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Characterization of Glycerol-Ester Emulsions from Transesterification with Different Alcohols Using the CLSM Technique

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Abstract-Confocal laser scanning microscopy (CLSM) was applied in order to observe the apparent characteristics of glycerol-ester emulsions in transesterification with methanol, ethanol, and mixtures of methanol/ethanol in the present study. The CLSM operated with an XY mode using a laser combiner, which featured a red helium-neon (543 nm) and Nile blue A that was used as dye. The confocal images show significant differences involved in the distribution of glycerol in ester obtained from different alcohol systems. In the case of methanolysis, drops of glycerol clearly separated from fatty acid methyl ester (FAME), which made phase separation easier between glycerol and FAME. In ethanolysis, an unclear formation of glycerol drops in fatty acid ethyl ester (FAEE) was visualized. When the amount of ethanol in mixtures of methanol/ethanol increased, the shape of glycerol drops became unstable. This resulted in a flock of glycerol being dispersed in ester, which made phase separation difficult. The confocal images strongly support the notion that alcohol type is a crucial factor in the formation of glycerol drops in ester, which can affect phase separation between glycerol and ester in biodiesel production.

Keywords-CLSM, FAME, FAEE, Glycerol, Emulsions.

1. Introduction

Due to a shortage of global petroleum resources since 2005, the production of biodiesel has significantly increased. Biodiesel can be produced through transesterification of renewable resources, such as vegetable oils or animal fats that use alkaline, acid, or enzymes as catalyzes. Generally, the base-catalyzed transesterification of vegetable oil is the most widely used method for biodiesel production [1,2]. Today, fatty acid methyl esters (FAME) are the only types of biodiesel that are sold commercially. In order to produce FAME, vegetable/animal oil must be mixed with methanol and base-catalyst using a methanolysis reaction. The utilized methanol is a chemical derivative, and in some regions methanol is imported for FAME production. For example, in 2009 Thailand imported methanol 95.6 kton for FAME production [3]. From an economic perspective, ethanol is attractive as an alternative alcohol for biodiesel production particularly in agricultural countries because ethanol can be produced from agricultural resources [4] such as sugars and starches. However, a limitation of using biodiesel from ethanol, the fatty acid ethyl esters (FAEE), as a commercial biodiesel is the formation of undesirable emulsions in the product after ethanolysis, which have been observed by various research groups [5-9].

Theoretically, both methanol and ethanol are immiscible with triglycerides in transesterification at ambient temperatures. Thus, to enhance mass transfer in reaction, the mechanical stirrer must be applied. Emulsions are usually formed in this step. During methanolysis, these emulsions are quickly and easily separated into a lower glycerol-rich layer and an upper methyl ester-rich layer. However, in ethanolysis, this emulsion is much

more stable leading to difficulty in phase separation and purification of the FAEE product [9]. Although literature has compared methanolysis and ethanolysis emulsions [5,6,8,9], their appearance characteristics are infrequently reported.

In this study, the confocal laser scanning microscopy (CLSM) technique is selected to observe FAME and FAEE emulsion characteristics. Normally, the CLSM is a high resolution fluorescence microscopy and becomes a very powerful tool that more effective than conventional microscopy. The CLSM contains special properties that make it more effective than a conventional one due to its point illumination and detection nature. In the CLSM, the point detection is usually achieved through the incorporation of a pinhole in front of the photo detector. Point illumination is achieved with a laser as a light source. The microscope is very effective at rejecting out-of-focus light with the confocal pinhole. The light from the focusing position is able to pass through the pinhole, while most of the light from the non-focusing position is blocked by the pinhole. As a result, the out-of-focus parts appear dark in color in the image making it possible to optically slice a thick tissue or drop of liquid sample without a glass cover. Light from a part other than the focusing position overlap with ordinary optical microscopes, and the overall image tends to be vague [10].

CLSM is wildly used for structural analysis of microorganisms [11-14] and food material (e.g. yam, wheat dough, and bread) [15-17] In membrane technology, CLSM is utilized to visualize the fouling materials [18,19]. This technique has been applied during observations of the emulsion characteristics of lipids or proteins in the food industry [20], oil-water-modified starch granules emulsions [21], stabilization of foams and emulsions of surface active particles and proteins mixture [22], microstructure of oil-in-water emulsions containing crayfish protein as emulsifier [23] and mineral-oil-water emulsion in the petroleum industry [24]. However, characteristics of emulsions from transesterification have been rarely examined in research.

According to the aforementioned performance of CLSM, this technique is shown to be a highly efficient tool for observing characteristics of emulsions from transesterification. Therefore, the present study focuses on utilizing the CLSM technique in order to observe different physical distributions between glycerol and ester obtained from methanolysis and ethanolysis and to examine the roles of methanol and ethanol on the formation of glycerol-ester emulsions that occur during biodiesel production.

