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# Transesterification reaction catalysed by Novozym 435 in supercritical carbon dioxide

Tahmina Yasmin, Tao Jiang *, Buxing Han **, Jicheng Zhang, Xiumin Ma

*Beijing National Laboratory for Molecular Sciences (BNLMS), Center for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China*

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## Abstract

Transesterification reaction between a substrate having two functional groups (ethylene glycol) with ethyl acetate in the presence of lipase Novozym 435 was carried out to study the effect of supercritical $CO_2$ ($scCO_2$) on the conversion and selectivity of the reaction. It was demonstrated that in the presence of $scCO_2$ the equilibrium conversion could be considerably higher than that in the absence of $scCO_2$. $scCO_2$ could also enhance the selectivity of ethylene glycol monoacetate (EGMA) and suppress the formation of ethylene glycol diacetate (EGDA), and selectivity could be tuned by $CO_2$ pressure.

*Keywords:* Supercritical; Carbon dioxide; Enzyme; Lipase; Novozym 435; Ethylene glycol; Ethyl acetate; Transesterification

## 1. Introduction

Biocatalysts have found widespread applications in organic chemistry over the last decade. Lipases are triacylglycerol hyrolases enzymes capable of catalyzing hydrolysis reactions of esters as well as their synthesis and transesterification. They can catalyze reactions in organic solvents [1,2] supercritical fluids (SCFs) [3–5], and ionic liquids (ILs) [6–8].

Non-conventional solvents are receiving more and more attention in biocatalysis. Solvents can modify the conformation of enzyme and hence altering its catalytic efficiency or specificity, which may increase regio- and enantioselectivity of reactions and also increase its stability. Furthermore, they can reduce the risk of microbial contamination in some cases [9]. Supercritical $CO_2$ ($scCO_2$), which is a promising alternative of conventional solvents, possesses many useful characteristics, such as nontoxic, non-flammable, inexpensive, available in large quantities, tunable solvent and solvation properties, and $CO_2$ has moderate critical temperature and pressure (31.1 °C and 7.38 MPa) [10]. Recently, Tewari et al. [11] carried out transesterification reaction with benzyl alcohol and butyl acetate in $scCO_2$ and found that the equilibrium constant of the reaction was 30% smaller than that of the reaction in *n*-hexane or toluene. However, the reaction proceeded more rapidly in $scCO_2$ than in *n*-hexane, toluene or in the absence of solvent. Romero et al. [3] studied the transesterification reaction of isoamyl acetate with acetic anhydride in the presence of immobilized *Candida antarctica* lipase in $scCO_2$ and in *n*-hexane. The esterification extent was found to be similar in both solvents, but the initial reaction rate was high in $scCO_2$. Madras and co-workers [12] reported the synthesis of commercially important flavor esters of isoamyl alcohol using hog pancreas lipase. The overall conversion under solvent-free conditions was lower than that in $scCO_2$ at similar enzyme concentrations. Zhang et al. [13] carried out the esterification of acetic acid with ethanol in the presence of ionic liquid 1-butyl-3-methylimidazolium hydrogen sulphate ([bmim][$HSO_4$]) and $scCO_2$. They found that $CO_2$ could induce phase separation of the reaction system and the conversion was different in different phase regions.

Up to now, most of the reversible reactions studied were performed with substrates containing only one functional group. To our knowledge, only one paper reported the etherification using chemicals with more functional groups [14]. The work described the effect of pressure on the formation of mono-ether

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[*] Corresponding author. Tel.: +86 10 62562821; fax: +86 10 62559373.
[**] Corresponding author.
*E-mail addresses:* Jiangt@iccas.ac.cn (T. Jiang), Hanbx@iccas.ac.cn (B. Han).

