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Recent production methodologies and advanced spectroscopic characterization of biodiesel: A review

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Abstract

The development and improvement of analytical methods such as chromatography and spectroscopy has been used for monitoring biodiesel production and determining the fuel quality. Spectroscopic techniques being able to be coupled with other separation methods have number of advantages over gas chromatography (GC) and high performance liquid chromatography (HPLC). Spectroscopic techniques are vital in analysis of biodiesel due to higher sensitivities, reproducibility, less time consumption, better characterization and identification of large number of chemical structures. Therefore, the present article is designed to review the various spectroscopic techniques that have been used to evaluate the quality of biodiesel as well as the process of transesterification. Nuclear magnetic resonance (NMR) spectroscopy, ultraviolet-visible spectroscopy, thermal lens spectroscopy (TL), infrared spectroscopy (IR) and Raman spectroscopy have been reported to be used for monitoring the quality control of feedstocks and end products of biodiesel. Their applications include determination of levels of blending of biodiesel, oxidative degradation, contamination analysis and transesterification monitoring.

Keywords: GC, HPLC, NMR, UV-Visible, TL, IR, Raman spectroscopy

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1. Introduction

Energy is an ultimate requirement for our daily activities and is needed in all of the sectors including transportation, agriculture and industry [1-3]. The need of energy is increasing rapidly due to increase in industrialization and number of vehicles. The main energy sources are non-renewable sources which include natural gas, coal, crude oil, propane and nuclear energy [4-5]. The large numbers of non-renewable sources are utilized by transport sector. Crude oil accounts for 35% of total energy utilization [6-7]. The major problem is that there are limited fossil fuel sources and their demand is increasing day by day. It is estimated that energy sources will be depleted soon due to increase in worldwide population to 8 billion by 2025 [8]. Non-renewable energy sources are not environment friendly as their consumption significantly contributes to acid rain and global warming. The biofuels are one of the alternatives of energy sources, they are renewable and have high potential [9-11]. Biodiesel and ethanol are two types of biofuels. Ethanol is basically an alcohol which is produced by the fermentation of any biomass, rich in carbohydrates. Biodiesel is advanced form of biofuels. It is biodegradable, non-toxic and renewable fuel, consisting of long chain methyl esters of fatty acids of fats or oil [7]. Biodiesel is helping many countries to overcome their demand of diesel. It can be used in any diesel engine with small or no modification in the engine system. In comparison with conventional diesel, it has high flash point, non-toxic, renewable and eco-friendly nature [12]. Biodiesel blends can be used in the transportation sector because it shows the properties similar to petroleum diesel but has low emission of greenhouse gas [13]. Biodiesel utilization can results in the reduced production of transferable carcinogens and pollutants [14].

Vegetable oils can be converted into methyl esters through the process of transesterification. Researchers of biodiesel have shown that fuel derived from vegetable oil can be properly used in the diesel engines [15-17]. The density of biodiesel is nearly close to the conventional fuel. Biodiesel can be produced by the transesterification of fat or vegetable oil with methanol. In the presence of acid catalysts, combustion properties of biodiesel has made it most promising sustainable and renewable source of energy for automobiles as compare to conventional petroleum fuel [18]. Biodiesel can be classified into three generations which depends on their source. First generation of biodiesel

is commonly derived from the edible feedstocks, for example soybean and rapeseed oil. The second generation of biodiesel is derived from the non-edible feedstock, for example jatropha or neem oil. The main benefits of second generation biodiesel are low cost of biodiesel production. It eliminates food imbalance and less need of cultivation land [8]. The main drawbacks of biodiesel from non-edible oils are: (i) high consumption of alcohol does not fulfil the commercial demand and (ii) highly viscous nature. The third generation of biodiesel is generated from microalgae. Information of merits and demerits of biodiesel generated from different feedstock could help to the focus on main problems faced in the process of biodiesel production [19].

Currently, there are more than 350 species of plants that are potential source of biodiesel. Feedstock selection for biodiesel is very important because it is related to the 75% of the total production cost. The quality of biodiesel mainly depends on the raw material used for its production, country of its origin and process of production. The selection of suitable feedstock is very important for biodiesel production as the 75 % cost of biodiesel depends on the feedstock [20]. The feedstock vary from country to country, such as in Europe main feedstock for biodiesel production is rapeseed, in Argentina, Brazil and US main source is soybean oil. However, in Indonesia and Malaysia, main feedstock of biodiesel is palm oil [21].