2. Experimental

2.1. Chemicals

The chemicals, methanol (99.5 % wt), ethanol (99.5 % wt), and potassium hydroxide (KOH, 95.0% wt) were purchased from LabChem (Pittsburgh PA. USA) and were financially supported by Specialized R&D Center for Alternative Energy from Palm Oil and Oil Crops, Prince of Songkla University, Thailand. The analytical grade nile blue A was also purchased from LabChem (Pittsburgh PA. USA).

2.2. Sample preparation

Samples were prepared from methanolysis and ethanolysis of refined palm oil containing 1% wt free fatty acid and alcohol (i.e. methanol, ethanol or methanol/ethanol mixture). The KOH was utilized as a catalyst. Normal transesterification conditions, the molar ratio of alcohol to oil of 6:1, KOH of 1 %wt, the reaction time of 30 minutes, and the temperature of 70 oC, were used for observe characterization of product mixtures with different alcohol. After the reaction, 10 mL portions of sample were kept in vials immediately and stained with Nile blue A (5 μg of dye per 1 mL of sample) to allow visualization of both glycerol and ester. In addition, the small amount of by products were neglected.

2.3. Confocal laser scanning microscopy (CLSM)

The stained samples were analyzed using a CLSM (FV300, Olympus) with a XY mode to find bright plane images. Images were taken using a 10X objective with additional zoom of 2 in 1 micrometer axial step and a laser combiner featuring a red helium-neon (543 nm). Each sample was dropped on a cover slip without covering it in order to prevent the reformation of the drop shape. To avoid glycerol-ester phase

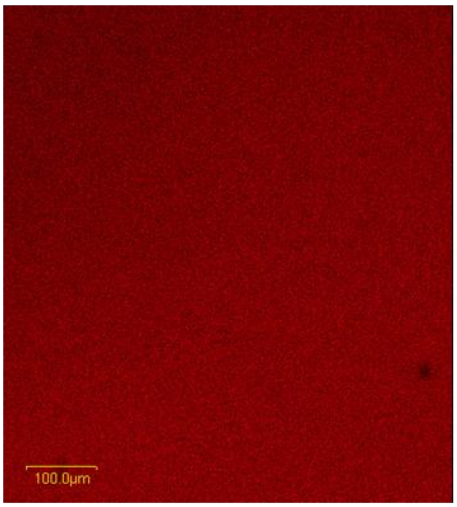
separation during analysis, it was required that the samples be shaken well to ensure homogeneity. The objective lens was located below the samples, covered with immersion oil, and allowed to approach the bottom of the glass slide.

Sample element was scanned point by point with a focused laser beam during the CLSM analysis. The reflected, or emitted, fluorescence light from the sample was detected by two photomultipliers, and out-of-focus information from the image was removed with an adjustable pinhole setting. The output data was transferred to a computer, and a confocal image was generated on a computer monitor.

3. Results and Discussions

3.1. Identification of glycerol and ester using CLSM

In order to characterize emulsions using the CLSM technique, all of the components had to be verified. All samples are measured at room temperature. Fig. 1 shows the confocal images of pure glycerol (95 %wt glycerol), FAME (97.5 %wt ester), and FAEE (96.5 %wt ester), respectively. Pure glycerol was visualized as a red color (Fig. 1a), and the esters FAME and FAEE were visualized as a darker color (Fig. 1b and 1c). The mass ratio of glycerol to FAME was 9:1 in the mixture of glycerol-ester.



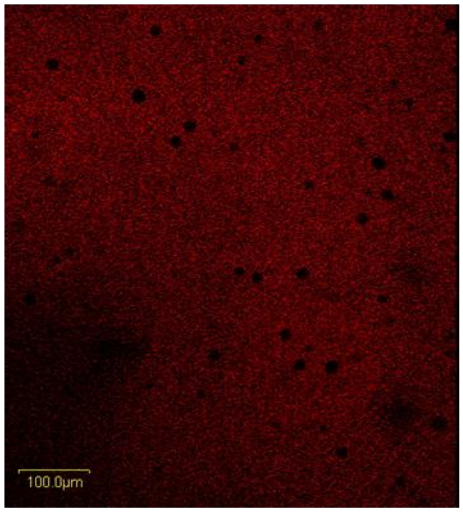
(a)

(b)

