CHAPTER IV
RESULTS AND DISCUSSION

4.1 Microemulsion Studies for Esterification Reaction

4.1.1 Effect of Salt

The NaDEHP/isooctane/water microemulsion systems were prepared by mixing 0.1 M of HDEHP in isooctane and 0.1 M of NaOH in aqueous solution with varying concentrations of NaCl from 0 to 4.0 M. NaDEHP was formed during the mixing of organic and brine phases. The reaction can be represented as:

$$HDEHP + NaOH \longrightarrow NaDEHP + H_2O.$$

NaDEHP/isooctane/water microemulsion does not formed without adding NaCl to the aqueous phase and the microemulsion system undergoes a series of phase transitions with increasing NaCl concentration in aqueous phase as shown in Figure 4.1.

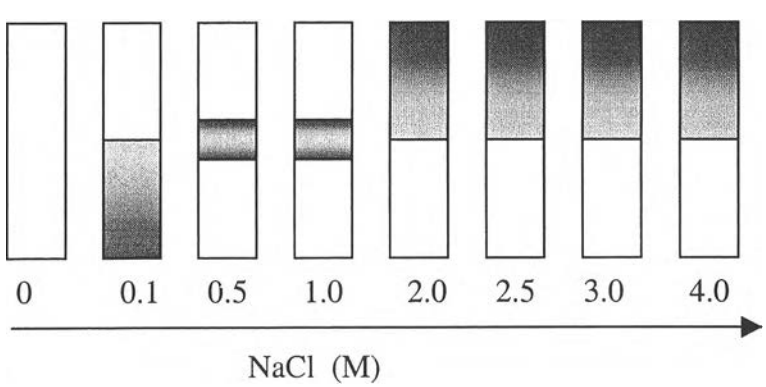


Figure 4.1 Phase behavior of NaDEHP/isooctane/NaCl aqueous solution as a function of NaCl concentration.

With increasing NaCl concentration, the microemulsion underwent a series of phase changes. At 0.1 M NaCl Winsor I (oil in water) microemulsion was formed. When NaCl concentration was increased to 0.5 and 1.0 M, a clear third phase was formed between excess organic and excess aqueous phases, indicating the formation of a Winsor III microemulsion (bicontinuous structure). When NaCl concentration was further increased to 2.0 M and higher, then the system was transformed to two transparent phases again and became a Winsor II (water in oil) microemulsion.

4.1.1.1 Micellar size in W/O microemulsion

Micellar size in W/O microemulsion (the upper phase) system at 100 mM NaDEHP/isooctane with varying NaCl concentration, 2.0-4.0 M was studied by dynamic light scattering (DLS). The results of hydrodynamic radius (Rh) of microemulsion droplets are shown in Figure 4.2. As NaCl concentration increases, the hydrodynamic radius of micellar structure decreases.

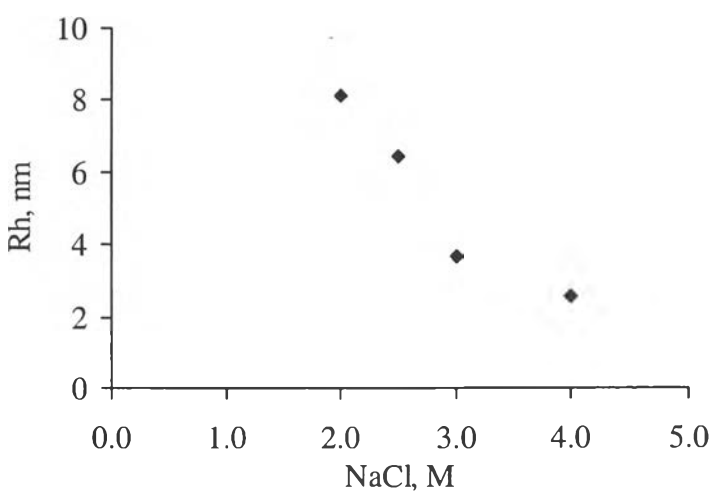


Figure 4.2 Effect of NaCl concentration on the hydrodynamic radius of 100 mM NaDEHP/isooctane/NaCl microemulsion.

4.1.1.2 Water content in W/O microemulsion

Water content (wt%) in the W/O microemulsion system of NaDEHP/isooctane with varying NaCl aqueous solution was measured by coulometer and the results are shown in Figure 4.3. After the formation of Winsor II above 2.0 M, the water content decreases as NaCl concentration increases.

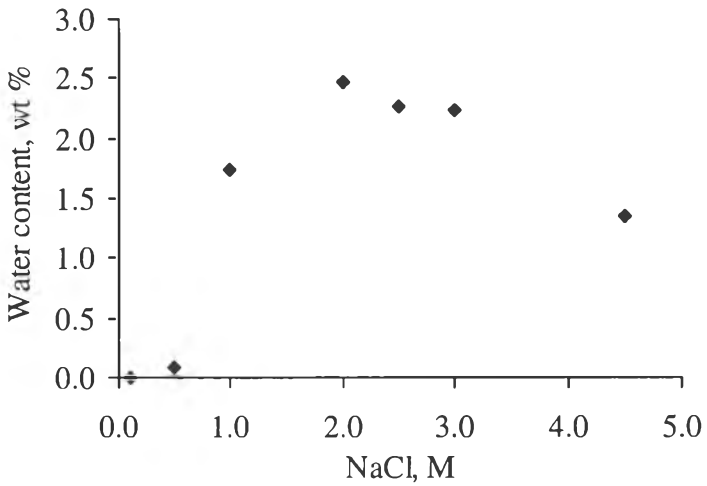


Figure 4.3 Effect of NaCl concentration on water content of in isooctane phase.

Decreases in the hydrodynamic radius of micellar size and water content in the upper phase of W/O microemulsion might be due to screening effect of NaCl, which decreases ionic repulsion between the surfactant head groups.

4.1.2 Effect of Alcohol as a Cosurfactant

When the systems of 100 mM NaDEHP/isooctane/0.1 M NaCl with varying the concentration of alcohols (1-propanol, 2-propanol, and hexanol) from 0 to 200 mM were studied, the microemulsion phases underwent a series of phase change (as shown in Fig. 4.4) in a similar maner as increasing NaCl concentration. For the long chain alcohol like hexanol, Winsor II microemulsion was formed at 20 mM hexanol. Hexanol acts as a good cosurfactant to ease the formation of Winsor II. For short chain alcohols, 1-propanol and 2-propanol were less effective cosurfactant in which Winsor II was formed at higher concentration of alcohols, 160 mM and 200 mM for 1-propanol and 2-propanol, respectively.

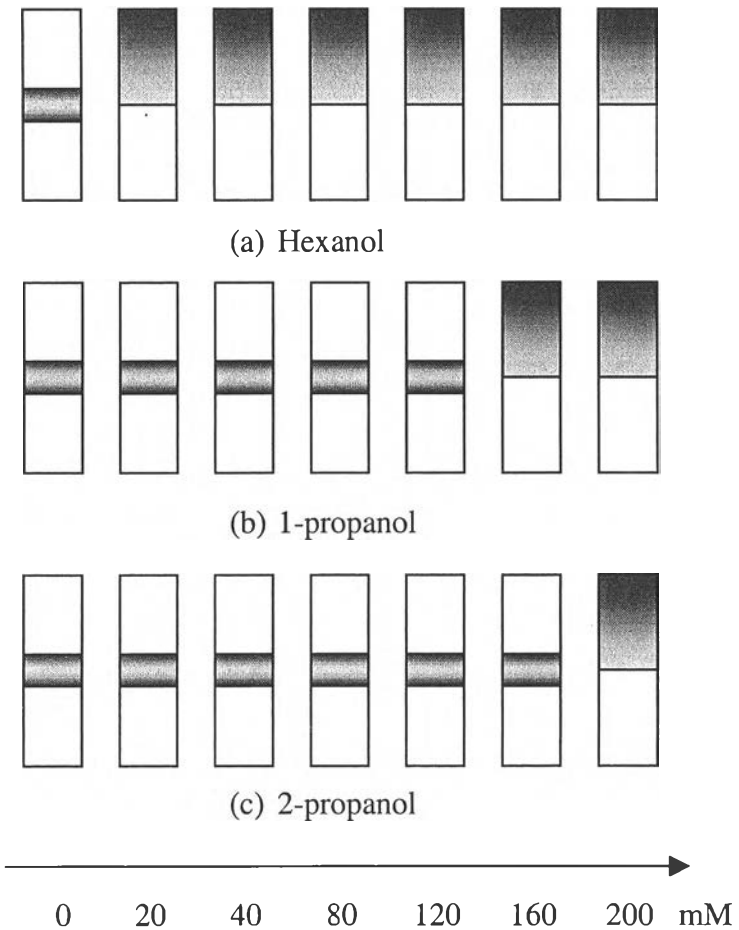


Figure 4.4 Effect of alcohols as a cosurfactant of 100 mM NaDEHP/isooctane/0.1 M NaCl; (a) hexanol (b) 1-propanol, (c) 2-propanol.

4.1.3 Determination of Water Content

4.1.3.1 Effect of NaDEHP on water content

The microemulsion systems of 50, 70, 100 mM NaDEHP/isooctane/40 mM hexanol prepared by mixing with varying concentrations of NaCl from 0 to 4.5 M, were studied for water content and the results are shown in Figure 4.5. with increasing the NaDEHP concentration, the water content was increased. However, in Winsor II, increasing NaCl concentration resulted in decreasing water content and hydrodynamic radius of microemulsion.

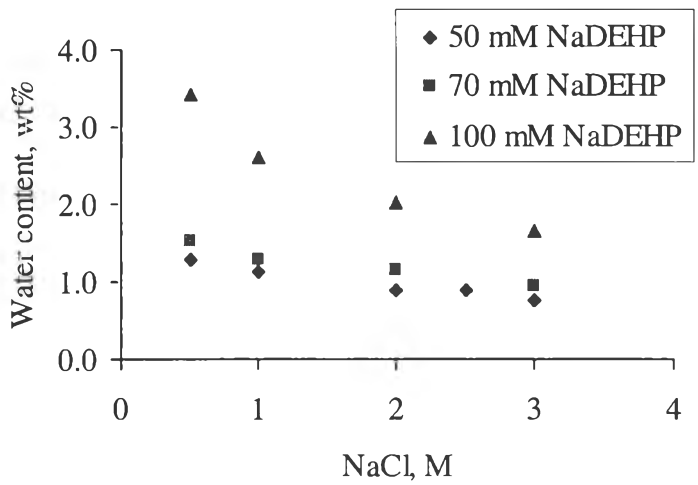


Figure 4.5 Effect of NaDEHP on water content of 50-100 mM NaDEHP/isooctane/1.0-4.5 M NaCl (aq) microemulsion system.

