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ORIGINAL PAPER

Fatty Acid Alkyl Esters as Feedstocks for the Enzymatic Synthesis of Alkyl Methacrylates and Polystyrene-*co*-alkyl Methacrylates for use as Cold Flow Improvers in Diesel Fuels

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Abstract The enzymatic transesterifications of fatty acid methyl esters (FAME) with hydroxyethyl methacrylate (HEMA) were carried out using the *Candida antarctica* lipase B immobilized within a porous polymethacrylate resin. The enzymatic activity in the transesterification reaction of FAME with HEMA depended on the polarity of the solvent and the highest yield was obtained in toluene (non-polar). The molar ratio of 1:4 (for methyl laurate:HEMA) and 1:2 (for methyl oleate:HEMA) was most favorable for the transesterification yield. The reaction condition (at 60 °C/24 h), and the enzyme concentration of 5% (w/w) for methyl laurate with HEMA, 2% (w/w) for methyl oleate with HEMA resulted in the highest final yield. Under these conditions, the maximum yields for the transesterification of methyl laurate with HEMA, methyl oleate with HEMA were 97 ± 5.4% and 91 ± 4.7%, respectively. After ten batches of transesterification of FAME with HEMA, enzyme activity was retained at the level of 88 ± 2.6% and 76 ± 2.3%, respectively, compared with their initial activity. Also, alkyl methacrylate/styrene copolymers were synthesized by radical polymerization of HEMA-LMA (or HEMA-OMA) and styrene. The prepared copolymers have average molecular weights from 2.6×10^4 to 5.5×10^4. Especially, the poly(styrene-*co*-alkyl methacrylate)s (PStmHAMAn) led to a reduction in the pour point in ultra low sulfur diesel (ULSD) treated with 200–1,000 ppm of poly(styrene-*co*-alkyl methacrylate). Diesel fuel containing 1,000 ppm of the copolymer (PSt2HLMA8) showed a 15 ± 1.25 °C reduction in its pour point.

Keywords Enzymatic synthesis · Fatty acid methyl esters · Hydroxy ethyl methacrylate · *Candida antarctica* lipase B · Poly(styrene-*co*-alkyl methacrylate)s · Pour point depressant

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Introduction

It is known that ultra low sulfur diesel (ULSD) may contain substantial amounts of waxy materials. When these materials are subjected to temperatures below their cloud points, these tend to deposit on cold surfaces or crystallize and agglomerate into a solid gel [1, 2]. The gelling or stiffening of ULSD introduces rheological problems that interfere with oil production or pipeline transportation and cause handling problems [3]. The high wax crude oils are characterized by high pour point, high viscosity, high gel strength, and abundant wax deposits. Several techniques such as the wintering process [4], blending process [5, 6], and additive addition [7] have been used to minimize the problems caused by wax deposition in diesel fuel. Among them, a few polymers having long alkyl side chains, so-called "comb-like" polymers, are known to decrease the rate of wax formation. Polymeric modifier such as poly (ethylene-*co*-vinyl acetate) (EVA) [8–10], poly(styrene-*co*-alkyl methacrylates) [11, 12], poly(fumarate-*co*-vinyl acetate) [13], poly(alkyl methacrylate-maleic anhydride) [14], polymethylmethacrylate [15–18], and their acid amide derivatives [19–21] have been known for several decades as commercial flow improvers and wax inhibitors of diesel fuel, but none of them are sufficiently effective in diesel fuel. Thus, it has become necessary to develop new cold

flow improver for ULSD. Moreover, considering cost and environmental issues, it is necessary to synthesize cold flow improvers based on biodiesel-like materials, fatty acid methyl esters (FAME), and enzymatic processes.

In order to prepare the compounds which can satisfy the above criteria of the monomers for synthesis of pour-point depressants, 2-(methacryloyloxy)ethyl alkyl esters were selected as the monomeric compounds, which could be prepared by lipase-catalyzed transesterification of fatty acid methyl esters (FAME) with hydroxyethyl methacrylate (HEMA). HEMA has a vinyl group that can be polymerized. FAME have various length of hydrocarbons. Therefore, 2-(methacryloyloxy)ethyl alkyl esters are expected to be efficient monomers for the synthesis of pour-point depressants. In this study, a lipase was used as catalyst for the transesterification reaction, because lipase-catalyzed transesterification reaction has advantages over conventional chemical-methods owing to mild reaction conditions and energy-saving process [22–24].

We investigated the reaction conditions such as the effect of methanol removal in the reaction system, molar ratio of substrates, enzyme amount, and reaction temperature for the transesterification of FAME with HEMA. And the copolymerization of these monomers with styrene was also performed.

Materials and Methods

Materials

HEMA, methyl laurate (99.5%), methyl oleate (99%), styrene, and 2,2′-azobisisobutyronitrile (AIBN) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Silica gel 60 (Merck Co.) was used for separation of product. Toluene, acetonitrile, and 1,4-dioxane were obtained form Merck Co. (Darmstadt, Germany). All other chemicals such as methanol, ethyl acetate, hexane, chloroform-d6, and TMS were analytical grade. Macroporous polymethacrylate resin (VPOC 1600, Lewatit, Germany), tris-HCl buffer (Sigma, St. Louis, MO, USA), Cal-B (Novozymes A/S, Bagsvaerd, Denmark) for immobilization of lipase were used.

