ORIGINAL PAPER

A Novel Method for Monitoring the Transesterification Reaction of Oil in Biodiesel Production by Estimation of Glycerol

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Abstract A quantitative method is reported for the estimation of glycerol during transesterification of oil to form biodiesel. The reagent used to derivatize glycerol was 9,9-dimethoxyfluorene. Glycerol is estimated by both UV–visible spectrophotometric and high performance liquid chromatography methods. Using the former method, detection limits of 0.05% w/w of glycerol in biodiesel was established. Validation of the developed method was done using the Greenhill method for determination of free glycerol formed during the transesterification reaction.

Keywords Non-edible oils · Transesterification reaction · Biodiesel · Glycerol · Estimation · 9,9-Dimethoxyfluorene · p-Toluenesulfonic acid · Jatropha curcas · UV–visible spectrophotometric · HPLC

Abbreviations

ASTM	American Society for Testing and Materials
ATR-FTIR	Attenuated total reflectance-Fourier transform infrared
CCDC	Cambridge Crystallographic Data Collection
CE	Capillary electrophoresis
FAME	Fatty acid methyl esters
FID	Flame ionization detection
HPLC	High performance liquid chromatography
HTGC	High temperature capillary gas chromatography
NIR	Near infrared spectroscopy
PTSA	p-Toluenesulfonic acid
SEC	Size exclusion chromatography
TG	Triglycerides
TLC	Thin layer chromatography
mp.	Melting point
NMR	Nuclear magnetic resonance
HRMS	High resolution mass spectra
RT	Retention time

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Introduction

Biodiesel is known for its environmental benefits such as biodegradability and its non-toxic nature. Moreover, it is derived from renewable resources [1]. Biodiesel is typically produced from neat oil or fat by transesterification with an alcohol, usually methanol, in the presence of an alkaline catalyst [2]. Alkali metal hydroxides are the most common catalysts employed in the transesterification reaction but other metal catalysts [3, 4] as well as biocatalysts [5, 6] have also been reported. Successful commercialization of biodiesel depends on a variety of parameters. One of these parameters is fuel quality as defined in a provisional standard for biodiesel by the American Society for Testing and Materials (ASTM) and existing standards in some European countries [7, 8]. The fuel standards address quality issues of biodiesel by contaminants [glycerol, mono-, di-, and triglycerides (TG) and alcohol] present in it [7]. Glycerol, a by-product of the

transesterification reaction, is the major constituent in determining fuel quality [9].

The first method for monitoring the transesterification reaction was thin layer chromatography with flame ionization detection (TLC/FID) [10]. It was used to determine the fatty acid methyl esters (FAME), mono-, di-, and triglycerides. Although its analysis time is quite short, this method was reported to be sensitive to humidity, showing material discrepancies and is not as accurate as gas chromatography (GC) and high performance liquid chromatography (HPLC) methods developed later. The cost of the instrument (TLC/FID) is high [7]. TLC on silica gel [11] developed later is based on the area of TG spot seen on the TLC in comparison to a standard and is merely qualitative and does not allow the exact determination of the degree of conversion. Bansal et al. [12] recently reported a TLC method and image analysis to detect glycerol in biodiesel. This method is based on the area of the spot seen on the TLC in proportion to the amount of glycerol in the sample, followed by image analysis using the Matlab program. Subsequently, high temperature capillary gas chromatography (HTGC) was developed to monitor esters, tri-, di-, and monoglycerides in the transesterification reaction in a single run [13]. A later protocol developed for determining total glycerol in biodiesel, using Plank's method (HTGC) [14] requires the hydroxyl groups of the glycerides and free glycerol to be derivatized in the presence of expensive reagents before analysis, limiting its use for routine analysis. HPLC methods have also been developed as an alternative to HTGC [15]. For this, derivatization of the samples is not necessary [16], the analysis time is shorter and all the reaction mixtures of oils and fats are readily quantitated [17]. However, both HTGC and HPLC methods require standards for quantification.

Gelbard et al. [18] described the first work on the utilization of nuclear magnetic resonance (NMR) spectroscopy. ^{1}H NMR, for monitoring the yield of the transesterification reaction and ^{13}C NMR for rapeseed oil transesterification [19]. The fiber-optic near infrared spectroscopy (NIR) method was developed by Knothe [7]. However, in this method, the presence of low levels of contaminants in biodiesel cannot be completely quantified. The insolubility of some contaminants (e.g., glycerol) in methyl esters contribute to this problem. Later, overcoming some of these limitations ATR-FTIR methods (attenuated total reflectance) were reported and results by these methods were validated with size exclusion chromatography (SEC) [7]. In addition, a SEC method was developed for monitoring the transesterification reaction of oil in biodiesel production [20].