4.1.3.2 Effect of alcohol on water content

For long chain alcohol, hexanol, the 100 mM NaDEHP/isooctane/ 1.0 M NaCl (aq) microemulsion with varying concentration of hexanol from 40 to 150 mM resulted in decreasing water content from 3.00 to 2.36 wt % (see Appendix A, Table A1.3). The decrease in water content a result of the closer packing of the hydrophilic head group of NaDEHP in the presence of hexanol molecules. On the other hand, an increase in concentration of the short chain alcohols from 150 to 200 mM caused water content to increase. 1-Propanol showed insignificant increase of water content from 1.11 to 1.17 wt% (Table A2.1) but 2-propanol showed significant increase from 1.13 to 2.89 wt % (Table A3.1). It could be due to solubilization of 2-propanol, causing loose packing of branching alcohol in the polar core of the surfactant in relation to 1-propanol.

4.1.3.3 Effect of fatty acid on water content

The effect of fatty acid on water content was investigated by adding 50 mM of caprylic acid into the different microemulsion conditions with different types of alcohol. The results showed that the addition of caprylic acid caused a dramatically decrease in water content in all microemulsion systems and all types of alcohol as shown in Table 4.1.

Table 4.1 Effect of caprylic acid to water content of microemulsion with different types of alcohol.

Type of alcohol	Water, % (w/w) no caprylic acid	Water, % (w/w) with 50 mM caprylic acid
hexanol *(a)*	2.84 ± 0.20	1.30 ± 8.63
1-propanol*(b)*	3.02 ± 8.89	2.03 ± 6.41
2-propanol*(c)*	1.95 ± 2.32	1.33 ± 4.22

(a) 70 mM NaDEHP/40 mM hexanol in isooctane/0.5 M NaCl (aq)
(b) 120 mM NaDEHP/200 mM 1-propanol in isooctane/1.5 M NaCl(aq)
(c) *100 mM NaDEHP/200 mM 2-propanol in isooctane/2.5 M NaCl(aq)*

Amount of water which controls a rate of catalytic esterification of encapsulated enzymes in the reverse micelle microemulsion can be affected by the change in any system parameters, i.e. NaDEHP concentration, NaCl concentration, types and concentrations of alcohol cosurfactant, and fatty acid substrates.

4.1.4 Determining Wo of Microemulsion Media for Esterification

The appropriate water content was initially selected based on the previous work on AOT system (Stamatis *et al.,* 1993; Hayes and Gulari, 1990). They found that the appropriate water content for esterification in AOT reverse micelle, Wo was about 9. Thus, for NaDEHP reverse micelle in this study, the water content was initially set closed to 9 by varying the concentrations of NaCl, NaDEHP, and alcohols in microemulsion system (see Appendices A to B) and initial concentration of substrate was fixed at 50 mM of fatty acid (caprylic acid). Since the water content increased with the increase in surfactant concentration but decrease with the decrease in salt, hexanol and fatty acid, for hexanol, 150 mM surfactant concentration was used to compensate for the water content decrease in the presence of hexanol and fatty acid concentrations, for 1-propanol and 2-propanol, the surfactant and salt concentration were reduced to obtained Wo~9 (Table 4.2).

Table 4.2 Conditions for esterification reaction with 50 mM fatty acid.

Alcohols	Conditions	Wo with out caprylic acid	Wo with 50 mM caprylic acid
hexanol	150 mM NaDEHP/ 150 mM hexanol/ isooctane/1.0 M NaCl	10.7 ± 0.21	9.0 ± 0.07
1-propanol	120 mM NaDEHP/ 200 mM 1-propanol/ isooctane/0.5 M NaCl	15.45 ± 0.05	8.7 ± 0.10
2-propanol	100 mM NaDEHP/ 200 mM 2-propanol/ isooctane/0.5 M NaCl	-	6.7 ± 0.22

The results showed that water content was dependent of the system parameters and significantly decreased in the presence of substrates both fatty acid and alcohol. So, the change in water content range 4<Wo<9 could be obtained by varying fatty acid concentration in a range of 50-100 mM.

4.2 Esterification Reaction

4.2.1 Effect of Lipase concentration on the Reaction Rate

The microemulsion system of 150 mM NaDEHP/isooctane/150 mM hexanol/50 mM caprylic acid/1.0 M NaCl was used to study the catalytic rate of lipase. The lipase concentrations of 0.021, 0.042, and 0.084 mg/ml in the aqueous phase were used. To make sure that lipase concentrations did not change when it was encapsulated in the reverse micelles, the aqueous phase of before and after lipase encapsulation were measured by UV-VIS absorption at 280 nm. The result showed no significant change in lipase concentration after encapsulation in the reverse micelles (Appendix I).

The rate of the catalytic esterification were observed at various concentrations by measuring the depletion of caprylic acid as a function of time as shown in Figure 4.6. The rate of reaction increased with increasing concentration of lipase but was not significantly different. Therefore, 0.042 mg/ml lipase was selected for furthur study of catalytic esterification.

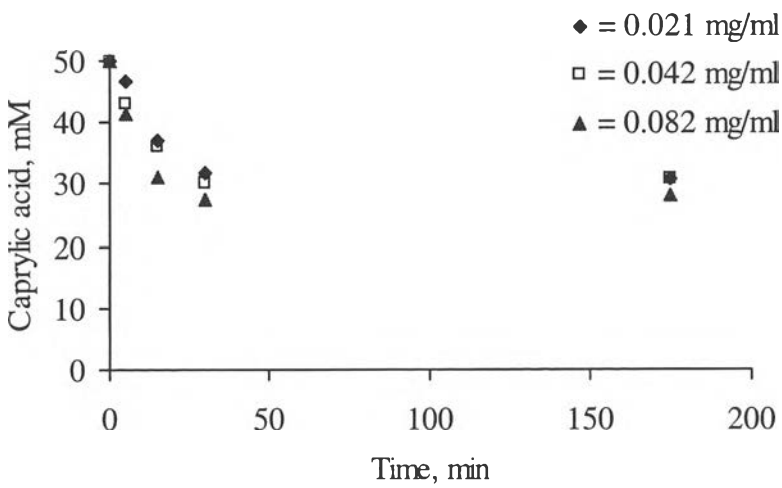


Figure 4.6 Effect of lipase concentration on the rate of depletion of caprylic acid in 150 mM NaDEHP/150 mM hexanol/ 50 mM caprylic acid/ isooctane/1.0 M NaCl (aq); Lipase concentrations: ◆ = *0.021 mg/ml of lipase,* □ = *0.042 mg/ml of lipase, and* ▲ = *0.084 mg/ml of lipase.*

4.2.2 Effect of Type of Fatty Acid Substrates on Conversion and Reaction Rate

The microemulsion system as suggested in the previous section (4.2.1) was used to study the effect of chain length of fatty acid substrates, i.e. caprylic acid ($C_8H_{16}O_2$), palmatic acid ($C_{16}H_{32}O_2$), and oleic acid ($C_{16}H_{34}O_2$). The concentration of each fatty acid was varied in the range of 50 to 150 mM and the rate of esterifications was determined.

4.2.2.1 Esterification of caprylic acid with hexanol

The caprylic acid concentrations were increased from 50 mM to 150 mM, the water content of the system, Wo value was decrease from 6.7 to 4.3. Figures 4.7 and 4.8 show the rates of depletion and conversion of caprylic acid with time, respectively.

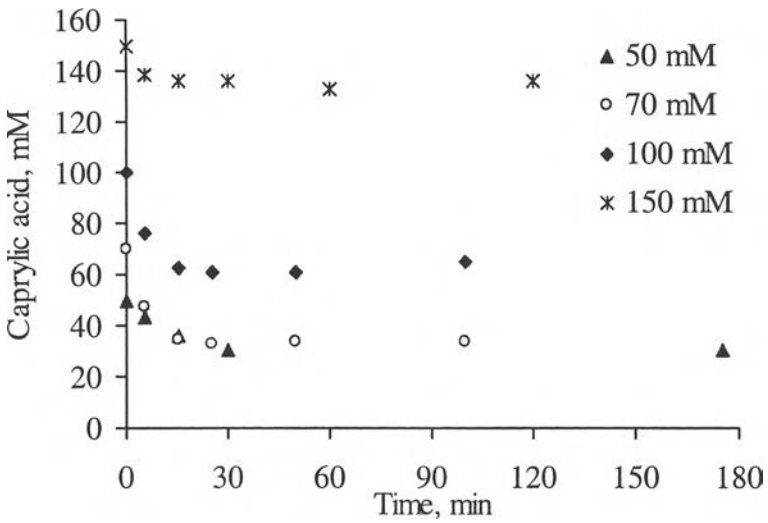


Figure 4.7 Depletion of caprylic acid in 150 mM NaDEHP/150 mM hexanol/ isooctane/1.0 M NaCl. Caprylic acid concentration was 50-150 mM and Wo was 4.2 to 7.5.

The initial velocity was then calculated by a fitting curve with excel program. The highest initial rate of reaction was obtained from the initial slope at 70 mM of caprylic acid at Wo about 6 and the highest conversion was also observed (Table 4.3). Water content played significant role in catalyzing the reaction. Increasing fatty acid concentration decreases water content in microemulsion. The high reaction rate and velocity was obtained when Wo before reaction was about 6 and maximum change in water content was about 10 %, corresponding to the esterification of 70-100 mM of caprylic acid. The results show the significance of water content to the rate of reaction. High water content when 50 mM caprylic acid was used, the initial rate and velocity took longer time to obtain high conversion, while too low water content (with 150 mM caprylic acid) resulted in slow initial rate and velocity, and low conversion. The results also showed that the caprylic acid did not only affect water content but also the conversion.