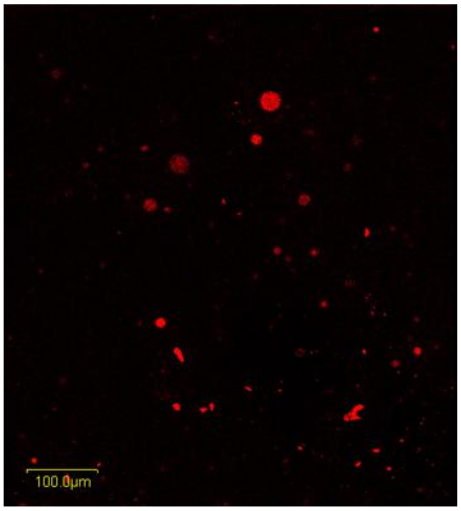
(c)

Fig. 1. Confocal images of a) 95 %wt of glycerol b) 97.5 %wt of FAME c) 96.5 %wt of FAEE

The confocal image was visualized as dark circular domains in the red area (Fig. 2a). On the other hand, the red circular domains in the dark area were visualized when the mass ratio of glycerol to FAME was 1:9 (Fig. 2b). It can be identified that the glycerol and esters, FAME and FAEE, were visualized as red and dark colors, respectively.



(a)



(b)

Fig. 2. Confocal images of mass ratio of glycerol to FAME at a) 9:1 b) 1:9

3.2. Effect of alcohol types on glycerol and ester distribution

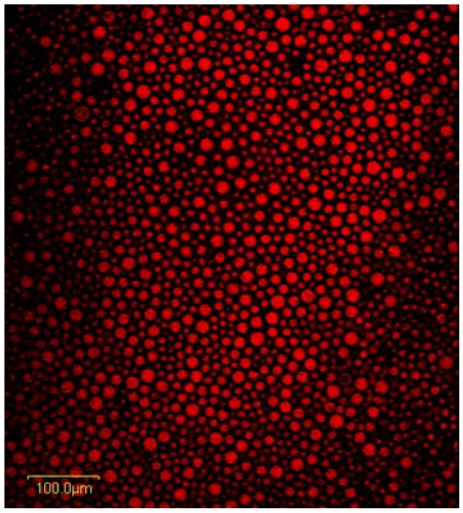
Many studies report that the type of alcohol used in transesterification can have an effect on phase separation between glycerol and ester [5-9]. Two main types of alcohols, methanol and ethanol, are used in the transesterification reaction. During methanolysis, the emulsions are formed during the mechanical stirring step. After the reaction is complete, the emulsions are quickly and easily separated, leading to the formation of a lower glycerol-rich layer and an upper FAME-rich layer. During ethanolysis, however, the generated emulsions are much more stable leading to a complicated separation of glycerol from FAEE. The causes of this occurrence have been rarely examined in research.

To elucidate this phenomenon, the products after methanolysis and ethanolysis were characterized by CLSM. The confocal images are shown in Fig. 3. The captured images clearly demonstrate the difference of glycerol-ester distribution in the product from methanolysis and ethanolysis. It can be seen in Fig. 3a that glycerol formed as spherical drops and plainly separated from FAME. Fig. 3b also illustrates that unclear formations of glycerol drops occurred and dispersed during the entire FAEE phase. This colloidal solution is strongly stable, which may cause drawbacks on subsequent FAEE purification processes.

Fig. 3 indicates that ethanol could be an important factor causing emulsions of glycerol in the FAEE product. To support this hypothesis, various mixtures of methanol/ethanol with molar ratios of ethanol to methanol at 1:5, 2:4, and 3:3 were used to observe the physical distribution of glycerol in ester. The total molar ratio of alcohol to oil was controlled at 6:1.

The confocal images show the effect of the molar ratio of ethanol to methanol on glycerol and ester distributions as illustrated in Fig. 4. It should be noticed that as the ethanol content increased, the flocculation of glycerol in ester also gradually promoted. Fig. 4a shows that at the molar ratio of 1:5, glycerol is generated as sharp spherical drops that are distributed in ester, and then the glycerol drops separate from the ester by gravity in a few minutes. This phenomenon is similar to the effects of methanolysis using pure methanol (Fig. 3a). However, when the molar ratio of ethanol to methanol increased from 1:5 to 3:3, the glycerol gradually formed a smaller drop size, stayed closer, and formed a flock of glycerol dispersed in ester (Fig. 4a-4c). The gravitational force rarely affected these fine droplets of glycerol. Finally, a very stable glycerol-ester emulsion was observed. The characteristics of the glycerol-ester emulsion with a high ethanol content are similar to the characteristics of the stable emulsions observed in ethanolysis using pure ethanol (Fig. 3b). Experimental results confirm that ethanol is an important factor affecting the drop size of generated glycerol resulting in suspension of glycerol in ester. This result supports findings from a study by Issariyakul et al. [6], which reports that glycerol-ester emulsions frequently occur when ethanol is used in transesterification. Besides, no

