# Chapter 4

# Development of a Novel Hollow-Fibre Membrane Reactor for Interesterification of Triglycerides and Fatty Acids Using Modified Lipase

## Introduction

The enzyme can be used in either free or immobilized form. Numerous methods have recently been applied to the immobilization of lipases, however, each involving a different complexity and efficiency for the biocatalyst[4,5,36,62,65,75,94]. Nevertheless, none of these approaches was found to be applicable for all of the enzymatic systems. Just in many cases when lipases were treated in one way or another, their performance with respect to either activity, specificity, stability or dispersibility was improved.

The use of membrane bioreactors for the enzymatic processing of fats and oils is becoming an increasingly attractive field of research to substitute stirred tank and fixed-bed reactors[32,44,89]. However, the low resistance of membrane reactors to organic solvents, the limited solubility of substrates either in organic or aqueous phases and achieving a suitable microenvironment for enzyme are normally the main obstacles encountered in using membrane reactors for oils and fats processing. Therefore, the lipase-catalyzed interesterification and hydrolysis reactions have been performed either in a two-phase membrane reactor system or in a membrane-emulsion reactor[85,100].

In Chapters 2 and 3, the surfactant-modified lipase has been characterized and mathematical models of the interesterification between triglycerides and fatty acids and the interesterification between triglycerides have been developed. In this section the utilization of a hollow-fibre membrane reactor to assess the interesterification of triglycerides and fatty acids using surfactant-modified lipase was studied. The interesterification of tripalmitin and stearic acid using a modified lipase in *n*-hexane was chosen as a model system. The use of a membrane reactor has never been reported for such a hydrophobic reaction system using modified lipases. Therefore, the feasibility of utilizing a hollow-fibre membrane reactor in a batch as well as continuous mode for the interesterification of triglycerides and fatty acids catalyzed by the modified lipase in a microaqueous *n*-hexane system was studied.

## Materials and Methods

### *Materials*

All chemicals and lipase used in this section were mentioned in Chapter 2-1.

### *Lipase modification*

Lipase Saiken 100 (*R. japonicus*) and Emazol S-10 (F) (sorbitan monostearate) were used for the modification. Lipase modification was carried out according to the method described in Chapter 2-2.

### *Experimental setup*

In a preliminary study a new hollow-fibre membrane reactor system (Chiyoda Seisakusho Co. Ltd., Tokyo, Japan) resistant to hydrophobic organic solvents, was used. The membrane module is being developed by Toray Co. Ltd., (Tokyo, Japan) for use of hydrophobic organic solvents. The module is made of polyphenylene sulfone membrane (PPSO) and the membrane has molecular weight cut off 50,000. It consisted of a bundle of 63 fibres each 13.5 cm in length, 1.2 mm outer diameter and 0.8 mm inner diameter. The membrane surface area of the module was 32 $cm^2$. The reactor system is shown in Fig. 4-1. This system could be operated either batchwise or continuously. The outlet of the membrane module was closed when operated batchwise and opened when operated continuously.

Four consecutive batch reactions using the same batch of the modified lipase were performed in the hollow-fibre membrane reactor system as follows: A solution of 660 mL *n*-hexane containing 6 mmol/L of tripalmitin and 12 mmol/L of stearic acid was magnetically stirred at 800 rpm and recirculated for 1 h in the system through the hollow fibre membrane module. The recirculation flow rate was 200 mL/min and the temperature in the reaction solution was maintained at 40°C. The reaction was initiated by adding 240 mg of the modified lipase (364 mg biocatalyst/L) into the recirculated solution, and samples were taken periodically. After 20 h of recirculation,