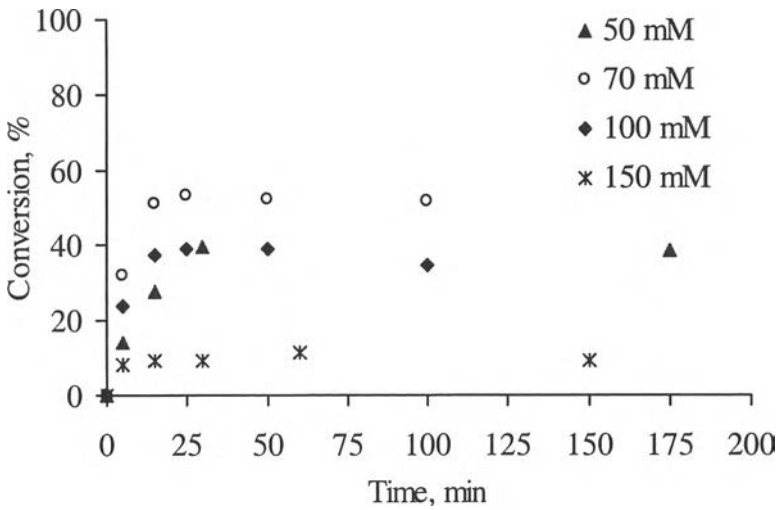


Figure 4.8 Conversion of caprylic acid in 150 mM NaDEHP/150 mM hexanol/ isooctane/1.0 M NaCl. Caprylic acid concentration was 50-150 mM and Wo was 4.2 to 7.5.

Table 4.3 Conversion and initial rate of esterification of caprylic acid with hexanol for 150 mM NaDEHP/isooctane/150 mM hexanol/1.0 M NaCl.

Caprylic acid, mM	Wo		Initial rate, mM/min	Conversion	Initial velocity, mM/min*mg
	Before reaction	After reaction			
50	6.70 ± 0.01	6.91 ± 0.01	1.9	38.7	9.05
70	6.27 ± 0.10	6.91 ± 0.08	5.3	51.8	25.24
100	5.81 ± 0.05	6.38 ± 0.04	5.0	35.0	23.81
150	4.23 ± 0.14	4.24 ± 0.21	2.8	9.2	13.33

4.2.2.2 Esterification of oleic acid with hexanol

The concentrations of oleic acid were varied from 50 to 150 mM to get Wo in the range of 4.7 to 7.2 (Appendix J2). Figures 4.9 and 4.10 show that in all cases the depletion rate of oleic acid and the conversion with time were higher than those of caprylic acid. The highest conversion was obtained at 50 mM

oleic acid and Wo~7.2. Table 4.4 also shows high initail rate and velocity in all cases. The highest initial rate of esterification was obtained. *R. delemar* lipase shows almost two times higher in initial rate at lower water content than those of caprylic acid system.

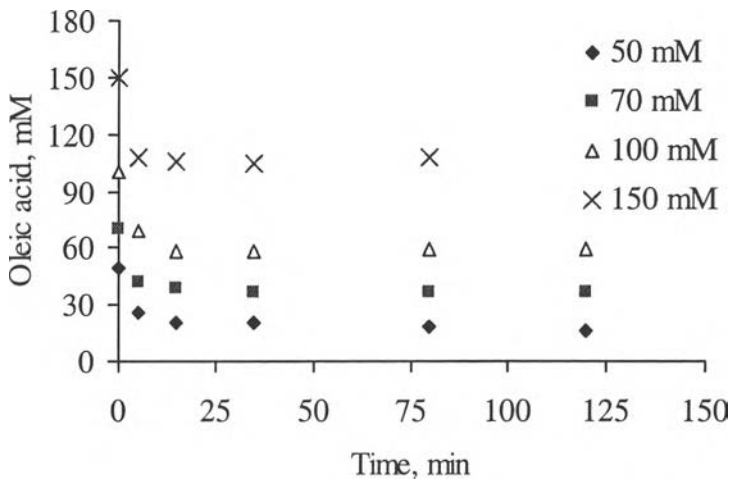


Figure 4.9 Depletion of olelic acid in 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl. Oleic acid concentration was 50-150 mM and Wo was 4.7 to 7.2.

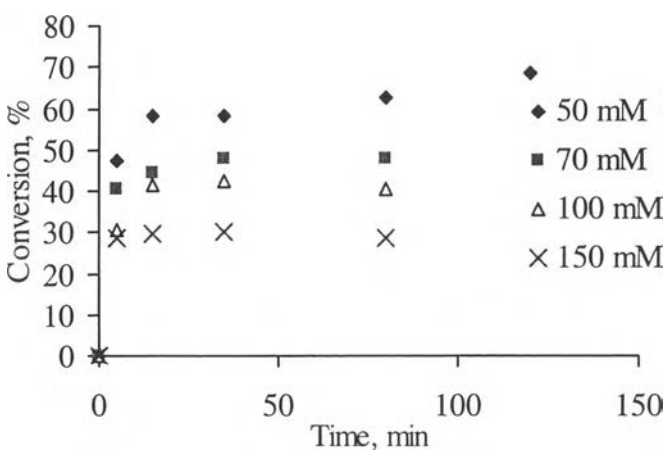


Figure 4.10 Conversion of olelic acid in 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl. Oleic acid concentration was 50-150 mM and Wo was 4.7 to 7.2.

Table 4.4 Conversion and initial rate of esterification of oleic acid with hexanol for 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl.

Oleic acid, mM	Wo		Initial rate, mM/min	Conversion	Initial velocity, mM/min*mg
	Before reaction	After reaction			
50	7.23 ± 0.08	8.05 ± 0.05	5.7	68.6	27.14
70	6.35 ± 0.05	6.90 ± 0.07	6.0	47.9	28.57
100	5.51 ± 0.04	5.92 ± 0.10	7.0	40.7	33.33
150	4.71 ± 0.23	4.82 ± 0.14	10.0	31.1	47.62

4.2.2.3 Esterification of palmatic acid with hexanol

In this section, the concentration of palmatic acid was varied from 50 to 100 mM and Wo~5.6 to 6.8 (Appendix J3). In all cases, the initial rate and velocity and the conversion were higher than those of caprylic acid (Figures 4.11-4.12 and Table 4.5). The highest conversion was obtained in all cases. At Wo~5.6 and 100 mM palmatic acid, the highest initial rate of esterification was obtained. Increasing fatty acid chain length increased the initial rate and velocity and the conversion of esterification, it might be due to the ability of long chain hydrophobic part of produced ester to penetrate well in to the organic bulk phase, and it was not locally concentrated at to the interface, which inhibited the substrates availability for esterification reaction, and enhance the conversion.

The overall initial rate and velocity of palmatic acid were better than oleic acid at high concentration (≥ 70 mM) and the appropriate Wo of system was closed to 6. It might be due to the difference in substrate structure because the presence of the internal double bond of oleic acid might enhance polarity to the hydrophobic portion and change water content in the system.

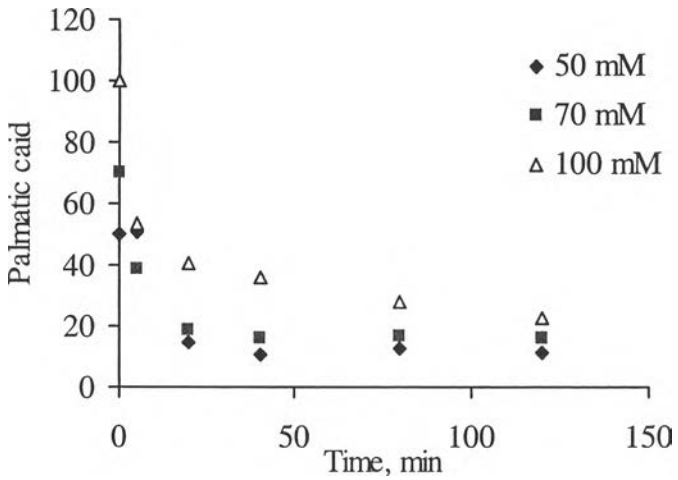


Figure 4.11 Depletion of palmatic acid in 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl. Palmatic acid concentration was varied from 50-100 mM and Wo was 5.6 to 6.8.

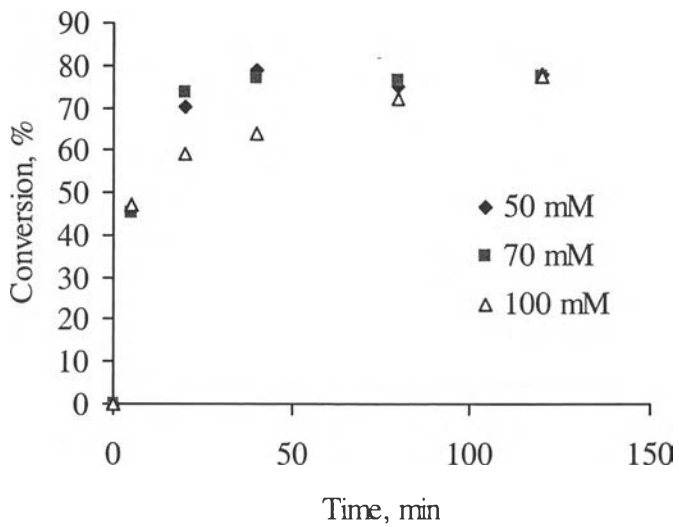


Figure 4.12 Conversion of palmatic acid in 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl. Palmatic acid concentration was 50-100 mM and Wo was 5.6 to 6.8.

Table 4.5 Conversion and initial rate of esterification of plamatic acid with hexanol for 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl.

Plamatic acid, mM	Wo		Initial rate, mM/min	Conversion	Initial velocity, mM/min*mg
	Before reaction	After reaction			
50	6.80 ± 0.05	7.52 ± 0.09	2.4	77.8	11.43
70	6.16 ± 0.05	6.78 ± 0.08	6.7	77.6	31.90
100	5.57 ± 0.08	5.99 ± 0.05	10.0	77.5	47.62

It was found that the rate of esterification of long chain fatty acids with alcohols catalyzed by *R. delemar* lipase in NaDEHP microemulsion was better than the short chain ones. The initial rate and velocity, and conversion were dependent of the types, structures, and concentrations of fatty acid. Stamatis *et al.* (1993) studied on *R. delemar* lipase in AOT microemulsion media. They reported a preference for esterification of medium chain length fatty acids with the esterification hexanol (oleic acid>caprylic acid>palmatic acid where the condition used was 120 mM hexanol and 50 mM fatty acid). However, in the study of NaDEHP microemulsion, the results agreed well with previous work only at low concentration of fatty acid (50 mM). For higher concentration of fatty acid, it can be seen that *R. delemar lipase* shows high initial rate of esterification with long chain fatty acid and exceptionally high rate in case of palmatic acid at the same Wo value (Table 4.6).

Table 4.6 Activity of *R. delemar* lipase from various fatty acids with hexanol for 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl.

