*J. Microbiol. Biotechnol.* (2009), **19**(12), 1596–1602
doi: 10.4014/jmb.0905.05051
First published online 7 November 2009

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY



# Lipase-catalyzed Esterification of (*S*)-Naproxen Ethyl Ester in Supercritical Carbon Dioxide

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Received: May 26, 2009 / Revised: July 7, 2009 / Accepted: July 13, 2009

**A lipase-catalyzed esterification reaction of (*S*)-naproxen ethyl ester by CALB (*Candida antarctica* lipase B) enzyme was performed in supercritical carbon dioxide. Experiments were performed in a high-pressure cell for 10 h at a stirring rate of 150 rpm over a temperature range of 313.15 to 333.15 K and a pressure range of 50 to 175 bar. The productivity of (*S*)-naproxen ethyl ester was compared with the result in ambient condition. The total reaction time and conversion yields of the catalyzed reaction in supercritical carbon dioxide were compared with those at ambient temperature and pressure. The experimental results show that the conversion and reaction rate were significantly improved at critical condition. The maximum conversion yield was 9.9% (216 h) at ambient condition and 68.9% (3 h) in supercritical state. The effects of varying amounts of enzyme and water were also examined and the optimum condition was found (7 g of enzyme and 2% water content).**

**Keywords:** Supercritical carbon dioxide, *Candida antartica* lipase B, naproxen, (*S*)-naproxen ethyl ester, esterification, statistical analysis method

Recently, supercritical fluids have been gaining attention as new reaction media owing to their high diffusivity. Supercritical carbon dioxide ($SCCO_2$) is a new reaction medium and offers several advantages as an alternative to organic solvents [3, 12, 16]. It has desired physical properties such as low viscosity, low surface tension, high diffusivity, and low critical temperature and pressure (304.15 K and 73.8 bar). Owing to the superior properties of supercritical carbon dioxide, enzymatic reactions can be achieved with high efficiency, resolution, and selectivity compared with conventional organic solvents [11].

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Naproxen (6-methoxyl-α-methyl-2-naphthaleneacetic acid) is a nonsteroidal anti-inflammatory drug (NSAID) and is widely used to treat human connective tissue diseases [2]. It is also used for painful and inflammatory rheumatic and certain non-rheumatic conditions. Only the (*S*)-enantiomer exhibits anti-inflammatory activity, whereas the (*R*)-enantiomer exhibits gastrointestinal (GI) side effects and toxicity [9]. Previous pharmaceutical studies of NSAIDs have indicated that GI side effects such as ulceration and hemorrhage are the most frequent adverse effects [19]. Naproxen ester prodrugs effectively reduce the GI side effects and deliver naproxen through the skin [2, 20]. Many naproxen ester prodrugs have been proposed in the literature [17, 19]. Production of (*S*)-naproxen prodrugs from racemic naproxen involves two reaction steps. In step (1), the (*S*)-enantiomer is generated by optical resolution of the racemate; and in step (2), (*S*)-naproxen prodrugs are synthesized by chemical esterification [23]. The esterification reaction in step (2) can be catalyzed using enzymes. Lipases are known to have the ability to catalyze enantioselective esterification in organic solvents. The enzymatic esterification reaction scheme is shown in Fig. 1. Tsai *et al.* [24] developed a lipase-catalyzed enantioselective esterification process in cyclohexane. Cui *et al*. [5] used *Candida cylindracea* to catalyze the esterification reaction in isooctane and reported that the conversion could be reached up to 33.5% for 11 days. Experiments of an enzymatic reaction of this type require at least 100–200 h for completion. As mentioned at the beginning of this section, several authors [3, 12, 16] have reported that an enzymatic reaction can be accelerated if supercritical fluids are used as reaction media. In this study, a lipase-catalyzed esterification reaction of (*S*)-naproxen ethyl ester by CALB (*Candida antarctica* lipase B) enzyme was performed in supercritical carbon dioxide to evaluate the effectiveness of using $SCCO_2$ as a reaction medium. It is well known that the use of supercritical fluids decreases mass transfer limitations owing to the high diffusivity, low surface tension, and low viscosity in the mixture. Furthermore, $SCCO_2$ has properties that can be

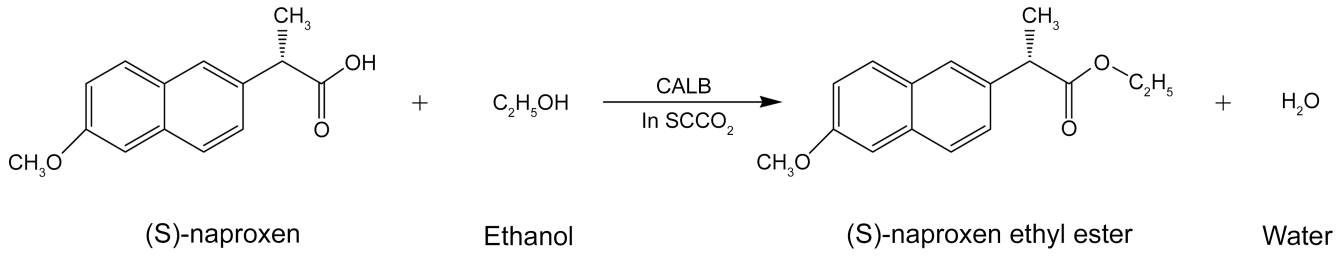


**Fig. 1.** The reaction scheme of enantioselective esterification of (*S*)-naproxen by *Candida antarctica* lipase B in supercritical carbon dioxide.