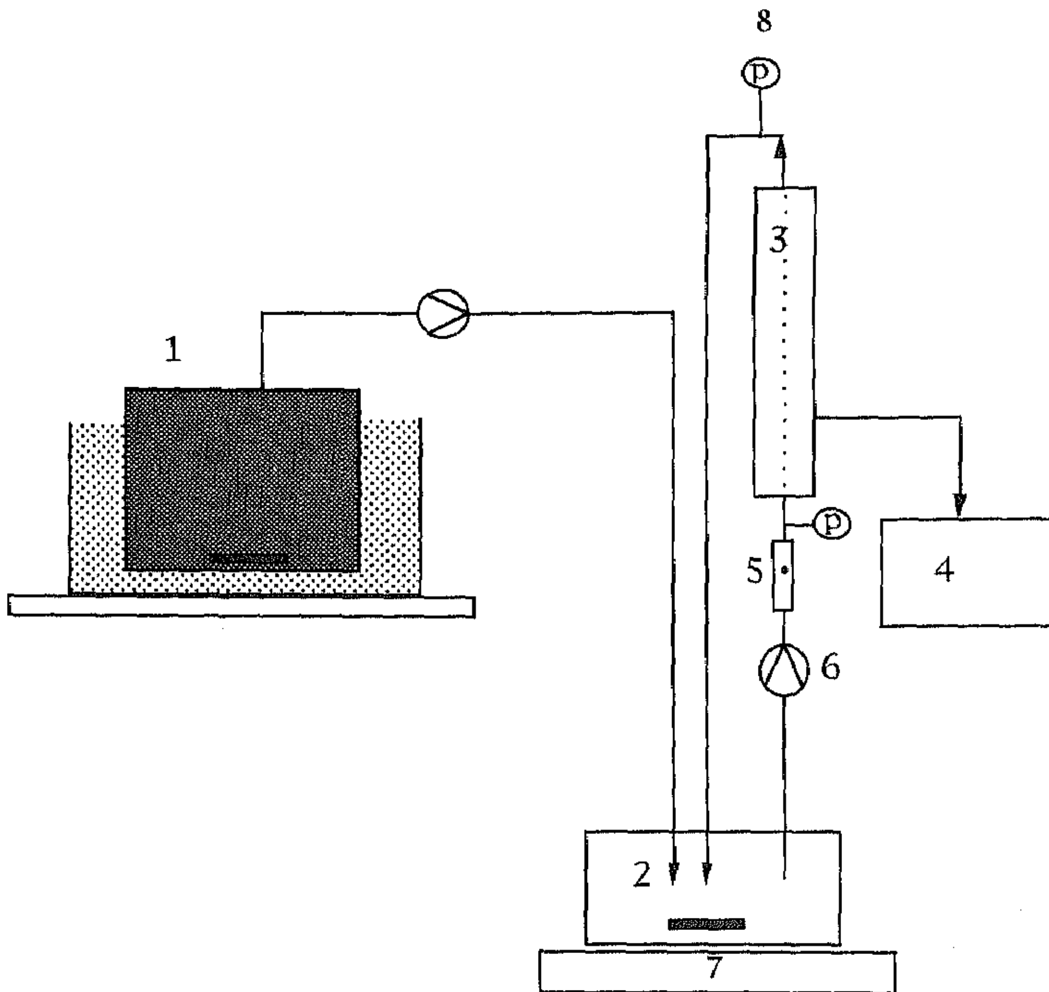


**Fig. 4-1** Flow diagram of the used hollow-fibre membrane reactor system. 1- Thermostated feed reservoir, 2- reaction flask, 3- hollow-fibre membrane module, 4- effluent, 5- rotameter, 6- pump, 7- magnetic stirrer, and 8- pressure gauge.

the outlet of the membrane module was opened and 0.5 bar of transmembrane pressure was applied in order to force 400 mL of the solution to permeate through the membrane [average flux was 2 mL/min or 10.4 mL/($m^2$·s)]. The membrane rejected the biocatalyst while the substrates and the products dissolved in *n*-hexane were allowed to permeate. Then the system was purged with 400 mL *n*-hexane and after that 400 mL of the recirculated solution was forced to permeate through the membrane module. The water content in *n*-hexane was reduced to 10 mg/L after drying over molecular sieve. This process was repeated once more and then, 400 mL *n*-hexane solution containing 6 mmol/L of tripalmitin and 12 mmol/L of stearic acid was added to the remaining solution in the system. The initial concentrations of tripalmitin and stearic acid after purging the system was 3.7 and 7.4 mmol/L, respectively. This process was repeated three times more using the modified lipase retained in the membrane system. The water concentration in all reaction solutions was about 90 mg/L.