Assay of Lipase Activity

Lipase activity was measured by spectrophotometric method using *p*-nitrophenyl palmitate (pNPP) as a substrate. The 10 mM pNPP dissolved in acetonitrile was mixed with ethanol and 50 mM sodium phosphate buffer (pH 7.0) to meet the final ratio of acetonitrile: ethanol: sodium phosphate buffer to 1:4:95 (v/v/v). The reaction was started by adding an appropriate amount of enzyme to 1,000 µl of reaction mixture at 45 °C and the release of *p*-nitrophenol product was monitored by measuring the absorbance at 405 nm with a spectrophotometer. One unit of lipase activity was defined as the amount of enzyme releasing 1 µmol of *p*-nitrophenol per min.

Immobilization of Lipase

Lipase immobilization was performed as previously described [25]. The macroporous polymethacrylate resin (30 g) was washed with 1 L of 25% methanol (3 times) sequentially prior to use. After removal of methanol by filtration, the resin was washed with 1 L of 50 mM Tris-HCl buffer (pH 7.5). The supernatant was removed and replaced with 1 L of fresh 50 mM Tris-HCl buffer (pH 7.5). Seven hundred ml of the commercial CalB solution The culture broth (the solution of CalB prepared from the culture supernatant of *A. oryzae*) [26] containing CalB (700 mL) was added and mixed at 150 rpm for 6 h at 25 °C by mechanical stirrer. The immobilized CalB was filtered, washed twice with 1 L of 50 mM Tris-HCl buffer (pH 7.5) for 15 min, and then dried at room temperature for 20 h. The activity of the immobilized CalB was 0.23 U/g dry resin (pNPP unit).

Enzymatic Transesterification

The transesterification reactions were performed following the same procedure published in the literature as follows [24]. Into a 100-mL round-bottomed flask equipped with a magnetic stirrer, thermometer, and a water cooled condenser, 16.0 mmol of FAME (methyl laurate and methyl oleate) and 32.0 mmol of HEMA was added along with 5% (based on FAME weight) of the immobilized CalB in 15 mL of solvent such as toluene, acetonitrile, and 1,4-dioxane. The transesterification reactions were carried out at the temperatures in the range of 25–80 °C with vigorous stirring for 24 h with removing methanol through N_2 purge. To optimize conditions, the reactions were performed under various conditions by changing substrate ratios of FAME and HEMA, enzyme concentrations. After the transesterification reaction, the immobilized CalB was separated from the reaction medium by filtration. The reaction mixture was concentrated under reduced pressure of 30 mmHg at 50 °C and the crude mixture was separated on a silica gel column (100–200 mesh) using ethyl acetate/hexane (4/1) as the eluent. Solvent was removed under reduced pressure.

Batch Operational Stability Tests

After each transesterification reaction, the lipase (1 g) was recovered by filtration and washed with a mixture (100 mL) of hexane and ethyl acetate (10:2 vol/vol) to get

rid of the synthetic products from the lipase. After washing, the enzyme was dried for 24 h at 25 °C in a vacuum oven (1 mmHg). After that, the enzyme was reused under the enzymatic transesterification conditions as described above.

GC-MS Analysis

The transesterification reactions of FAME with HEMA were monitored by gas chromatography (HP 5890 Model). A DB-Wax capillary column with dimensions of 0.25 mm × 30 m i.d. × 0.25 μm film thickness (Agilent Technologies, Palo Alto, CA, USA) was used for the separation of the products. The initial temperature of 115 °C was raised to 230 °C at a rate of 20 °C/min, and kept at 230 °C for 20 min. The split ratio was 1:50, and helium was used as the carrier gas with a flow rate of 1 mL/min. The injector and detector temperatures were 200 and 230 °C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV with an ion source temperature of 230 °C, a quadrupole temperature of 150 °C, and a translating line temperature of 270 °C. The mass scan ranged from 50 to 550 *m*/*z* with an Em voltage, 1,035 V.

Copolymerization and Analysis

As shown in Fig. 1a, the structures of synthetic monomers were confirmed by ^{1}H-NMR and FT-IR spectrum analyses. ^{1}H-NMR spectra were obtained with 300 MHz BRUKER DPX-300 spectrometer using $CDCl_3$. FT-IR spectra were measured on a Bio-RAD FTS165 spectrometer. Also, the detailed procedures to synthesize copolymers (Fig. 1b) are described as follows: a synthesized monomer such as HEMA-LMA (or HEMA-OMA) and styrene with different monomer feed ratios (mol% HEMA-LMA/mol% Styrene viz. 40/60, 50/50, 60/40, 70/30, and 80/20) was dissolved in 100 mL toluene and the solution was poured into a