Recently, methods for the determination of small amounts of free glycerol present in commercial biodiesel samples, using the periodate oxidation of glycerol followed by UV–visible spectrophotometry, capillary electrophoresis (CE) and HPLC [16, 21, 22] have been developed. Furthermore, Sigma-Aldrich Fine Chemicals developed a kit (NBB method BQP-02—Greenhill method) for enzymatic determination of free and total glycerol in biodiesel [23]. This method is based on coupled enzyme reactions of glycerol leading to a spectrophotometric detection (at 540 nm) of quinonimine dye. Although this method is faster and simpler than chromatographic methods, the commercially available free glycerol kit is expensive for routine analysis. As part of an ongoing project in our laboratory on biodiesel from non-edible local oils [5, 24, 25], herein we report an efficient and novel method for monitoring transesterification and assessing biodiesel production by the estimation of glycerol.

Experimental Procedures

Materials

Melting points of the synthesized compounds were determined using a Toshniwal melting point apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 400 instrument at 100 MHz. The chemical shifts are quoted in parts per million (δ) relative to tetramethyl silane for ^{1}H and ^{13}C NMR using CDCl$_3$ as solvent and the infrared (IR) spectra were recorded on a Nicolet 6700 spectrophotometer. Mass spectra were recorded on a Q TOF micromass spectrometer. Flash chromatography was performed on silica gel (100–200 mesh) using hexane and ethyl acetate as eluent. TLC was done using Kieselgel 60 F254 aluminium sheets (Merck 1.05554). Chemicals, 9-fluorenone, trimethyl orthoformate, p-toluenesulfonic acid (PTSA) and free glycerol kit were purchased from Sigma Chemical Co. All reactions were carried out under an inert atmosphere. The spectrophotometry analysis was carried out using a UV–visible spectrophotometer (JASCO, V530). The yield was determined by HPLC analysis on a JASCO PU-1580 liquid chromatograph equipped with PDA detector. The column used to monitor derivatized glycerol was C$_{18}$ (Qualisil BDS; 4.6 × 250 mm) and the absorbance was monitored at 280 nm. Acetonitrile: water (85:15) was used as the mobile phase at a flow rate of 1 mL/min. Crystallographic data were collected on a APEX CCD diffractometer equipped with Mo–Ka ($l = 0.7107$ Å) radiation. GC analysis was performed with Clarus 500 Gas Chromatograph Perkin Elmer-USA. Sample analysis was carried out on a Zebron ZB-5HT fused silica capillary column (15 m × 0.32 mm × 0.10 μm). Samples (5 μL) were injected by an auto sampler injector at an oven temperature of 50 °C. After an isothermal period of 1 min at 50 °C, the GC oven was heated at 15 °C min^{-1} to 170 °C, then at

3 °C min^{-1} to 200 °C and at 20 °C min^{-1} to 350 °C (hold for 10 min for a total run time of 36.5 min). Flow rates of the gases: Nitrogen—1.5 mL/min, Hydrogen—45.0 mL/min, Air—450.0 mL/min. The injector and detector temperatures were 360 and 360 °C respectively. Biodiesel was prepared from *Jatropha curcas* oil and TG was separated from *Jatropha curcas* oil by column chromatography prior to use (Ravikumar et al., unpublished data).

Methods

Synthesis of 9,9-Dimethoxyfluorene *1*

A mixture of 9-fluorenone **3** (9.0 g, 50 mmol), *p*-toluenesulfonic acid (PTSA, 0.38 g, 1.95 mmol), trimethyl orthoformate (12 mL) and CH_3OH (60 mL) was stirred for 36 h at room temperature. The clear yellow solution was made neutral with triethylamine and the suspension was filtered to remove the tosylate salt. The filtrate was evaporated to dryness, and the residue was recrystallized from CH_3OH to give 8.8 g (78%) of colorless needles of compound **1**.

^{1}H NMR (CDCl$_3$, 400 MHz) δ_H (ppm) : 7.2–7.7 (m, 8 H, ArH) and 3.3 (s, 6 H).

^{13}C NMR (CDCl$_3$, 100 MHz) δ_C (ppm) : 141.5, 130.0, 127.8, 124.5, 120.1, 107.9, 52.0

Melting point (mp.): Observed 85–86 °C (reported mp. 86–88 °C) [26].

