specific gravity bottle with oil at 30°C, B is the weight in g of specific gravity bottle at 30°C, C is the weight in g of specific gravity bottle with water at 30°C.
3.6.7 **Total Moisture (Karl Fisher Method)**

The determination of total moisture by Karl Fisher (KF) titration is a calculation based on the concentration of iodine in the KF titrating reagent (i.e. titer) and the amount of KF reagent consumed in the titration. The endpoint of the titration is determined by the dead-stop end-point method.

### 3.6.7.1 Procedure

Fresh solvent was added into the titration vessel. The solvent was titrated till dryness (drift $<20 \ \mu\text{mL min}^{-1}$ is used as stop criteria). The sample was transferred to a closed glass-weighing spoon. The amount of sample depends on the water content; an expected amount of 10 to 50 mg water is suitable. The weighing spoon with a sample was placed on the balance. The balance was tared. The sample was dosed into the titration vessel. Close the titration vessel as soon as possible with the help of stopper. The weighing spoon with remaining powder was placed on the balance. The sample weight was calculated and titration was executed. When the endpoint was reached (drift $<20 \ \mu\text{mL min}^{-1}$) the volume of titrant was noted in mL. If the amount of KF reagent added is less than 0.5 mL, the amount of sample to be analyzed was increased. All measurements were made in duplicate. Using Equation (3.19) the total moisture content in weight percent was calculated.

\[
H_2O \ % = \frac{V \times F \times 100}{1000 \times W} \quad (3.19)
\]

Where, $V$ stands for mL KF reagent used for sample, $F$ stands for factor mg $H_2O \ \text{mL}^{-1}$ KF reagent and $W$ for weight in g.

3.6.8 **Fatty Acid Profile (Gas Chromatography Method)**

The determination of the fatty acid composition present in oil mainly as TG was determined by Gas Chromatogram (GC). This is generally achieved by open
tubular column gas chromatography through methanolic transesterification of the lipidic matrix. This is a well established conventional GC method that produces effective results.

3.6.8.1 Preparation of FAME

FAME of the samples were prepared according to AOAC Official Method 969.33 (Preparation of methyl esters by Boron Trifluoride method). Glycerides and phospholipids are saponified and fatty acids were esterified in the presence of BF₃ catalyst and were analyzed. 200 mg of oil sample was taken in a 100 mL round bottom flask and then 4 mL of methanolic sodium hydroxide (0.5 M) was added along with boiling chips. Attached condenser and refluxed until oil globules disappear (10 min). 5 mL of BF₃ solution from auto pipette was added through condenser and boiled for 2 min. 2 mL heptane was added through condenser and boiled for 1 min. Heat was removed and then added 15 mL saturated NaCl solution. Stoppered flask was shaken vigorously for 15 s. Additional saturated NaCl solution was added to float heptane solution into flask neck. Upper layer of heptane solution was transferred into glass-stoppered test tube and added small portion of anhydrous Na₂SO₄ to remove water. FAMEs were collected by means of syringe and diluted to a concentration of 10% for GC determination. It was then kept in closely tight glass vial in refrigerator.

3.6.8.2 Procedure

The fatty acid profile of oil was quantified and qualified using CHEMITO GC 8610 instrument with a flame ionization detector. The column was packed with BPX-70 phase (50% cyanopropyl and 50% methylsiloxane). The injection port was maintained at 250°C and the detector port was maintained at 260°C. The temperature of the oven was initially 160°C and was increased by 7.5°C per minute to a final oven temperature of 240°C. Total run time was 120 min. The career gas flow rate used here was 0.3 mL min⁻¹ (Nitrogen) and 15 mL min⁻¹ make
up gas (Nitrogen). Hydrogen and oxygen were used as flame gas at 35 and 350 mL min\(^{-1}\) respectively. A 1 \(\mu\)L syringe from Hamilton Co. was employed for injection.