Fatty acid, mM	Initial velocity, mM/min*mg caprylic acid	Initial velocity, mM/min*mg oleic acid	Initial velocity, mM/min*mg palmatic acid
50	9.05	27.14	11.43
70	25.24	28.57	31.90
100	23.81	33.33	47.62
150	13.33	47.62	-

It might be due to the catalytic rate of enzyme followed the first order of Michaelis-Menton rate expression where the rates of reaction depended on the fatty acid concentration. However, the ester products might be an important factor that affect the rate and conversion of esterification. In case of medium chain length of ester produced from caprylic acid substrate could penetrate closely to interface of oil and water and prohibited the conversion. On the other hand, the long chain ester produced from palmatic and oleic acids substrates could diffuse away from the interface of oil and water, stayed in bulk medium thus enhanced the conversion.

4.2.3 Effect of Types of Cosufactant

In order to elucidate the role of alcohol structure on lipase activity and selectivity, the esterfication of short chain alcohols both primary and secondary propanols was investigated and compared with the results of long chain, one hexanol, in previous section.

4.2.3.1 Esterification of caprylic acid with propanol

For 1-propanol system with Wo varied 3.0-6.0, the microemulsion was 120 mM NaDEHP/isooctane/200 mM hexanol/50-150 mM caprylic acid/0.5 M NaCl, and for 2-propanol system with varied Wo 3.3-6.21, the microemulsion was 100 mM NaDEHP/isooctane/200 mM 2-propanol/50-150 mM caprylic acid/1.0 M NaCl. Figure 4.13 shows very slow depletion rate of caprylic acid. The similar trend was also observed for both 1-propanol and 2-propanol in all conditions.

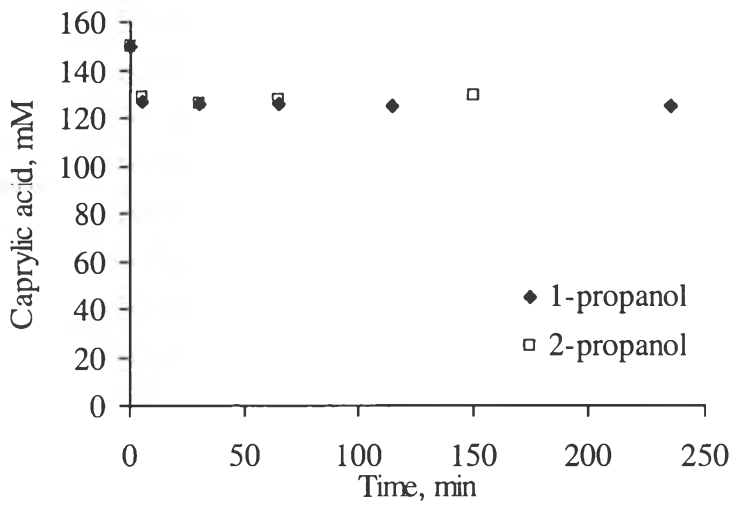


Figure 4.13 Depletion of palmatic acid with 1-propanol and 2-propanol.

4.2.3.2 Esterification of oleic acid with propanols

Only 200 mM 1-propanol in the 120 mM NaDEHP/70 mM oleic acid/isooctane/0.5 M NaCl system and 200 mM 2-propanol in the 100 mM NaDEHP/70 mM oleic acid/isooctane/0.5 M NaCl system were studied and the results showed that the rates of esterification were so slow that the change in the UV-VIS absorption when mornitoring the depletion of oleic acid with time could not be detected (Appendix D).

4.2.3.3 Esterification of palmatic acid with propanols

The same systems as shown in section 4.2.3.3, but 70 mM palmatic was used instead. The results of UV-VIS absorption showed no change in fatty acid concentration (Appendix E).

The esterification of three fatty acids with various alcohols showed that the highest esterification rate occurred with alcohol containing high carbon number (C=6). The result contrasted with the previous works of Stamatis (1993) and Hayes and Gulari (1990) which they reported the higest rate of alcohol with carbon number equal 3 and profoundly decrease of esterification activity with increasing chain length of alcohol. It could be explained in terms of different enzyme localization in different microstructure. The chain length and molecular structure of alcohol affected micellar structure and hence enzyme activity and selectivity. To further explain our results, we categorized alcohol into two groups, the small and

polar amphiphile (1-propanol and 2-propanol) and large and apolar amphiphile (hexanol). Since primary and secondary alcohols are polar in relation to hexanol and have no significant amphilicity, they do not partition strongly to the interface, but remain inside the water pools, while hexanol, more amphiphilic character partitions at the interface. The results showed that the activity of *R. delemar* lipase was enhanced as the amphiphilic structure of alcohol increased. The preference of *R. delemar* lipase localized close to the interface of oil and water was because at the interface both long chain alcohol and fatty acid were abundance for lipase to catalyze the esterification. Moreover, the selectivity of *R. delemar* lipase can be attributed to various factors such as the effect of shape and size of revere micelles and the availability of the substrates as well.

4.2.4 Effect of Wo on Conversion and Reaction Rate

Figure 4.14 presents the initial rate of esterification of hexanol with three types of fatty acid. The initial rates of long chain alcohols were decreased as Wo increased. For caprylic acid, the initial rate was maximum between Wo = 6-7 and decreased again for Wo>7. The maximun conversion of all fatty acids occurred at Wo~6 (Figure 4.15). Wo has a significant effect on the activity of lipase as reported by Stamatis *et al.* (1993) and Hayes and Gulari (1990) for the activity of different micellar-encapsulated enzymes.

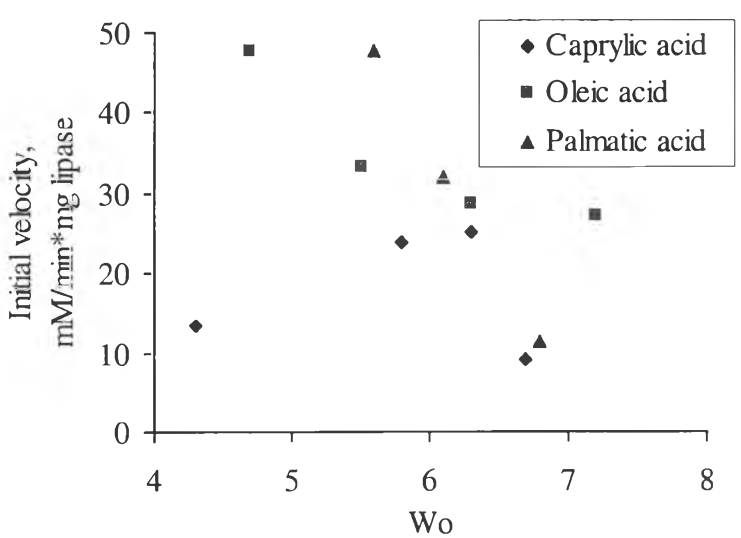


Figure 4.14 Effect of Wo on initial velocity of three types of fatty acid with hexanol in microemulsion media.

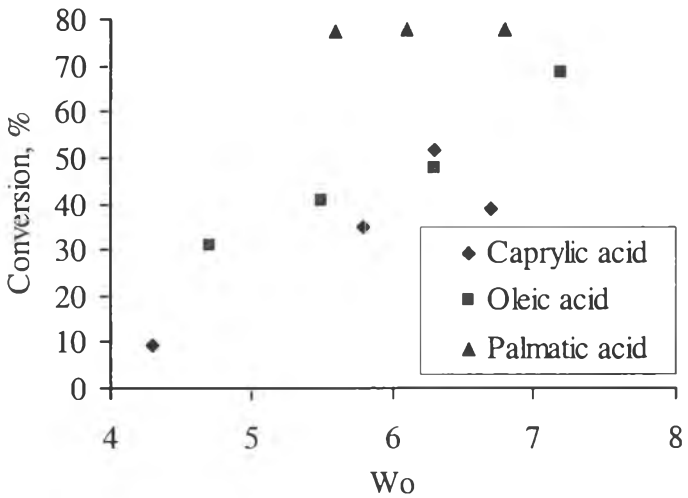


Figure 4.15 Effect of Wo on conversion of three types of fatty acid with hexanol in microemulsion media.

4.2.5 Comparison of Reverse Micelles with General Oil/Water System

The depletion rate of three different fatty acids with different general oil/water media are shown in Figures 4.16-4.18. The results showed no selective esterifications with fatty acid types because there was no significant difference in reaction rate of both alcohol and fatty acid types.

Esterification of fatty acids with hexanol in microemulsions media was compared with the reaction in general oil and water media and the depletion rates of three types of fatty acid are shown in Figures 4.19-4.21. The results showed that the esterification rate obtained from the microemulsion media was about 100 times faster than from that of the general oil/water media.

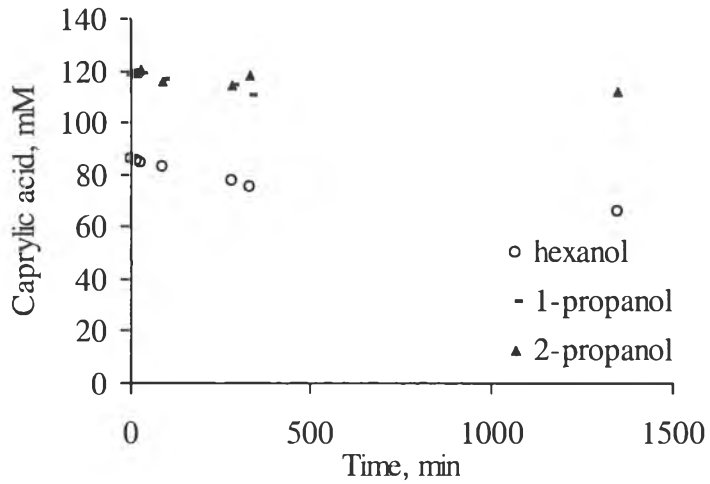


Figure 4.16 Depletion of caprylic acid in esterification with hexanol, 1-propanol, and 2-propanol in general water/oil media.

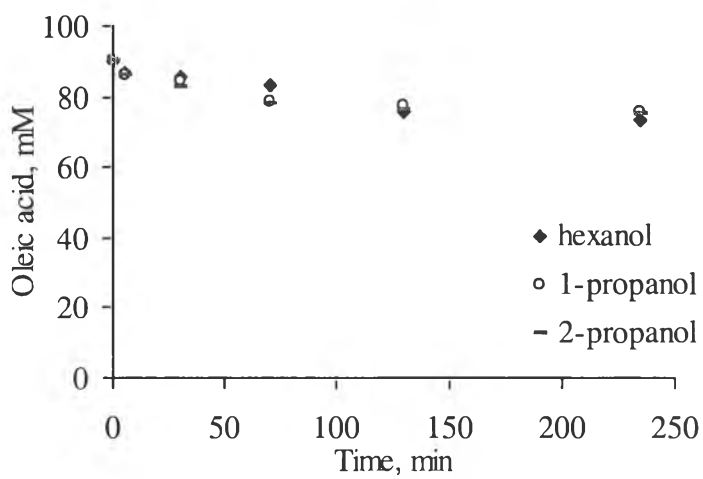


Figure 4.17 Depletion rate of olelic acid in esterification with hexanol, 1-propanol, and 2-propanol in general water/oil media.