tuned by changing the temperature and pressure, allowing easy separation after the reaction. In other words, the reaction rate and selectivity are enhanced in supercritical carbon dioxide.

Moreover, the optimum condition for maximum conversion yields is to be sought. Optimum conditions for the products of the lipase-catalyzed reaction in $SCCO_2$ were investigated using Response Surface Methodology (RSM). RSM is the most widely used statistical technique for optimization. It can be used to evaluate the relationship between a set of controllable experimental factors and observed results, and to design effective experiments [4, 6, 14, 15, 21, 22]. The regression equations generated by RSM allow the process to be modeled in terms of temperature and pressure dependence. The effects of water and enzyme contents are also discussed.

## Materials and Methods

### Materials

Liquefied carbon dioxide at high pressure with a purity greater than 99.9 mass% was purchased from PS Chem. Co. (South Korea). *Candida antarctica* lipase B (200 U/g matrix) was purchased from NOVO Nordisk (Denmark). Optically pure (*S*)-naproxen was purchased from Sigma (U.S.A.). All other chemicals were obtained from commercial suppliers and were of analytical grade.

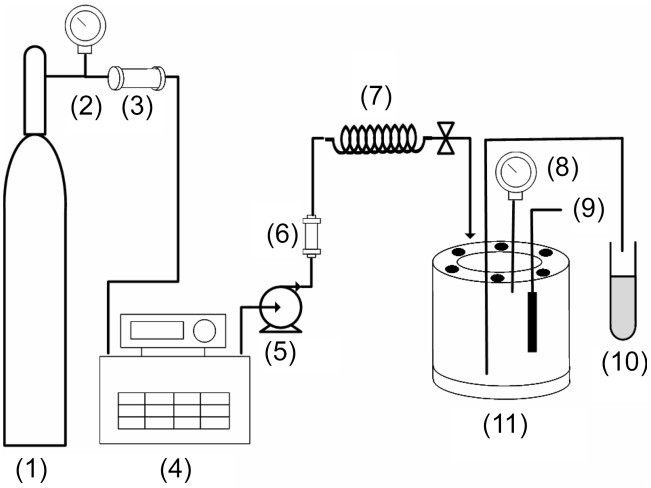


**Fig. 2.** The experimental apparatus of the enzyme-catalyzed esterification reaction by CALB.

### Experimental

A schematic diagram of the experimental apparatus is shown in Fig. 2. The reaction was carried out for 10 h at temperatures ranging from 303.15 to 343.15 K and pressures ranging from 75 to 175 bar with a constant stirring rate of 150 rpm. Experiments were performed in a high-pressure cell [(11) in Fig. 2]. Then, 0.2 mM (*S*)-naproxen and water was added to the cell and sonicated for 20 min using the ultrasonic processor (Cole-Parmer Inc.) to ensure thorough dispersion of water. In this study, a very low concentration of (*S*)-naproxen (0.2 mM) was used because this value is the maximum saturation condition for the substrate found in the previous study [13]. Isooctane and ethanol were then added to the cell and sonicated again for 20 min. The ratio of ethanol to water was 94:6 and isooctane to ethanol was 7:3 by volume. Lipase (1,000 U) was added just before the reaction. The total volume in the cell was 100 ml. Samples were taken from the reactor [(10) in Fig. 2] on an hourly basis. The esterification reaction products were analyzed by HPLC (YOUNG-LIN Instrument Co., Ltd., Korea) using a chiralcel OD column (Daicel Chemical Industries, Japan) capable of separating the (*R*)- and (*S*)-enantiomers. A UV detector with wavelength of 254 nm was used for composition analysis. The mobile phase was a mixture of *n*-hexane:2-propanol:acetic acid (97:3:1, by volume) at a flow rate of 1.0 ml/min. To determine the (*S*)-naproxen ethyl ester composition, (*S*)-naproxen ethyl ester standards of each concentration were ethylated by adding 2 ml of 10% boron trifluoride in ethanol at 333.15 K for 15 min. The results in triplicate were reproducible to within 5% and the conversion yields were calculated by the average value from three independent measurements. The conversion yield (percent conversion) of the process was defined as the maximum molar conversion per unit time based on the amount of the final product, (*S*)-naproxen ethyl ester.

