

# Lipase-Catalyzed Synthesis of Fatty Acid Sugar Ester Using Extremely Supersaturated Sugar Solution in Ionic Liquids

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**ABSTRACT:** The low solubility of sugars has hampered the lipase-catalyzed synthesis of fatty acid sugar esters in organic solvents and ionic liquids (ILs), because several solvents that are able to effectively dissolve sugars are detrimental to enzymes. In this work, in order to prepare a high concentration of sugars in ILs, we have developed a new procedure that entails mixing an aqueous sugar solution into ILs followed by removal of the water from the solution. The glucose concentrations in the supersaturated [Emim][TfO] and [Bmim][TfO] were 19 and 10 times higher, respectively, than the solubilities (6.1 and 4.8 g/L) of glucose in the ILs at 25°C. Furthermore, the supersaturated glucose solutions in ILs were maintained over a long period of time without any significant loss of glucose. In ILs that were extremely supersaturated with glucose, lipase-catalyzed esterifications of glucose with vinyl laurate, and lauric acid were successfully carried out. The conversion increased from 8% to 96% at 1 day of reaction by using supersaturated solution in [Bmim][TfO] which had dissolved glucose concentration of 400% higher than its solubility, compared with the reaction using saturated glucose solution. By making the glucose concentration in ILs much higher than the solubility through our novel and simple method, the initial rate and conversion of the lipase-catalyzed reaction were significantly improved.

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**KEYWORDS:** ionic liquid; lipase; sugar ester; supersaturation; water

## Introduction

Fatty acid sugar esters offer a wide range of commercial applications (pharmaceuticals, cosmetics, food industry, etc.) due to their surfactant properties (Flores et al., 2002). While most of these compounds are chemically synthesized, the enzymatic synthesis of sugar esters has garnered considerable interest with regard to the creation of environment-friendly processes (Liu et al., 2005). However, a major problem in synthesizing sugar esters by using enzymes in non-aqueous media is the selection of an appropriate solvent to dissolve sugars. To dissolve sugars such as glucose, sucrose, fructose, etc., hydrophilic organic solvents are the preferred reaction media. However, most enzymes are quickly inactivated under hydrophilic organic solvents. Therefore, a partially dissolved or a solid-phase system or a metastable supersaturated solution can be used for the enzymatic reaction in less harmful organic solvents such as acetonitrile, acetone, *t*-butanol, and 2-methyl 2-butanol (Degn et al., 1999; Flores et al., 2002; Zhang and Hayes, 1999). Alternatively, the solubility can be increased by using protected glucoses or alkyl glycosides. However, the use of these substrates requires extra steps; otherwise the products show different properties compared to non-derivatized glucose esters (Ganske and Bornscheuer, 2005a).

Ionic liquids (ILs) are organic salts that melt below 100°C. Interest in ILs stems from their potential as “green solvents” (Sheldon et al., 2002). Specifically, their non-volatile character and thermal stability make them attractive alternatives for volatile organic solvents. In chemical processes, ILs exhibit excellent physical characteristics including the ability to dissolve polar and non-polar organic, inorganic, and polymeric compounds. Moreover, the combinations of anions

Abbreviations: [Emim][MS], 1-ethyl-3-methylimidazolium methylsulfate; [Emim][TfO], 1-ethyl-3-methylimidazolium trifluoromethanesulfonate; [Bmim][TfO], 1-butyl-3-methylimidazolium trifluoromethanesulfonate; [Emim][BF<sub>4</sub>], 1-ethyl-3-methylimidazolium tetrafluoroborate; [Bmim][BF<sub>4</sub>], 1-butyl-3-methylimidazolium tetrafluoroborate; [Omim][BF<sub>4</sub>], 1-octyl-3-methylimidazolium tetrafluoroborate; [Bmim][PF<sub>6</sub>], 1-butyl-3-methylimidazolium hexafluorophosphate.

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and cations encompassed by ILs are vast. In addition, owing to their associated synthetic flexibility, ILs are referred to as “designer solvents” (Freemantle, 1998).