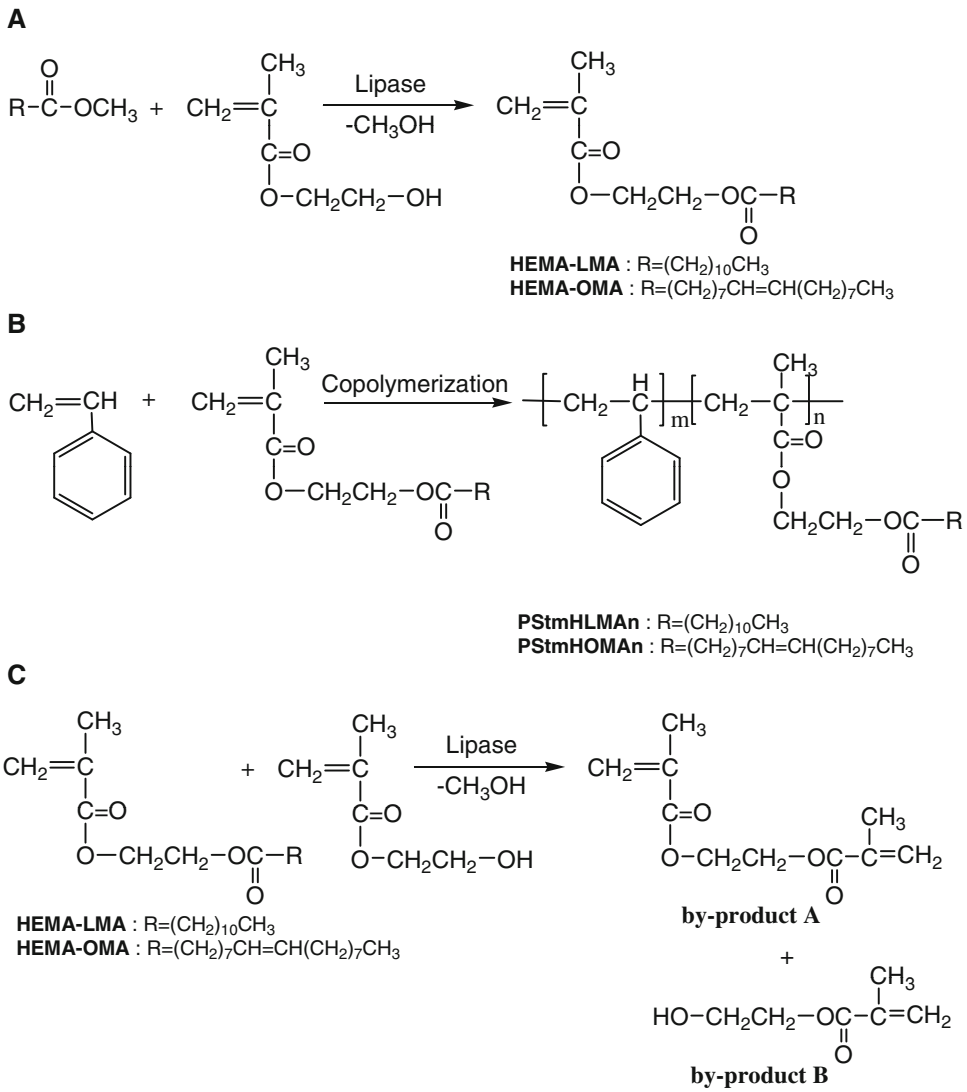

Fig. 1 Synthesis of monomers (**a**) and copolymers (**b**) for cold flow improvers and synthetic route of by-product **a** and **b** (**c**)

round-bottom flask under N_2 atmosphere. The monomers were mixed together with AIBN initiator 0.2% (w/w). The solution was then heated to 60 °C and was reacted for 24 h. After the completion of copolymerization, the reaction mixtures were poured into excess methanol with stirring. The obtained precipitate was filtered and dried in vacuum (1 mmHg) at 60 °C to a constant weight.

The prepared copolymers were analyzed using ^{1}H-NMR spectroscopic technique for determining the chemical structure of the copolymers. ^{1}H-NMR spectra were obtained at 300 MHz on a BRUKER DPX-300 spectrometer using $CDCl_3$ as a solvent and TMS as an internal reference. The molecular weights of copolymers were determined using a size exclusion chromatograph consisting of a Waters 2690 HPLC pump, three Ultrastyragel columns (two mixed-B and one 500-Å column), and a Waters refractive index detector, using THF as solvent at a flow rate of 1 mL/min, and narrow disperse polystyrene (PS-1, Mw: 580–7,800,000 g/mol, Part No 2010-0501, PL, UK) as calibration standards. Cold flow properties of copolymers were characterized by measuring pour point of diesel fuel containing copolymers through ASTM D97 method using Automated Pour Point tester (CPP 5Gs, ILS Co, France) [27].

Results and Discussion

Enzymatic Synthesis of 2-(Methacryloyloxy)Ethyl Alkyl Esters

The monomers, HEMA-LMA and HEMA-OMA, for cold flow improvers were synthesized as shown in Fig. 1a. 2-(Methacryloyloxy)ethyl alkyl esters (HEMA-LMA and HEMA-OMA) were selected as the monomeric compounds, which could be prepared by lipase-catalyzed transesterification of fatty acid methyl esters (FAME-L and FAME-O) with hydroxyethyl methacrylate (HEMA). GC-MS was used to identify the reaction products. In the gas chromatogram of the reaction to synthesize HEMA-LMA, methyl laurate and HEMA presented retention times of 7.76 and 7.83 min, respectively. Three new peaks appeared at 8.03, 12.97, and 13.49 min. According to GC-MS analysis, the compound with a retention time of 8.03 min was identified as compound of [$M^+ = m/e$ 198]. This compound shows 100% intensity at m/z 69, $[CH_2=C(CH_3)C=O]^+$. The compound with a retention time of 12.97 min was identified as compound of [$M^+ = m/e$ 244]. This compound shows 100% intensity both at m/z 104 and m/z 183. The peak at m/z 104 could be assigned as $[CH_2C(=O)OCH_2CH_2OH]^+$ and the peak at m/z 183 could be assigned as the fragment of $[CH_3(CH_2)_{10}C(=O)O]^+$. The compound with retention time of 13.49 min was identified as the product (HEMA-LMA) of [$M^+ = m/e$ 312]. This compound shows 100% intensity at m/z 113, $[CH_2=C(CH_3)C(=O)OCH_2CH_2]^+$, 80% intensity at m/z 69, $[CH_2=C(CH_3)C=O]^+$, and 50% intensity at m/z 183, $[CH_3(CH_2)_{10}C(=O)O]^+$. From these results, we concluded that the compound with retention time of 8.03 min was identified as the compound B, $CH_2=C(CH_3)C(=O)OCH_2CH_2O(O=)C(CH_3)C=CH_2$, and the compound with retention time of 12.97 min was identified as the compound A, $CH_3(CH_2)_{10}C(=O)OCH_2CH_2OH$, judging from the fact that the higher molecular weight compounds have longer retention time.