The operational stability of the modified lipase was checked in a long-term experiment using the membrane reactor system operated continuously. The system was operated under the conditions described previously. The feed rate of the substrate solution was in average 0.75 mL/min (residence time 14.7 h). The reaction was initiated by the addition of 240 mg modified lipase.

As a comparison reaction system, the interesterification reaction of tripalmitin (PPP) and stearic acid (S) catalyzed by the modified lipase to produce 1,2-dipalmitoyl-3-stearoyl glycerol (PPS) and 1,3-distearoyl-2-palmitoyl glycerol (SPS) was performed in a 60-mL stirred batch reactor. The reaction was initiated by adding 20 mg modified lipase into 55 mL *n*-hexane under the same previous conditions. The concentration of water in the reaction solution was about 25 mg/L.

*Analytical methods*

The time course of the interesterification reactions was followed by determining the concentrations of substrates and all possible products with time. Samples (0.5 mL)

were periodically withdrawn from the reaction systems, filtered using disposable Millipore filters (pore size 0.5 μm) and kept in screw cap vials. GC and HPLC analysis followed the method described in detail in Chapters 2-1 and 2-2

Water concentrations in the reaction system were measured by Karl Fischer Titrator.

The protein content of the modified lipase was measured by nitrogen analyzer (Chapter 2-1).

**Results and Discussion**

*The interesterification of triglycerides and fatty acids in n-hexane using the modified lipase in the stirred tank reactor*

Figure 4-2 shows the concentration profiles of tripalmitin (PPP) and the products 1,2-dipalmitoyl-3-stearoyl glycerol (PPS) and 1,3-distearoyl-2-palmitoyl glycerol (SPS) in the reaction system performed in the stirred tank reactor. It can be seen that the modified lipase catalyzed predominantly the interesterification reaction. GC and HPLC analysis results showed that the modified lipase catalyzed the hydrolysis reaction of triglycerides and therefore around 6 wt% of diglycerides (of the initial concentration of tripalmitin) were produced as byproducts. Glycerol and monoglycerides were not detected in the reaction system under these experimental conditions. Tristearin was also not detected in the reaction system and this indicates that the modified lipase has 1,3-positional specificity. It can also be seen that the reaction system reached steady state after about 2 h, and no subsequent changes in concentration occurred even after adding more biocatalyst to the reaction system 5 h after the start of the reaction.

*The interesterification of triglycerides and fatty acids in n-hexane using the modified lipase in the hollow-fibre membrane reactor system*

Figures 4-3 and 4-4 show that the modified lipase used in four consecutive runs catalyzed predominantly the interesterification reaction to produce PPS and SPS.