### Experimental Design for Statistical Analysis

Response Surface Methodology (RSM) was employed to determine the design parameters of the lipase-catalyzed reaction in order to optimize the reaction temperature and pressure. Table 1 shows the coded values of the experimental variables between the minimum and maximum ranges. All the variables were taken at a central coded value (dimensionless) set as zero. With these specifications, experiments were performed randomly with given coded levels. The design of the experiments is given in Table 1. A total of 11 experiments were performed, and the experimental results are also summarized in Table 1. The variables were coded according to the following equation:

**Table 1**. Range of variables at different levels for experimental design and results.

| Factors | Symbol | Coded values | | | | |
|---|---|---|---|---|---|---|
| | | −2 | −1 | 0 | +1 | +2 |
| Temperature (K) | $X_1$ | 303.15 | 313.15 | 323.15 | 333.15 | 343.15 |
| Pressure (bar) | $X_2$ | 75 | 100 | 125 | 150 | 175 |

| Runs | Coded values | | |
|---|---|---|---|
| | $X_1$ | $X_2$ | Conversion yield (%) |
| 1 | +1 | +1 | 56.4 |
| 2 | +1 | −1 | 52.7 |
| 3 | −1 | +1 | 54.7 |
| 4 | −1 | −1 | 50.3 |
| 5 | +2 | 0 | 42.3 |
| 6 | −2 | 0 | 65.8 |
| 7 | 0 | +2 | 66.1 |
| 8 | 0 | −2 | 58.6 |
| 9 | 0 | 0 | 64.6 |
| 10 | 0 | 0 | 68.9 |
| 11 | 0 | 0 | 60.7 |

$$x_i=(X_i-X_0)/\Delta X \quad (1)$$

where $x_i$ is the coded value of the variable $X_i$ (i=1, 2, 3 . . . , j), $X_0$ is the value of $X_i$ at the center point, and $\Delta X$ is the step change.

A second-order polynomial equation was then fitted to the data through multiple regressions.

$$y = \beta_0+\sum\beta_i x_i+\sum\beta_{ii} x_i^2+\sum\beta_{ij} x_i x_j \quad (2)$$

where y is the predicted response, $\beta_0$ is the constant, and $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ represent the coefficients of the linear, quadratic, and cross product terms, respectively.

## RESULTS

### Time-Course Analysis

(*S*)-Naproxen ethyl ester with a small quantity of (*R*)-naproxen ethyl ester was obtained through the esterification of (*S*)-naproxen in $SCCO_2$. The time-courses and conversion yields at 323.15 K and 125 bar are presented in Fig. 3. The total reaction time was 10 h, but the maximum conversion yield of (*S*)-naproxen ethyl ester in this reaction was 68.9% over 3 h. As shown in Fig. 3 and Fig. 6A, a small amount of (*R*)-naproxen ethyl ester was also produced with (*S*)-naproxen ethyl ester. In our previous study [13], enantioselectivity of *Candida antartica* lipase B was examined using molecular dynamics (MD) simulation and the result showed the enzyme−substrate formed a hydrogen bond and the strength of the hydrogen bond was affected by the structure of substrate molecules. Although the MD study proves that the formation of (*S*)-naproxen ethyl ester is preferential, there are still possibilities to form (*R*)-naproxen ethyl ester during the complex formation steps. Hydrogen bond lengths in the enzyme−substate intermediates were compared in the previous study [13].

### Effects of Temperature and Pressure

In a previous study, the effects of pressure and temperature were insignificant in enantioselectivity. However, an accurate effect for lipase activity can be not proved [25] because the reaction conditions were fixed. Moreover, in some studies, temperature and pressure are the most important parameters for enzyme-catalyzed reactions, and reactions can be improved by tuning these parameters [7, 26]. It is known that the dielectric constant of $CO_2$ dramatically changes in supercritical conditions [7]. Recently, Yasmin *et al*. [26] reported the effects of temperature and pressure on the