1\(\mu\)L each of FAME standard solutions and sample solution (saturated, unsaturated and trans) was injected in GC. FAME standard solutions were optimized for chromatographic response before injecting the test solution. The data obtained were collected by Win-Chrom software and compared with standards. The mean molecular weight of the oil and FAME were calculated from the fatty acid profile using the Equation (3.20) and (3.21) respectively.

\[
MW_{\text{oil}} = 3 \sum (MW_i \times X_i) + 38 \quad (3.20)
\]

\[
MW_{\text{FAME}} = \sum (MW_i \times X_i) + 14 \quad (3.21)
\]

where, \(MW_{\text{oil}}\) stands for average molecular weight of the oil, \(MW_{\text{FAME}}\) stands for average molecular weight of FAME, \(MW_i\) and \(X_i\) stand for molecular weight and mass fraction of the \(i^{th}\) fatty acid, respectively.

### 3.7 CATALYST

The alkali methoxide was prepared by dissolving required amount of the high pure alkali hydroxide (NaOH or KOH) in anhydrous methanol. The methoxide ion (-OCH\(_3\)), is the active catalyst for the production of methyl esters. It is this chemical unit that attacks the triglyceride molecules and produces the methyl esters. It is regenerated at the end of each reaction step when a hydrogen ion is stripped from a nearby methanol molecule. If ethanol is being used, then the corresponding catalyst is called ethoxide (-OCH\(_2\)CH\(_3\)).
The methoxide ions needed for the reaction by dissolving NaOH or KOH in methanol. When this is done, the hydroxide splits apart or dissociates and then undergoes the following reaction as shown in Figure 3.2.

\[
\text{NaOH} + \text{CH}_3\text{OH} \rightarrow \text{NaOCH}_3 + \text{H}_2\text{O}
\]

Figure 3.2 Formation of sodium methoxide from NaOH and methanol

This reaction produce methoxide ion needed for homogeneous alkali catalyzed biodiesel production reaction. Unfortunately, the reaction also creates a molecule of water. Water causes the formation of soap through a chemical reaction called saponification. When alkali is used as catalysts, the soap production will be similar to what happens when oil or fat containing water is used.

Commercial producers prefer to get their methoxide from solutions of sodium (or potassium) methoxide already dissolved in methanol. Sodium methoxide, also known as sodium methylate can be purchased as a 25% or 30% concentration and is made from a water free process, so the catalyst does not contribute to soap formation.

3.7.1 Catalyst Preparation

The NaOH or KOH was preferred homogeneous alkali catalyst for catalyzing transesterification reaction. KOH is readily soluble in methanol and the reaction rate was slightly higher when compared to equimolecular weight of NaOH. Preparation of the catalyst was done by dissolution of alkali hydroxide with excess methanol. A stock of catalyst solution was prepared by dissolving 15 g of hydroxide in 100 mL of anhydrous methanol under cold condition. The
dissolution of hydroxide in methanol produces the methoxide and water. Therefore the solution was dried by the addition of 5 g of previously dried anhydrous sodium sulphate. The prepared solution was mixed well, allowed to settle and filtered through a Whatman filter paper. The weight percentage of the catalyst required for the reaction was added in volume from the stock based on their weight proportions.

Similarly homogeneous acid catalyst was prepared by dissolving 10 g of concentrated H\textsubscript{2}SO\textsubscript{4} in unhydrous methanol. The dissolution of of H\textsubscript{2}SO\textsubscript{4} is an extremely exothermic reaction. So the acid was added slowly and cooled simultaneously.