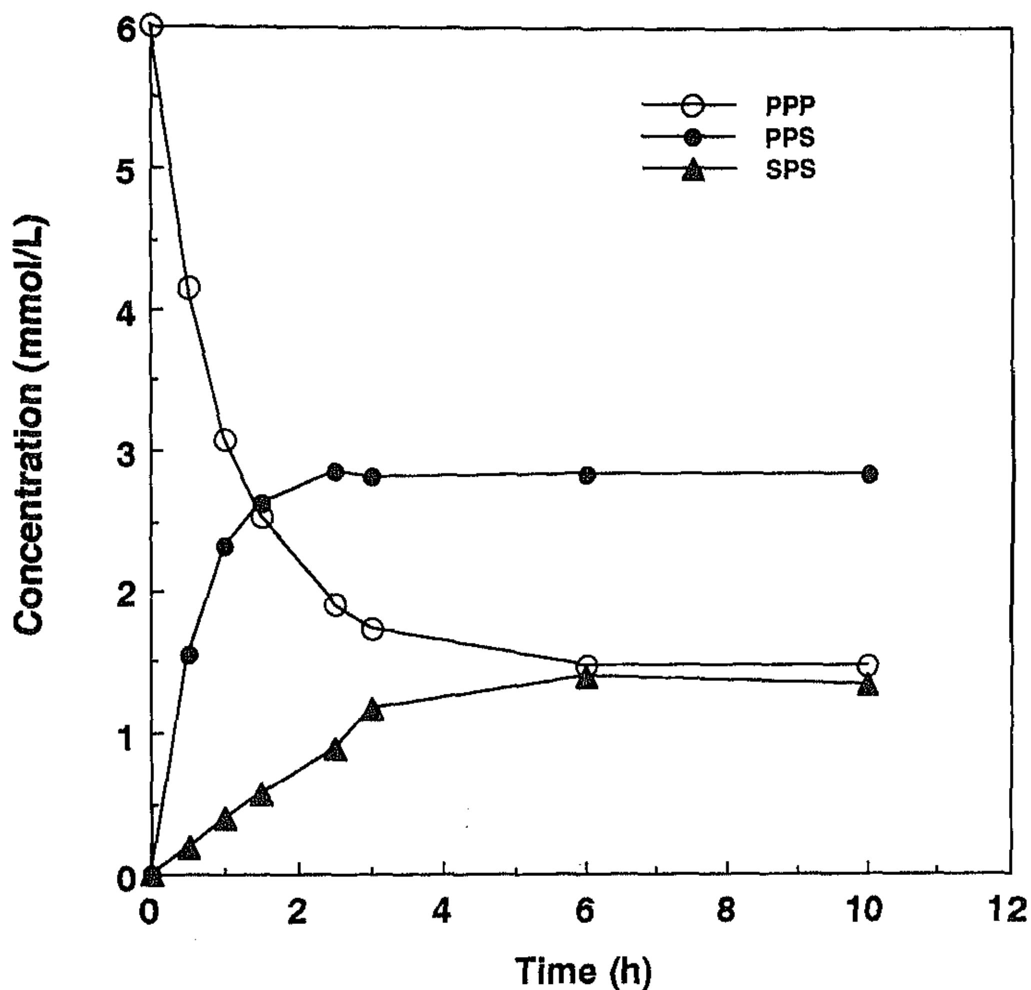


Fig. 4-2 Triglycerides concentration profiles resulting from the modified lipase catalyzed interesterification of tripalmitin and stearic acid in the stirred tank reactor. The reaction was carried out in 55 mL *n*-hexane containing 6 mmol/L tripalmitin, 12 mmol/L stearic acid and 30 mg modified lipase. The reaction mixture containing 25 mg/L water was magnetically stirred at 800 rpm and thermostated at 40°C.