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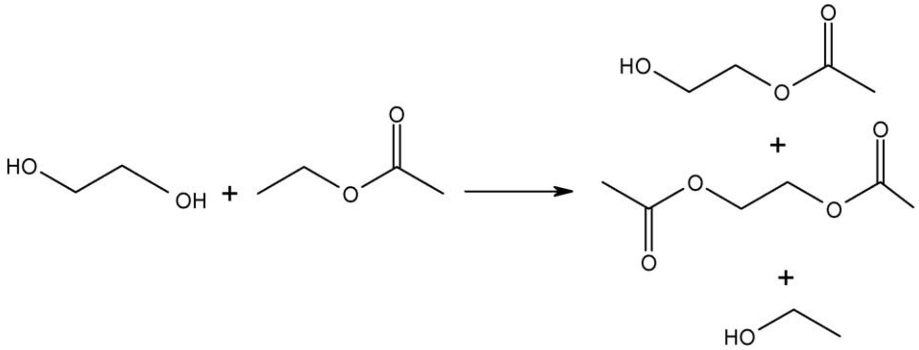

Scheme 1. Chemical reaction between ethylene glycol and ethyl acetate.

and bis-ether from 1, *n*-terminal diols ($n = 3$ or 6) with simple alcohols in $scCO_2$ over an acid catalyst (Amberlist 15). However, study on the transesterification with substrates having more than one functional group is of importance for providing more information about the effect of $scCO_2$ on chemical equilibrium and product distribution. In this paper we studied the transesterification (Scheme 1) of ethylene glycol (EG) with ethyl acetate (EA) using *C. antarctica* lipase (Novozym 435) as a catalyst in $scCO_2$ to investigate the effect of solvent properties on the conversion and selectivity.

## 2. Experimental

### 2.1. Materials

Ethyl acetate, ethylene glycol and *N*,*N*-dimethyl formamide having purities of 99.5, 96 and 99.5%, respectively, were obtained from Beijing Chemical Reagent Plant and were used without further purification. Lipase (Novozym 435) was purchased from Novozymes in Beijing. $CO_2$ with 99.995% purity was obtained from Beijing Analytical Instrument Factory.

### 2.2. Apparatus and procedures

The reaction was performed in a 12 mL stainless steel batch reactor. The reactor was first loaded with reactants and enzyme, immersed in a water bath of desired temperature, which was controlled with a temperature controller (Haake D3) with an accuracy of $\pm 0.1\,^{\circ}C$. $CO_2$ was compressed into the system up to a desired pressure. The pressure gauge was composed of a pressure transducer (FOXBORO/ICT Model 93) and an indicator. Its accuracy was $\pm 0.025$ MPa in the pressure range 0–20 MPa. After the desired reaction time the reactor was rapidly cooled with ice water and depressurized by releasing the gas slowly through an absorbing tube containing 6 mL *N*,*N*-dimethyl formamide to capture the reactants and products entrained by $CO_2$. When depressurization was complete the reactor was opened and *N*,*N*-dimethyl formamide in the absorbing tube was added into the reactor and the reaction mixture was centrifuged to separate the enzyme. The reaction mixture was then analyzed by gas chromatography (Agilent 4890D) with nitrogen as a carrier gas. Reactions without $CO_2$ were carried out in the same stainless steel batch reactor at the desired temperature.

## 3. Results and discussions

### 3.1. Effect of enzyme concentration with and without solvent

The effect of enzyme concentration on the conversion was studied at solvent-free condition and in $scCO_2$ in the enzyme concentration range from 1 to 11 g of enzyme/mole of EG. The results are shown in Fig. 1. The conversion increases with increasing enzyme concentration from 1 to 7 g/mole of EG in both cases. The main reason is that at the lower enzyme concentration active sites of the enzyme are not enough and the reaction rate is controlled by number of the active sites of the enzyme. As the enzyme concentration is high enough the diffusion steps become dominant. Results in Fig. 2 indicate that the selectivity is sensitive to enzyme concentration when enzyme is below 7 g/mole of EG, and the selectivity is nearly constant at higher enzyme concentration at solvent-free condition. But the selectivity does not change with enzyme concentration in $scCO_2$. Based on the results above, a concentration of 7 g enzyme/mole of EG was selected for the following work.