The main advantages of biodiesel are low aromatic and sulfur contents, eco-friendly nature, easy availability, high biodegradability, high cetane number and renewability. The main disadvantages of biodiesel are low energy contents, high nitrogen oxide emissions (NOx), high viscosity, injector coking, low speed and power of engine, high price and compatibility of engine [22-23]. Present review is the compilation of advanced spectroscopic and chromatographic techniques used for complete chemical characterization and separation of essential classes of compounds using hyphenated techniques such as GC, HPLC, NMR, UV-Visible, TL, IR, Raman spectroscopy.

2. Production of biodiesel

There are many problems related to the biodiesel production among them most common are methods of biodiesel production and cost of biodiesel feedstock. Many techniques have been used for biodiesel production such as transesterification, pyrolysis, reactive distillation, micro-emulsion and distillation. Every technique has its own pros and cons, and its own specific suitable feedstock. Reaction conditions are also important in each technique, these conditions include alcohol to oil molar ratio, reaction time, reaction temperature, type and amount of solvents and catalyst, and reaction medium [24].

2.1. Pyrolysis

In thermal cracking or pyrolysis, organic mass is converted into the fuel by heating it in the absence of oxygen. In many studies, it is reported that fuel produced from this method contains a tolerable quantity of sulfur, less viscosity and less ignition delay. Pyrolysis process can be divided into three classes, flash pyrolysis, conventional pyrolysis and fast pyrolysis [25]. The classification of pyrolysis process with difference in heating rate, temperature, residence time and major product is shown in Table 1.

Table 1: Classification of pyrolysis methods with differences in temperature, residence time, heating rate and major products

Method	Heating Rate (℃/s)	Residence Time (Seconds)	Temperature (°C)	Major Products
Fast pyrolsis	High 100	Short than 5-2s	Med-high (400-650)	Bio-oil (thinner) Gases and Char
Ultra-fast/flash pyrolysis	Low 10	Very short<0.5s	High (1000)	Gases and Bio-oil
Conventional/slow pyrolysis	Low 10	Long 5-30 min	Med-high (400-500)	Gas, Char and Bio-oil

The liquid fraction produced by the pyrolysis of bio-oils and vegetable oils is likely to reach conventional fuel characteristics and properties. The disadvantage of pyrolysis process is requirement of distillation equipment to separate various fractions. The product produced is comparable to gasoline having sulfur, making it less eco-friendly [14]. The equipment for this process is expensive, the products of reactions are also chemically similar to the diesel fuel and oxygen removal during this process also eliminates the eco-friendly benefits of oxygenated fuel. It generates some lower value materials and sometimes it produces gasoline instead of diesel fuel [26].

2.2. Micro-emulsification

The vegetable oils cannot be used directly as a fuel due to its high viscosity. It is observed that this problem can be solved by formation of Microemulsion [26]. The biodiesel micro-emulsion consists of vegetable oil, diesel fuel, surfactants, alcohol and cetane improver. Methanol and ethanol additives are used to reduce the viscosity, alkyl nitrate can be used as cetane improver and high molecular weight alcohols can be used as surfactants [27-28]. The spray properties are improved by micro-emulsions as micelle vaporized explosively. It also resulted in high cetane number, reduced viscosity, better spray characteristics of biodiesel [29]. The regular use of micro-emulsion causes problems such as incomplete combustion, sticking of injector needle and carbon deposit [30].

2.3. Dilution

Generally, the direct use of vegetable oils is not feasible for both indirect and direct use in diesel engines. The free fatty acid contents, high viscosity, formation of gum due to polymerization and oxidation during combustion and storage, thickening of lubricating oil and deposits of carbon are critical problems. In these conditions, dilution of vegetable oils with solvent, diesel fuels or ethanol is helpful. The dilution resulted in decrease density and viscosity of vegetable oils. It has been reported that addition of (4%) ethanol in fuel significantly enhanced the thermal brake efficiency, brake power and brake torque, while decreased the specific fuel combustion in brake [31].