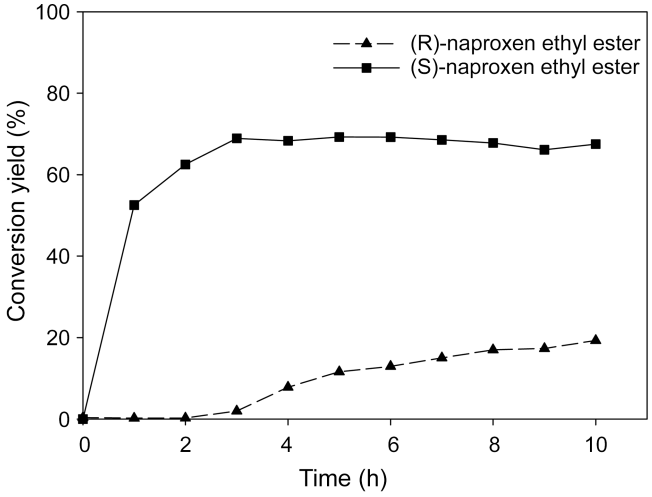


**Fig. 3.** Esterification of (*S*)-naproxen using enantioselective lipase at 323.15 K and 125 bar.

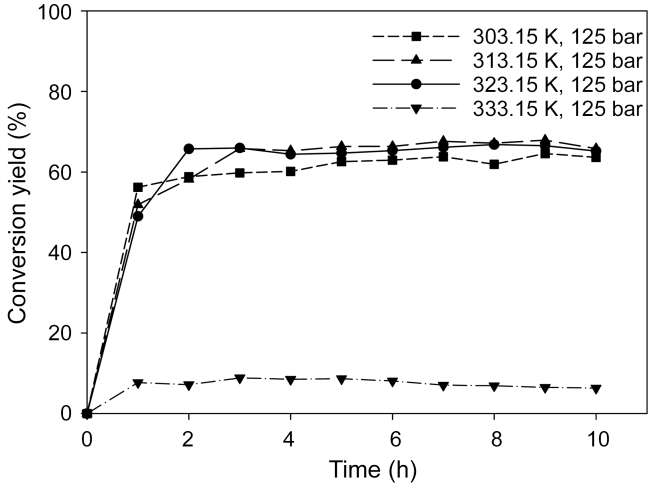


**Fig. 4.** Production of (*S*)-naproxen ethyl ester from *Candida antarctica* lipase B with increasing temperature at 125 bar.

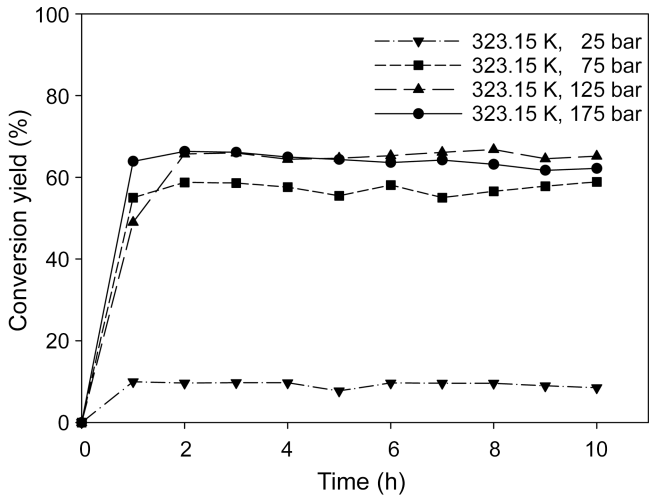


**Fig. 5.** Production of (*S*)-naproxen ethyl ester from *Candida antartica* lipase B by increasing pressure at 323.15 K.

transesterification reaction catalyzed by Novozyme 435 in $SCCO_2$.

The effect of temperature was studied in the range from 303.15 to 323.15 K at constant pressure (125 bar); the experimental results are shown in Fig. 4. The conversion of (*S*)-naproxen ethyl ester increased with temperature at constant pressure, but the productivity of the reaction was reduced at temperatures above 323.15 K. Above 323.15 K, the conversion yield was decreased significantly owing to decreased enzyme activity at high temperature.

The effect of pressure was also studied over the range of 25 to 175 bar at 323.15 K, and the experimental results are shown in Fig. 5. As shown in this figure, the conversion yield was increased as the pressure increased, but the effect of increasing pressure diminished above 125 bar. To compare the conversion yield at ambient pressure (1 bar), we also performed a separate experiment with extended reaction time. The conversion yields of (*S*)- and (*R*)-naproxen ethyl esters at atmospheric pressure for 216 h are shown in Fig. 6A. The maximum conversion was 9.9% in ambient condition. Consequently, a higher enantiomeric excess of product ($ee_p$), 59.1%, was obtained under optimized condition (325.15 K, 130 bar).

As shown in Fig. 6B, the maximum conversion yield in supercritical state was 67.38% over 3 h. Thus, it can be concluded that the reaction proceeded more rapidly and more favorably in supercritical condition than that in ambient condition.

## Determination of Optimum Reaction Conditions Using Statistical Analysis

The optimum reaction condition was found using RSM as described in a previous section. The experimental data of (*S*)-naproxen ethyl ester production were obtained over the temperature range from 303.15 K to 343.15 K and the

**Table 2.** Statistical analysis for the model of (*S*)-naproxen ethyl ester conversion at different conditions of temperature and pressure.

| Source | Sum of squares | Degrees of freedom | Mean square | *F*-value | *P*>*F* |
|---|---|---|---|---|---|
| Model | 403.044 | 5 | 80.609 | 1.21 | 0.4193 |
| Error | 332.802 | 5 | 66.560 | | |
| Corrected total | 735.845 | 10 | | | |

Coefficient of variation (CV)=14.06%; Coefficient of determination ($R^2$)=0.548.