Estimation of Glycerol in Biodiesel

A mixture of 0.9 g of biodiesel (from *Jatropha curcas* oil), glycerol (100 mg, 1.01 mmol), 9,9-dimethoxyfluorene (339 mg, 1.5 mmol) and dry toluene (3 mL) were placed in a 10 mL single neck round bottom flask under an inert atmosphere. PTSA (30 mg, 0.157 mmol) was added and stirred for 2.5 h at 80 °C (Scheme 1). The reaction was monitored by TLC using hexane/ethyl acetate (7:3) as eluent. Then, the reaction mixture was diluted with ethyl acetate and treated with 2 mL of saturated aq. NaHCO$_3$. The separated organic phases were washed with brine, dried over Na$_2$SO$_4$, and concentrated. The product was then purified by column chromatography on silica gel, using hexane/ethyl acetate (7:3) as the solvent to give a white colorless solid **2** in 0.265 mg (96%) of isolated yield. The structure of the derivatized glycerol **2** was confirmed from their spectral data from IR, NMR, HRMS and single crystal XRD. The reaction mass was analyzed by UV–visible spectrophotometry and HPLC methods.

^{1}H NMR (CDCl$_3$, 400 MHz) δ_H (ppm) : 7.44–7.14 (m, 8H), 4.72 (m, 1H), 4.45 (dd, $J = 6.4$, 8.4 Hz, 1H), 4.32 (dd, $J = 4.4$, 11.6 Hz, 1H), 4.24 (dd, $J = 5.6$, 11.6 Hz,
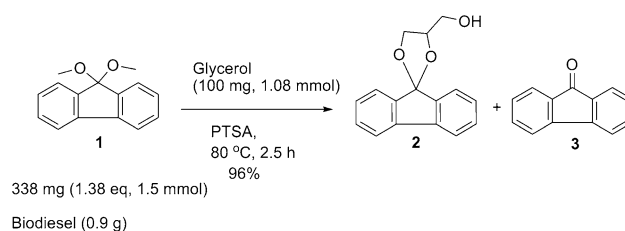
Scheme 1 Estimation of glycerol in biodiesel

1H), 4.06 (dd, $J = 6.8$, 8.4 Hz, 1H), 2.02 (t, $J = 6.0$ Hz, 1H).

^{13}C NMR (CDCl$_3$ 100 MHz) δ_C (ppm) : 144.3, 143.3, 140.0, 139.3, 131.0, 130.2, 128.4, 128.3, 124.0, 123.5, 120.0, 120.0, 113.1, 77.3, 67.0, 62.3.

IR (cm^{-1}): 3408, 3063, 2926, 1611, 1482, 1450, 1254, 1209, 1115, 1057, 993, 936, 732.

HRMS (M + Na$^+$) : Calc. 277.0841, Obs. 277.0846 (C$_{16}$H$_{14}$O$_3$ Na), mp. 72–75 °C.

Crystallographic data for derivatized glycerol **2**: CCDC # 738893. Intensity data were collected on an APEX CCD diffractometer equipped with Mo–Ka ($l = 0.7107$ Å) radiation. The intensity data were corrected for Lorentzian, polarization and absorption effects. C$_{16}$H$_{14}$O$_3$, $M = 254.10$, tetragonal, $a = 22.6156(9)$ Å, $b = 22.6156(9)$, (3) Å, $c = 11.6576$ Å, $\alpha = \beta = \gamma = 90°$, $V = 5962.5(5)$ Å^3, space group *P4nc*, $Z = 2$, $d$calc. = 1.226 g/cm^3, 20874/3928 reflections collected/unique, final R indices [$I > 2s(I)$] $R1 = 0.0835$, $wR2 = 0.2214$, R indices (all data) $R1 = 0.1063$,

$wR2 = 0.2483$. The crystallographic data for derivatized glycerol **2** have been deposited with the Cambridge Crystallographic Data Centre. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [Fax: +44-(1223)336033, E-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk].

Control Experiment with TG and Biodiesel in the Absence of Glycerol

A mixture of TG (500 mg), biodiesel (500 mg) and 9,9-dimethoxyfluorene (339 mg, 1.5 mmol) and dry toluene (3 ml) was placed in a 10 mL single neck round bottom flask under an inert atmosphere. PTSA (30 mg, 0.157 mmol) was added and stirred for 2.5 h at 80 °C (Scheme 2). Then, the reaction mixture was diluted with ethyl acetate and treated with 2 mL of saturated aq. NaHCO$_3$. The separated organic phase was washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. Samples were analyzed by using UV–visible spectrophotometry and HPLC.