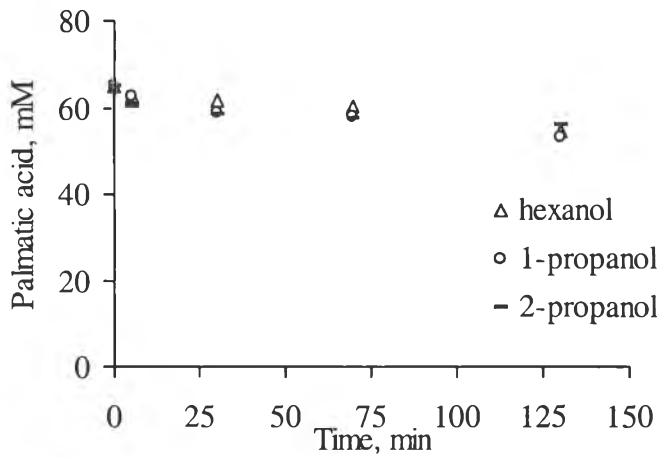


Figure 4.18 Depletion rate of palmatic acid in esterification with hexanol, 1-propanol, and 2-propanol in general water/oil media.

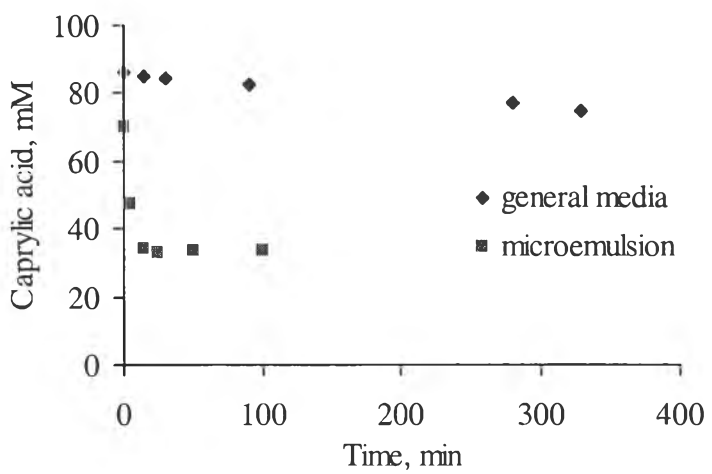


Figure 4.19 Comparison of the depletion of caprylic acid in microemulsion of 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl with general water/oil media.

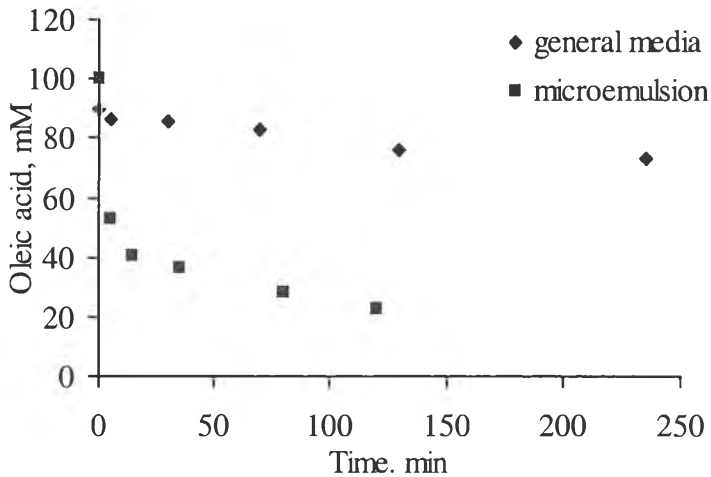


Figure 4.20 Comparison of the depletion of olelic acid in microemulsion of 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl with general water/oil media.

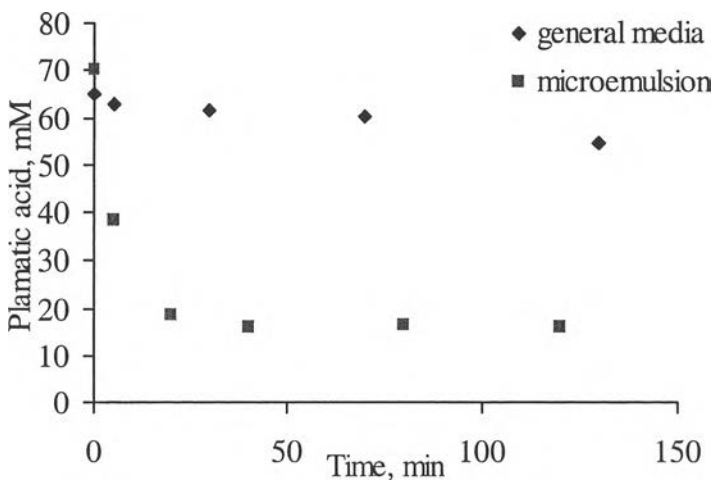


Figure 4.21 Comparison of the depletion of palmatic acid in microemulsion of 150 mM NaDEHP/150 mM hexanol/isooctane/1.0 M NaCl with general water/oil media.

During this study, it was found that the rate of esterification of fatty acids with alcohols catalyzed by *R. delemar* lipase in NaDEHP microemulsion was varied depending on the type and structure of substrates. In the previous study on *R. delemar* lipase in AOT microemulsion, Stamatis *et al.* (1993) showed a preference for esterification of medium chain length of fatty acids with short chain of primary alcohols. In this study with NaDEHP microemulsion, it can be seen that *R. delemar lipase* gave high initial rate of esterification with long chain length of fatty acid and exceptionally high rate with palmatic acid at the same Wo value. Moreover, the long chain alcohol can act as a good cosurfactant as well as substrates and also promote the esterification rate. *R. delemar* lipase seems to show selectivity on long chain length alcohol, hexanol, better than the short chain 1-propanol and 2-propanol. It could be the effect of a type of cosurfactant which changed the localization of lipase in microemulsion. Localization of lipase close to the oil and water interface was due to the long chain length of fatty acid and alcohol acting as good cosurfactants and could penetrate well to the oil and water interface and resulted in fast esterification reaction, comparing with the short chain ones. Stamatis *et al.* (1993) also reported that the reaction system used in esterification reaction plays an important role for *R. delemar* lipase as far as the chain length of primary alcohols concern. The results obtained in this study also agreed with their report.

R. delemar lipase showed a strong dependence on the water content in microemulsion and the optimun Wo was about 6 for case of caprylic acid. Stamatis (1993) reported Wo~9 for the AOT microemulsion system. This also confirmed the effect of different microemulsion systems on Wo. The change in microemulsion system also affected the change in size, water content, and the localization of *R. delemar* lipase.

4.3 Chemical Analyses

4.3.1 Fatty Acid Determination by UV-VIS

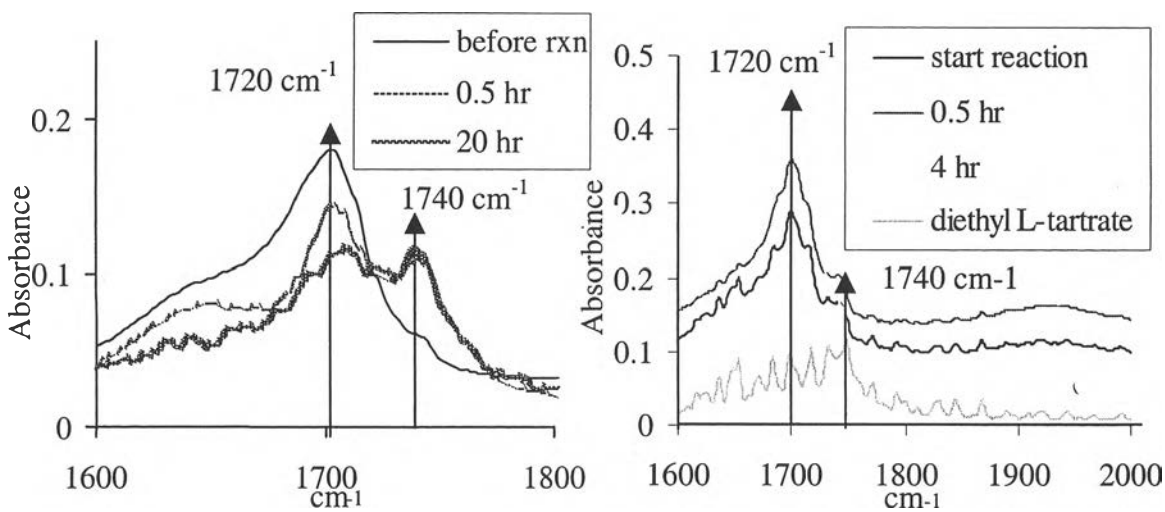
Investigation of all components in reverse micelle was verified by UV-VIS spectrophotometer. The results were found that absorbance of lipase, acetate buffer, and three types of alcohol did not interfere with the absorbance of fatty acid (Appendix G) except NaDEHP, which absorbs the light (713 nm) near fatty acid range. Thus, calculation of the actual rate of reaction, the absorbance of microemulsion without fatty acid was used to substract from absorbance measured during the reaction.

4.3.2 Ester Determination by FT-IR

All other components in microemuslsion such as alcohols, buffer solution, and lipase were measured from 1000 to 4000 cm^{-1} by FT-IR and the results showed no IR absorption overlapping with ester spectra (Appendix G), except the NaDEHP at high salt concentration.

The upper phase of microemulsion before and after catalytic esterifications were taken to verify by FT-IR. The interested range was between 1600 and 1800 cm^{-1}, the same the absorbance peaks of carbonyl of ester bond at 1740 cm^{-1} and fatty acid bond at 1720 cm^{-1}. The peak observed from the esterification of caprylic acid with hexanol (Figure 4.22) showed the shift of carbonyl peak of fatty acid at 1720 cm^{-1} to ester peak at 1740 cm^{-1} at low salt concentration (0.1 M). As the reaction proceeded, the intensity of fatty acid peak was decreased and the ester peak intensity was increased. It indicated that ester was produced in the reverse micelles. At higher salt concentration the ester peak at 1740 cm^{-1} was not clearly shown (Figures 4.23-4.25). However, the peak of fatty acid observed was significantly decreased in all cases of fatty acids and the shift of fatty acid intensity to ester was varied with time. It might be a result of low concentration of ester produced from different water content at higher salt concentration. To prove that the concentration of the ester product from esterification was too low, its spectrum was compared with the ester peak of 70 mM diethyl L-tartrates at the same wavenumber. The intensity of the ester peak of 70 mM diethyl L-tartrates was very low (Figure 4.29), thus the result confirmed that the ester concentration is too low to be observed. Figures 4.26-

4.28 show the absorbances of esterification product of fatty acids with short chain 1-propanol and 2-propanol. The results show that the rate of reaction was very slow in all conditions, no significantly reduced in fatty acids concentrations. Figures 4.30-4.32 show IR spectra of ester products obtained from general water/oil media and esterification of the fatty acids with three types of alcohol in microemulsion. The results showed clear ester peak and, clearly shifting of fatty acid to ester peak.