2.4. Catalytic distillation

Biodiesel can be produced by reactive distillation method as it involves the use of multifunctional reactor. It combines the thermodynamic separation and chemical reaction in a single unit; thus, it improves the general process of distillation. This process can be successfully used in the biodiesel production and the chemical equilibrium is also maintained, in the end product. The main advantage of this method is that feedstock having high free fatty acid contents can easily be used in this process. In this process, there is no need of extra methanol to move the equilibrium towards ester formation, which is the main product. It can be done by continuous removal of water which is one of the by-products [32].

2.5. Transesterification

It is the most common method for biodiesel production. This process involves the conversion of oil and fats using alcohol (methanol or ethanol) in the presence of catalyst [33]. Transesterification mechanism is shown in Fig.1.

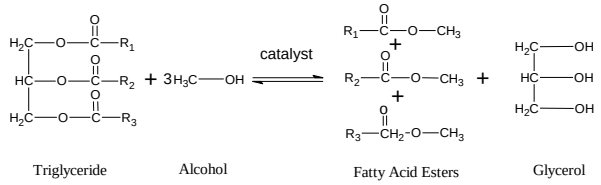

Fig.1 Mechanism of transesterification reaction

2.5.1. Acid catalyzed transesterification

In history of biodiesel production, it was the first method to produce ethyl esters from palm oil using sulfuric acid and ethanol [34]. This process is acid catalyzed as reaction occurs in the presence of acid catalyst to produce esters and glycerol as by-product. This method is specifically economical for biodiesel production from feedstock having high contents of free fatty acids. However, this reaction requires high temperature and long reaction time as compared to alkali catalyzed reaction [35].

2.5.2. Alkaline catalyzed transesterification

In this process, alkali catalyst is used in the transesterification of fat or oil with alcohol, esters and glycerol as the byproducts. Alkali catalyst can be alkoxides or hydroxides of alkaline metals, sodium or potassium carbonates. Alkali catalyzed reaction is much faster than acid catalyzed reaction and is not corrosive to commercial equipment, thus this process is commonly used in commercial production of biodiesel [14-36]. However, free fatty acids of feedstock and water lead towards the saponification reaction which is the cause of incomplete reaction and emulsion formation and difficulty in the separation of glycerol from product [30].

2.5.3. Ultrasound-assisted transesterification

In this process, cavitation is used to mix the alcohol and oil phase, which is promising for the reaction. It enhances the mass transfer rate, which results in better yield. In this method, catalyst reactive surface area is increased with the help of low frequency. High temperature and pressure is the need of this process, which is achieved by ultrasonic wave. The main drawback of this process is the production of unwanted products such as soap. Furthermore, waste water is produced in large amount when excess of catalyst, glycerol and soap is washed after the reaction completion [13-32].

2.5.4. Enzyme catalyst transesterification reaction

In the transesterification reaction, use of enzyme eliminates the problems of soap formation, washing, purification and neutralization thus use of enzyme is preferred. The feedstock having high free fatty acid contents can be easily converted into biodiesel with the use of enzyme as a catalyst. The use of lipase in transesterification is very efficient as it can work in mild reaction conditions and can easily be separated out and reused. The major disadvantages related with the use of enzymes in transesterification are high price of enzymes and small range of working temperature due to enzymes denaturation at high temperature [37]. The problem with use of enzymes as a catalyst is long reaction time and high prices [38]. The most commonly used enzyme in transesterification reaction is lipase. The lipase catalyzed the process of transesterification between triglycerides and alcohol to produce esters and glycerol [34].

2.6. Supercritical technology

In this method, oil and alcohol that are immiscible at room temperature form a homogenized mixture. It happened due to decrease in dielectric constant and significant change in the solubility of alcohol, which makes the alcohol non-polar solvent [38]. In this process there is no transfer of mass takes place. Supercritical transesterification process occurs under high pressure and temperature. When the temperature and pressure of liquid or gas cross the

critical point, unusual changes are shown in their properties. This method is feasible for continuous production on commercial scale. The main drawback of this method is high energy requirement to achieve the supercritical conditions of solvent.

In this case, liquid and vapor phase are no longer confined under these conditions and single supercritical fluid phase is generated. This method is easy to use in continuous production on a large scale. The major disadvantage of this option is the high energy demand for the solvent to achieve supercritical conditions. The need of high pressure and temperature depends on the solvent, so special solvent fabrication is required, which leads to an unsustainable process with high cost [39]. Another limitation of this process is corrosion and salt deposition. The corrosion occurs due to the fact that supercritical water has increases the level of dissolved oxygen and inorganic ions. It can be due to change in pH values at elevated temperature and pressure [40].