Several groups have reported that ILs can be used as an alternative reaction media for biocatalysis. It was observed that their use enhanced the reactivity, selectivity, and stability of enzymes (Kragl et al., 2002; Lee and Kim, 2002; Park and Kazlauskas, 2003). Spear and coworkers reported the high solubility of various mono and disaccharides in ILs containing  $[\text{Cl}]^-$  (Murugesan and Linhardt, 2005). However, ILs containing  $[\text{Cl}]^-$  cannot be used to carry out enzyme reaction due to the inactivation of most enzymes (Lee et al., 2006). Only ILs containing the dicyanamide ( $[\text{dca}]$ ) anion have been reported to be good solvents for sugar dissolution and enzyme reaction, but *Candida antarctica* lipase B irreversibly deactivated in  $[\text{Bmim}][\text{dca}]$  after reaction (Liu et al., 2005; van Rantwijk et al., 2006). Recently, pure  $[\text{Bmim}][\text{BF}_4]$  and  $[\text{Bmim}][\text{PF}_6]$  were used as reaction media in the lipase-catalyzed transesterification of glucose with fatty acid vinyl ester. However, the solubility of glucose in these ILs is very low (Ganske and Bornscheuer, 2005a).

In this study, we investigated the solubility of sugars in various ILs that can perform enzyme reactions and prepared a supersaturated solution in ILs to increase the dissolved content of sugar. Lipase-catalyzed esterifications of glucose were also performed with a supersaturated glucose solution in ILs.

## Materials and Methods

### Materials

Novozym 435 (*C. antarctica* type B lipase immobilized on acrylic resin) was provided by Novo Nordisk (Bagsvaerd, Denmark). All ILs were synthesized and purified by C-TRI (Suwon, Korea) and had a residual chloride content of less than 30 ppm. ILs were dried in a vacuum oven at 60°C for 3 days before use. Glucose, fructose, sucrose, vinyl laurate, and lauric acid were purchased from Sigma (St. Louis, MO). All other chemicals used in this work were of analytical grade and used without further purification.

### Solubility of Sugar

Glucose (250 mg), fructose (250 mg), and sucrose (50 mg) were added to glass vials containing ILs (1 mL), respectively. The suspension was stirred for 12 h at 25°C, 50°C, and 60°C. After centrifugation for 5 min at 14,000 rpm, supernatant was obtained to determine sugar content. The glucose and fructose concentration were determined by the dinitrosalicylic acid (DNS) method (Miller, 1959) with a glucose and fructose standard. To measure the concentration of sucrose, it was first hydrolyzed by adding 1N HCl solution (0.2 mL) to sample (1 mL) and heating for 10 min and then analyzed by the DNS method. The interference of ILs in the analytical

process was also carefully investigated and no influence of ILs on the DNS methods in the measured concentration range (0.1–1.0 g/L) was observed.

### Supersaturated Sugar Solution by Conventional Method

Glucose (250 mg), fructose (250 mg), and sucrose (50 mg) were added to glass vials containing ILs (1 mL) and the suspension was stirred for 12 h at 60°C. The saturated solution in the presence of excess sugar was slowly cooled to 25°C and then incubated for 2 h at 25°C. After centrifugation, supernatant was obtained to analyze sugar concentration.

### Supersaturated Sugar Solution by Water-Mediated Method

First, glucose (50 mg), fructose (50 mg), and sucrose (50 mg) were dissolved in water (0.3 mL), respectively. ILs (1 mL) were then added to these solutions at room temperature. In order to dissolve sugars in a quantity exceeding 50 mg, the water content has been increased. After clear solutions were obtained, the water in the mixtures was removed by vacuum evaporation for 12 h at 60°C. The saturated sugar solutions were slowly cooled to 25°C and then incubated for 2 h at 25°C. After centrifugation, the supernatant was obtained to analyze sugar concentration. The residual water content was measured from the weight difference of the solution and confirmed to be lower than 0.1% (w/w) by Karl-Fischer Titration (756 KF Coulometer, Metrohm Ltd., Herisau, Switzerland) using HYDRANAL-Coulomat AK reagent.