(a) (b)

Figure 4.22 Spectra of before and after esterification of caprylic acid with hexanol in microemulsion system of 150 mM NaDEHP/isooctane/100 mM caprylic acid/0.1 M NaCl: (a) 0.02 ml sample and (b) 0.05 ml sample.

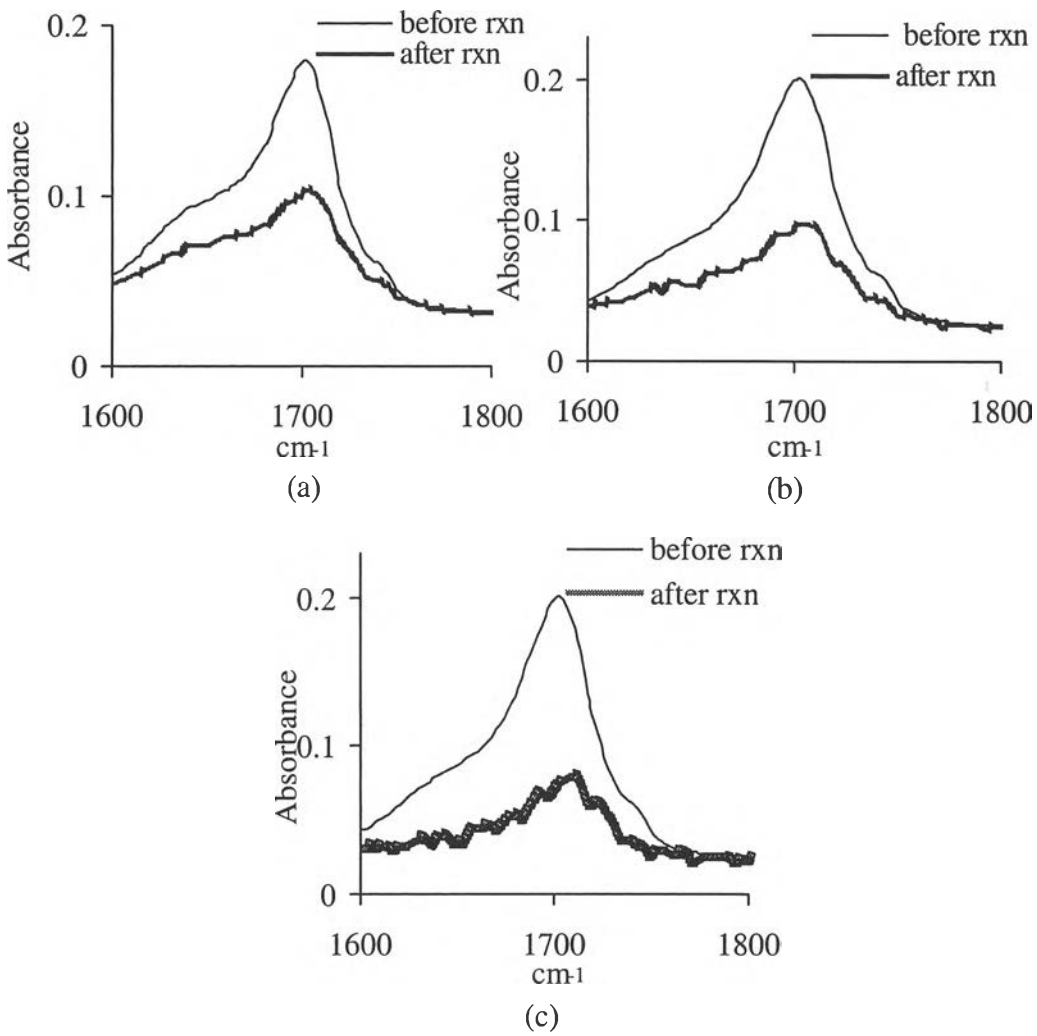


Figure 4.23 Spectra of before and after esterification of caprylic acid with hexanol in microemulsion system of 150 mM NaDEHP/isooctane/150 mM hexanol/1.0 M NaCl: caprylic acid (a) 70 mM (b) 100 mM and (c) 150 mM.

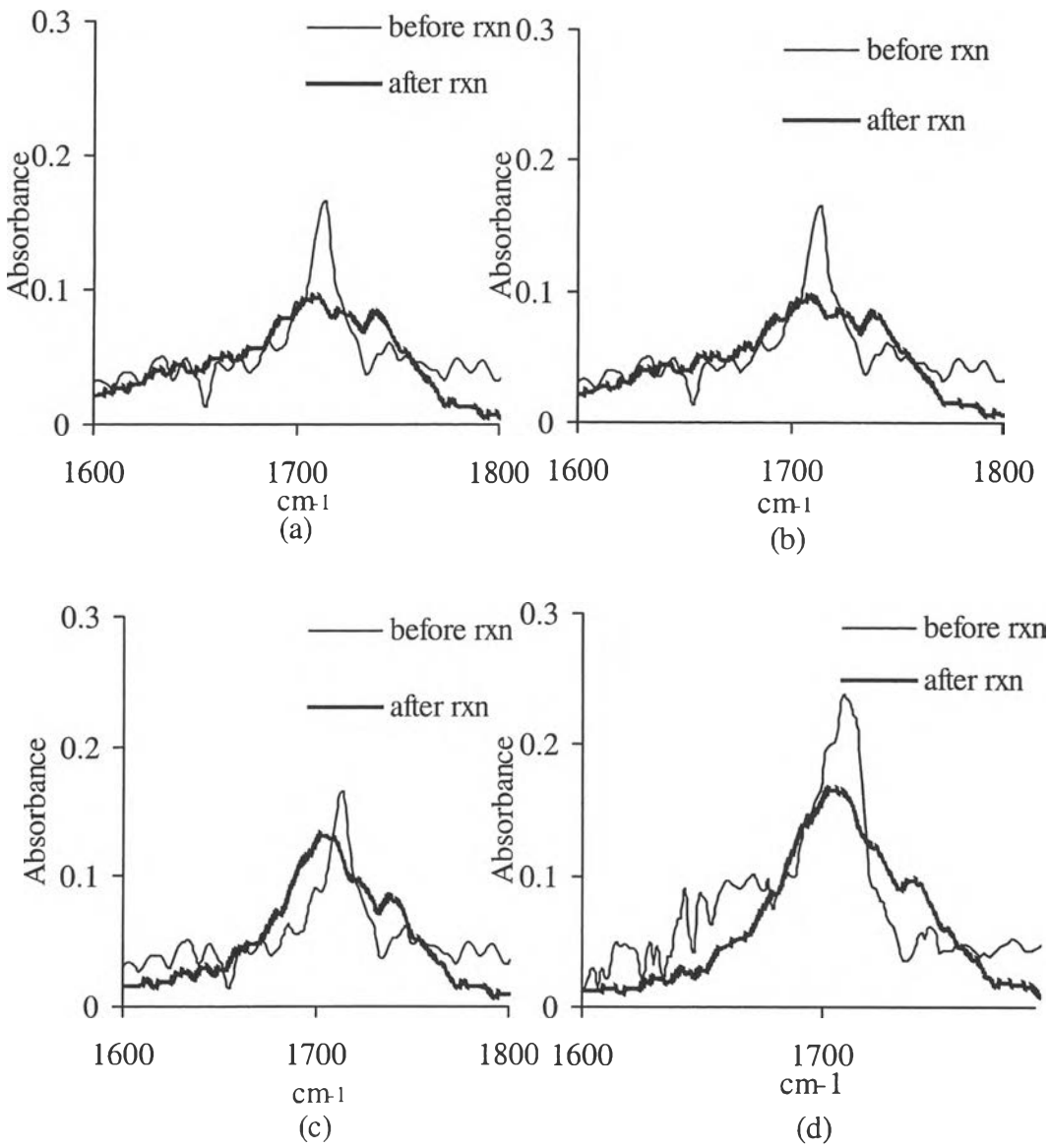


Figure 4.24 Spectra of before and after esterification of oleic acid with hexanol in microemulsion system of 150 mM NaDEHP/isooctane/150 mM hexanol/1.0 M NaCl: caprylic acid (a) 50 mM (b) 70 mM and (c) 100 mM (d) 150 mM.