The analysis of the reaction product for synthesizing HEMA-OMA was similar to that of HEMA-LMA.

The structure of the prepared monomers was confirmed by ^{1}H-NMR spectral and FT-IR spectroscopic analysis. ^{1}H-NMR spectra of HEMA-LMA and HEMA-OMA monomers are shown in Fig. 2. As can be seen in Fig. 2A (HEMA-LMA), peaks at 6.2 and 5.6 ppm were assigned as the protons of the CH= in vinyl group of HEMA. The methylene proton adjacent to the oxygen atom of HEMA shows a chemical shift of 4.3 ppm; the proton of the methylene adjacent to carbonyl group of laurate shows a chemical shift of 2.3 ppm; the proton in the methyl group of HEMA exhibits a chemical shift of 1.95 ppm, and the chemical shifts of the methylene of lauryl unit are ranged within 1.6–1.25 ppm; the proton in the methyl group of lauryl shows a chemical shift of 0.87 ppm. This indicates that there exists an alkyl chain moiety of laurate and a methacrylate moiety of HEMA in the prepared monomer, HEMA-LMA. As seen in Fig. 2b (HEMA-OMA), the vinyl proton of the oleyl unit shows a chemical shift of 5.6 ppm; the chemical shifts of the methylene of oleyl unit are ranged within 2.0–1.25 ppm; the other protons in HEMA-OMA show similar chemical shift as seen in HEMA-LMA.

FT-IR spectra of HEMA-LMA and HEMA-OMA monomers are shown in Fig. 3. CH_3 and CH_2 absorption peaks of long-chain alkyl at 2,923 and 2,853 cm^{-1}; C=O stretching peaks of methacrylate at 1,732 cm^{-1}; C=C stretching vibration peaks of methacrylate at 1,639 cm^{-1}. This also indicates that there exists an alkyl chain fragment of FAME and a methacrylate fragment of HEMA in the prepared monomers, HEMA-LMA and HEMA-OMA.

2-(Methacryloyloxy)ethyl Lauryl Ester (HEMA-LMA)

^{1}H-NMR ($CDCl_3$): $\delta = 6.2$ (1H, s, =CH), 5.6 (1H, s, =CH), 4.3 (4H, t, $2OCH_2$), 2.3 (2H, t, $C(=O)CH_2$), 1.95 (3H, s, $CH_3C=$),

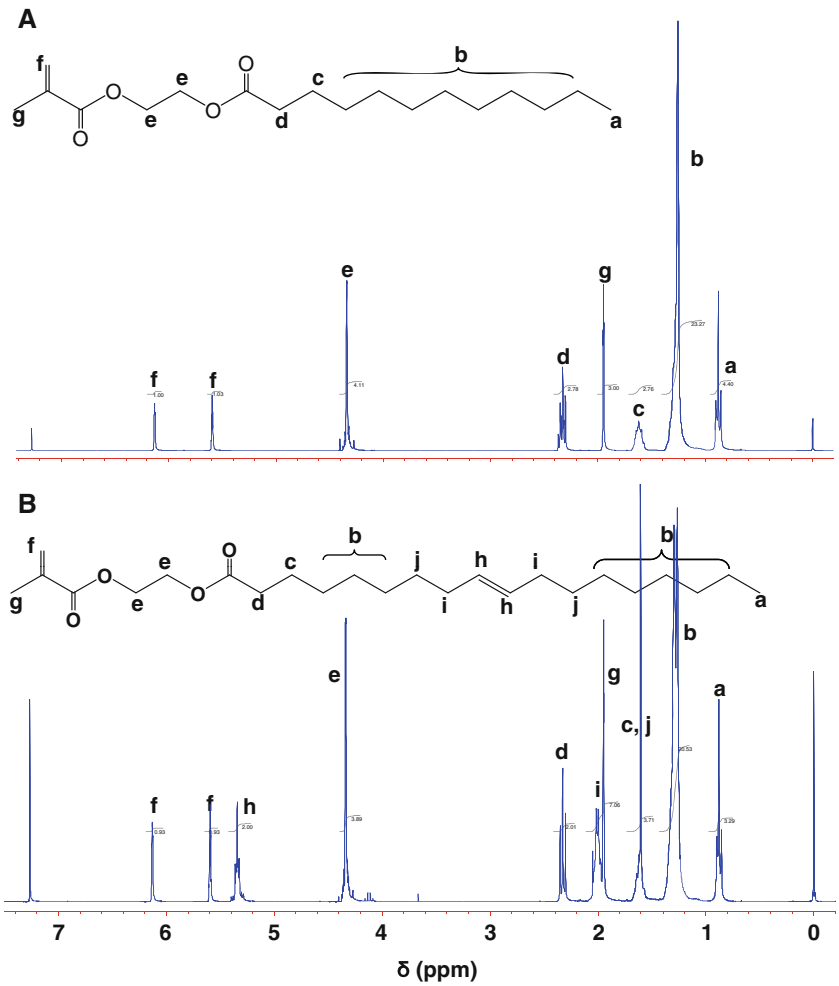

Fig. 2 ^{1}H-NMR spectra of HEMA-LMA (**a**) and HEMA-OMA (**b**)

