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New Ether-Functionalized Ionic Liquids for Lipase-Catalyzed Synthesis of Biodiesel

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Abstract Ionic liquids (ILs) are being explored as solvents for the enzymatic methanolysis of triglycerides. However, most available ILs (especially hydrophobic ones) have poor capability in dissolving lipids, while hydrophilic ILs tend to cause enzyme inactivation. Recently, we synthesized a new type of ether-functionalized ionic liquids (ILs) carrying anions of acetate or formate; they are capable of dissolving a variety of substrates and are also lipase-compatible (*Green Chem.*, 2008, **10**, 696–705). In the present study, we carried out the lipase-catalyzed transesterifications of Miglyol® oil 812 and soybean oil in these novel ILs. These ILs are capable of dissolving oils at the reaction temperature (50 °C); meanwhile, lipases maintained high catalytic activities in these media even in high concentrations of methanol (up to 50% *v*/*v*). High conversions of Miglyol oil were observed in mixtures of IL and methanol (70/30, *v*/*v*) when the reaction was catalyzed by a variety of lipases and different enzyme preparations (free and immobilized), especially with the use of two alkylammonium ILs **2** and **3**. The preliminary study on the transesterification of soybean oil in IL/methanol mixtures further confirms the potential of using oil-dissolving and lipase-stabilizing ILs in the efficient production of biodiesels.

Keywords Biodiesel · Enzymatic transesterification · Ionic liquid · Lipase · Vegetable oil · Triglyceride

Introduction

There is an increasing demand for renewable energy sources, fearing the depletion of crude oil in the next 50 years. As one of the alternative biological sources, biodiesel (fatty acid monoester) is becoming an attractive, renewable, and biodegradable fuel for diesel engines and heating systems as it can be produced from vegetable oils or animal fats [1]. Other sources of lipids are also being considered, such as oleaginous microbial biomass [2],

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soybean oil deodorizer distillate (SODD) [3], and pine trees (Arizona Chemical Company, www.azchem.com). Biodiesel has comparable fuel economy as petroleum-based diesel, and can reduce the emissions of polluting substances (such as particulate matter, carbon monoxide, and hydrocarbon) [4].

The common synthetic route for biodiesel production is the transesterification of vegetable oils (or animal fats) with methanol (or ethanol). This reaction can be catalyzed by acids, alkaline metal hydroxides, alkoxides, and non-ionic bases (such as amines and amidines) [5]. However, these methods have several drawbacks: (1) acid/base processes are often related to corrosion and emulsification problems; (2) acid-catalyzed reactions are usually much slower than base-catalyzed processes [6, 7]; however, the base-catalysis technology may cause unnecessary saponification of fatty acids [8]; (3) a large excess of alcohol is required to drive the equilibrium to the ester formation and to achieve the facile separation of biodiesel from the glycerol; however, it creates the recycling problems (such as cost and recovery of methanol); (3) practically, oils and fats are not soluble in alcohols, resulting in barriers for triglyceride conversions; (4) other issues include being energy intensive, alkaline waste-water treatment, and interference of free fatty acids and water [1]. Therefore, a number of new approaches have been vigorously pursued to circumvent these problems, such as the development of heterogeneous catalysts [9–15], alcoholysis in supercritical methanol [16–19], ionic liquids (ILs)-catalyzed transesterification [20–22], and the lipase-catalyzed transesterification [1, 3, 23, 24].

In particular, the enzymatic transesterification method offers many advantages over chemical methods such as mild reaction conditions, low energy demand, low waste treatment, the reusability of enzymes, flexibility in choosing different enzymes for different substrates, also allowing small amount of water in substrates, etc. [23]. Unfortunately, the current lipase-catalyzed method exhibits several downsides that prevent this promising approach from being commercialized. These disadvantages include the high cost of enzymes, lipase inactivation by acyl acceptors such as methanol, lipase inactivation by impurities in crude and waste oils, etc. [23]. In addition, due to the poor miscibility between oils/fats and methanol, many enzymatic transesterification reactions are heterogeneous systems involving a complicated liquid–liquid interface [23]. To address the issue of methanol inhibition of lipases, a number of approaches have been studied, for example, a stepwise addition of methanol during the reaction [25], the use of other acyl acceptors such as methyl and ethyl acetate [26, 27], enzyme immobilization [23, 28], the use of other organic solvents such as *t*-butanol, hexane, *n*-heptane, and 1,4-dioxane [23, 28, 29], the use of fatty acid-containing feedstock [3], and the genetic modification of lipases for higher methanol tolerance [23].