TG + Biodiesel $\xrightarrow[\substack{\text{PTSA, Toluene,} \\ \text{80 °C, 2.5 h.}}]{\textbf{1}}$ **3**
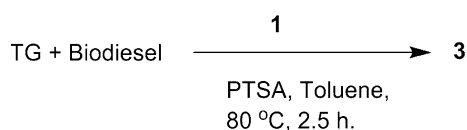
**Scheme 2** Compound **1** with TG and biodiesel in the absence of glycerol

Estimation of Glycerol in Biodiesel with One Pot Real System Experiments

One gram of *Jatropha curcas* oil, KOH (1%, 10 mg) and dry methanol (1:10 M ratio 0.45 mL) was placed in a 10 mL single neck round bottom flask under a nitrogen atmosphere. The reaction mass was stirred for 30 min at 60 °C for complete conversion (Scheme 3). The pH of the reaction mixture was adjusted between six and seven using glacial acetic acid and 1 mL of toluene was added (to remove trace amounts of moisture and methanol) and concentrated under reduced pressure. To this reaction mass, 9,9-dimethoxyfluorene (339 mg, 1.5 mmol), 3 mL of dry toluene and PTSA (30 mg, 0.157 mmol) were added under an inert atmosphere. The reaction mass was stirred at 80 °C for 2.5 h (Scheme 3). The reaction was monitored by TLC using hexane/ethyl acetate (7:3) as eluent. Then, the reaction mixture was diluted with ethyl acetate and treated with 2 mL of saturated aq. NaHCO$_3$. The separated organic phase was washed with brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. Samples were analyzed by using UV–visible spectrophotometry and HPLC methods.

Synthesis of Spiro[1,3]dioxolane-2,9'-fluorene]-4-ylmethyl Palmitate **4**

A mixture of derivatized glycerol **2** (254 mg, 1 mmol), palmitic acid (256 mg, 1 mmol) and dry dichloromethane (10 mL) was placed in a 50 mL single neck round bottom flask under a nitrogen atmosphere. Dicyclohexylcarbodiimide (DCC) (226.6 mg, 1.1 mmol) and 4-(dimethylamino)-pyridine (DMAP) (6 mg, 0.04 mmol) was added to the reaction mass at 0 °C for 5 min. After the addition, the reaction mass was stirred for 4 h at room temperature under an inert atmosphere (Scheme 4). The suspension was filtered, and the filtrate was treated with 5 mL of saturated aq. NaHCO$_3$ and extracted with dichloromethane. The separated organic phase was washed with brine, dried over
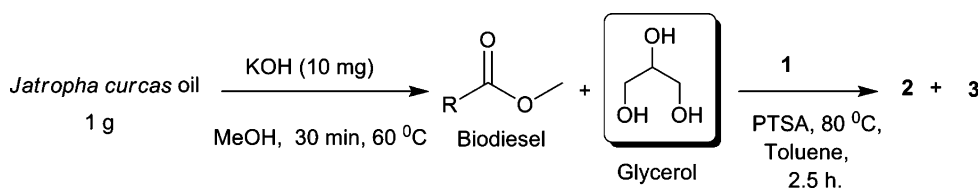
anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, using hexane/ethyl acetate (99:1) as the solvent to give a colorless solid **4** in 354 mg (72%) of isolated yield. The structure of the derivatized monoglyceride **4** was confirmed based on the spectral data from IR, NMR and HRMS.

^{1}H NMR (CDCl$_3$, 400 MHz) δ_H (ppm): 7.55–7.25 (m, 8H), 4.85 (m, 1H), 4.56 (m, 1H), 4.40 (m, 2H), 4.19 (m, 1H), 2.38 (t, 2H, $J = 7.48$), 1.25–1.65 (m, 26H), 0.88 (t, 3H, $J = 6.52$)

^{13}C NMR (CDCl$_3$ 100 MHz) δ_C (ppm): 173.7, 144.3, 142.9, 140.0, 139.4, 130.6, 130.2, 128.3, 128.2, 124.1, 123.5, 120.1, 120.0, 113.6, 74.8, 67.7, 64.2, 34.1, 32.0, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 24.9, 22.7, 14.1