3.8 TRANSESTERIFICATION SETUP

The transesterification reaction was carried out in a 250 mL double-necked round flat bottom flask, equipped with a thermometer, condenser and magnetic stirring systems as shown in Figure 3.3. To prevent the loss of methanol during reaction, a water-cooled condenser was used to condense the vapors and reflux it back into the reactor. In order to achieve perfect contact between the reagents and the oil during transesterification, they must be stirred well at constant rate. The reactor containing 100 mL oil was immersed in a water bath, heated by a hot plate to a predetermined temperature at 600 rpm to promote a sufficient agitation. Required amount of catalyst previously dissolved in methanol was charged into the reactor. After the reaction was completed, heating and stirring were stopped. Experiments were performed in triplicate and the results shown were from the mean of three independent experiments.
3.9 DOWNSTREAM PROCESS

The ASTM standards ensure that the following important factors in the fuel production process by transesterification are satisfied:

(i) Complete transesterification reaction

(ii) Complete esterification of FFA

(iii) Removal of glycerin

(iv) Removal of catalyst

(v) Removal of alcohol

Lower value of the specific gravity of the final product is an indication of completion of reaction and removal of heavy glycerin (Sharma et al 2008). As a
thumb rule, if the difference in specific gravity of 0.1 in a mixture of immiscible liquids will result in phase separation by gravity (Luo et al 2008). Gravity separation is suitable to recover biodiesel from the process byproducts (glycerin and alcohol), as shown in Table 3.4.

3.9.1 Separation

The FAME and glycerol separation is typically the first step of product recovery in biodiesel processes. The separation process is based on the facts that FAME and glycerol are sparingly mutually soluble and there is a significant difference in density between the ester and glycerol phases. The presence of methanol in one or both phases affects the solubility of biodiesel in glycerol and glycerol in biodiesel. The washing step is used to neutralize any residual catalyst, to remove any soap formed during the transesterification reaction and to remove residual free glycerol and methanol.

FAME has a lower density in comparison to glycerol, which has a density in the order of 1.05 g cm\(^{-3}\) or more. The glycerol density depends on the amount of methanol, water and catalyst in the glycerol. This density difference is sufficient for the use of simple gravity separation techniques for the two phases. However, the rate of separation is affected by several factors. In biodiesel processes intense mixing were used to incorporate the sparingly soluble alcohol into the oil phase. The mixing continues for the entire reaction and the glycerol can be dispersed in very fine droplets throughout the mixture. This dispersion requires several minutes to several hours to allow the droplets to coalesce into a distinct glycerol phase.
Table 3.4   Physical properties of chemicals related to transesterification

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Specific Gravity</th>
<th>Melting Point (K)</th>
<th>Boiling Point (K)</th>
<th>Solubility (&gt;10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl myristate</td>
<td>0.875</td>
<td>291.0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Methyl palmitate</td>
<td>0.825</td>
<td>303.8</td>
<td>469.2</td>
<td>Benzene, ether, ethanol</td>
</tr>
<tr>
<td>Methyl stearate</td>
<td>0.850</td>
<td>311.2</td>
<td>488.2</td>
<td>Ether, chloroform</td>
</tr>
<tr>
<td>Methyl oleate</td>
<td>0.875</td>
<td>253.4</td>
<td>463.2</td>
<td>Ethanol, water</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.792</td>
<td>176.2</td>
<td>337.9</td>
<td>Water, ether, Ethanol</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.789</td>
<td>161.2</td>
<td>351.6</td>
<td>Water, ether</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.26</td>
<td>255.3</td>
<td>563.2</td>
<td>Water, ether</td>
</tr>
</tbody>
</table>

The reaction mixture was transferred into a decanter (separating funnel) and left for 12 h to separate into two distinct phases as shown in Figure 3.4, ester phase (biodiesel) and glycerol phase. It took approximately 10 min for phase separation, but the biodiesel layer was translucent. After 12 h, the ester phase became transparent and the separation was completed. Further reaction may happen during the settling time, but the process is slow because of a low temperature, lack of stirring and presence of low amounts of catalyst and methanol. However, it is said that even longer settling time is favorable for the separation (Leung and Guo 2006). The lower glycerol layer was decanted.
The temperature in the decanter affects the solubility of the alcohol in both phases and the viscosity of the two liquids. Too high temperature in the decanter can cause residual alcohol to flash. On the other hand, too low a temperature increases the viscosity in both phases. The increased viscosity will slow the coalescence rate in the system. So a warm temperature of approximately 40 to 50°C was maintained throughout the process.