2.7. Nano-catalytic technology for biodiesel production

From the past few years, use of nano-sized catalyst has gained special attention for production of biodiesel due to high efficiency [41]. Nanoscale catalyst have high surface to volume ratio which results in increase catalytical activity when compared to other catalysts [42]. As these catalysts possess high surface area, many bottle neck difficulties related with transesterification reactions have been eliminated [13]. The characteristics of nano catalysts include high activity, reusability, high surface area and resistance to saponification reaction [13-43]. These catalysts can be used either in the supported form with solids such as oxides, carbon, zeolites or without support where catalyst is used directly [41].

3. Characterization of biodiesel

In the process of biodiesel production, many intermediates and by-products are formed such as glycerol, mono-acyglycerols and di-acyglycerols, small quantity of these might remain in biodiesel. These intermediates and by-products contaminate the biodiesel. While using biodiesel such contaminations can be the cause of serious operational problems including filter clogging, engine deposits or fuel deterioration [35]. Therefore, for the successful commercialization of biodiesel, quality control factors have much importance. Some of important issues of quality control of biodiesel involve the check on transesterification reaction, quantification of glycerol and unreacted triglycerides as well as determination of unreacted alcohol and catalyst. Most commonly used analytical methods for biodiesel characterization are spectroscopy and chromatography [44]. Most widely used method is GC because it has high accuracy for the quantification of minor components. HPLC is also common. Biodiesel analysis with GC is time consuming as well as expensive [45]. The spectroscopic techniques show many positive points about the use of analytical methods such as high sensitivity, requirement of small amount of sample, identification of chemical structures and reproducibility [46].

3.1. Introduction to spectroscopy

Spectroscopy involves the study of interaction between electromagnetic radiations and matter. The base of spectroscopic analysis is the measurement of radiation absorbed or emitted by the species of interest. It is the most common technique used for the identification of the sample through the spectrum, generated by the emitted or absorbed radiation by the sample. In spectroscopy analysis, different regions of electromagnetic radiations can be used. It regions include radio waves, microwave, IR, visible, UV, x-rays and gamma rays [47].

Spectroscopic techniques have many applications including analysis of biological and elemental abundance in astrophysical objects [48-49]. It is mandatory to ensure the quality of fuel before its acceptance in market and commercialization [50-52]. The properties of biodiesel are largely influenced by the concentration and type (chain length, unsaturation), of the fatty acids used in the production of biodiesel [6-7]. The presence of contaminants in the final product depends on the raw material and method used for the production of biodiesel [53]. That is the reason behind the regular monitoring of fuel quality and presence of contaminants [37-53].

3.2. Spectroscopy techniques for biodiesel characterization

3.2.1. UV/Vis spectroscopy

The spectroscopic analysis is fast and provides informative data when biodiesel sample is decomposed [54]. This technique uses electromagnetic radiation in the range of 190 nm to 800 nm, ultraviolent (190-400 nm) and visible (400-800 nm). This technique provides the information about conjugated unsaturated, conjugated non-bonding electron systems, π-electron systems and aromatic compounds. It studies the energy changes in the electronic energy levels of molecules, movement of electrons from non-bonding or π orbitals. Thus, UV-Vis spectroscopy can monitor the conjugate dienes presence in the oxidized biodiesel [55].

3.2.1.1. Oxidative degradation of biodiesel

The fatty acid composition has great influence on the oxidative stability of biodiesel, stability decreases with the increased contents of linolenic and linoleic acid [56]. Linoleic and linolenic acid contain two and three double bonds respectively. When the biodiesel is degraded, unwanted compounds are formed such as organic acids, gums and aldehydes. These compounds cause many problems such as blockage of filter and engine. Thus, the feedstock having double bond fatty acids are of main concern, particularly when biodiesel is to be stored for long

period of time [57]. Oxidation of biodiesel is initiated by the abstraction of hydrogen atom, results in the formation of radical. This radical further combines with oxygen and produce peroxy radical (ROO·), this radical gains the hydrogen atom and form hydroperoxide (ROOH). In biodiesel, after the peroxide formation, non-conjugated double bonds are converted into the conjugated double bonds [58]. It is important to note that this analytical technique provides information about the quality and oxidative products of the biodiesel. It determines the different primary and secondary oxidation products such as ketones, peroxides, aldehydes and others, for this purpose absorbance at 232nm was used to detect the presence of peroxidation products of sunflower biodiesel [57].