Recently, ionic liquids (ILs), a new class of non-volatile organic solvents, have evolved as suitable media for many enzymatic reactions [30]. The enzymatic transesterification of vegetable oils has been demonstrated by several groups in producing biodiesel. Ha et al. [31] screened 23 ILs[1] for methanolysis of soybean oil catalyzed by immobilized *Candida antarctica* lipase (Novozym® 435), and identified the hydrophilic IL (i.e., [EMIM][OTf]) as the best solvent for achieving the highest yield (80%) of fatty acid methyl esters at 12 h. On the other hand, Sunitha et al. [32] obtained 98–99% yields of fatty acid methyl esters within 10 h of methanolysis of sunflower oil in hydrophobic [BMIM][PF_6] and [EMIM][PF_6] when catalyzed by Novozym 435. Gamba et al. [33] also utilized several ILs (i.e., [BMIM][Tf_2N], [BMIM][BF_4], and [BMIM][PF_6]) as solvents in the enzymatic transesterification

[1] Most imidazolium ILs are based on anions of BF_4^-, OTf^-, $MeSO_4^-$, Tf_2N^-, PF_6^-, and SbF_6^-. Poor yields were seen in most hydrophobic ILs except in [OMIM][Tf_2N].

of soybean oil, achieving over 90% biodiesel yield in 48 h. However, hydrophobic ILs usually do not dissolve triglycerides and the lipase, resulting in a multi-phase reaction [31–33]; on the other hand, hydrophilic ILs often cause enzyme dissolution and denaturation [34–40].

To seek ILs with higher substrate solubility and higher enzyme compatibility, we recently synthesized a series of new ether-functionalized ILs that are able to dissolve a variety of substrates (such as sugars, cellulose, ascorbic acid, and amino acids), but do not inactivate the lipase [35, 41]. In addition, these ether-functionalized ILs have relatively low viscosities (50–150 mPa s at 20 °C) [35, 41]. In this study, we intend to dissolve oils in these new ILs (Scheme 1), i.e., [$Me(OEt)_3$-Et-Im][OAc] (**1**), [$Me(OEt)_3$-Et_3N][OAc] (**2**), and [$Me(OEt)_3$-Et_3N][HCOO] (**3**). The homogeneous substrate–solvent system is then subject to the lipase-catalyzed transesterification to produce fatty acid monoester. The use of ILs is expected to improve the methanol tolerance of lipases, and to dissolve oils without the involvement of other more harmful volatile organic solvents. The model lipid selected for this study, Miglyol® oil 812, is an oily liquid (~28 mPa·s at 20 °C) widely used in food industry as a release agent, lubricant, glazing agent, or carrier substance. This oil mainly contains four different saturated triglycerides of caprylic acid (C8) and capric acid (C10), and can be easily identified by HPLC analysis. Furthermore, we also demonstrate the enzymatic transesterification of soybean oil in these ionic media.

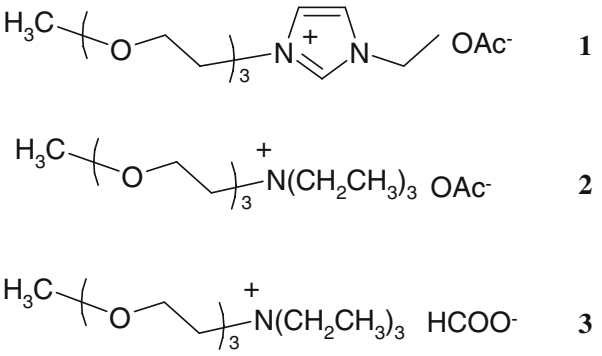
Materials and Methods

Miglyol® oil 812 NF/USP from Sasol was supplied by Fisher Scientific. Methyl octanoate and methyl decanoate were obtained from Sigma-Aldrich. The following enzymes were also purchased from Sigma-Aldrich: free lipase B from *C. antarctica* (CALB, ~9 U/mg, product #62288, LOT #067K3522), CALB immobilized on acrylic resin (Novozym 435®, ≥10,000 U/g, product #L4777, LOT #067K3522), porcine pancreas lipase type II (PPL, 30–90 U/mg using triacetin,[2] product #L3126), Amano lipase A from *Aspergillus niger* (≥12,000 U/g, optimum pH 6.5, optimum temperature 45 °C, product #534781), lipase immobilized in Sol–Gel-AK from *Pseudomonas cepacia* (≥40 U/g,[3] product #62279), and lipase immobilized in Sol–Gel-AK from *Candida cylindracea* (≥10 U/g,[4] product #62278). The following Amano lipases were kind gifts from Amano Enzyme USA: lipase PS from *Burkholderia cepacia* (≥30,000 U/g, optimum pH 7.0, optimum temperature 50 °C, Lot #LPSAC0750102), lipase PS-D I (immobilized on diatomite, Lot #ILPSAB0152305R), lipase PS-C I (immobilized on ceramic, Lot #ILPSAC0350403R), lipase AK 20 from *Pseudomonas fluorescens* (lipolytic activity at pH 7.0 ≥20,000 U/g, optimum pH 8.0, optimum temperature 55 °C, Lot #LAKAFC0452302R), and Newlase F from *Rhizopus niveus* (lipase activity ≥30,000 U/g, protease activity ≥7,000 U/g, Lot #NC0951704).