3.9.2 Methanol Recovery

An excess of alcohol is normally used in biodiesel production, to ensure that the oil or fat used will be fully converted to esters. But the amount of alcohol in the system has to be minimized for good phase separation. Once the glycerol and biodiesel phases have been separated. The excess alcohol in each phase was removed by using RV10 control V-C rotary vacuum evaporator (Figure 3.5) that
was purchased from IKA, China. The alcohol that was recovered can be reused. Care must be taken to ensure no water accumulates in the recovered alcohol stream.

Figure 3.5 Rotary vacuum distillation unit

3.9.3 Washing and Drying

The primary purpose of washing step is the removal of any soap formed during the transesterification reaction. In addition, the water provides a medium for addition of acid to neutralize the remaining catalyst and a means to remove the product salts. The residual methanol should be removed before the wash step.
Washing was performed in a 1 L three neck flask equipped with stirrer and thermometer. The flask was immersed in a constant temperature water bath and the temperature was maintained between 40 to 50ºC. Three-neck flask was initially charged with 100 mL of crude biodiesel and then placed in constant temperature bath. A 200 mL of water was poured while mixing. The use of warm condition prevents precipitation of saturated fatty acid esters and retards the formation of emulsions with the use of a gentle washing action. Softened water (slightly acidic) eliminates calcium and magnesium contamination and neutralizes remaining alkali catalysts. Weak acid like acetic acid was used for this purpose. Mechanical mixing is normally applied to increase the mass transfer rate. Therefore for all experiments on biodiesel washing, a maximum rotation of 100 rpm was used. Gentle washing prevents the formation of emulsions and results a rapid and complete phase separation.

After one hour, the mixture was transferred into a separating funnel and allowing the water to separate by gravity. This was kept for three hours without any disturbance for phase separation. Then the bottom aqueous layer was removed and separated from biodiesel layer. The biodiesel was washed for two cycles with acidic water followed by three cycles with soft water. The pH of the water layer was analyzed and washing was further continued till the pH reaches around 7 pH. The phase separation between esters and water is typically very clear and complete as shown in Figure 3.6 and Figure 3.7. However, the equilibrium solubility of water in FAME is higher than the specified water content for B100. Therefore, after the washing step there will be more than the equilibrium amount of water present.
Figure 3.6 Water and biodiesel phase separated in initial wash

Figure 3.7 Water and biodiesel phase separated in final wash
Biodiesel drying is required to meet the stringent limits on the amount of water present in the final biodiesel product. Finally, the product was dried for 1 h in a hot air oven at 105°C or till moisture content reaches below 0.05 wt%. The final product yield was determined gravimetrically and the biodiesel yield was calculated using the Equation (3.22).

\[
Biodiesel\ yield\ (wt\%) = \frac{\text{Mass of Biodiesel\ produced\ (g)}}{\text{Mass of oil\ taken\ (g)}} \times 100 \tag{3.22}
\]

### 3.10 PROTON NUCLEAR MAGNETIC RESONANCE (H\textsuperscript{1}NMR)

H\textsuperscript{1}NMR analysis is a reliable and rapid analysis in high priority for quality control in biodiesel production. Quantifying biodiesel with alternative H\textsuperscript{1}NMR can provide total methyl esters distributions without significant sample pretreatment, which identify and quantify relative and absolute concentrations of methyl esters in a biodiesel.

#### 3.10.1 H\textsuperscript{1}NMR Analysis

Proton NMR provides a good probe for biodiesel since H\textsuperscript{1} is the most naturally abundant and most sensitive NMR active isotope. Relatively narrow line widths of a few Hertz are obtained for H\textsuperscript{1} spectra so that magnetically unique nuclei are resolved at many field strengths.