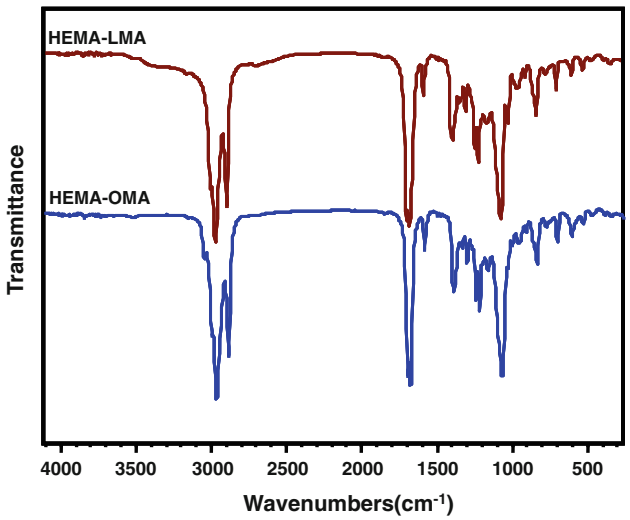

Fig. 3 FT-IR spectrum of HEMA-LMA and HEMA-OMA

1.6 (2H, m, CH_2), 1.25 (16H, m, $8CH_2$), 0.87 (3H, t, CH_3).

FT-IR (KBr) 2,923 (C-H stretching), 1,732 (C=O), 1,639 (C=C), 1,080 (C–O), cm^{-1}.

2-(Methacryloyloxy)Ethyl Oleyl Ester (HEMA-OMA)

^{1}H-NMR ($CDCl_3$): $\delta = 6.1$ (1H, s, =CH), 5.6 (1H, s, =CH), 5.3 (2H, m, 2CH=), 4.3 (4H, t, $2OCH_2$), 2.3 (2H, t, $C(=O)CH_2$), 2.0 (4H, m, $2CH_2$), 1.95 (3H, s, $CH_3C=$), 1.6 (6H, m, $CH_2 + 2CH_2$), 1.2 (16H, m, $8CH_2$), 0.8 (3H, t, CH_3).

FT-IR (KBr) 2,923 (C–H stretching), 1,732 (C=O), 1,639 (C=C), 1,080 (C–O), cm^{-1}.

Effects of Solvents in Transesterification

The effect of solvents in the transesterification of FAME with HEMA was investigated (reaction condition: FAME (1 mol), HEMA (2 mol), enzyme (5 wt%), 24 h, 50 °C). We selected transesterification solvents such as toluene (log $P = 2.5$), acetonitrile (log $P = 0.3$), and 1,4-dioxane (log $P = -0.3$), all of which have higher boiling point than that of methanol (log $P = -0.75$) [28]. In the transesterification reaction of FAME-L with HEMA, the yields of HEMA-LMA were 89.6 ± 4.5, 87.5 ± 3.9 and 83.5 ± 3.3% in toluene, acetonitrile, and 1,4-dioxane, respectively

(Fig. 4). The yields of the by-product were 8.2 ± 0.3, 1.4 ± 0.1 and 1.0 ± 0.05% in toluene, acetonitrile, and 1,4-dioxane, respectively. In the transesterification reaction of FAME-O with HEMA, the reaction yields seem to be solvent dependent compared with FAME-L with HEMA. The yields of HEMA-OMA were 83.9 ± 3.4, 70.1 ± 2.1, and 29.8 ± 0.3% in toluene, acetonitrile, and 1,4-dioxane, respectively. The yields of the by-product were 7.6 ± 0.2, 15.8 ± 0.6, and 0.7 ± 0.02% in toluene, acetonitrile and 1,4-dioxane, respectively. From these results, we could conclude that the transesterification reaction yields of FAME-L with HEMA did not largely depend on solvent, whereas that of FAME-O with HEMA largely depended on the polarity of the solvent. Transesterification reaction yields in toluene (relatively nonpolar) were higher than those in acetonitrile and 1,4-dioxane, suggesting that nonpolar solvent is more favorable and effective for transesterification of more lipophilic HEMA-OMA, compared with that of HEMA-LMA.

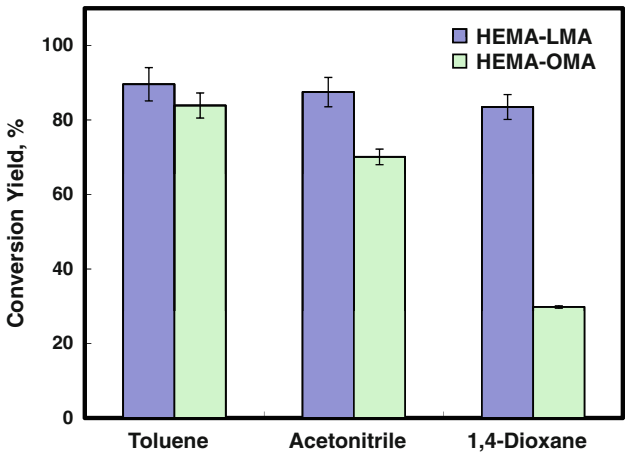

Fig. 4 Effect of solvents in the transesterification of FAME with HEMA

Effect of the Molar Ratio of the Substrate

The effect of the molar ratio was investigated in the transesterification reaction of FAME with HEMA (reaction condition: toluene, enzyme (5 wt%), 24 h, 50 °C), we tried FAME:HEMA molar ratios from 1:1 to 1:5 (Fig. 5). In the range of 1:2 and 1:5, the yields are considered to be statistically the same since the p values (the significance level = 0.05) calculated from analysis of variance (ANOVA) were found to be 0.76 and 0.15 for the product (HEMA-LMA) and by-product, respectively, by the use of one-way ANOVA using Microsoft Excel 2007. The average yields of product (HEMA-LMA) and by-product in transesterification of FAME-L with HEMA were obtained in 91.1 ± 4.4 and 8.4 ± 0.4%, respectively, (Fig. 5a). In case of the synthesis of HEMA-OMA, the yields were statistically the same at ratios of methyl oleate to HEMA between at least 1:2 to 1:5.