3.2.1.2. Quantitative analysis of biodiesel in diesel-biodiesel blends

The UV-Vis spectroscopy has been used for the quantitative analysis of biodiesel in the blends of biodiesel. This technique provides the information of biodiesel concentration in their blends. The complete method for analysis of biodiesel consists of two steps. The first step is the quantification of biodiesel contents in blends by univariate calibration using the information of UV-Vis spectra, obtained at 365nm. If it is confirmed that the blend is standard, it is considered that analytical analysis is completed. However, if there is chance of alteration in sample due to presence of vegetable oil then partial least square model (PLS), is used for further analysis. For this purpose more biodiesel contents are added into the blend. The same spectroscopic data from the first step is used, however the wavelength ranges from 350-750 nm is used. The partial least square (PLS) model and UV-Vis spectroscopy can differentiate between vegetable oil and biodiesel because it uses range of wavelength instead of one variable. It means if the difference between biodiesel and vegetable oil is not noted by UV-Vis analysis, it can be noticed by the model [59].

3.2.2. Thermal lens spectroscopy (TL)

It is highly sensitive technique which can be applied to study the samples with small optical absorptivity and it is feasible because it is neither invasive nor destructive and can directly analyze biofuels at gas stations. TL dual beam configuration can be applied to study the biodiesel and its blends. It can be used to analyze different biodiesel samples produced from different feedstocks such as biodiesel derived from castor bean, turnip oils, sunflower and soy oils. Researchers analyzed two samples of castor oil; one of them was oxidized, to correlate the composition parameters. They concluded that because of the simplicity of this method, it can be used for guarantee purposes and for investigation of physiochemical properties [60].

TL spectroscopy with single beam configuration can also be used for the identification of impurities such as alcohol, unreacted catalyst and antioxidants [61]. Recently, it is used to differentiate biodiesel and its blends. It can also characterize biodiesel-oil blends because this method permits the determination of mass and thermal diffusivities. When moves from pure biodiesel to blend having 2%, soybean oil and 98% biodiesel, the mass diffusivity are increased upto 59% and thermal is increased by 15%. It indicates that both parameters are significant to provide the information of impurity in biodiesel. This technique is also significantly used to indicate the presence of oil as an adulterant in the biodiesel [62].

3.2.3. Infrared spectroscopy

The IR spectral region has longer wavelength than visible region and smaller than radio wave region. The IR spectra covers wide range, further divided into the three regions, far infrared (far-IR), middle infrared (mid-IR) and near infrared (near-IR). Mid-IR shows the absorption of all the chemical bonds such as C–H, N–H, OH and so on. This spectrum is sensitive to chemical and physical states of every components of sample between 4000 to 400 cm^{-1}. Its common use is identification of functional groups as different functional groups absorb the specific frequencies of radiations. The mid-IR region between 1500 and 800cm^{-1} is known as fingerprint region. In this region, complex interacting vibrations also contribute in the absorption, which provide unique information about each compound [63]. It can be used for both qualitative and quantitative analysis, however the low concentration of substance is difficult to identify because the noise level can cause problem.

Near-IR spectroscopy is classical method to analyze the sample as it is non-destructive, no or minor need of sample preparation. On the other hand, it uses low cost equipment with high signal to noise ratio [64-65]. Near-IR also has many limitations such as it is not sensitive technique as compared to mid-IR and components with low concentration cannot be determined by use of near-IR. On the whole, IR can be used to analyze all types of samples such as solutions, paste, films, powders and fibers. Recently, this technique is using successfully to characterize the samples of biodiesel.

3.2.3.1. Applications in feedstock selection

Selection of suitable feedstock is very important because it greatly affect the cost of biodiesel. It accounts for the 80% of total cost of biodiesel production [66]. High cost of feedstock is not beneficial for economics of the country. Further, final physiochemical properties are also affected by it. Thus, there is a need to analyze the feedstock before biodiesel production. Among all the methods of analysis, mid-IR is most commonly used to characterize feedstock. Commonly, it determines the free fatty acids present in feedstock which is important parameter for the conversion of oils into biodiesel [66-67].