The peaks at 5.35 ppm, 2.8 ppm and 2.1 ppm are related to the H\textsuperscript{1} located at or near the double bond(s) within the unsaturated methyl esters. The sharp peak at 3.7 ppm is due to the ester methyl located next to the carbonyl carbon and the triplets around 0.9 ppm are from the terminal alkyl methylin each of the methyl esters. The methylene alpha to the ester group is at 2.3 ppm and the beta group is at 1.6 ppm. The remaining CH\textsubscript{2} group protons have similar resonance frequencies and overlap in the range of 1.2 to 1.4 ppm. The total intensity in this region is the
sum of the individual contributions from the remaining CH\textsubscript{2} groups in the molecule.

Analyses were performed on a Bruker AVANCE III 500 MHz (AV 500), at 300 K using a 5 mm probe head and CDCl\textsubscript{3} as solvent. All samples were dissolved in deuterated chloroform with 1\% v/v TMS (1,3,5-tris [trifluoro methyl] benzene). A standard TMS was used to quantify the exact concentration of the methyl esters in the NMR tube. The protons of the methylene group adjacent to the ester moiety in triglyceride and the protons in the alcohol moiety of the product methyl esters were used to calculate the conversion of oil to biodiesel. The conversion was calculated using the prescribed Equation (3.23).

\[
\text{Conversion(\%)} = 100 \times \frac{2A_{\text{ME}}}{3A_{\text{\alpha-ME}}}
\]  

(3.23)

where A\text{ME} stands for the integration value of the methoxy protons of the methyl esters and A\text{\alpha-ME} is the integration value of methylene protons.

3.11 CHARACTERIZATION OF BIODIESEL

The values of the various biodiesel properties specified by ASTM D6751 are listed in Table 3.5. These properties were analyzed in Petroleum Testing Laboratory, Department of Chemical Engineering, Anna University, Chennai; Sophisticated Analytical Instrument facility, Indian Institute of Technology Madras, Chennai and Sargam Lab, Maedavakkam, Chennai. Each of these properties and the test methods used to measure are described below.
Table 3.5  ASTM D6751 requirements

<table>
<thead>
<tr>
<th>Properties</th>
<th>Units</th>
<th>Test methods</th>
<th>ASTM D6751 Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>---</td>
<td>ASTM D4052</td>
<td>---</td>
</tr>
<tr>
<td>Flash point</td>
<td>ºC</td>
<td>ASTM D93</td>
<td>130 Min</td>
</tr>
<tr>
<td>Cloud point</td>
<td>ºC</td>
<td>ASTM D2500</td>
<td>Report</td>
</tr>
<tr>
<td>Pour point</td>
<td>ºC</td>
<td>ASTM D2500</td>
<td>Report</td>
</tr>
<tr>
<td>Oxidation Stability</td>
<td>h</td>
<td>EN 14112</td>
<td>3 Min</td>
</tr>
<tr>
<td>Cold Soak Filtration Time</td>
<td>second</td>
<td>ASTM D7501</td>
<td>360 Max</td>
</tr>
<tr>
<td>Viscosity@40ºC</td>
<td>mm²s⁻¹</td>
<td>ASTM D445</td>
<td>1.9-6</td>
</tr>
<tr>
<td>Acid number</td>
<td>mgKOHg⁻¹</td>
<td>ASTM D664</td>
<td>0.05 Max</td>
</tr>
<tr>
<td>Cetane number</td>
<td></td>
<td>ASTM D613</td>
<td>47 Min</td>
</tr>
<tr>
<td>Water &amp; sediments</td>
<td>% volume</td>
<td>ASTM D2709</td>
<td>0.05 Max</td>
</tr>
<tr>
<td>Sulfated ash</td>
<td>% weight</td>
<td>ASTM D874</td>
<td>0.02 Max</td>
</tr>
<tr>
<td>Copper strip corrosion</td>
<td>---</td>
<td>ASTM D130</td>
<td>Number 3 Max</td>
</tr>
<tr>
<td>Na &amp; K combined</td>
<td>ppm</td>
<td>EN 14538</td>
<td>5 Max</td>
</tr>
<tr>
<td>Ca &amp; Mg combined</td>
<td>ppm</td>
<td>EN 14538</td>
<td>5 Max</td>
</tr>
</tbody>
</table>