ILs, i.e., 1-ethyl-3-(2-(2-methoxyethoxy)ethoxy)ethylimidazolium acetate (**1**, [$Me(OEt)_3$-Et-Im][OAc]), triethyl (2-(2-methoxyethoxy)ethoxy)ethylammonium acetate (**2**, [$Me(OEt)_3$-Et_3N][OAc]), triethyl (2-(2-methoxyethoxy)ethoxy)ethylammonium formate (**3**, [$Me(OEt)_3$-Et_3N][HCOO]), and 1-butyl-3-methylimidazolium dicyanamide (**4**,

[2] One unit hydrolyzes 1.0 microequivalent of fatty acid from triacetin in 1 h at pH 7.4 at 37 °C.
[3] One unit is the amount of immobilized enzyme which forms 1% octyl laurate (GC, area percent) from 0.5 mmol lauric acid and 1.0 mmol 1-octanol in 10 mL water-saturated isooctane in 1 h at 20 °C.
[4] One unit corresponds to the amount of enzyme which liberates 1 μmol oleic acid from triolein per minute at pH 7.5 and 37 °C.

Scheme 1 Imidazolium- and ammonium-based ILs consisting of alkyloxyalkyl-substituted cation (short as $[Me(OEt)_3\text{-}Et\text{-}Im][OAc]$ (**1**), $[Me(OEt)_3\text{-}Et_3N][OAc]$ (**2**), and $[Me(OEt)_3\text{-}Et_3N][HCOO]$ (**3**))



[BMIM][dca]) were prepared and characterized following our previously reported method [35]. All ILs were dried in an oven at 100 °C over 24 h. Acros® 3A molecular sieves were added into ILs during storage.

Enzymatic Transesterification of Miglyol Oil

Miglyol oil (0.11 g) or soybean oil (0.11 g) was added into a micro-reaction vessel (5 mL volume, Sigma-Aldrich product #33299), containing 1.0 mL anhydrous mixture of IL and methanol. The oil fully dissolved in the IL/MeOH mixture upon a gentle agitation at 50 °C. Before the addition of lipase, 40 μL solution of reaction mixture was withdrawn for determining the initial concentrations of triglycerides by HPLC. Then, Novozym 435 (40 mg) was added into the mixture to initiate the reaction. The reaction mixture was sealed and incubated at 50 °C in a water bath. Periodically, the reaction vessel was taken out of the water bath and chilled briefly in an ice bath to condense the volatiles. A sample of 40 μL was withdrawn and diluted with 80 μL methanol, followed by a centrifugation to precipitate the enzyme. The clear supernatant was injected into a LC-10AT Shimadzu HPLC equipped with a SPD-10Avp UV–visible dual wavelength detector. The injection-loop volume was 20 μL. The isocratic HPLC eluent consisted of 95% (*v*/*v*) MeOH and 5% (*v*/*v*) water. The flow rate was 1.0 mL min^{-1}. The column was a Shimadzu Premier C18 column (150 mm×4.6 mm, particle size 5 μm). The UV detection wavelength was 210 nm. The integration of each triglyceride peak at time *t* was compared with its initial peak area (*t*=0) to calculate its conversion. The product peaks were quantified through comparing them with those of standard solutions of methyl octanoate and methyl decanoate. All experiments were run at least in duplicate. The percent errors were less than 5%.

To purify the product for ^1H NMR analysis, 30 mL methanol was added into the reaction mixture to precipitate the free lipase. The enzyme was removed by filtration, and methanol was removed through a vacuum evaporation. Distilled water (50 mL) was then mixed with the residue to dissolve IL and glycerol, and 50 mL ethyl acetate was added to extract the fatty acid ester. The ethyl acetate layer was further washed with water (50 mL each) twice to remove trace IL and glycerol. After drying the organic layer with sodium sulfate and filtering off sodium sulfate, the product was collected after a vacuum evaporation of ethyl acetate. The product was weighed and then dissolved in $CDCl_3$ for NMR analysis (JEOL ECX-300 MHz).

Miglyol Oil 812 ^1H NMR (300 MHz, $CDCl_3$, [ppm]) δ=0.85 (9H, t, —CH_3), 1.24 (29H, m, aliphatic CH_2), 1.58 (6H, m, —$O_2CCH_2CH_2$—), 2.30 (6H, t, —O_2CCH_2—), 4.09–4.30

(4H, d,d,d, $RCO_2CH_2CH(O_2CR)CH_2O_2CR$), 5.24 (1H, m, $RCO_2CH_2CH(O_2CR)CH_2O_2CR$).

Methyl Octanoate and Methyl Decanoate Produced from Miglyol Oil 812 ^{1}H NMR (300 MHz, $CDCl_3$, [ppm]) δ=0.87 (9H, t, —CH_3), 1.24 (32H, m, aliphatic CH_2), 1.61 (6H, m, —$O_2CCH_2CH_2$—), 2.30 (8H, t, —O_2CCH_2—), 3.66 (6H, s, CH_3O_2CR).

Vegetable Oil (Soybean Oil) ^{1}H NMR (300 MHz, $CDCl_3$, [ppm]) δ=0.87 (9H, m, —CH_3), 1.29 (50H, m, aliphatic CH_2), 1.60 (6.5H, m, —$O_2CCH_2CH_2$—), 2.02 (8.5H, m, —HC=$CHCH_2CH_2$—), 2.30 (6H, t, —O_2CCH_2—), 2.76 (4H, t, —HC=$CHCH_2CH$=CH—), 4.20 (4H, d,d,d, $RCO_2CH_2CH(O_2CR)CH_2O_2CR$), 5.34 (10H, m, $RCO_2CH_2CH(O_2CR)CH_2O_2CR$ and vinyl).