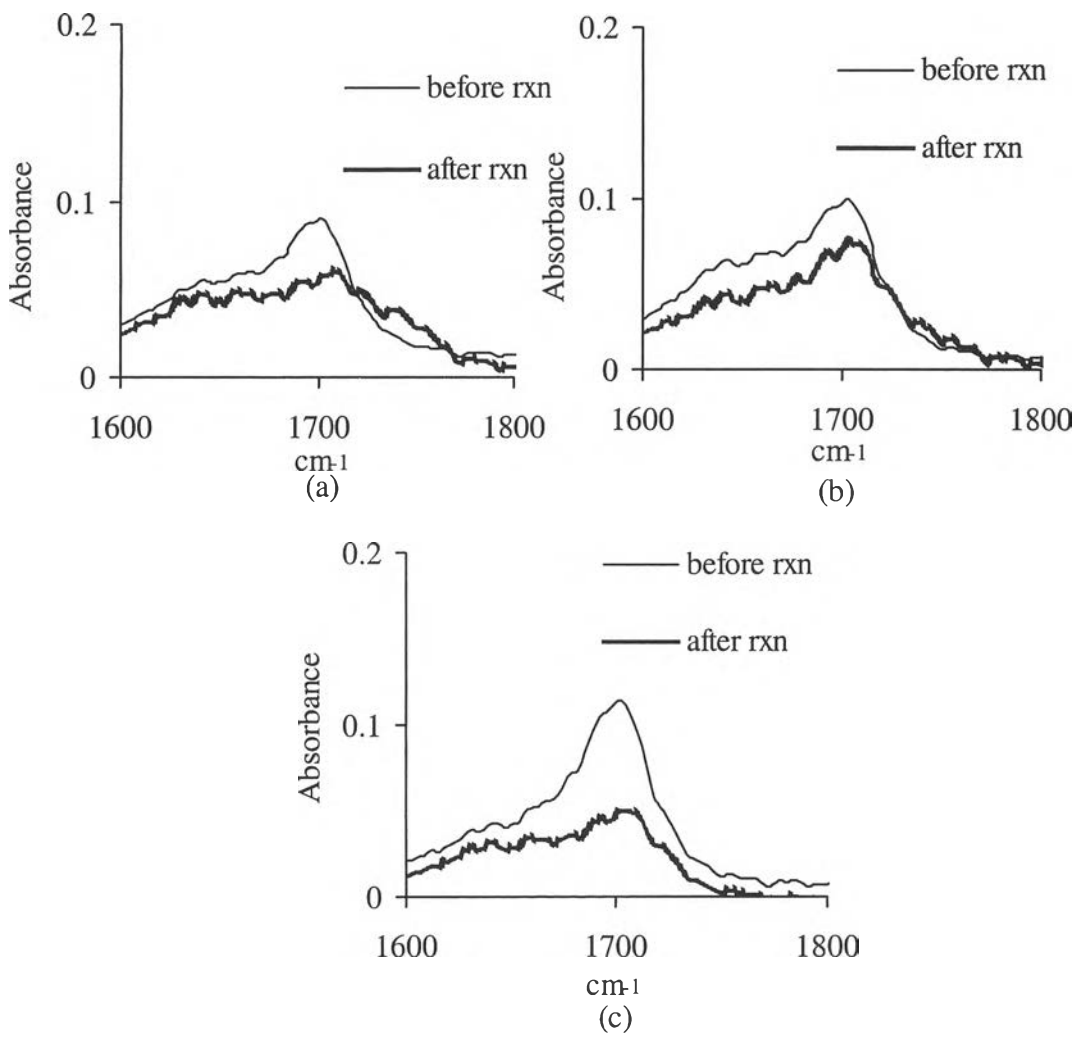


Figure 4.25 Spectra before and after esterification of palmatic acid with hexanol in microemulsion system150 mM NaDEHP/isooctane/150 mM hexanol/1.0 M NaCl: palmatic acid (a) 50 mM (b) 70 mM and (c) 100 mM.

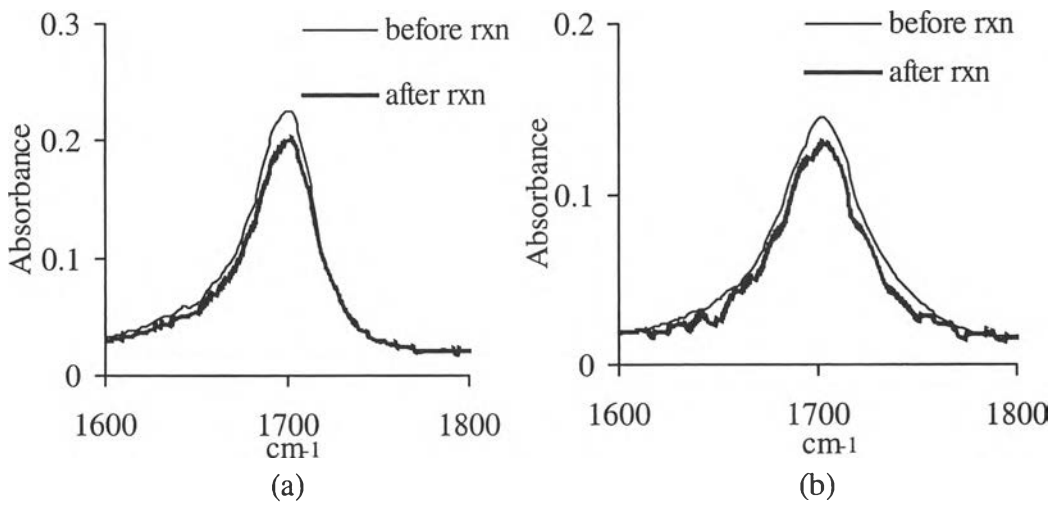


Figure 4.26 Spectra of before and after esterification of 70 mM caprylic acid with: (a) 120 mM NaDEHP/isooctane/200 mM 1-propanol /0.5 M NaCl and (b) 100 mM NaDEHP/isooctane/200 mM 2-propanol /0.5 M NaCl.

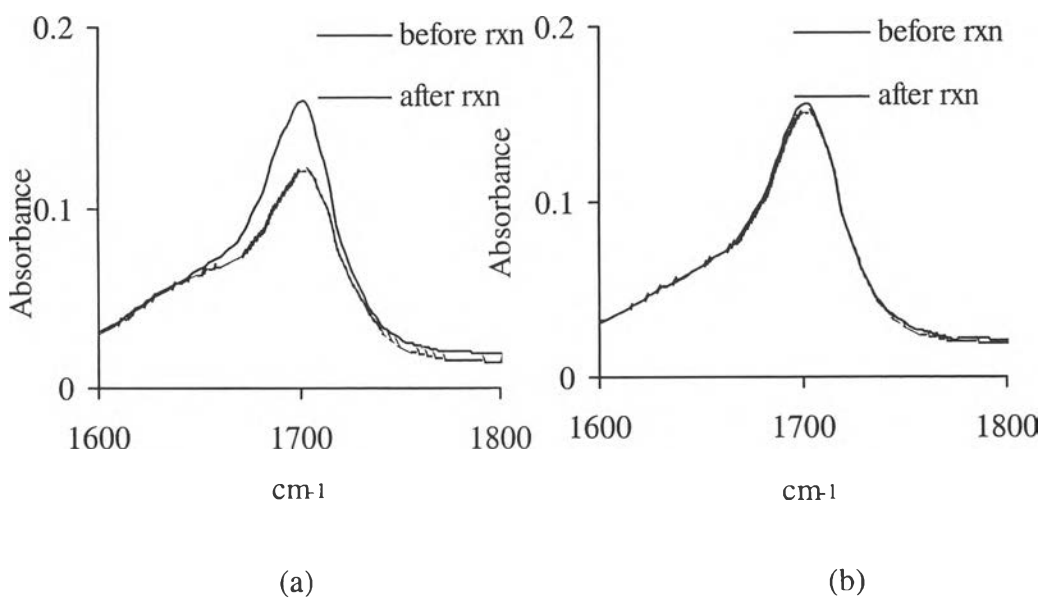


Figure 4.27 Spectra of before and after esterification of 70 mM oleic acid with: (a) 120 mM NaDEHP/isooctane/200 mM 1-propanol /0.5 M NaCl and (b) 100 mM NaDEHP/isooctane/200 mM 2-propanol /0.5 M NaCl.

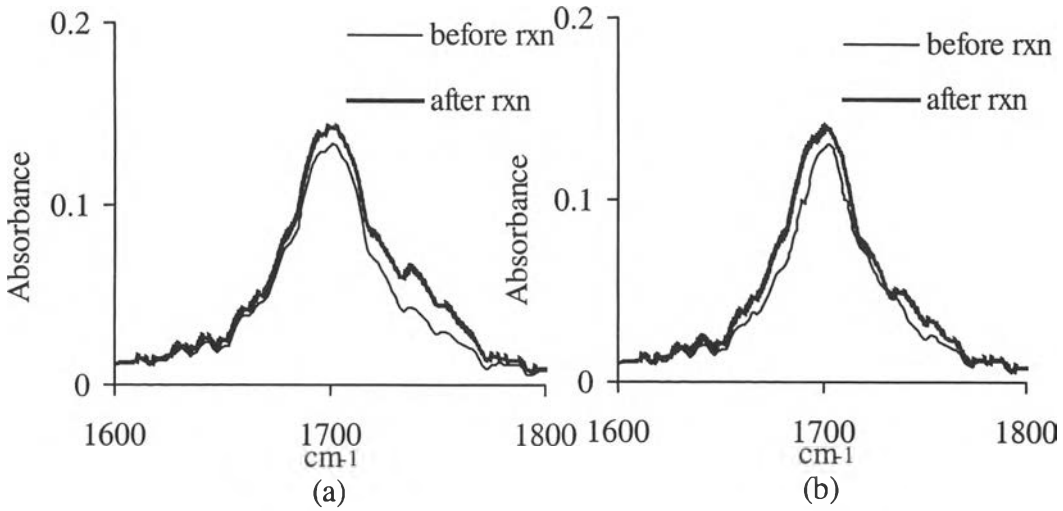


Figure 4.28 Spectra of before and after esterification of 70 mM palmatic acid with: (a)120 mM NaDEHP/isooctane/200 mM 1-propanol /0.5 M NaCl and (b) 100 mM NaDEHP/isooctane/200 mM 2-propanol /0.5 M NaCl.

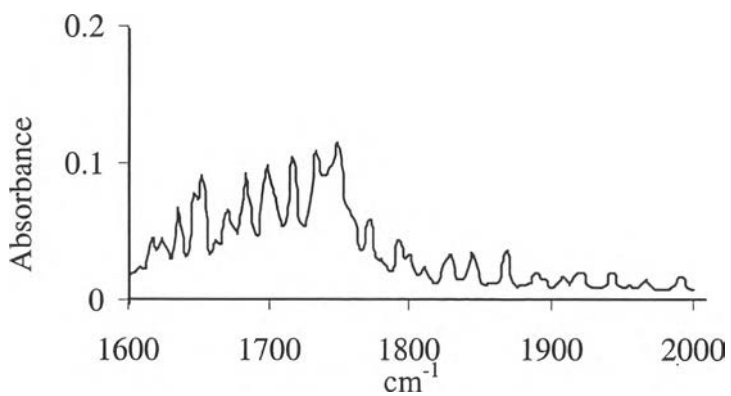


Figure 4.29 Spectrum of 70 mM diethyl L-tartrate (as standard ester).