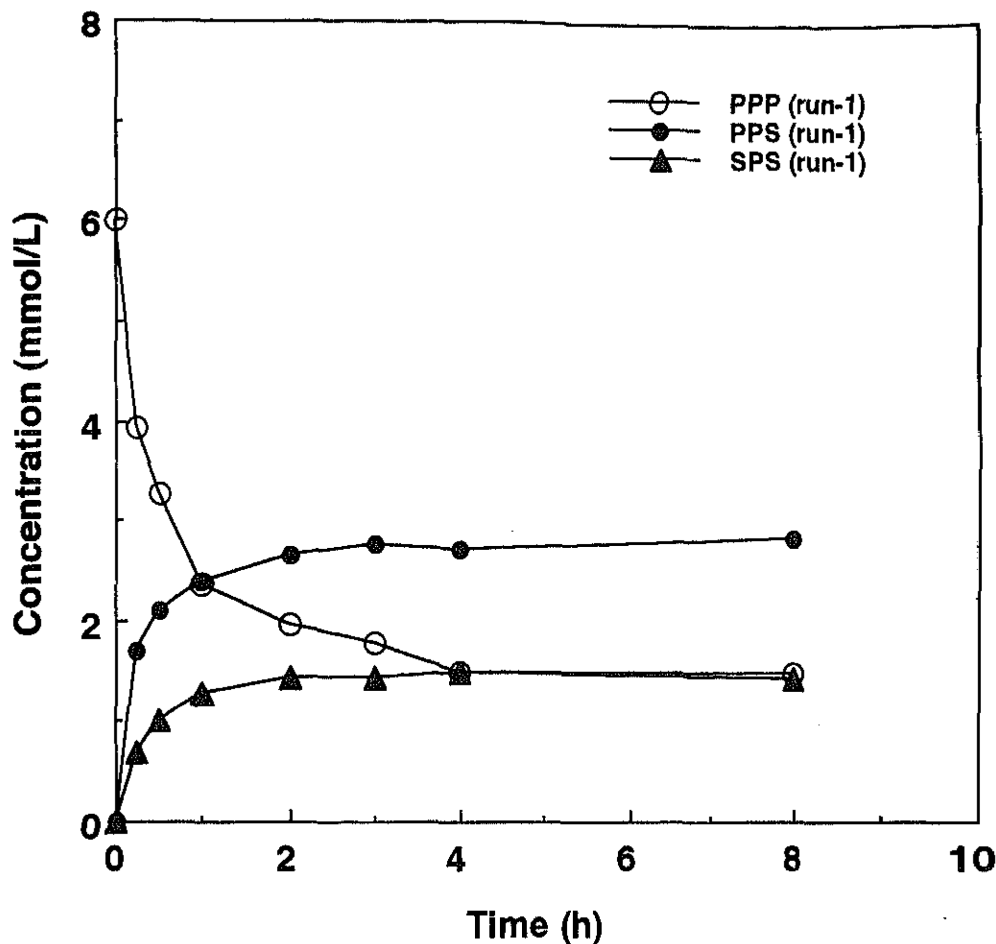


Fig. 4-3 The concentration profiles of triglycerides in the first run (run-1) using the hollow-fibre membrane reactor system. The reaction was carried out in 660 mL *n*-hexane containing 6 mmol/L of tripalmitin and 12 mmol/L of stearic acid. The reaction solution was magnetically stirred at 800 rpm and recirculated through the membrane module at 200 mL/min. The temperature of the system was maintained at 40°C.