3.11.1 Method: ASTM D93 - Flash point using Pensky-Martens Flash Cup Tester (Requirement: 130 ºC minimum)

The flash point is defined as the lowest temperature corrected to a barometric pressure of 101.3 kPa (760 mm Hg), at which application of an ignition source causes the vapors of a specimen to ignite under specified conditions of test. This test, is a measure of residual methanol in the B100.
The flash point is a determinant for flammability classification of materials. The typical flash point of pure methyl esters is >200°C (classifying them as non-flammable). However, during production and purification of biodiesel, all the methanol may not be removed, making the fuel flammable and more dangerous to handle and store. If the flash point falls below 130°C, excess methanol in the fuel may also affect engine seals and elastomers and corrode metal components.

3.11.2 Method: ASTM D2500 - Cloud point using Cloud and Pour Point Bath
(Requirement: Reported in °C to customer)

The cloud point is the measure of temperature at which a cloud of wax crystals first appears in a liquid when it is cooled down under conditions prescribed in this test method. The cloud point is a critical factor in cold weather performance for all diesel fuels. The chemical composition of some biodiesel feedstock leads to a B100 that may have higher cloud points. The saturated methyl esters, methyl palmitate and methyl stearate are the determining factors for the cloud point.

3.11.3 Method: ASTM D2500 - Pour point using Cloud and Pour Point Bath
(Requirement: Reported in °C to customer)

Cloud point is the temperature at which the wax is first precipitated and the fuel began to appear ‘cloudy’. It is the temperature at which wax first becomes visible to the observer when the fuel is cooled in a glassy vessel at a specific rate. Cloud point depends on boiling range of biodiesel. Saturated methyl esters are first components to come out as wax. One of the drawbacks of cloud point measurement is its depended on the testers judgment. Usually the cloud point of the diesel fuel is in the range between 10 and 20°C depending upon location and season.
3.11.4 Method: EN 14112 - Oxidation Stability using Rancimat Method
(Requirement: 3 h minimum)

The measure of resistance to oxidative degradation during storage is Oxidation stability. Compared to petroleum diesel fuel, biodiesel oxidizes easily and forms reaction products shown to have unfavorable effects on vehicle fuel systems. Biodiesel molecules typically have several double bonds, and oxidize easily. Initially, the oxidation process generates peroxides (hydrogen peroxide), which subsequently forms acids. The acid itself plays a major role in the acceleration of oxidation. In the end, the acid reacts with sludge that deposits or other undissolved chemical compounds and accumulate as deposit. Therefore, oxidation stability is one of the most important characteristics for practical use of biodiesel.

3.11.5 Method: ASTM D613 - Cetane number using (Requirement: 47 minimum)

The cetane number is a measure of the ignition performance of a diesel fuel obtained by comparing it to reference fuels in a standardized engine test. For B100, the cetane number is seldom an issue because all of the common fatty acid esters have cetane numbers near or above 47. The cetane number can be predicted ± 10 % using the esters composition.

3.11.6 Method: ASTM D2709 - Water and sediment using Bench Top Centrifuge (Requirement: 0.05% volume maximum)

Water and sediment is a test that determines the volume of free water and sediment in middle distillate fuels having viscosities at 40°C in the range 1.0 to 4.1 mm² s⁻¹ and densities in the range of 700 to 900 kg m⁻³.