### 3.2. Effect of reaction time

By keeping the EG:EA mole ratio at 1:3 the effect of reaction time on conversion and selectivity with and without $scCO_2$

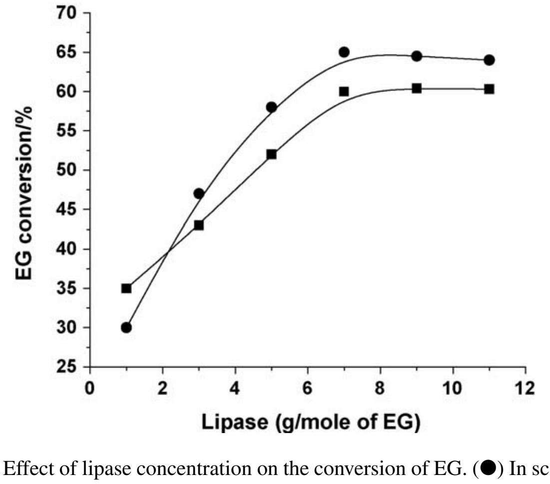

Fig. 1. Effect of lipase concentration on the conversion of EG. (●) In $scCO_2$ at 10 MPa; (■) solvent-free EG:EA mole ratio 1:3, 50 °C, 1 h.

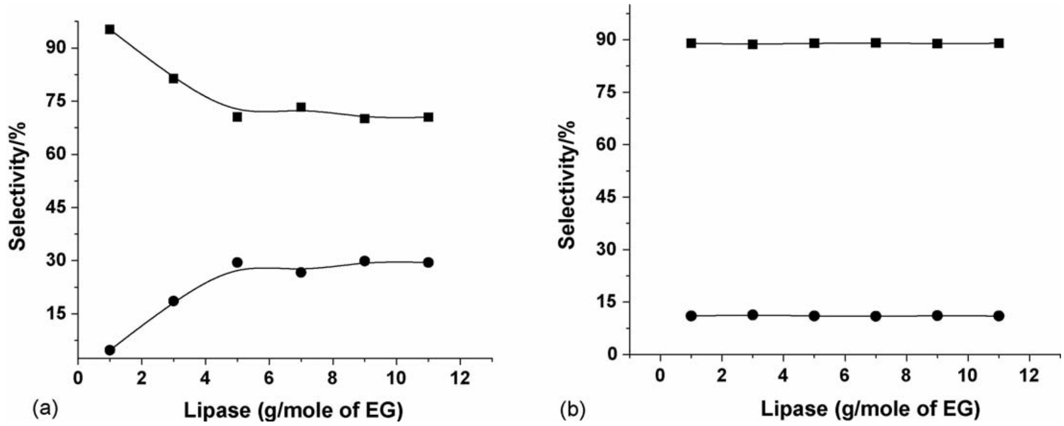

Fig. 2. Effect of lipase concentration on the selectivity (selectivity in all experiments was calculated as the percentage of number of moles of EGMA or EGDA in the total number of moles of the two species). (a) Solvent-free; (b) 10 MPa $CO_2$; (■) ethylene glycol monoacetate (EGMA); (●) ethylene glycol diacetate (EGDA); EG:EA mole ratio 1:3; 50 °C; 1 h.

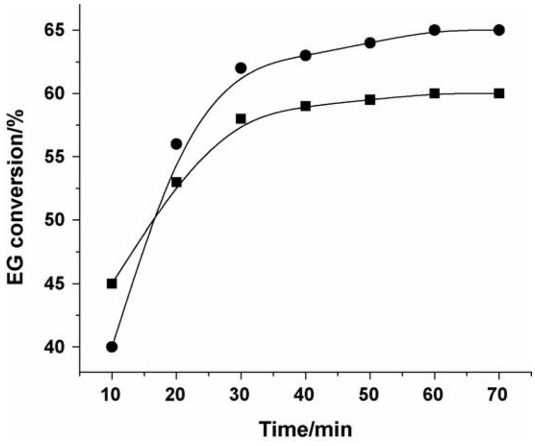

Fig. 3. Effect of reaction time on the conversion of EG. (●) In $scCO_2$ at 10 MPa; (■) solvent-free; EG:EA mole ratio 1:3, 7 g lipase/mole of EG, 50 °C.