IR (cm^{-1}): 2922, 2852, 1738, 1450, 1302, 1253, 1210, 1172, 1172, 1115, 1057, 994, 834, 760.
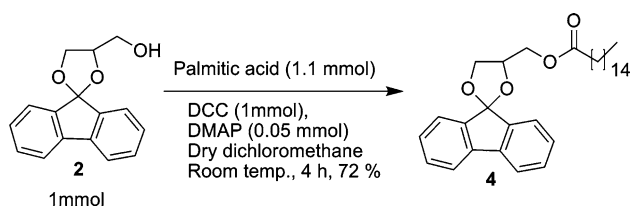
HRMS (M + H$^+$): Calc. 493.3319, Obs. 493.3318 (C$_{32}$H$_{45}$O$_4$), mp. 50–55 °C.

Detection of Various Concentrations of Glycerol in Biodiesel

To each of seven 10 mL single neck round bottom flasks, was added different amounts of glycerol (5, 10, 20, 30, 40, 50 and 100 mg) and biodiesel (995, 990, 980, 970, 960, 940 and 950 and 900 mg) respectively, to make a final volume of reaction mass of one gram. To each flask 9,9-dimethoxyfluorene (339 mg, 1.5 mmol) and dry toluene (3 mL) was added under inert atmosphere. PTSA (30 mg, 0.157 mmol) was added to the reaction mass and stirred for 2.5 h at 80 °C. Then, the reaction mixture was diluted with ethyl acetate and treated with 2 mL of saturated aq. NaHCO$_3$. The separated organic phase was washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. Samples were analyzed by using UV–visible spectrophotometry.

Progress of Transesterification as a Function of Time

Progress of transesterification reaction of *Jatropha curcas* oil to biodiesel formation by estimation of the glycerol in one pot real system experiments was carried out for 5, 10, 15, 20 and 30 min. Five 10 mL single neck round bottom flasks were taken and to each flask 1 g of oil, KOH (1%, 10 mg) and dry methanol (1:10 M ratio 0.45 mL) was

Scheme 3 One pot real system experiment for the monitoring transesterification reaction

Jatropha curcas oil $\xrightarrow[\substack{\text{MeOH, 30 min, 60 °C}}]{\text{KOH (10 mg)}}$ Biodiesel + Glycerol $\xrightarrow[\substack{\text{PTSA, 80 °C,} \\ \text{Toluene,} \\ \text{2.5 h.}}]{\textbf{1}}$ **2** + **3**

1 g

Scheme 4

added and heated to 60 °C. Reactions were stopped at 5, 10, 15, 20 and 30 min. The pH of the reaction mixture was adjusted between 6 and 7 using glacial acetic acid and 1 mL of toluene was added (to remove trace amounts of moisture and methanol) and the reaction mass was concentrated under reduced pressure. To each flask 9,9-dimethoxyfluorene (339 mg, 1.5 mmol) and dry toluene (3 mL) were added under an inert atmosphere. PTSA (30 mg, 0.157 mmol) was added to the reaction mass and stirred for 2.5 h at 80 °C. Then, the reaction mixture was diluted with ethyl acetate and treated with 2 mL of saturated aq. $NaHCO_3$. The separated organic phase was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Samples were analyzed by using UV–visible spectrophotometry.

Sample Analysis by UV–Visible Spectrophotometry

Five milligrams of reaction mixture was dissolved in 10 mL of ethanol and the absorbance was measured for compound **3** at 380 nm using 1 mL quartz cuvettes. The yield of derivatized glycerol **2** was calculated based on the formation of **3**.

Sample Analysis by the HPLC Method

Five milligrams of reaction mixture was dissolved in 10 mL of acetonitrile, from this 20 μL was taken for the analysis. The derivatized glycerol **2** was monitored at 280 nm and the yield of derivatized glycerol **2** was calculated from the standard graph.

Results and Discussion

Estimation of glycerol formed during the transesterification of oil using 9,9-dimethoxyfluorene **1** as a reagent and PTSA as a catalyst by UV–visible spectrophotometry and HPLC was carried out. The compound **1** was synthesized from commercially available inexpensive materials [26] in 78% yield. Most importantly, compound **1** and catalyst (PTSA) were stable, solid and easy to use at room temperature. Earlier compound **1** was used for the synthesis of *p*-nitrophenyl *β*-D-ribofuranoside derivatives, which were

used as substrate analogues for nucleoside glycosylase enzymes [26, 27]. Another derivative of compound **1** i.e. (4S, 6S)-4-(2-piperidyl)spiro[1,3-dioxolane-2,9′-[9H]-fluorene] which is an analogue of the dioxolanes dexoxadrol and etoxadrol was used as potential phencyclidine-like agent [28]. In addition, recently compound **1** was coupled with 1,2-, *syn*-, *anti*-1,3-diols and 1,3-sulfanyl alcohols to produce flexible and optically transparent molecules [in UV–visible molecules] which shows remarkable changes in the optical rotation values [29].