Effect of Reaction Temperature

To determine the effect of temperature on the transesterification of FAME with HEMA, reactions were performed at 40, 50, 60, 70 and 80 °C, respectively. The transesterification reactions below 40 °C were not carried out, because methanol was not removed in the reaction system below 40 °C. In transesterification of both HEMA-LMA and HEMA-OMA, the best yields were obtained at 60 °C in 97 ± 5.4 and 89 ± 4.1%, respectively (Fig. 6). At 70 °C, catalytic activity decreased presumably due to thermal deactivation of the biocatalysts and polymerization of HEMA.

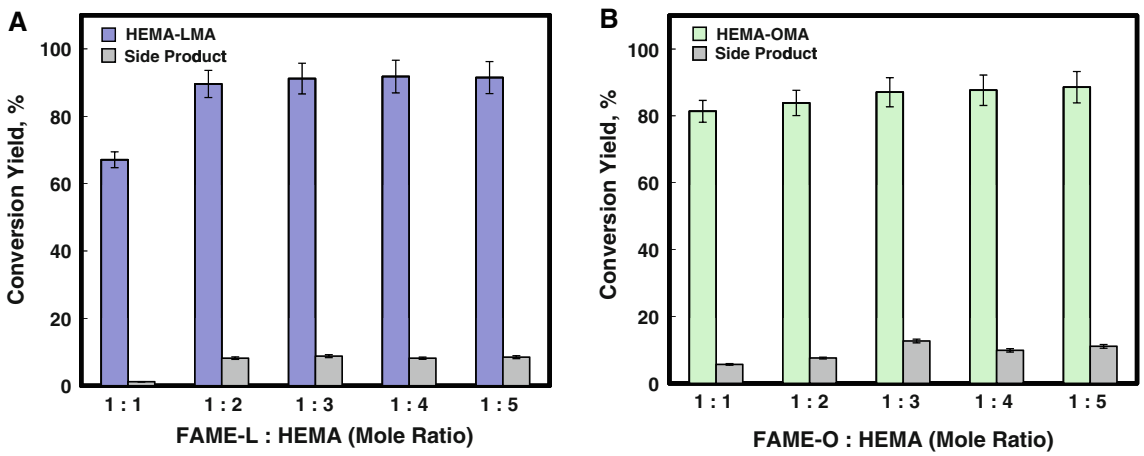

Fig. 5 Effect of molar ratios of substrate in the transesterification of FAME-L (**a**) and FAME-O (**b**) with HEMA

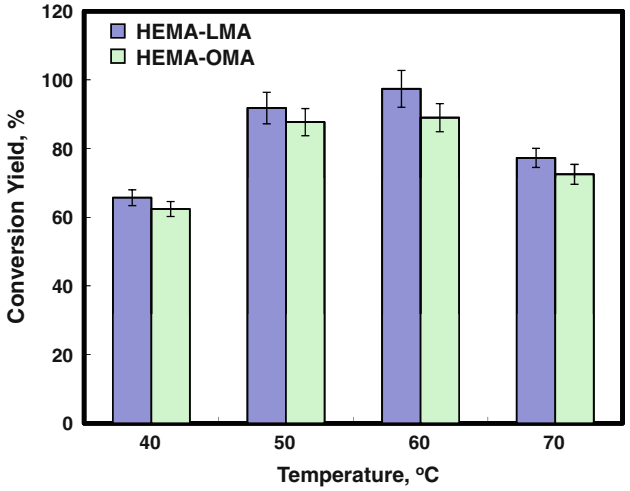

Fig. 6 Effect of reaction temperature in the transesterification of FAME with HEMA

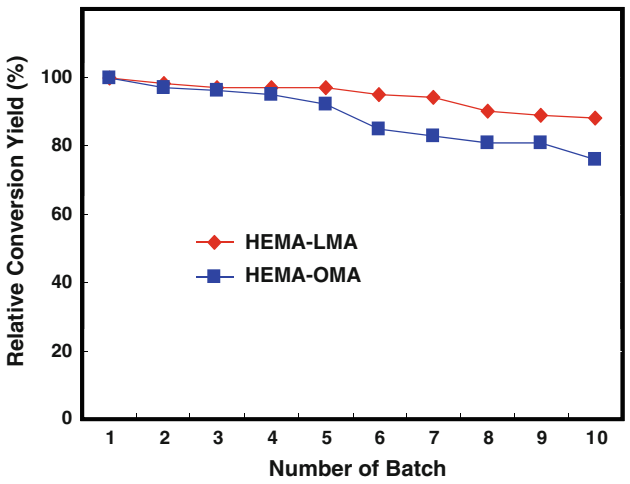

Fig. 8 Enzyme stability in the transesterification reaction of FAME with HEMA

Effect of Enzyme Concentration

The effect of enzyme concentration on the catalytic activity of the immobilized lipase in the transesterification of FAME with HEMA was studied. Enzyme concentration on the basis of the mass of substrates varied in range from 1 to 7 wt%, with 1 wt% increments at the same condition selected in the previous experiment. As shown in Fig. 7, the highest yield of HEMA-LMA and HEMA-OMA was in 97.4 ± 5.4% (enzyme 5 wt%) and 91.0 ± 4.7% (enzyme 3 wt%), respectively. Above 5 wt% enzyme concentrations, the transesterification yields decreased because the product (HEMA-LMA and HEMA-OMA) could be easily transformed to the by-products (Fig. 1c).