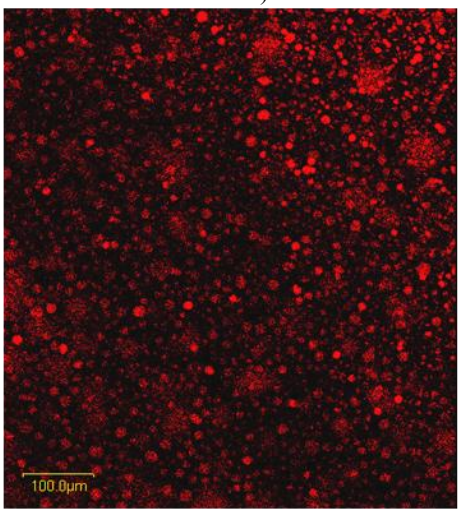
emulsification problem occurred with the use of methanol or small amounts of ethanol in mixtures of methanol/ethanol.



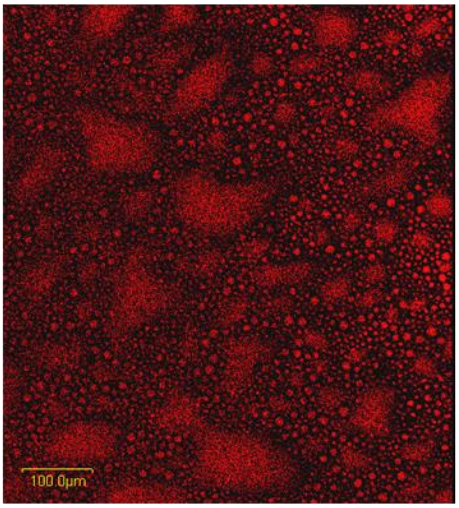
(a)

(b)

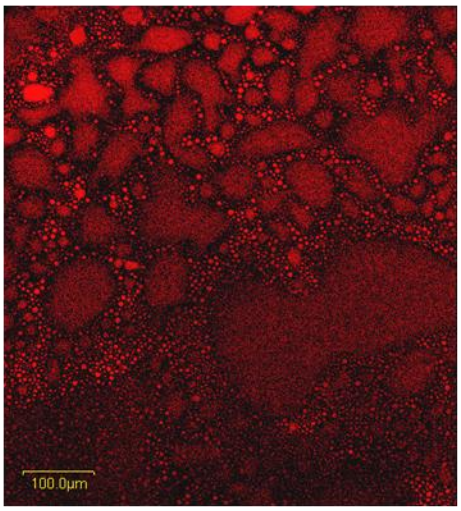
Fig. 3. Confocal images of glycerol-ester distribution after a) methanolysis b) ethanolysis (reaction conditions: molar ratio of alcohol to oil 6:1, KOH 1 %wt, reaction temperature of 70ºC, and reaction time of 30 min)



(a)



(b)



(c)

Fig. 4. Confocal images showing the effect of molar ratio of ethanol to methanol on glycerol and ester distribution a) 1:5 b) 2:4 c) 3:3 (reaction conditions: molar ratio of alcohol to oil 6:1, KOH 1%, reaction temperature of 70 ºC, and reaction time of 30 min)

The glycerol-ester separation process in the conventional methanolysis is generally based on the fact that by-product glycerol and esters are sparingly soluble due to significant differences in their polarities and densities [25]. However, retained alcohol in the product mixture could have an affect on these properties. In ethanolysis, it might be possible that the remaining ethanol in the product decreases differences of polarity and density between glycerol and FAEE ester because its polarity is lower than methanol. Therefore, low amounts of glycerol could be isolated from the FAEE products. It is obvious from confocal images that glycerol tends to form fine droplets and suspend in FAEE ester causing the stable glycerol-ester emulsion. CLSM images clarify

different characteristics of glycerol in ester obtained from utilization of different alcohols in transesterification. This could serve as supportive evidence for solving the problem of emulsion in FAEE. However, other amphiphilic compounds, such as soap, mono-glyceride, and di-glyceride, are also commonly used as emulsifiers for promoting a stable glycerol-ester emulsion in FAEE [1,9,26], which is a research area that should be examined further.

4. Conclusion

CLSM appears to be an effective technique for characterizing the glycerol-ester emulsions obtained from the transesterification reaction. The Confocal images clearly show the different distribution characteristics of glycerol in ester, FAME and FAEE. With FAME, drops of glycerol were sharp and easily separated from FAME resulting in complete phase separation. On the other hand, an unclear formation of glycerol drops in FAEE was visualized, and finally, a very stable glycerol-ester emulsion was observed. This strongly supports the notion that different types of alcohol in transesterification affect phase separation between glycerol and ester.

Acknowledgements

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