In this study, compound **1** was coupled with glycerol in the presence of biodiesel using a catalytic amount of PTSA and dry toluene (solvent) at 80 °C for 2.5 h to give the corresponding derivatized glycerol **2** in 96% yield (Scheme 1). This is the first ever report on synthesis of derivatized glycerol **2** and the crystal structure of derivatized glycerol **2** is shown in Fig. 1. The yield of derivatized glycerol **2** was calculated using UV–visible spectrophotometry and HPLC methods from the crude reaction mixture. In the UV–visible spectrophotometric method, the yield was calculated based on formation of **3** at 380 nm (Fig. 2), since the product **2** and the byproduct **3** had a common λ_{max} at 290 nm. In the HPLC method, the yield was calculated based on formation of derivatized glycerol **2** using the standard graph (Fig. 3). The real system experiment was performed from one gram of *Jatropha curcas* oil. Biodiesel formation in this was followed by glycerol estimation (Scheme 3). The derivatized glycerol **2** (RT 3.8 min) and the byproduct fluorenone (RT 4.8 min) were monitored at 280 nm using acetonitrile/water (85:15) (Fig. 4). The monoglyceride derivative **4**, was synthesized using the reported procedure [30] (Scheme 4). The compound **4** on HPLC analysis had a retention time of 144 min.
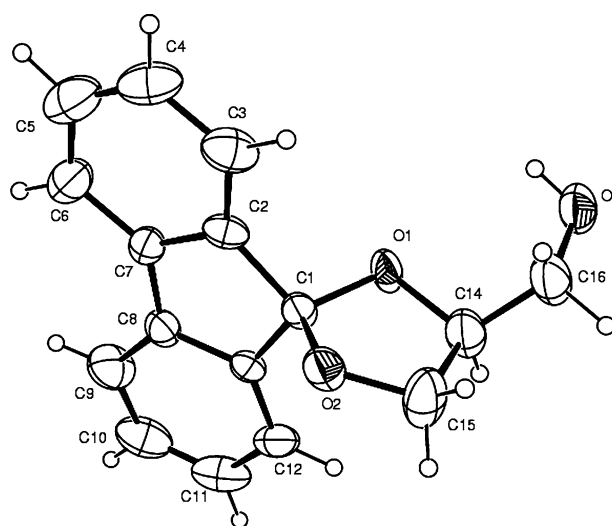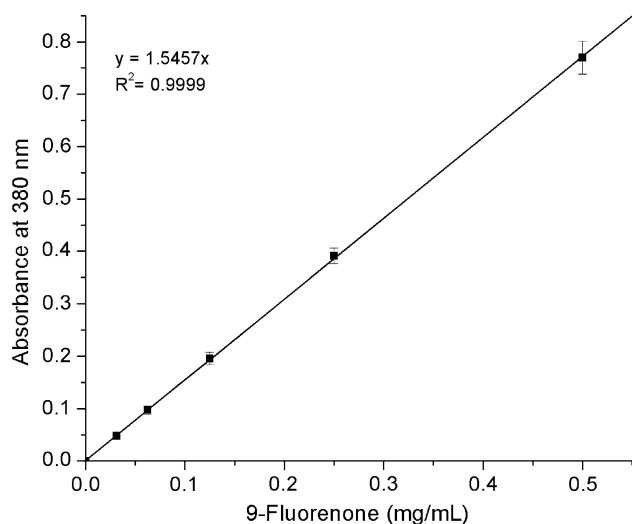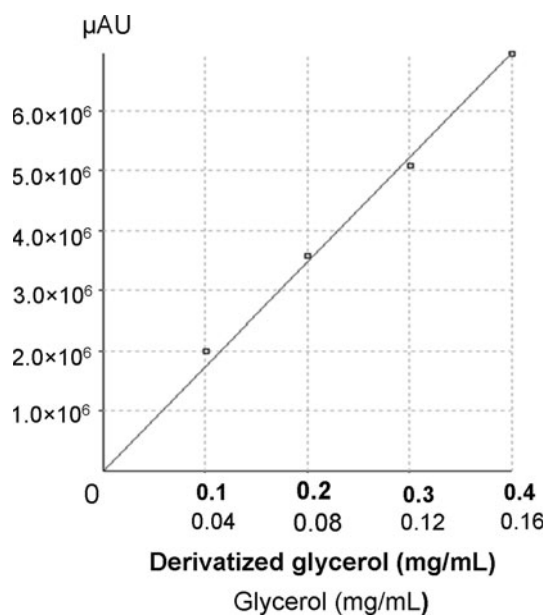