was investigated, and the results are illustrated in Figs. 3 and 4, respectively. It can be known from the figures that reaction equilibrium can be reached after 60 min. In the presence of $CO_2$, the equilibrium conversion is higher than that in the absence of $CO_2$. The equilibrium conversion is 65% in $scCO_2$, while that is 60% at solvent-free condition. The selectivity to ethylene glycol monoacetate (EGMA) decreases with increasing reaction time in both cases. However, the selectivity to EGMA is considerably higher in the presence of $scCO_2$ than that in the absence of $scCO_2$. In other words, the conversion of EGMA into ethylene glycol diacetate (EGDA) is suppressed when the reaction is carried out in $scCO_2$. One of the reasons may be that some EGMA formed exists in the $CO_2$-rich phase, while the enzyme is in the liquid phase, which is favorable to producing EGMA. $CO_2$ may also influence the properties of the enzyme, which makes the enzyme more selective to EGMA.

### 3.3. *Effect of $CO_2$ pressure*

Effect of $CO_2$ pressure was studied in the pressure range of 8–12 MPa, and the results are given in Figs. 5 and 6. The conversion of EG increases with pressure in low-pressure range, but further increase in pressure reduces the EG conversion. This

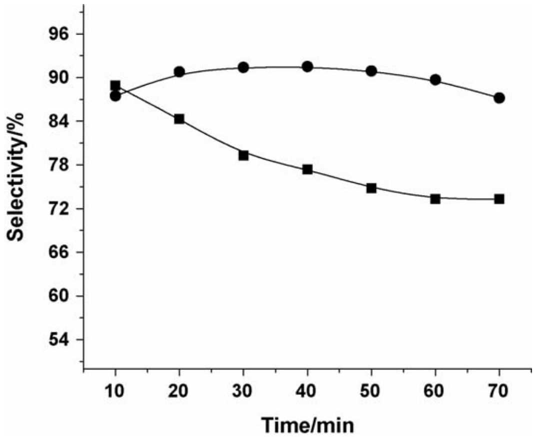

Fig. 4. Effect of reaction time on the selectivity to EGMA. (●) In $scCO_2$ at 10 MPa; (■) solvent-free; EG:EA mole ratio 1:3, 7 g lipase/mole of EG, 50 °C.

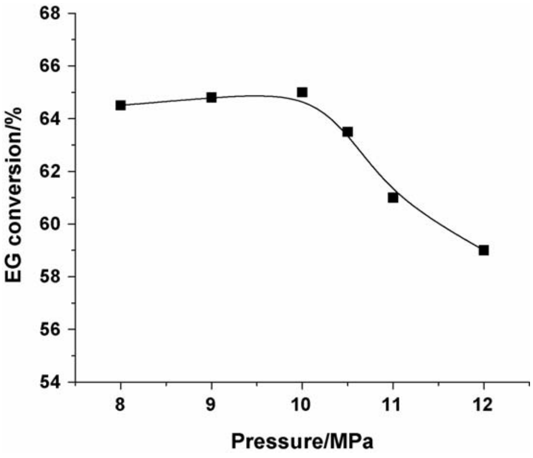

Fig. 5. Effect of pressure on EG conversion. EG:EA mole ratio 1:3, 7 g lipase/mole of EG, 50 °C.

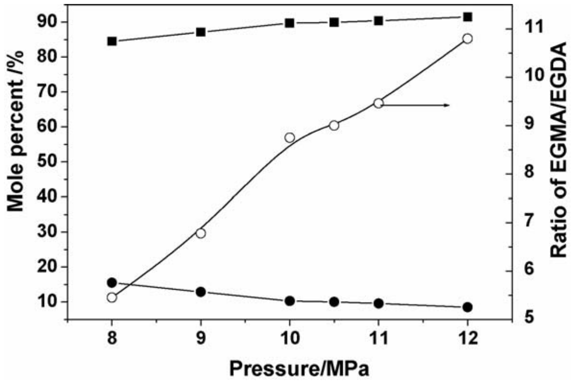

Fig. 6. Effect of pressure on the selectivity. (■) EGMA; (●) EGDA; EG:EA mole ratio 1:3, 7 g lipase/mole of EG, 50 °C.