This test is a measure of cleanliness of the fuel. For B100 it is particularly important because water can react with the esters, making FFAs and can support
microbial growth in storage tanks. Water is usually kept out of the production process by removing it from the feedstock. However, some water may be formed during the process by the reaction of the NaOH or KOH catalyst with methanol. If FFAs are present, water will be formed when they react to either biodiesel or soap. Finally, water is deliberately added during the washing process to remove contaminants from the biodiesel. This washing process was followed by a drying to ensure the final product will meet ASTM D2709. Sediments may plug fuel filters and may contribute to the formation of deposits on fuel injectors and engine damage. Sediment levels in biodiesel may increase over time as the fuel degrades during extended storage.

3.11.7 Method: ASTM D874 - Sulfated Ash using Basic Muffle Furnace

(Requirement: 0.02 wt% maximum)

Sulfated ash is the residue remaining after a fuel sample has been carbonized and the residue subsequently treated with sulfuric acid and heated to a constant weight. This test monitors the mineral ash residual when a fuel is burned. For biodiesel, this test is an important indicator of the quantity of residual metals present in the fuel that came from the catalyst used in the esterification process. Many of these spent sodium or potassium salts have low melting temperatures and may cause engine damage in combustion chambers.

3.11.8 Method: ASTM D445 - Kinematic viscosity at 40ºC using Brookfield Viscometer (Requirement: 1.9 - 6.0 mm²s⁻¹)

Kinematic viscosity is the resistance to flow of a fluid under gravity. The kinematic viscosity is equal to the dynamic viscosity/density. The kinematic viscosity is a basic design specification for the fuel injectors used in diesel engines. The injectors do not perform properly for high viscosity fuel.
3.11.9 Method: ASTM D664 - Acid Number using Standard Acid Base Titration (Requirement: 0.50 mg KOH g\(^{-1}\) maximum)

The acid number is the quantity of base (expressed as milligrams of KOH per gram of sample), required to titrate a sample to a specified end point. The acid number is a direct measure of FFA in B100. The FFA can lead to corrosion and may be a symptom of water in the fuel. Usually, for an alkali catalyzed process, the acid value after production will be low since the alkali catalyst will strip the available FFAs. However, the acid value may increase with time as the fuel degrades due to contact with air or water.

3.11.10 Method: ASTM D130 - Copper Strip Corrosion using Copper Strip Test Bath (Requirement: No. 3 maximum)

The copper strip corrosion is used for the detection of corrosiveness to copper by fuels and solvents. This test monitors the presence of acids in the fuel. For B100, the most likely source of a test failure would be excessive FFAs, which are determined in accordance with an additional specification.

3.11.11 Method: EN 14538 - Na & K combined using Laser Ablation Inductively Coupled Plasma Mass Spectrometer (Requirement: 5 ppm maximum)

The evaluation of Na and K is also important since some production processes employ KOH or NaOH as catalysts. Alkali earth metals can be found in biodiesel at very low levels. Na and K can form abrasive solids or metallic soaps, which may cause abrasion and filter plugging.
3.11.12 Method: EN 14538 - Ca & Mg combined using Laser Ablation Inductively Coupled Plasma Mass Spectrometer (Requirement: 5 ppm maximum)

Calcium and magnesium may be present in biodiesel as abrasive solids or soluble metallic soaps. Abrasive solids can contribute to injector, fuel Pump, piston, and ring wear, as well as to engine deposits. Soluble metallic soaps have little effect on wear, but they may contribute to filter plugging and engine deposits. High levels of calcium and magnesium compounds may also be collected in exhaust particulate removal devices, are not typically removed during passive or active regeneration, and can create increased back pressure and reduced time to service maintenance. Engine manufacturers were concerned that even very small amounts of minor compounds like Ca and Mg could build up in particulate traps and eventually cause the traps to clog particulate filter.