**Fig. 1** Crystal structure of spiro[[1, 3]dioxolane-2,9′-fluorene]-4-ylmethanol **2**

Fig. 2 Standard graph for 9-fluorenone ($\varepsilon_{380} = 281$ M^{-1} cm^{-1}) by UV–visible spectrophotometry

Fig. 3 Standard graph for derivatized glycerol **2** by HPLC

A mixture of **2**, **3** and **4** were subjected to HPLC using acetonitrile/water (85:15) and this was used as a standard reference for further analysis.

The sample from the real system experiment (Scheme 3) did not show **4** (derivative of monoglyceride). Furthermore, a known amount of glycerol (90 mg) and monoglyceride 2,3-dihydroxypropyl palmitate (10 mg) in one gram of biodiesel containing compound **1**, under conditions shown in Scheme 1 did not show the formation of **4**. All this indicates the absence of derivatized monoglyceride.
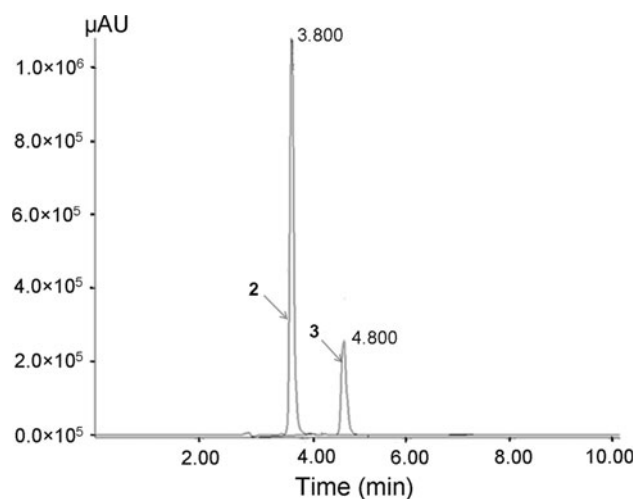
Fig. 4 HPLC chromatogram of the crude reaction mixture (transesterification followed by derivatization) of real system experiment from one gram of *Jatropha curcas* oil for biodiesel formation in 30 min, monitored by estimation of the derivatized glycerol **2**

Standard Graph for 9-Fluorenone Using a UV–Visible Spectrophotometer

The standard graph for compound **3** is shown in Fig. 2. The correlation between concentration and recorded absorbance is linear up to 0.76 units, absorbance measured at 380 nm and the calculated R^2 is 0.99. The molar extinction coefficient for compound **3** was determined to be $\varepsilon_{380} = 281$ M^{-1} cm^{-1}.

Standard Graph for Derivatized Glycerol **2** by HPLC

Figure 3 shows the standard graph for derivatized glycerol **2** in acetonitrile, at varying concentrations. The correlation between concentration and recorded area is linear up to 7×10^6 μAU], the calculated R^2 is 0.99. The solvent used was acetonitrile: water (85:15) at a flow rate of 1 mL/min. The derivatised glycerol **2** was monitored at 280 nm using a PDA detector.

### Control Experiment with TG and Biodiesel in the Absence of Glycerol

The control reaction was examined in the absence of glycerol using the 500 mg of TG and 500 mg of biodiesel in the presence of compound **1** as described in Scheme 2. From this experiment it was observed that compound **1** was completely converted into compound **3** without forming any derivatized glycerol. It was concluded that compound **1** has no effect on TG and biodiesel and the reaction is between glycerol and **1** (Scheme 2). This was confirmed by both UV–visible spectrophotometry and HPLC methods.

One-Pot Real System Experiments for the Estimation of Glycerol

The parameters of the glycerol estimation were optimized and used for a one pot real system experiment starting from the *Jatropha curcas* oil to biodiesel formation followed by glycerol estimation (Scheme 3). Biodiesel was prepared by transesterifying *Jatropha curcas* oil using the reported procedure [31]. The total content of biodiesel in the final product was 99%, as confirmed by GC and ^{1}H-NMR analysis. The proposed method using UV–visible spectrophotometry and HPLC, revealed that the amount of glycerol formed from one gram of *Jatropha curcas* oil on complete conversion to biodiesel gave 110 ± 2.0 and 109 ± 1.5 mg respectively, whereas the expected yield for 100% conversion was 115 ± 2.0 and 114 ± 1.5 mg.