Biodiesel Produced from Soybean Oil ^{1}H NMR (300 MHz, $CDCl_3$, [ppm]) δ=0.87 (9H, m, —CH_3), 1.29 (50H, m, aliphatic CH_2), 1.60 (6.5H, m, —$O_2CCH_2CH_2$—), 2.02 (8H, m, —HC=$CHCH_2CH_2$—), 2.30 (6H, t, —O_2CCH_2—), 2.76 (4H, t, —HC=$CHCH_2CH$=CH—), 3.66 (3H, s, CH_3O_2CR), 5.34 (8H, m, vinyl).

Results and Discussion

Miglyol® oil 812 is a mixture of triglycerides of caprylic acid (C8) and capric acid (C10). From LC–MS analysis, there are four major triglycerides: three caprylic acid groups (C8C8C8), two caprylic and one capric acid groups (C8C8C10), one caprylic and two capric acid groups (C8C10C10), and three capric acid groups (C10C10C10). Based on the HPLC chromatogram integration (Fig. 1a), their contents in Miglyol oil 812 were calculated as 24.0%, 43.3%, 26.9%, and 5.8%, respectively. Upon completion of the reaction, the triglyceride peaks are expected to disappear, and there are two new peaks for methyl octanoate (methyl caprylate) and methyl decanoate (methyl caprate), respectively (Fig. 1b). Therefore, despite the fact that there are four major components in Miglyol oil 812, it is still relatively straightforward to monitor the reaction progress via the HPLC analysis.

Control Reactions

Through our observation, Miglyol oil 812 (0.1 g) is fully soluble in three ILs in Scheme 1, i.e., [$Me(OEt)_3$-Et-Im][OAc] (**1**), [$Me(OEt)_3$-Et_3N][OAc] (**2**), and [$Me(OEt)_3$-Et_3N][HCOO] (**3**), at 50 °C (reaction temperature). In addition, this oil can be dissolved in IL–methanol mixtures (up to 30/70 (*v*/*v*) of IL/MeOH) at 50 °C. On the other hand, Miglyol oil is only partially soluble in pure methanol.

In the absence of lipase, we detected no transesterification reaction of Miglyol oil in pure methanol. However, in 70/30 (*v*/*v*) mixture (1.0 mL) of IL/MeOH (IL=**1**, **2**, or **3**), we observed less than 5% oil (total 0.11 g) being converted into fatty acid ester in 24 h at 50 °C without the addition of any lipase. Although the conversion is not considerable, it suggests that these ILs are able to catalyze the transesterification reaction. This observation is consistent with previous reports from our group [41] and others [42] that acetate- and formate-based ILs might catalyze the transesterification reactions. We also examined another popular IL, 1-butyl-3-methylimidazolium dicyanamide (**4**, [BMIM][dca]), which could dissolve significant amounts of sugars [43]. But we observed a very poor solubility of Miglyol oil in this IL at 50 °C, and most of the oil was floating on the top of IL after days