| Model term | Parameter estimate | Degree of freedom | Computed t | P (P>\| t \|) |
|---|---|---|---|---|
| Intercept | 61.5315 | | 14.70 | <.0001 |
| $X_1$ | −4.025 | 1 | −1.71 | 0.1481 |
| $X_2$ | 1.925 | 1 | 0.82 | 0.4509 |
| $X_1*X_2$ | −0.175 | 1 | −0.04 | 0.1909 |
| $X_{11}$ | −2.808 | 1 | −1.51 | 0.8397 |
| $X_{22}$ | −0.396 | 1 | 1.86 | 0.9674 |

pressure range from 75 bar to 175 bar. The coefficients of regression equation are obtained as follows;

$$y=61.532-4.025x_1+1.925x_2-2.808x_1^2-0.396x_2^2 -0.175x_1x_2 \quad (3)$$

Analysis of variance (ANOVA) for the selected model is shown in Table 2. The model was statistically analyzed with Fisher's statistical test for ANOVA, and the results of various statistical parameters are shown in Table 2. Based on the results of ANOVA, the isoresponse mesh and the circular contour plot of the calculated response surface were obtained as shown in Fig. 7A and 7B. The optimal points for maximum (*S*)-naproxen ethyl ester production were 325.15 K ($x_1$=0.21) and 130 bar ($x_2$=0.23), and the predicted maximum value of (*S*)-naproxen ethyl ester production was 65.65% (Table 3). A higher enantiomeric excess of product ($ee_p$), 59.1%, was obtained under optimum condition (325.15 K, 130 bar). This obtained optimum condition was used for the final verification experiment and the conversion yield was measured as 67.38%, a close value compared with that predicted.

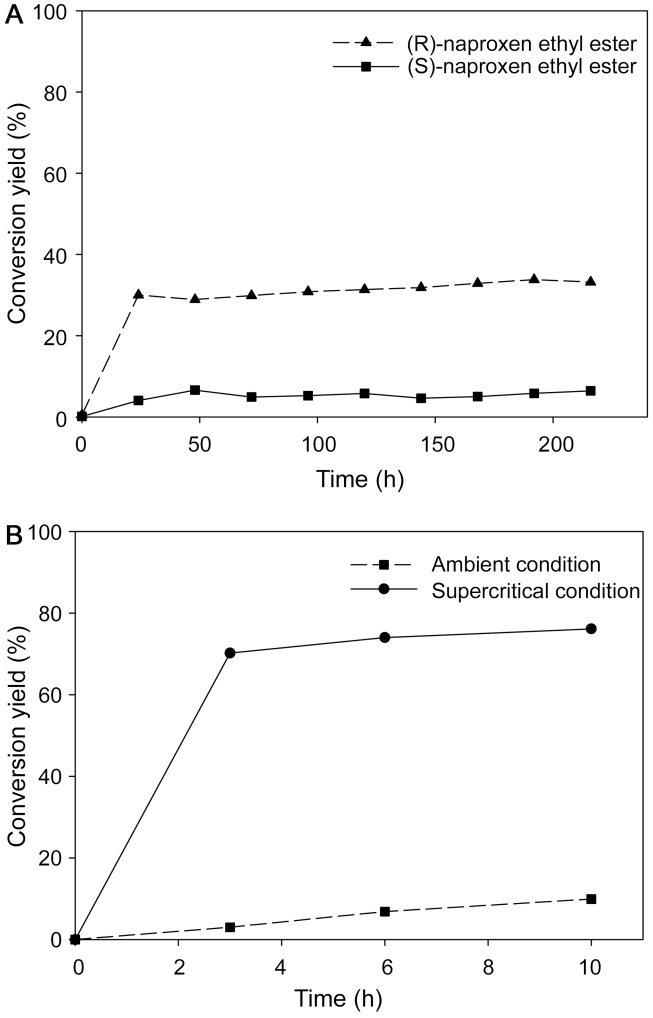


**Fig. 6. A**. Esterification of (*S*)-naproxen using enantioselective lipase at 323.15 K and atmospheric pressure; and **B**. Comparison of experimental results under ambient conditions (323.15 K, atmospheric pressure) and supercritical conditions (323.15 K, 130 bar).

**Effect of the Amount of Enzyme and Water**

The contents of enzyme and water are also important factors affecting the enzymatic-catalyzed reactions. As discussed in Cui *et al.* [5], in the presence of higher amounts of