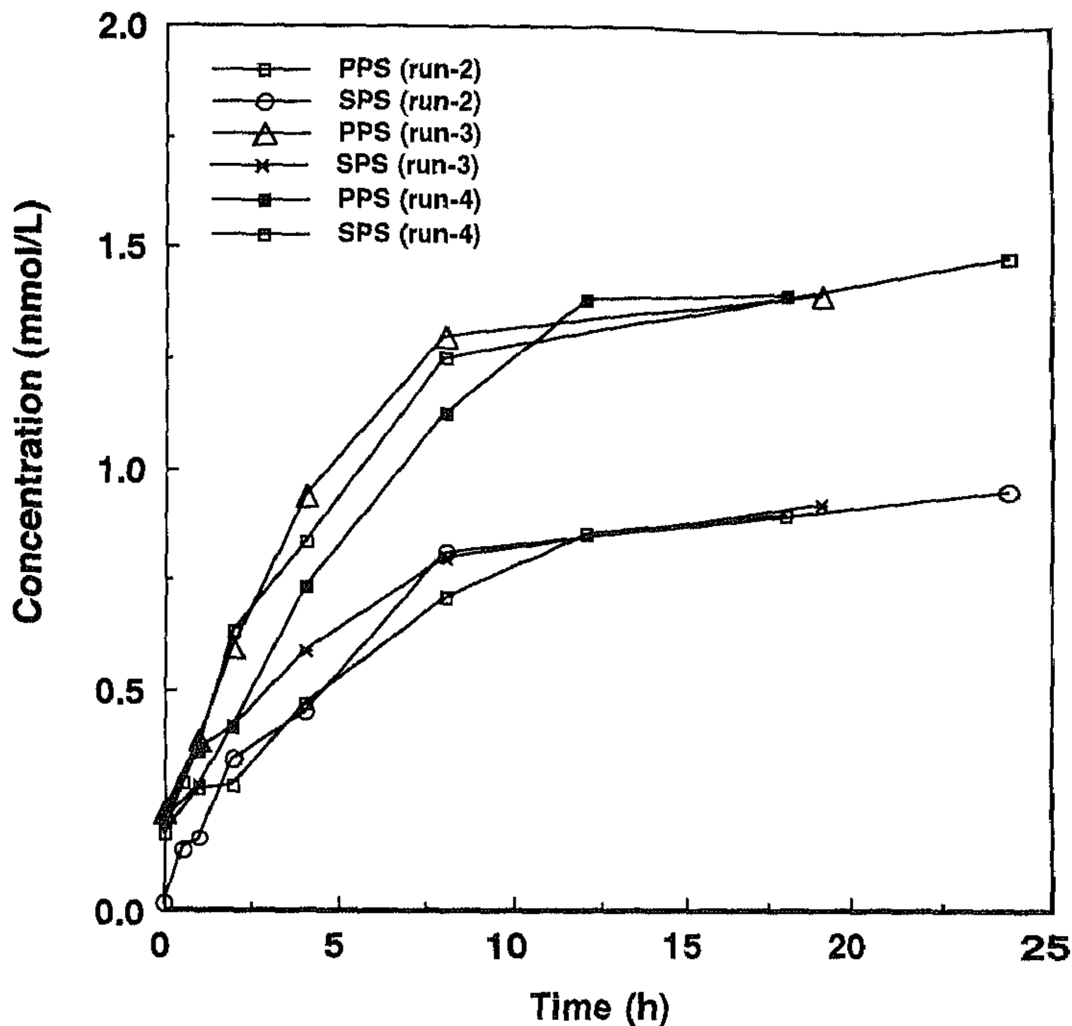


Fig. 4-4 The concentration profiles of the interesterification products, PPS and SPS, in run-2, 3 and 4 in the hollow-fibre membrane reactor system using the same modified lipase in Figure 4-3. The reaction was carried out in 660 mL *n*-hexane containing 3.7 mmol/L of tripalmitin and 7.4 mmol/L of stearic acid. The reaction solution was operated under the same condition in Figure 4-3.

Similarly to the stirred tank reactor, it can be seen in Fig. 4-3 that steady state in the first run (run-1) was reached after about 2 h, and after that there was no substantial changes in the concentration of the components involved in the interesterification. Steady state was reached in all of the last three runs after about 10 h. This difference might be ascribed to the permeation of the free surfactant during the process of forcing the reaction solution to permeate through the membrane module after the first run was completed. Based on this result it is speculated that the free surfactant which is not attached to the lipase-surfactant complex has an important role in determining the activity of the modified lipase. It can also be seen that the steady state in the first run differs from that of the last three runs. This difference in the concentrations at the steady state positions was caused mainly because the concentration of substrates in the last three runs was lower than that in the first run. However, under the operation conditions, there was no significant enzyme activity losses during 100 h operation in the 2nd, 3rd and 4th runs when the same batch of the modified lipase was used.

Figure 4-5 shows the operational stability of the modified lipase. It can be seen that steady state in the system was reached after about 4 h, and the system continued to operate for 76 h without any loss in enzyme activity. The residence time used in the continuous operation was 14.7 h and this ensured that steady state was reached in the continuous system (compared to 2 h the time required to reach steady state in the stirred tank reactor and batch membrane reactor).

Around 10 wt% of diglycerides were produced in both batchwise and continuous membrane reactor systems as byproducts compared to 6 wt% in the stirred tank reactor. This is mainly attributed to the higher water content in the membrane system.