The rice barn has free fatty acids so it is not suitable for food oil, but it can be a potential source of biodiesel. FTIR spectroscopy was used to analyze the free fatty acids of rice barn. In the diffuse reflectance IR Fourier transform spectra (DRIFTS) of rice barn, the significant spectral changes were obtained in the region of carbonyl (1760–1700 cm^{-1}) due to the presence of peaks of acyl glyceride carbonyl (1743 cm^{-1}) which decreased and the free fatty acids carbonyl peaks (1712 cm^{-1}) which increased. Models of calibration were applied by using the region of carbonyl (1731–1631 cm^{-1}) and mid-IR (4000–400 cm^{-1}). From the calibration models, the coefficient of correlation obtained were 0.88 and 0.96, the root mean square were 5.80 and 3.64 respectively. It was concluded that FTIR can be successfully used for the characterization of free fatty acids present in rice barn. Researchers also used near-IR spectroscopy, thus the important quality parameters of oil such as water contents, iodine value and acid number can be quantitatively analyzed by IR spectroscopy [68].

3.2.3.2. Transesterification reaction monitoring

Transesterification reaction of feedstock with alcohol in the presence of catalyst produces fatty acid alkyl esters. The products of incomplete transesterification reaction, such as un-separated glycerol, unreacted triglycerides and catalyst, residual alcohol can contaminate the final product [37]. Thus, it is important to quantify and identify the products and by-products of the reaction. Transesterification reaction can be monitored by IR spectroscopy, this technique is fast, reliable, easy to operate and nondestructive in nature. In a report, near-IR was first used to monitor the transesterification process [37]. Besides this spectroscopy, ^{1}H NMR has also been used to cross check and co-relate the results.

3.2.3.3. Determination of biodiesel blend level

Generally, blends of biodiesel with conventional fuel are common in use [69]. The concentration of biodiesel in blend varies from 2 to 35% and it depends on the country from where feedstock is collected [70]. It is important to determine the concentration of biodiesel in blends to ensure its use according to expected blend levels and standards. The fiber-optic near-IR spectroscopy has also been used to analyze the level of biodiesel blend in conventional fuel. The near-IR region was preferentially used because it showed the differences at 4600–4800 cm^{-1} and 6005 cm^{-1}, which allows the determination of biodiesel in their blend with conventional fuel [37-52-70-73].

3.2.3.4. Analysis of biodiesel properties

The most of biodiesel applications depend on the properties of biodiesel such as viscosity, density and flashpoint etc. There are two quality standards of biodiesel, United States Standard (ASTM D 6751) and European Standard (EN 14214). These standard methods are slow and time consuming which also requires high cost equipment. IR technique is an alternative method to analyze the quality parameters it is also non-destructive and time saving. The researchers reported that FT-NIR and FTIR together with the PLS and network of artificial methods has been used successfully to analyze the biodiesel properties. The properties such as viscosity, cetane number and density can be determined by the calibration models [74-75].

The first important analysis of biodiesel is identification and quantification of the ester contents. It is important to analyze the ester contents because overall biodiesel properties depend upon them [52]. This property is determined by the structures of alcohol moieties and fatty acids that form the fatty ester [73]. Researchers reported the use of IR spectroscopy to analyze the ester contents. Near-IR technique combined with multivariate calibration has been used for the identification of ester contents in biodiesel, also the linolenic acid methyl esters contents in laboratory and commercial scale samples of biodiesel. PLS and PCA were used to qualitatively analyze the spectra and formulation of calibration models within the range 4500 to 9000 cm^{-1}.

In the determination of linolenic acid methyl ester contents, the obtained error was (0.18%), which was slow approximately more than three times than the error reported in EN14103 [68]. In another study, near-IR was used to analyze important biodiesel properties such as kinematic viscosity at 40°C, cold filter plugging point, iodine value and density at 15°C. Qualitative analysis of spectra was performed by the PCA, and calibration models between spectral and analytical data were developed by the squares regression. Results of analysis supported the use of near-IR spectroscopy along with multivariate calibration to control the quality of biodiesel in both commercial and laboratory scale samples [76].