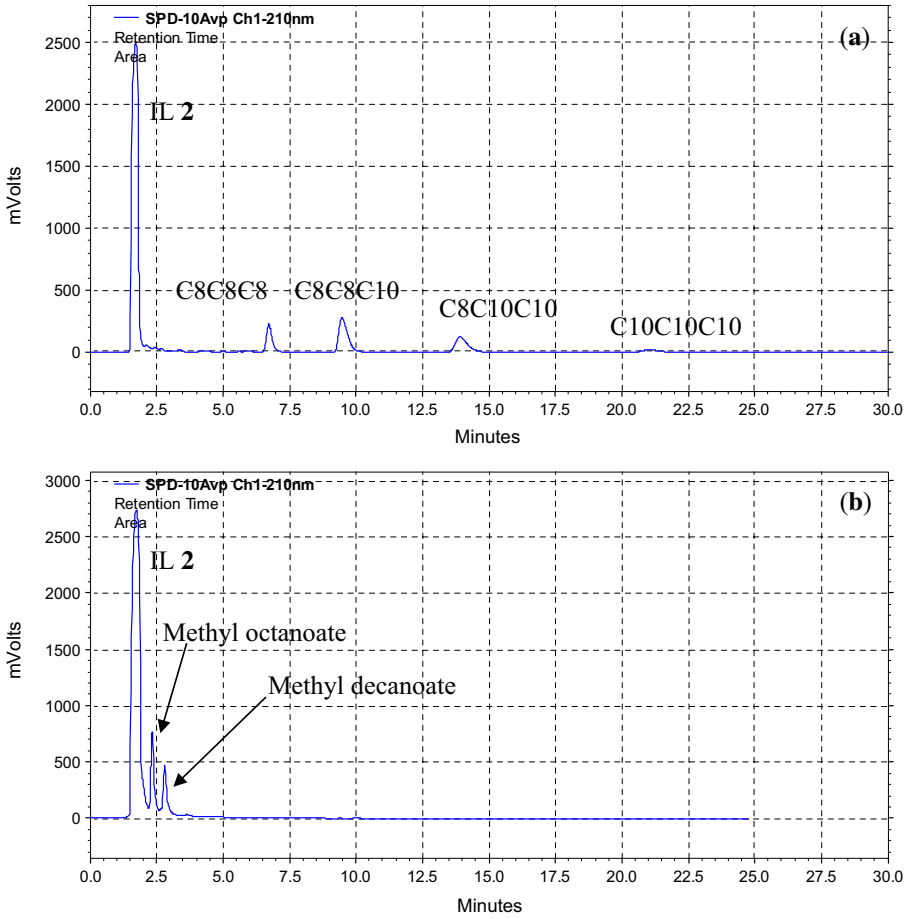


Fig. 1 HPLC chromatograms of transesterification of Miglyol oil 812: **a** before adding the lipase, the reaction mixture contained four triglycerides; **b** upon completion of the reaction, the reactant peaks disappeared and two product peaks (methyl octanoate and methyl decanoate) are shown

of agitation. Without the use of enzyme, we observed no transesterification activity of Miglyol oil in 70/30 (*v*/*v*) mixture of [BMIM][dca]/MeOH.

In summary, ether-functionalized ILs (**1**, **2**, and **3**) based on acetate or formate are able to dissolve Miglyol oil 812 and weakly catalyze the transesterification reaction during biodiesel synthesis.

Effect of IL/MeOH Ratio on the Transesterification

When the transesterification of Miglyol oil (0.11 g) was carried out in 1.0 mL of pure IL (**1**, **2**, or **3**) catalyzed by Novozym 435 (along with only 0.08 g methanol), we observed that most oil absorbed on the surface of enzyme particles. As a result, little triglycerides were detected in the solution after 24 h, nor much of the products (monoesters). Therefore, it seems that the oil aggregation on particles of immobilized CALB inhibited the product formation as well as the product migration into the solvent. To overcome this hurdle, we substituted the immobilized enzyme with free CALB (10 mg fully dissolved at 50 °C)

under the same reaction condition. Since the free lipase is soluble in IL after gentle agitation at 50 °C, there is no aggregation of substrate and products on the enzyme. However, we observed little monoesters were produced through HPLC analysis. The oil aggregation problem was solved by using at least 30% (*v*/*v*) of methanol in the reaction mixture. Figure 2 demonstrates that three major triglycerides in Miglyol oil were converted into methyl esters at about same reaction rates in 70/30 (*v*/*v*) mixture of IL **1**/MeOH. Therefore, the total triglyceride conversion is used in this study to quantify the overall reaction rate. As shown in Fig. 2, the enzymatic reaction in IL was rapid and most of the conversion finished in the first hour, but a longer reaction time (24–48 h) ensured a more complete reaction.

Figure 3 further illustrates the effect of methanol concentration (30–70%, *v*/*v*) on the triglyceride conversion. The immobilized CALB seems very stable in the presence of methanol up to 50%, which is almost impossible in conventional organic solvent systems. It has been documented that the presence of as low as 1.5–3 molar methanol (vs. 1 molar triglycerides) may lead to a significant loss of lipase activity [25, 44, 45] (the methanol/substrate ratio is higher for immobilized CALB such as >5/1 [46] or >6/1 [47]). Our molar ratio of methanol/substrate (~30 for 30% methanol) is much higher than these values, which further confirms our previous conclusion [35, 41] that these ether-functionalized ILs are capable of stabilizing the enzyme. When the methanol concentration was above 60% (*v*/*v*), lower oil conversions were seen (Fig. 3). The addition of methanol as co-solvent serves at least two purposes: (1) solving the oil aggregation on the enzyme's surface and (2) reducing the cost of overall process although ILs are expected to be recycled and reused. However, using too much methanol reduces the solubility of oils or fats in the solvent mixture; therefore, the ratio of 70/30 (*v*/*v*) IL/MeOH was chosen for further experiments.

The overall triglyceride conversion is 70% at 48 h in 70/30 (*v*/*v*) mixture of IL **1**/MeOH (Fig. 3). Further studies suggest that, in the presence of same methanol concentration, the conversions in **2** and **3** are 85% and 88% at 48 h, respectively (same reaction conditions as Fig. 3). However, in 70/30 (*v*/*v*) mixture of [BMIM][dca] (**4**)/MeOH, only 1% yield of fatty acid monoester was detected at 48 h as the oil is not soluble in the solvent and the oil absorption on Novozym 435 particles was observed.