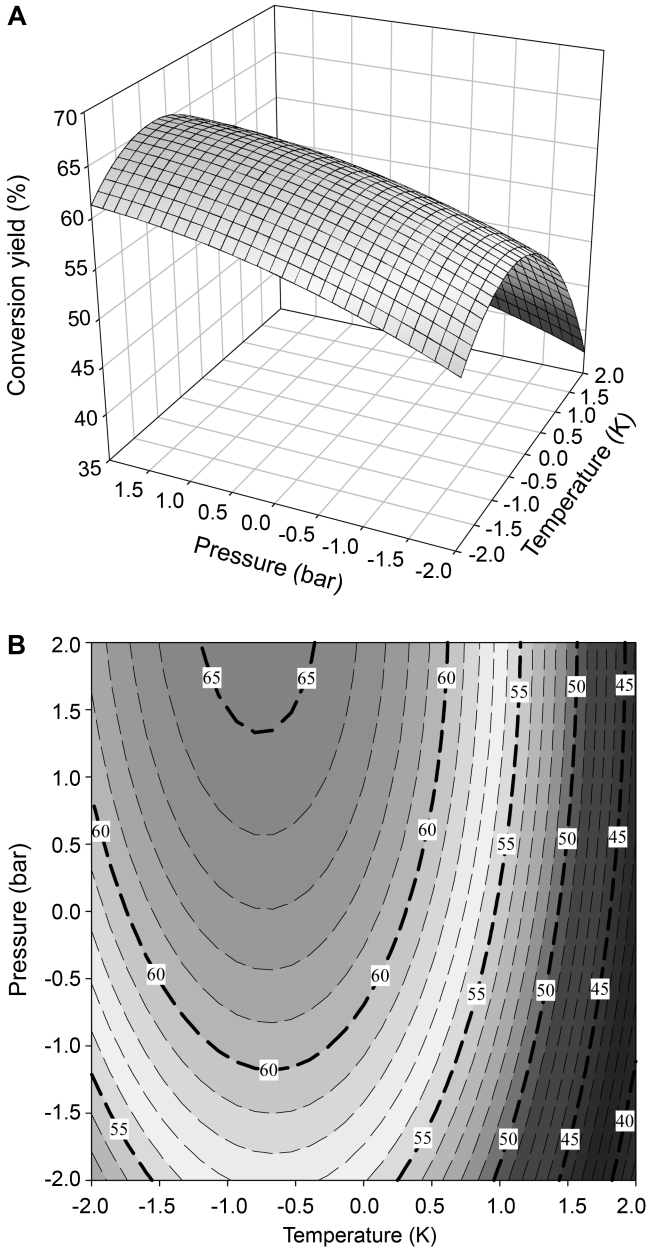


**Fig. 7.** Isoresponse mesh (**A**) and contour plot (**B**) of the calculated response surface on the naproxen ethyl ester conversion yield.

**Table 3.** The optimum condition for the reaction, and comparison of the predicted value with the experimental value.

| | Temperature | Pressure |
|---|---|---|
| Optimum point | 325.15 K | 130 bar |
| Coded value | 0.21 | 0.23 |
| Maximum value (conversion yield) | Predicted value 65.65% | Experimental value 67.38% |

enzyme, the conversion yield of the esterification reaction becomes higher. Fig. 8A represents the conversion yield when varying amounts (1 to 11 grams) of enzymes were used. A high conversion yield was obtained by increasing the amount of enzyme, as expected. The average conversion yields with increasing amounts of enzyme are shown in Fig. 8B. The conversion yields obtained when increasing the amounts of enzyme from 1 g to 11 g were 10.0%, 36.8%, 66.3%, 78.3%, 82.3%, and 83.1%, respectively.

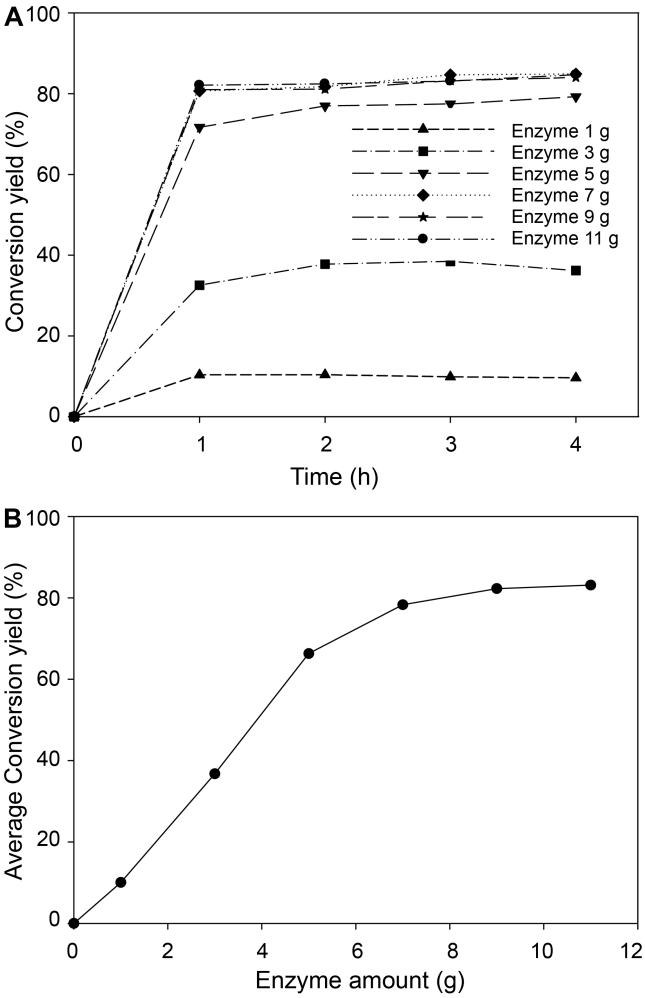


**Fig. 8. A**. Esterification of (*S*)-naproxen with different amounts of enzyme under optimized conditions; and **B**. Average conversion yield with increasing amounts of enzyme.