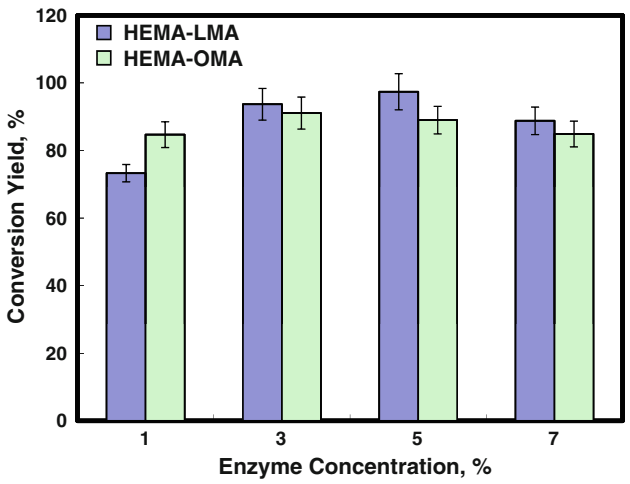

Fig. 7 Effect of enzyme concentration in the transesterification of FAME with HEMA

Enzyme Stability

Immobilized enzymes can be recycled, but the activity eventually decreases due to several factors, such as desorption, substrate deactivation, and product inhibition. Therefore, we investigated the stability of the immobilized lipase after each use. Figure 8 shows the results of the relative conversion yields of HEMA-LMA and HEMA-OMA, expressed as a percentage of the yield of the first batch reaction. After ten times transesterification, enzyme activity was retained at 88 ± 2.6% (HEMA-LMA) and 76 ± 2.3% (HEMA-OMA), indicating that the immobilized lipase can be recycled at least 10 times.

Synthesis of Copolymers

It is well known that cold flow improvers should have a hydrophobic character to be soluble in the fuel medium and a polar character to adhere to the wax crystals and saturated fatty acid methyl esters. In this study, we synthesized alkyl methacrylate/styrene copolymers by radical polymerization of HEMA-LMA (or HEMA-OMA) and styrene (Fig. 1b). The structure of the prepared copolymers was confirmed by ^{1}H-NMR spectral and FT-IR spectroscopic analysis. Figure 9 shows the ^{1}H-NMR spectrum of HEMA-LMA/Styrene copolymer. As seen in Fig. 9, comparing the ^{1}H-NMR spectrum with styrene and HEMA-LMA with the copolymer, the signals at 5.76 and 5.42 ppm disappeared, which are attributable to CH_2=CH–Ar of styrene and the peaks at 6.69 and 5.54 ppm are attributable to CH_2=CH- of HEMA-LMA increased the signals at 1.26 ppm attributable to aliphatic H of the copolymer.