Detection of Various Concentrations of Glycerol Biodiesel

The efficiency of the proposed method was determined by varying concentrations of glycerol ranging from 5 to 100 mg/g of biodiesel. Furthermore, the detection limits of glycerol as low as 0.05% w/w (5 mg/10 g) of glycerol in biodiesel was established. The correlation between actual glycerol taken in the reaction and the reacted glycerol starting from 5 mg to 100 mg is shown in Fig. 5 (R^2 is 0.99). The yields (Table 1) of these reactions were analyzed based on UV–visible spectrophotometry since the standard deviation between both methods (UV–visible spectrophotometry and HPLC) was less than one. In addition to this, UV–visible spectrophotometry was faster, easy to use and all further experimental analysis were carried out by this method.
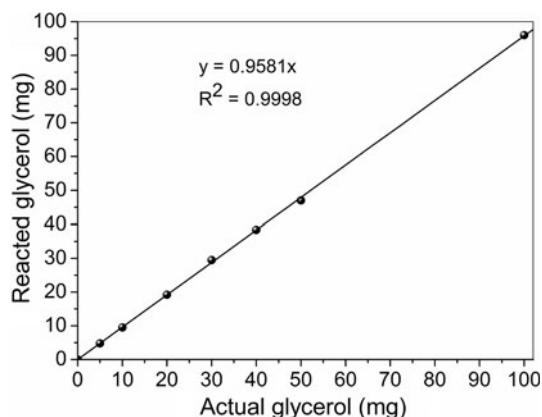
Fig. 5 Actual glycerol (mg) versus reacted glycerol

Table 1 Results on various concentrations of glycerol detection in biodiesel

Entry	Glycerol (mg)	2 (mg)	Reacted glycerol (mg)[a]	Yield (%)
1	5	13	4.79	96.0
2	10	26	9.50	95.0
3	20	53	19.2	96.0
4	30	82	29.5	98.3
5	40	106	38.4	96.0
6	50	130	47.0	94.0
7	100	265	96.0	96.0

[a] Amount of reacted glycerol was measured by the UV–visible spectrophotometric method

Table 2 Progress of transesterification reaction as a function of time

Entry	Time (min)	Glycerol (mg)[a]	Biodiesel (%)[b]
1	5	68	61
2	10	93	84 (83)
3	15	95	86
4	20	105	95 (94)
5	30	110	>99 (>99)

[a] The amount of glycerol formed was measured by the UV–visible spectrophotometric method

[b] The yield of biodiesel formed was determined based on glycerol formed as a function of time

[c] The yield of biodiesel as determined by GC

Progress of the Transesterification Reaction as a Function of Time

Progress of the transesterification reaction was monitored for biodiesel formation from the *Jatropha curcas* oil using the developed method. The glycerol formed was estimated at different time intervals of 5–30 min (Table 2). It was found that the concentration of glycerol and biodiesel increased with time as expected. These results were validated by GC estimation of biodiesel at different time intervals of 10, 20 and 30 min (Table 2).

Validation of the Proposed Method by the Greenhill Method

Validation of the proposed method was carried out using the Greenhill method for determination of free glycerol [23]. One gram of oil on complete conversion to biodiesel, yields 115 mg of glycerol (based on 800 as the MW of *Jatropha curcas* oil) [32]. It must be mentioned here that the molecular weight of *Jatropha curcas* oil varies from region to region i.e. 800 and 887.7 [33]. The Greenhill method gave 117 ± 1.5 mg of glycerol while the developed method gave 115 ± 2.0 mg of glycerol.

Conclusions

The method developed in this work is novel, efficient and uses stable solid reagents which are easy to handle at room temperature (9,9-Dimethoxyfluorene and PTSA). Glycerol was estimated both by UV–visible spectrophotometry and HPLC. Using this method quantitative estimation of glycerol and therefore biodiesel was carried out up to a detection limit of 0.05% w/w of glycerol in biodiesel. Progress of transesterification reaction as a function of time based on glycerol estimation, revealed that the concentration of glycerol and biodiesel increased with time as expected. Validation of the developed method was done using the Greenhill method.

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