We also explored the possibility of using *t*-butanol as co-solvent for the enzymatic reaction. In the presence of 40% (*v*/*v*) *t*-BuOH and 60% IL **1** (0.19 g methanol and other

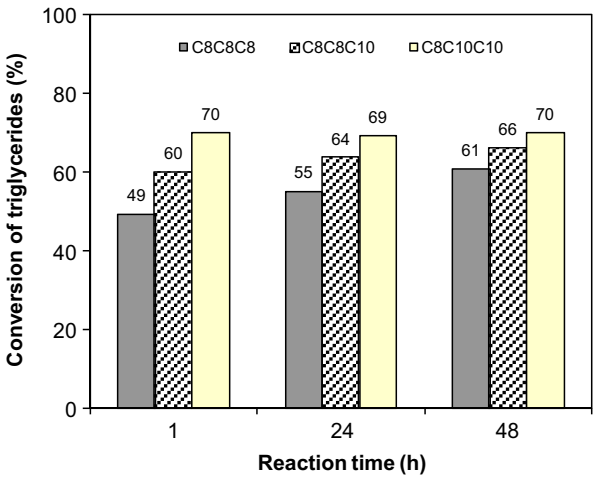


Fig. 2 Enzymatic conversion of individual triglycerides in Miglyol oil 812 (Miglyol oil 0.11 g, 700 μL [$Me(OEt)_3$-Et-Im][OAc] (**1**)+300 μL MeOH, 40 mg Novozym 435, 50 °C)

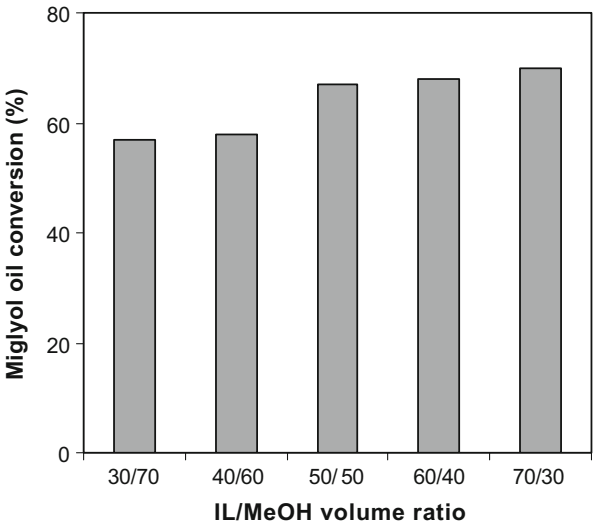


Fig. 3 Effect of IL/MeOH ratio on the enzymatic conversion of Miglyol oil 812 (Miglyol oil 0.11 g, [Me(OEt)$_3$-Et-Im][OAc] (**1**)+MeOH total volume 1.0 mL, 40 mg Novozym 435, 50 °C, reaction time 48 h)

conditions were same as Fig. 3), the oil conversions were 38% at 24 h and 70% at 96 h. The same reaction in 40/60 MeOH/IL **1** achieved conversions of 71% at 24 h and 80% at 96 h. We suspected that the differences could be due to the poor dissolution ability of *t*-BuOH towards the substrate (when comparing with that of methanol). Therefore, the IL/*t*-BuOH mixture is not advantageous over the IL/MeOH combination for the transesterification reaction. However, a future study could include other long-chain alcohols as co-solvents for easy product separation and stoichiometric addition of methanol.

Transesterification of Miglyol Oil Catalyzed by Other Lipases

To further examine the compatibility of our IL/MeOH systems for other lipases, we conducted the transesterification in 70/30 (*v*/*v*) mixtures of IL **2**/MeOH catalyzed by ten enzyme preparations (Table 1). It is important to notice that when free lipases were used they were also soluble in the ionic media at 50 °C after a few hours of gentle agitation (thus a truly homogeneous reaction). In most cases, most of the triglyceride conversions were completed in the first hour, implying a fast enzymatic process in ILs. Comparing with immobilized CALB (Novozym 435), the free CALB catalyzed reaction was slightly slower (trials 1 and 2 in Table 1). At 48 h, the highest yields were achieved in Novozym 435 (85%) and PS-D I (81%). With a further extension of reaction time to 96 h, the conversions of Miglyol oil were very close to 100% completion except 76% by Amano lipase A from *A. niger* and 86% by lipase from *C. cylindracea* immobilized on Sol–Gel-AK. These exciting results suggest that the IL/MeOH media are very suitable for many lipases and different enzyme preparations, which hold enormous potential for biodiesel production from a variety source of oils and fats [23].

Biodiesel Synthesis from Soybean Oil

To further apply our new solvent system to a real scenario, we conducted the transesterification of soybean oil (0.11 g) in 1.0 mL mixture of 70/30 (*v*/*v*) IL **2**/MeOH at

Table 1 Transesterification of Miglyol oil 812 catalyzed by different lipases.