The stability of biodiesel has been analyzed by mid-IR and near-IR techniques and multivariate calibration of three factors; water contents, acid number and oxidative stability index. The biodiesel samples were collected at the intervals of 3 to 4 days, from the biodiesel produced from the different feedstock. AFTIR Perkin Elmer Spectrum, GX spectrometer was applied to obtain the mid-IR (4000 to 600cm^{-1}) and near-IR (12,000 to 4000cm^{-1}) spectra. In near-IR spectroscopy, spectra were collected using quartz flow cell and in mid-IR region using ATR probe. The results showed that the IR spectroscopy is economical and fast way to analyze the biodiesel stability. Researcher also determined the distillation temperature, sulphur contents and the density of blends with help of near-IR and mid-IR spectroscopy.

The mid-IR (4000–600cm^{-1}) and near-IR (12,000–4000cm^{-1}) spectra were collected with a FTIR Perkin-Elmer Spectrum, GX spectrometer that used an ATR probe and quartz flow cell, respectively. Calibration models were applied in accordance to spectra obtained from PLS method

and the performance of models was analyzed respectively. The results collected from the RMSEP calculation showed that the models for calibration, applying mid-IR and near-IR regions can be easily used to estimate the quality parameters of biodiesel and its blends [68].

3.2.3.5. Analysis of contaminants present in biodiesel

Biodiesel can be easily contaminated by many factors, commonly at high temperature in the presence of oxygen or at low temperature. All contaminants affects the performance of biodiesel, most of them are related to engine. Thus, the contaminants analysis of biodiesel is important to control its quality. From the literature, it is reported that role of IR spectroscopy is significant to analyze the contaminants. The spectra of biodiesel precipitates, collected from FTIR spectroscopy was compared with steryl glucoside standard, the absorption peaks of biodiesel overlapped with steryl glucoside standard, which indicated that contamination was due to the presence of steryl glucosides. In another investigation, near-IR was used to analyze the methanol and water contents in biodiesel samples. The spectra of near-IR were related with analytical data with help of calibrated models. It was reported that peak of hydroxyl group of methanol was appeared at 4885–4480 cm^{-1} and that of water O–H group was appeared at 5200 cm^{-1} [77].

3.2.4. Nuclear magnetic resonance (NMR)

NMR spectroscopy can be used to analyze either raw material used for fuel generation or final product (biodiesel). The quality of raw material is directly related to the quality of biodiesel. NMR is an excellent technique but the cost of equipment maintenance is very high. This technique is fast and easy to use as compare to HPLC and GC. The transesterification reaction has been monitored by the ^{13}C NMR. The signal of terminal methyl group at 14.5 ppm was selected as the internal standard and signal of methoxy carbon of esters at 51 ppm along with glyceridic carbons signals at 62–71 ppm was used to determine the rate of conversion [78].

^{1}H NMR spectroscopy was first time used to monitor the yield of trans-esterification reaction. In triglycerides, the peaks of methylene group next to the ester (a-CH_2, 2.3 ppm,) and methoxy group of esters (OCH_3, 3.7 ppm, s) were observed to monitor the transesterification reaction. From the area of peaks, reaction conversion was calculated with help of following equation:

$$\mathbf{C} = \mathbf{100} \times \frac{\mathbf{2A_{Me}}}{\mathbf{3A_{CH2}}}$$

Where C shows the percentage change of triglycerides to representative methyl esters; A_{CH2} is the value of integration of methylene protons and A_{Me} is the value of integration of methoxy protons of methyl esters.

The ^{1}H NMR technique was applied to observe, soybean oil transesterification reaction and to determine the methyl esters and average unsaturation degree of fatty acids in biodiesel. This technique was also applied to monitor the reaction of ethanolysis and to quantify the fatty ethyl ester contents in biodiesel [79]. For the quantitative analysis of reaction, 4.05–4.40 ppm region was chose (glycerol methylene hydrogen and ester ethoxy). Hydrogen nuclear magnetic resonance technique (^{1}H NMR) was also projected to quantitatively analyze the biodiesel contents in biodiesel-diesel blends, this technique can detect the presence of raw material and can quantify its amount. This method consists of few steps, it does not require specific software, chemometric tools or complex equation and it can directly differentiate vegetable oil and biodiesel.