can be partially explained on the basis of phase behavior of the reaction system determined in this work using an optical cell [15]. The total composition in the system in the phase behavior measurement was the same as that in the reactor. The results indicated that below 10 MPa there were two phases, a liquid phase and a $scCO_2$-rich phase, and there was one phase in the system as pressure was higher than 10 MPa. In the two-phase region, with increasing pressure the solubility of the products in $scCO_2$ phase increases, which is favorable to enhancing the conversion and the conversion increases as pressure rises. The conversion reached maximum at the phase transition. In the one-phase region, all the components exist in one phase. It is well known that properties of $scCO_2$ change with pressure. Therefore, the intermolecular interaction in the reaction system changes with pressure, which results in the variation of conversion with pressure.

Fig. 6 indicates that the selectivity to EGMA increases and that to EGDA decreases with the increasing pressure, and the ratio of EGMA to EGDA increases considerably with increasing pressure. In other words, the selectivity of the product can be tuned by changing $CO_2$ pressure. This is easy to understand because, as discussed above, some EGMA produced can be extracted into $scCO_2$ phase, which avoids further reaction of EGMA. The solvent power of $CO_2$ is stronger at the higher pressure, and selectivity to EGMA is higher.

### 3.4. *Effect of temperature*

The effect of temperature was studied in 30–70 °C range in $scCO_2$ at 10 MPa and solvent-free condition, and the results are listed in Table 1. It is shown that EG conversion and selectivity to EGDA increase with increasing temperature at solvent-free condition. The selectivity to EGMA is relatively high at the lower temperatures, and more and more EGMA is converted to EGDA with temperature increasing. The selectivity to EGMA in $scCO_2$ is higher than at solvent-free condition i.e., $CO_2$ can enhance the formation of EGMA. In $scCO_2$, the effect of temperature on the conversion and selectivity is not significant, the reason for this phenomenon is not clear, and further studies need to be conducted in future.

Table 1
Effect of temperature on the conversion of EG and selectivity in $scCO_2$ and solvent-free condition[a]

| *T* (°C) | EG conversion (%) | | Selectivity (%) | | | |
|---|---|---|---|---|---|---|
| | In $scCO_2$ (10 MPa) | Solvent-free | In $scCO_2$ | | Solvent-free | |
| | | | EGMA | EGDA | EGMA | EGDA |
| 30 | 58.2 | 51.7 | 85.7 | 14.3 | 77.7 | 22.3 |
| 40 | 64.5 | 58.5 | 85.3 | 14.7 | 76.7 | 23.3 |
| 50 | 65.0 | 60.0 | 89.7 | 10.3 | 73.3 | 26.7 |
| 60 | 64.0 | 62.0 | 88.5 | 11.5 | 72.6 | 27.4 |
| 70 | 63.5 | 63.0 | 88.1 | 11.9 | 71.6 | 28.4 |

[a] EG:EA mole ratio 1:3, 7 g lipase/mole of EG, 1 h.

## 4. Conclusions

In this work, we investigated the effect of $scCO_2$ on the transesterification reaction of ethylene glycol with ethyl acetate in the presence of *C. antarctica* lipase (Novozym 435) at different temperatures and pressures. In the presence of $CO_2$, the equilibrium conversion is higher than that in the absence of $CO_2$. At 10 MPa and 50 °C, the equilibrium conversion can be 65%, while at solvent-free condition the equilibrium conversion is 60%. The enzyme selectively facilitates the production of EGMA and suppresses the production of EGDA in the presence of $scCO_2$. The selectivities of EGMA and EGDA are 89.7 and 10.3%, respectively at 10 MPa and 50 °C, while at solvent-free condition the selectivities are 73.3 and 26.7%, respectively. The enhancement of the selectivity is partially attributed to the dissolution of EGMA produced in $scCO_2$.

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