Trial	Lipase	Miglyol oil conversion (%)		
		1 h	48 h	96 h
1	40 mg Novozym 435	70	85	98
2	10 mg free CALB	10	74	96
3	15 mg Amano lipase PS-D I	67	81	98
4	20 mg Amano lipase PS-C I	64	77	99
5	10 mg Amano lipase PS	65	74	99
6	10 mg lipase PPL	64	72	99
7	15 mg Amano lipase AK 20	52	77	99
8	15 mg Amano Newlase F	69	74	99
9	10 mg Amano lipase A from *Aspergillus niger*	52	73	76
10	40 mg lipase from *Pseudomonas cepacia* immobilized on Sol–Gel-A	45	78	96
11	40 mg lipase from *Candida cylindracea* immobilized on Sol–Gel-AK	38	59	86

Reaction conditions: 0.11 g Miglyol oil, 700 μL [$Me(OEt)_3$-Et_3N][OAc] (**2**)+300 μL MeOH, and 50 °C

50 °C. After 96 h, the isolated product was 0.07 g when the reaction was catalyzed by Novozym 435 (40 mg), and 0.09 g by lipase PS-D I. The NMR spectra (see Materials and Methods) confirmed the structures of fatty acid monoesters. Although further systematic investigation is needed to optimize other reaction parameters, our preliminary study indicated that the oil-dissolving and enzyme-stabilizing IL systems are promising alternative media for the enzymatic synthesis of biodiesel.

Conclusions

The new ether-functionalized ILs were found capable of dissolving triglycerides. Various lipases maintained high catalytic activities in mixtures of IL (**1**, **2**, or **3**) with methanol, even at high concentrations (up to 50% *v*/*v*) of methanol. Higher triglyceride conversions were observed in alkylammonium ILs **2** and **3** than that in imidazolium IL **1**. The high lipase activities can be attributed to the enzyme compatible nature of these ILs. Quantitative conversions of Miglyol oil 812 were achieved at 96 h in the mixture of 70/30 (*v*/*v*) IL/MeOH when catalyzed by most lipases investigated. The preliminary studies on the transesterification of soybean oil in IL/MeOH mixtures suggest that the new solvent system can be further explored as promising media for biodiesel production.

In addition to their use as biodiesel, fatty acid esters can have other important industrial applications. For example, medium chain length fatty acid esters (such as caprylic and capric acid esters) have important applications in medical, nutritional, and dietetic uses [48]. Fatty acid sugar esters, as biodegradable non-ionic surfactants, have wide applications in cosmetic, pharmaceutical, and food industry [49]. Commercially, long-chain fatty acids are also extracted from pine trees, and their esters are used as synthetic lubricants including gear oils, hydraulic fluids, metal-working fluids, greases, and fuel additives (for example, Arizona Chemical Company, www.azchem.com). Therefore, the enzymatic transesterification (or esterification) of triglycerides (or fatty acids) in ILs can be explored in a wide range of industrial applications.

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References

1. Fukuda, H., Kondo, A., & Noda, H. (2001). *Journal of Bioscience and Bioengineering, 92*, 405–416.
2. Liu, B., & Zhao, Z. (2007). *Journal of Chemical Technology and Biotechnology, 82*, 775–780.
3. Du, W., Wang, L., & Liu, D. (2007). *Green Chemistry, 9*, 173–176.
4. Wang, W. G., Lyons, D. W., Clark, N. N., & Gautam, M. (2000). *Environmental Science and Technology, 34*, 933–939.
5. Schuchardt, U., Sercheli, R., & Vargas, R. M. (1998). *Journal of the Brazilian Chemical Society, 9*, 199–210.
6. Formo, M. W. (1954). *Journal of the American Oil Chemists' Society, 31*, 548–559.
7. Freedman, B., Pryde, E. H., & Mounts, T. L. (1984). *Journal of the American Oil Chemists' Society, 61*, 1638–1643.
8. Vicente, G., Martínez, M., & Aracil, J. (2004). *Bioresource Technology, 92*, 297–305.
9. Liu, Y., Lotero, E., Goodwin, J. G., & Lu, C. (2007). *Journal of Catalysis, 246*, 428–433.
10. Chai, F., Cao, F., Zhai, F., Chen, Y., Wang, X., & Su, Z. (2007). *Advanced Synthesis & Catalysis, 349*, 1057–1065.
11. Abreu, F. R., Alves, M. B., Macêdo, C. C. S., Zara, L. F., & Suarez, P. A. Z. (2005). *Journal of Molecular Catalysis. A, Chemical, 227*, 263–267.
12. Abreu, F. R., Lima, D. G., Hamú, E. H., Einloft, S., Rubim, J. C., & Suarez, P. A. Z. (2003). *Journal of the American Oil Chemists' Society, 80*, 601–604.
13. Toda, M., Takagaki, A., Okamura, M., Kondo, J. N., Hayashi, S., Domen, K., et al. (2005). *Nature, 438*, 178.
14. DaSilveira Neto, B. A., Alves, M. B., Lapis, A. A. M., Nachtigall, F. M., Eberlin, M. N., Dupont, J., et al. (2007). *Journal of Catalysis, 249*, 154–161.
15. Lapis, A. A. M., de Oliveira, L. F., Neto, B. A. D., & Dupont, J. (2008). *ChemSusChem, 1*, 759–762.
16. Demirbas, A. (2005). *Progress in Energy and Combustion Science, 31*, 466–487.
17. Saka, S., & Kusdiana, D. (2001). *Fuel, 80*, 225–231.
18. Kusdiana, D., & Saka, S. (2001). *Fuel, 80*, 693–698.
19. He, H., Wang, T., & Zhu, S. (2007). *Fuel, 86*, 442–447.
20. Wu, Q., Chen, H., Han, M., Wang, D., & Wang, J. (2007). *Industrial & Engineering Chemistry Research, 46*, 7955–7960.
21. Han, M., Yi, W., Wu, Q., Liu, Y., Hong, Y., & Wang, D. (2009). *Bioresource Technology, 100*, 2308–2310.
22. Liang, X., Gong, G., Wu, H., & Yang, J. (2009). *Fuel, 88*, 613–616.
23. Akoh, C. C., Chang, S. W., Lee, G.-C., & Shaw, J.-F. (2007). *Journal of Agricultural and Food Chemistry, 55*, 8995–9005.
24. Zhao, X., El-Zahab, B., Brosnahan, R., Perry, J., & Wang, P. (2007). *Applied Biochemistry and Biotechnology, 143*, 236–243.
25. Shimada, Y., Watanabe, Y., Samukawa, T., Sugihara, A., Noda, H., Fukuda, H., et al. (1999). *Journal of the American Oil Chemists' Society, 76*, 789–793.
26. Du, W., Xu, Y., Liu, D., & Zeng, J. (2004). *Journal of Molecular Catalysis. B, Enzymatic, 30*, 125–129.
27. Modi, M. K., Reddy, J. R. C., Rao, B. V. S. K., & Prasad, R. B. N. (2007). *Bioresource Technology, 98*, 1260–1264.
28. Iso, M., Chen, B., Eguchi, M., Kudo, T., & Shrestha, S. (2001). *Journal of Molecular Catalysis. B, Enzymatic, 16*, 53–58.
29. Royon, D., Daz, M., Ellenrieder, G., & Locatelli, S. (2007). *Bioresource Technology, 98*, 648–653.
30. van Rantwijk, F., & Sheldon, R. A. (2007). *Chemical Reviews, 107*, 2757–2785.
31. Ha, S. H., Lan, M. N., Lee, S. H., Hwang, S. M., & Koo, Y.-M. (2007). *Enzyme and Microbial Technology, 41*, 480–483.
32. Sunitha, S., Kanjilal, S., Reddy, P. S., & Prasad, R. B. N. (2007). *Biotechnology Letters, 29*, 1881–1885.
33. Gamba, M., Lapis, A. A. M., & Dupont, J. (2008). *Advanced Synthesis & Catalysis, 350*, 160–164.
34. Lau, R. M., Sorgedrager, M. J., Carrea, G., van Rantwijk, F., Secundo, F., & Sheldon, R. A. (2004). *Green Chemistry, 6*, 483–487.