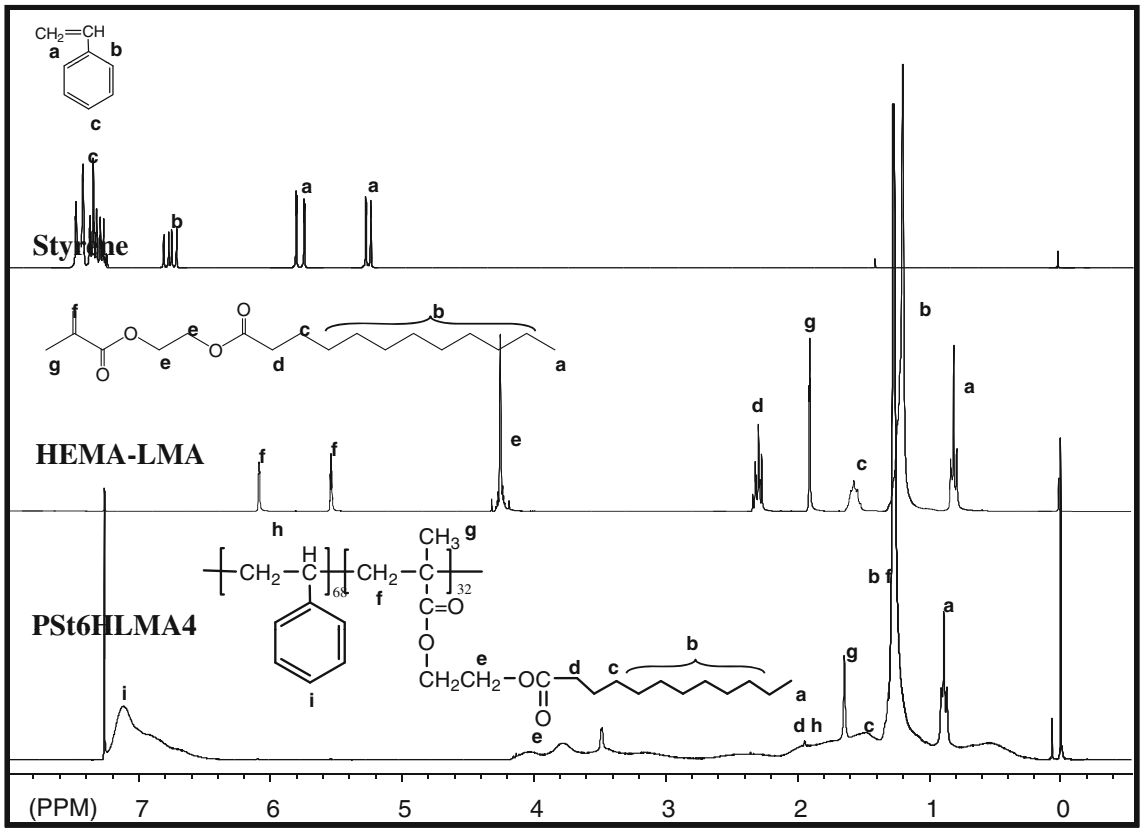

Fig. 9 [1]H-NMR spectra of **(a)** styrene, **(b)** HEMA-LMA and **(c)** PSt6HLMA4

The prepared copolymers were characterized by determining the weight average molecular weight (Mw), number average molecular weight (Mn), and polydispersity molecular distribution (PD = Mw/Mn), using GPC analysis, and the results are listed in Table 1. The results indicate that the prepared copolymers have an average molecular weight from 2.6×10^4 to 5.5×10^4.

Cold Flow Properties

The cold flow property was significantly influenced of *n*-paraffin and saturated bio-diesel contained in ULSD, therefore cold flow low properties might deteriorate due to an increase in the formation of the *n*-paraffin and a saturated bio-diesel crystal nucleus. The mechanism of the

Table 1 Synthetic results of styrene-alkyl methacrylate copolymers

Polymers	Reactant (mol %)		Composition[a] (mol %)		GPC, molar mass		
	St	AM	St	AM	Mn	Mw	PD
PSt6HLMA4	6	4	67	33	20,200	45,600	2.25
PSt5HLMA5	5	5	55	45	19,500	55,200	2.83
PSt4HLMA6	4	6	45	55	20,000	41,500	2.07
PSt3HLMA7	3	7	35	65	16,300	33,400	2.04
PSt2HLMA8	2	8	26	74	13,200	26,500	2.00
PSt6HOMA4	6	4	68	32	16,700	54,000	3.22
PSt5HOMA5	5	5	57	43	17,000	50,500	2.95
PSt4HOMA6	4	6	46	54	14,700	43,600	2.95
PSt3HOMA7	3	7	36	64	15,700	48,600	3.09
PSt2HOMA8	2	8	25	75	17,500	48,400	2.75

[a] Calculated from [1]H-NMR results

Table 2 Pour point properties of copolymers in ULSD

Polymers	Pour point depression (°C)		
	200 ppm	500 ppm	1,000 ppm
PSt6HLMA4	2.5	2.5	5.0
PSt5HLMA5	2.5	5.0	7.5
PSt4HLMA6	5.0	7.5	10.0
PSt3HLMA7	2.5	7.5	12.5
PSt2HLMA8	5.0	7.5	15.0
PSt6HOMA4	0	0	2.5
PSt5HOMA5	0	2.5	2.5
PSt4HOMA6	0	2.5	2.5
PSt3HOMA7	0	2.5	5.0
PSt2HOMA8	0	2.5	5.0

formation of the crystal nucleus is unknown, but a variety of additives applied as an inhibitor to retard the formation of the crystal nucleus. In this work, synthesized polymers were added to ULSD at a level of 200, 500 and 1,000 ppm, respectively. Pour points of ULSD containing synthesized copolymers were evaluated. The results are listed in Table 2. It is seen that pour point depression performance (ΔPP) of the PStmHLMAn copolymers (m = moles of styrene and n = moles of HLMA unit) possess better pour point depression capabilities than that of the copolymer with HOMA unit (ΔPP 5.0–15.0 vs. 2.5–5.0 at 1,000 ppm). Also, ΔPP of the copolymers with more HLMA showed lager ΔPP than that of the copolymers with more styrene unit. An increasing number of polar HLMA or HOMA moieties showed the better cold flow properties. Compared to HOMA, more polar HLMA was much more effective for the cold flow properties at the same content in the copolymer. According to the general knowledge of the pour point depression, the copolymer can co-crystallize with the wax in the ULSD to possess good cold flow performance if the structure of the copolymer is suitable for the composition of the wax in the ULSD. It is thus concluded that the HLMA unit is the optimal matching alkyl chain for preparing the cold flow improvers suitable for the tested basic fuels.

It is thus concluded that HLMA unit is the optimal matching alkyl chain for preparing the cold flow improvers suitable to the tested basic fuels.

Conclusions

In summary, the enzymatic synthesis of alkyl methacrylates was carried out using the *Candida antarctica* lipase B immobilized within the porous polymethacrylate resin. The reactivity depended on the polarity of the solvent, the molar ratio of FAME:HEMA, the reaction temperature, and the enzyme concentration. The highest yields, 97 ± 5.4% for methyl laurate/HEMA and 91 ± 4.7% for methyl oleate/HEMA, were obtained at 60 °C in toluene as the solvent. The copolymers, polystyrene-*co*-alkyl methacrylates, were synthesized by radical polymerization of HEMA-LMA (or HEMA-OMA) and styrene and have average molecular weight from 2.6×10^4 to 5.5×10^4. The poly(styrene-*co*-alkyl methacrylate)s (PStmHAMAn) led to a reduction in the pour point in ultra low sulfur diesel (ULSD) and the best one, PSt2HLMA8, showed a 15 ± 1.25 °C reduction in their pour points at 1,000 ppm concentration.

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