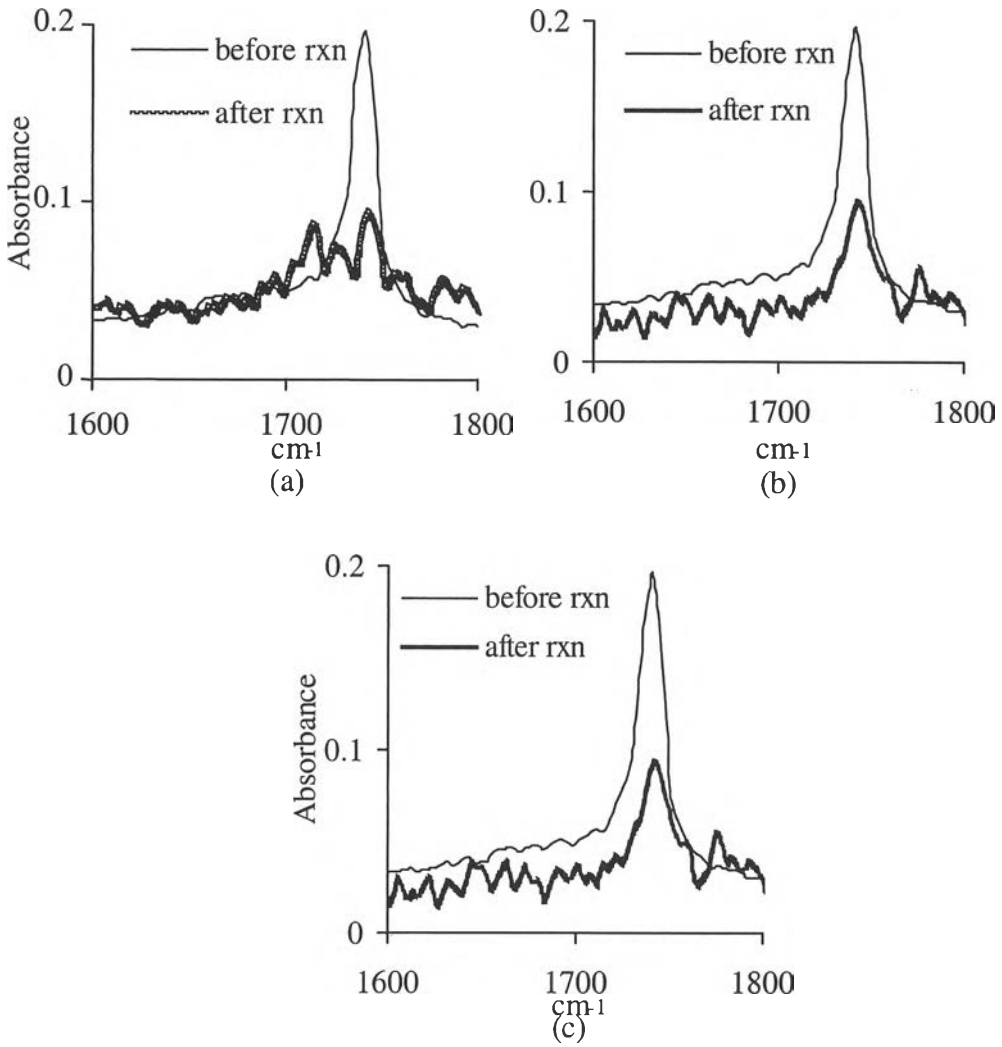


Figure 4.30 Spectra of before and after esterification of 70 mM caprylic acid in general water/oil system with: (a) 150 mM hexanol (b) 200 mM 1-propanol and (c) 200 mM 2-propanol.

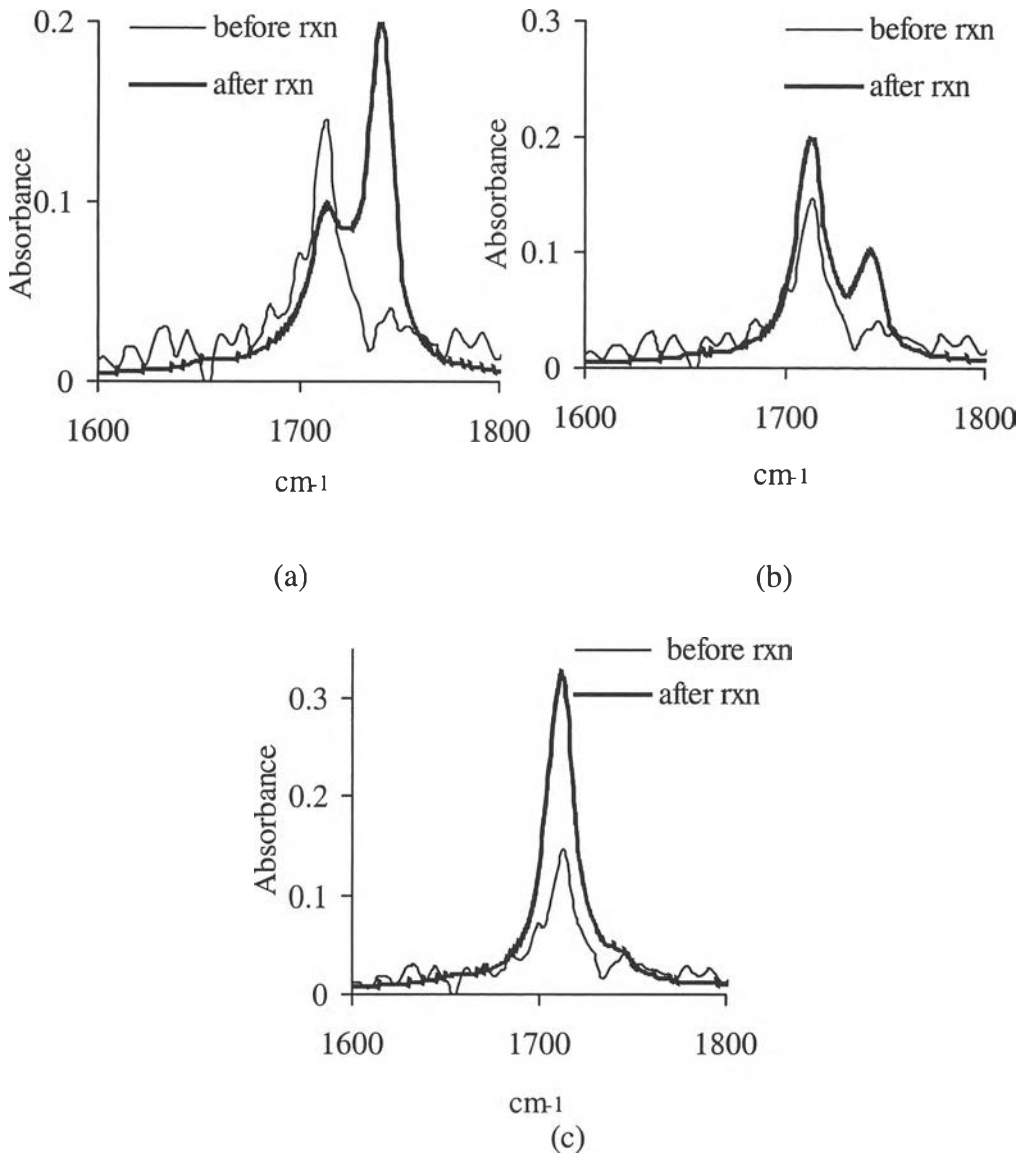


Figure 4.31 Spectra of before and after esterification of 70 mM oleic acid with hexanol in general water/oil system with: (a) 150 mM hexanol (b) 200 mM 1-propanol and (c) 200 mM 2-propanol.

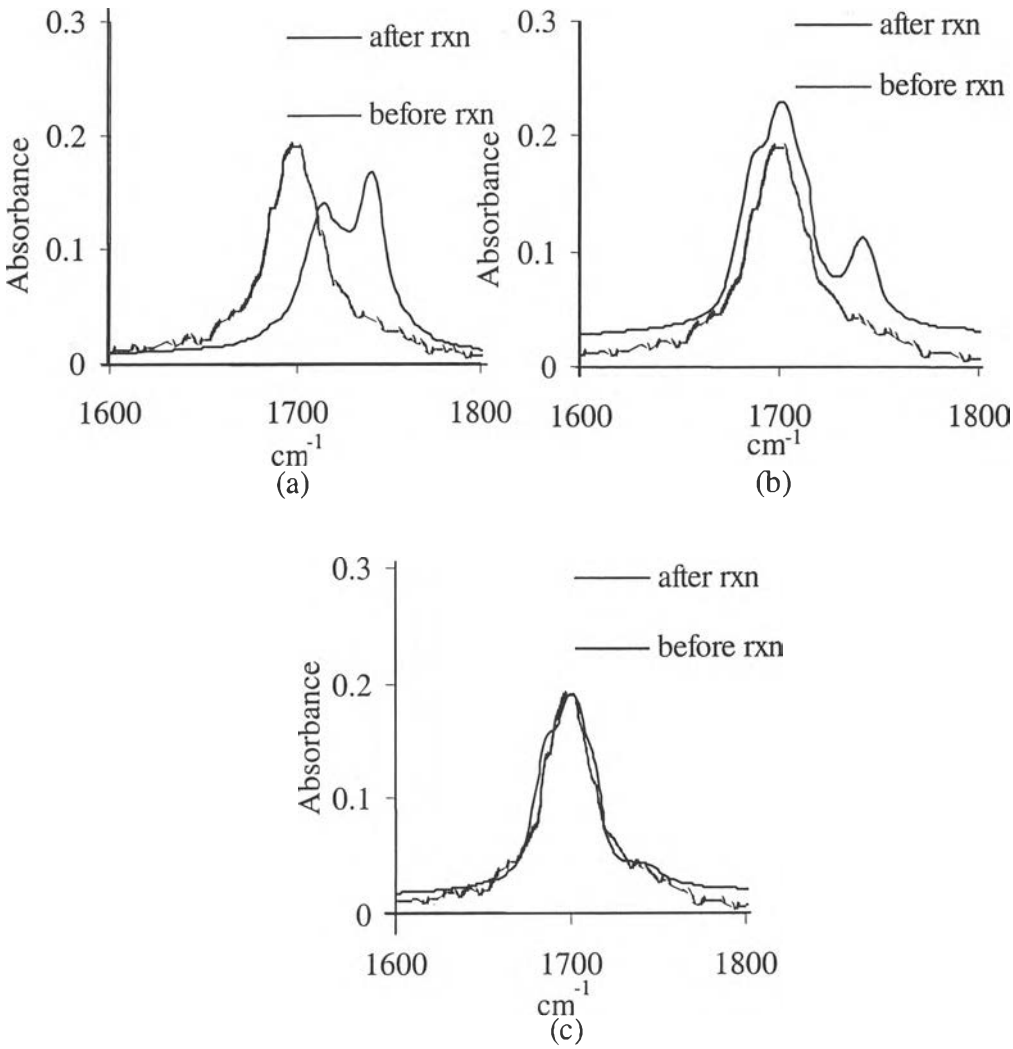


Figure 4.32 Spectra before and after esterification of 70 mM palmatic acid in general water/oil system with: (a) 150 mM hexanol (b) 200 mM 1-propanol and (c) 200 mM 2-propanol.