35. Zhao, H., Baker, G. A., Song, Z., Olubajo, O., Crittle, T., & Peters, D. (2008). *Green Chemistry, 10*, 696–705.
36. Turner, M. B., Spear, S. K., Huddleston, J. G., Holbrey, J. D., & Rogers, R. D. (2003). *Green Chemistry, 5*, 443–447.
37. Toral, A. R., de los Rios, A. P., Hernández, F. J., Janssen, M. H. A., Schoevaart, R., van Rantwijk, F., et al. (2007). *Enzyme and Microbial Technology, 40*, 1095–1099.
38. de los Ríos, A. P., Hernández-Fernández, F. J., Martínez, F. A., Rubio, M., & Víllora, G. (2007). *Biocatalysis and Biotransformation, 25*, 151–156.
39. Moniruzzaman, M., Kamiya, N., & Goto, M. (2009). *Langmuir, 25*, 977–982.
40. Zhao, H., Baker, G. A., Song, Z., Olubajo, O., Zanders, L., & Campbell, S. M. (2009). *Journal of Molecular Catalysis. B, Enzymatic, 57*, 149–157.
41. Zhao, H., Jones, C. L., & Cowins, J. V. (2009). *Green Chemistry, 11*, 1128–1138. doi:10.1039/b905388c.
42. MacFarlane, D. R., Pringle, J. M., Johansson, K. M., Forsyth, S. A., & Forsyth, M. (2006). *Chemical Communications*, 1905–1917.
43. Liu, Q., Janssen, M. H. A., van Rantwijk, F., & Sheldon, R. A. (2005). *Green Chemistry, 7*, 39–42.
44. Watanabe, Y., Shimada, Y., Sugihara, A., Noda, H., Fukuda, H., & Tominaga, Y. (2000). *Journal of the American Oil Chemists' Society, 77*, 355–360.
45. Soumanou, M. M., & Bornscheuer, U. T. (2003). *Enzyme and Microbial Technology, 33*, 97–103.
46. Köse, Ö., Tüter, M., & Aksoy, H. A. (2002). *Bioresource Technology, 83*, 125–129.
47. Yang, J.-S., Jeon, G.-J., Hur, B.-K., & Yang, J.-W. (2005). *Journal of Microbiology and Biotechnology, 15*, 1183–1188.
48. Babayan, V. K. (1981). *Journal of the American Oil Chemists' Society, 58*, 49A–51A.
49. Flores, M. V., Naraghi, K., Engasser, J.-M., & Halling, P. J. (2002). *Biotechnology and Bioengineering, 78*, 815–821.