The hollow-fibre membrane module used in this study showed a high resistance to the hydrophobic *n*-hexane solution in both batchwise and continuous experiments. No membrane damage, membrane swelling or enzyme leakage were observed under the experimental conditions. The results presented in this section demonstrate the use of a promising hollow-fibre membrane reactor system for lipase-catalyzed reactions in

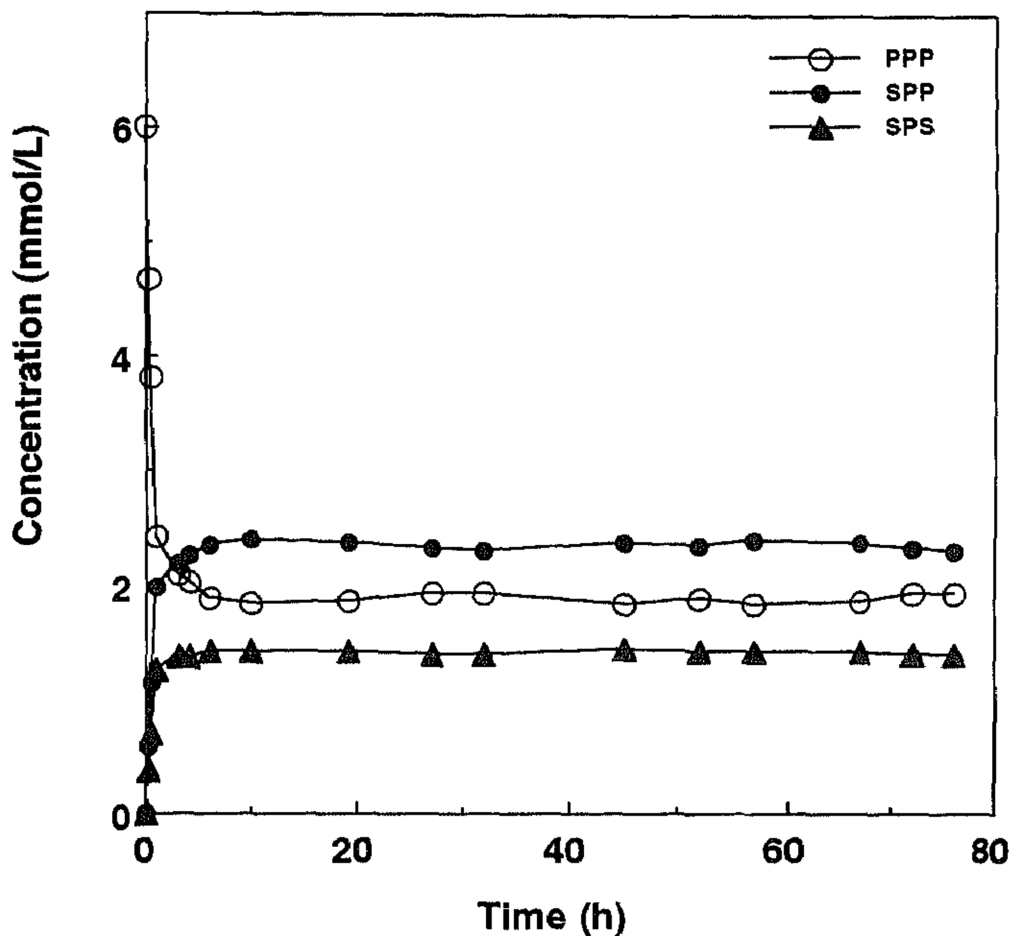


Fig. 4-5 Operational stability of the modified lipase in the continuous hollow-fibre membrane reactor system. The reaction flask was filled with 660 mL *n*-hexane solution containing 6 mmol/L of tripalmitin and 12 mmol/L of stearic acid. The feed rate was 0.75 mL/min of substrate solution. The reaction solution was operated under the same condition in Figure 4-3.

microaqueous hydrophobic organic solvent systems as a potential alternative to stirred tank and fixed-bed reactors.