A small amount of water is also necessary for the catalytic function of enzymes because it participates, directly or indirectly, in all interactions that maintain the conformation of the catalytic site of the enzyme [8]. On the other hand, in esterification/ hydrolysis reactions, the water content is also known to affect the equilibrium conversion of the reaction, as well as the distribution of products in the media [1]. Some authors agree that optimum water contents exist and generally range from 0.2–3% [10, 18]. The conversion yields when changing the water content from 1% to 5%, under optimized conditions, are shown in Fig. 9. The conversion yield did not vary significantly within 1–5% in our observations. The conversion yields of all water content systems ranged from 70–80%, but the conversions in the 1–3% range were a little higher than those in the 4–5% range. Based on these results, 2% water content was selected as the optimum.

## Discussion

A lipase-catalyzed enantioselective esterification process in supercritical carbon dioxide was examined for the synthesis of (*S*)-naproxen ethyl ester. The effects of temperature and pressure were also studied over a temperature range of 313.15–333.15 K and a pressure range of 50–175 bar. Both variables have significant effects on (*S*)-naproxen ethyl ester production. The reaction time for optimal conversion yield of (*S*)-naproxen ethyl ester production was estimated by 3 h in supercritical carbon dioxide. The production condition for (*S*)-naproxen ethyl ester was optimized through statistical analysis using RSM and is reported as 325.15 K and 130 bar. The productivity of (*S*)-naproxen ethyl ester increased with temperature at constant pressure, but the productivity of the reaction was reduced

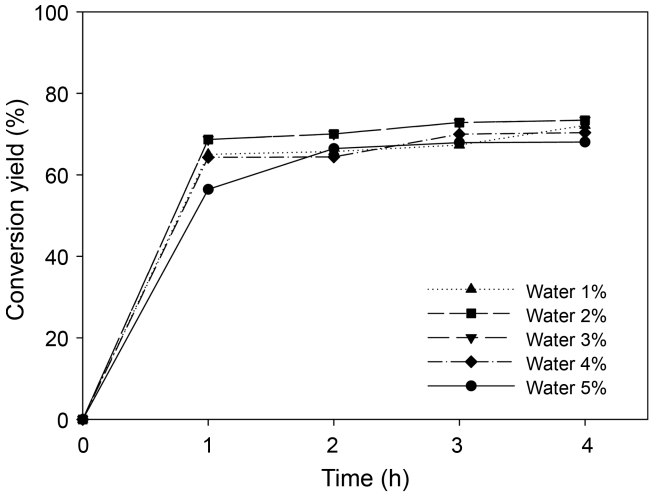


**Fig. 9.** Conversion yields with increasing water content under optimized conditions.

at temperatures above 323.15 K. The conversion yield was increased as the pressure increased, but the effect of increasing pressure diminished above 125 bar. Based on these experimental results, the maximum conversion was 9.9% over 216 h in ambient condition and 67.38% over 3 h only in supercritical state. Besides this, 7 g enzyme amount and 2% water content were selected as optimum conditions. Compared with ambient conditions, the reaction proceeds rapidly and effectively in supercritical carbon dioxide, as a result.

## Acknowledgment

The authors gratefully acknowledge financial support from the Korea Ministry of Commerce, Industry and Energy and the Korea Energy Management Corporation.