NMR spectroscopy can provide information of structural, geometrical and molecular formula of organic compounds [80]. This technique has many advantages inspite of the fact that this is expensive. For example, quantitative and qualitative analysis can be performed simultaneously, quick measurement (for an abundant nucleus, such as ^{1}H), separation of analyte is not required and single spectrum analysis can be performed for different analytes simultaneously [74]. From the above considerations, it can be concluded that ^{1}H NMR spectroscopy is efficient technique to quantify the biodiesel contents in blends [81].

3.2.5. Raman spectroscopy

Raman spectroscopy is potential technique to estimate the transesterification reaction yield, by observing the characteristic stretching mode of (C=O), present in the range of 1730–1750 cm^{-1} [82]. In a research investigation, FT-Raman spectroscopy along with partial least squares (PLS) multivariate analysis was effectively applied to quantify the mixtures of soybean oil/ethyl ester. Transesterification reaction was monitored by the FT-Raman/PLS methods, where mixture of ethanol and soybean oil produced the esters (biodiesel) in the presence of Lewis acid heterogenous catalyst. The heterogenous reaction was performed in glass round bottom flask of 50 mL capacity, stirred under reflux conditions at 100°C. Total six reactions (22, 18, 14, 10, 6 and 2 h) were conducted under same conditions. The results collected from the spectroscopy analysis were correlated with other methods to validate their values. The equation of two Hydrogen Nuclear Magnetic Resonance (^{1}H NMR) and Near Infrared Spectroscopy (NIR) spectroscopy was correlated with good agreement [37].

The curve of ^{1}H NMR was correlated with the measurement of viscosity, obtained a linear coefficient correlation of 0.998113. Fourier Transform Infrared Spectroscopy/Partial Least Square Regression (FTIR/PLS) methods were validated by use of GPC, R2 0.9837 was obtained between both techniques [83]. The results collected from the four methods (three ^{1}H NMR and FT-Raman/PLS methods) showed the reliable correlation with each other,

which indicated their mutual validation. It can be concluded that FT-Raman/PLS) models are attractive ways to analyze the biodiesel production, because they are cheap as compared to other methods, including NMR and inherent qualities. However, the ^{1}H NMR methods are feasible for ethanolysis and methanolysis reactions of vegetable oils. Both of the process explains here, were successfully correlated, and reported methods proved their validity, evidencing and enhancing their potential for biodiesel synthesis and quality monitoring [84].

In another research, adulterations produced by vegetable oil in B5 and B2 blends were determined by the vibrational spectroscopy (FT-Raman and Fourier transform (FT) near-infrared spectrometry). Artificial neural network (ANN), principal component regression (PCR) and Partial least square regression (PLS) models were used and their relative results were estimated by external validation with the help of F-test. The calibration models were designed using 120 samples. Castor and soybean oil were obtained from the commercial sources. Palm oil was also used without purification. FT-Raman and FT-NIR spectra were collected from Equinox 55 Fourier transform equipment from Bruker. Root mean square error of prediction (RMSEP) values of calibration models were compared with the help of F-test, according to chemometric methods, that are ANN, PLS or PCR. Results concluded that FT-Raman spectroscopy as well as FTNIR along with PLS, PCR and ANN chemometric methods can successfully analyze the adulteration of biodiesel blends with oil. The accuracy of model depends on the chemometric method and spectroscopic technique.

The spectral regions have shown that PCR and (PLS/FTNIR) calibration models are capable to analyze the adulteration of diesel-biodiesel blends with oils having an accuracy better than 0.05% w/w. However, the accuracy was better than 0.03% w/w for Artificial Neural Network/Fourier Transform Raman spectroscopy (ANN/FT-Raman) [85].

Conclusion

Biodiesel is a sustainable, significant as well as promising alternate of fuel that is used all over the world. During biodiesel production process contaminants are formed as by product which leads to several operational problems. Therefore, biodiesel quality is monitored by various analytical methods. Spectroscopic techniques are superior to others due to nondestructive and fast analysis. Different spectroscopic techniques such as UV/visible, thermal lens, nuclear magnetic resonance spectroscopy, infrared and Raman spectroscopy are used for monitoring reaction of transesterification, adulteration of biodiesel blends, oxidative stability, analysis of biodiesel characteristics and contaminants as glycerol and water contents, etc. Vibrational spectroscopy (Raman, IR and NIR) has extensive application in analysis of biodiesel as this analytical technique is nondestructive which allows fast, reliable and direct determination of many properties without pretreatment of sample.

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