### REFERENCES

1. Basheer, S., K. Mogi, and M. Nakajima. 1995. Surfactant-modified lipase for the catalysis of the interesterification of triglycerides and fatty acids. *Biotechnol. Bioeng.* **45:** 187–195.
2. Chang, C. S. and S. W. Tsai. 1997. A facile enzymatic process for the preparation of (*S*)-naproxen ester prodrug in organic solvents. *Enzyme Microb. Technol.* **20:** 635–639.
3. Chi, Y. M., K. Nakamura, and T. Yano. 1988. Enzymic interesterification in supercritical carbon dioxide. *Agric. Biol. Chem.* **52:** 1541–1550.
4. Chiang, K. T. 2007. Modeling and optimization of designing parameters for a parallel-plain fin heat sink with confined impinging jet using the response surface methodology. *Appl. Therm. Eng.* **27:** 2473–2482.
5. Cui, Y.-M., D.-Z. Wei, and J.-T. Yu. 1997. Lipase-catalyzed esterification in organic solvent to resolve racemic naproxen. *Biotechnol. Lett.* **19:** 865–868.
6. Dominguez, A. P. M., D. Bizani, F. Cladera-Olivera, and A. Brandelli. 2007. Cerein 8A production in soybean protein using response surface methodology. *Biochem. Eng. J.* **35:** 238–243.
7. Franck, E. U. and R. Deul. 1978. Dielectric behavior of methanol and related polar fluids at high pressures and temperatures. *Faraday Discuss. Chem. Soc.* **66:** 191–198.
8. Goldberg, M., D. Thomas, and M. D. Legoy. 1990. The control of lipase-catalysed transesterification and esterification rates. Effects of substrate polarity, water activity and water molecules on enzyme activity. *Eur. J. Biochem.* **190:** 603–609.
9. Hayball, P. J. 1996. Chirality and nonsteroidal anti-inflammatory drugs. *Drugs* **52:** 47–58.
10. Iso, M., B. Chen, M. Eguchi, T. Kudo, and S. Shrestha. 2001. Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase. *J. Mol. Catal. B Enzym.* **16:** 53–58.
11. Junco, S., T. Casimiro, N. Ribeiro, M. D. Ponte, and H. M. Marques. 2002. Optimisation of supercritical carbon dioxide systems for complexation of naproxen: Beta-Cyclodextrin. *J. Incl. Phenom. Macrocycl. Chem.* **44:** 69–73.
12. Kamat, S. V., E. J. Beckman, and A. J. Russell. 1995. Enzyme activity in supercritical fluids. *Crit. Rev. Biotechnol.* **15:** 41–71.
13. Kwon, C. H., D. Y. Shin, J. H. Lee, S. W. Kim, and J. W. Kang. 2007. Molecular modeling and its experimental verification for the catalytic mechanism of *Candida antarctica* lipase B. *J. Microbiol. Biotechnol.* **17:** 1098–1105.
14. Lim, J. S., M. C. Park, J. H. Lee, S. W. Park, and S. W. Kim. 2005. Optimization of culture medium and conditions for neo-fructooligosaccharides production by *Penicillium citrinum*. *Eur. Food Res. Technol.* **221:** 639–644.
15. Mundra, P., K. Desai, and S. S. Lele. 2007. Application of response surface methodology to cell immobilization for the production of palatinose. *Bioresour. Technol.* **98:** 2892–2896.
16. Nnakamura, K., Y. M. Chi, Y. Yamada, and T. Yano. 1986. Lipase activity and stability in supercritical carbon dioxide. *Chem. Eng. Commun.* **45:** 207–212.
17. Ranucci, E., L. Sartore, I. Peroni, R. Latini, R. Bemasconi, and P. Ferruti. 1994. Pharmacokinetic results on naproxen prodrugs based on poly(ethyleneglycol)s. *J. Biomater. Sci. Polym. Ed.* **6:** 141–147.
18. Rocha, J., M. Gil, and F. Garcia. 1999. Optimisation of the enzymatic synthesis of noctyl oleate with immobilised lipase in the absence of solvents. *J. Chem. Technol. Biotechnol.* **74:** 607–612.
19. Shanbhag, V. R., A. M. Crider, R. Gokhale, A. Harpalani, and R. M. Dick. 1992. Ester and amide prodrugs of ibuprofen and naproxen: Synthesis, anti-inflammatory activity, and gastrointestinal toxicity. *J. Pharm. Sci.* **81:** 149–154.
20. Shang, C. S. and C. S. Hsu. 2003. Lipase-catalyzed enantioselective esterification of (*S*)-naproxen hydroxyalkyl ester in organic media. *Biotechnol. Lett.* **25:** 413–416.
21. Shieh, C. J., H. F. Liao, and C. C. Lee. 2003. Optimization of lipase-catalyzed biodiesel by response surface methodology. *Bioresource Technol.* **88:** 103–106.
22. Sunitha, K., J. K. Lee, and T. K. Oh. 1999. Optimization of medium components for phytase production by *E. coli* using response surface methodology. *Bioprocess. Biosyst. Eng.* **21:** 477–481.
23. Tammara, V. K., M. M. Narurkar, A. M. Crider, and M. A. Khan. 1993. Synthesis and evaluation of morpholinoalkyl ester prodrugs of indomethacin and naproxen. *Pharm. Res.* **10:** 1191–1199.
24. Tsai, S. W., S. F. Lin, and C. S. Chang. 1999. Lipase-catalyzed enantioselective esterification of *S*(+)-naproxen ester prodrugs in cyclohexane. *J. Chem. Technol. Biotechnol.* **74:** 751–758.
25. Wu, J.-Y. and M.-T. Liang. 1999. Enhancement of enantioselectivity by altering alcohol concentration for esterification in supercritical $CO_2$. *J. Chem. Eng. Japan* **32:** 338–340.
26. Yasmin, T., T. Jiang, B. Han, J. Zhang, and X. Ma. 2006. Transesterification reaction catalysed by Novozym 435 in supercritical carbon dioxide. *J. Mol. Catal. B Enzym.* **41:** 27–31.