### Reaction With Saturated Glucose Solution

Glucose and vinyl laurate (lauric acid for direct esterification) were added to 0.5 mL of IL and stirred for 12 h at 40°C (50°C for direct esterification) to allow the dissolution of glucose. After 12 h, Novozym 435 (50 mg) was added to the mixture. A molecular sieve (4 Å, 15% w/v) was also added to remove water for direct esterification. At the end of the reaction, deionized water (1 mL) was added to the reaction vials in order to remove unreacted glucose. The supernatant was obtained after centrifugation of the reaction media. The concentration of unreacted glucose in the supernatant was determined by the DNS method with a glucose standard. As the solubilities of product (6-O-lauroyl-D-glucose) in water,  $[\text{Bmim}][\text{TfO}]$ , and  $[\text{Bmim}][\text{BF}_4]$  are lower than 0.01 g/L at 25°C, glucose concentrations measured by DNS method were not influenced by the product dissolved in supernatant. The conversion was calculated from the concentration of consumed glucose. The precipitated product (6-O-lauroyl-D-glucose) was dissolved in tetrahydrofuran and the concentration was determined by HPLC analysis to calculate product yield and confirm the conversion determined by remained glucose. The differences of conversion values

which were determined by the concentration of unreacted glucose and produced ester, respectively, were less than 5%.

### Reaction With Supersaturated Glucose Solution

Novozym 435 of 50 mg was added to supersaturated glucose solutions prepared by the conventional and water-mediated method, respectively. A molecular sieve (4 Å, 15% w/v) was also added for direct esterification. The reactions were started by adding vinyl laurate (lauric acid for direct esterification) and the mixtures were incubated in a shaking incubator at 40°C (50°C for direct esterification). At the end of the reaction, the same procedures previously described were used to analyze the concentrations of unreacted glucose and produced ester. For the preparative reaction, 0.2 g D-glucose (1.1 mmol) and 0.58 mL vinyl laurate (2.2 mmol) were reacted with 0.5 g Novozym 435 in 5 mL [Bmim][TfO].

### Structure Determination

<sup>1</sup>H-NMR spectra of 6-O-lauroyl-D-glucose were recorded on a 400 MHz FT-NMR spectrometer (Varian Inova 400, Walnut Creek, CA) in CD<sub>3</sub>OD and DMSO-d<sub>6</sub>/TMS. The results were in accordance to those in the literature (Adelhorst et al., 1990; Ganske and Bornscheuer, 2005a). The molecular weight of the product was analyzed by LC-MS (Varian 1200L). Calculated molecular weight for C<sub>18</sub>H<sub>34</sub>O<sub>7</sub>Na (M + Na<sup>+</sup>) is 385.4 and its measured value was 385.0.

### HPLC Analysis

The concentrations of 6-O-lauroyl-D-glucose were measured by HPLC. Separation was accomplished using a Shimadzu HPLC system (Model LC-10A, Japan) equipped with a reverse-phase C<sub>18</sub> column (SYMMETRY, Waters, Milford, MA) and a RI detector (Waters 410). The mobile phase consisted of methanol/water = 90:10 with a flow rate of 1.0 mL/min. The retention time for 6-O-lauroyl-D-glucose was 4.5 min.

## Results and Discussion

### Solubility of Sugars in ILs

The solubilities of D-(+)-glucose in the various hydrophilic and water miscible ILs at 25, 50, and 60°C are shown in Table I. In the case of ILs containing [Emim]<sup>+</sup>, the solubility of glucose was highly influenced by the anion structure of the ILs. Some researchers have asserted that the high solubility of carbohydrates in ILs containing several anions such as [dca]<sup>−</sup>, Br<sup>−</sup>, and Cl<sup>−</sup> can be attributed to the

**Table I.** Solubility and supersaturated concentration of glucose in ionic liquids.

Solvent	Solubility (g/L)			Concentration in supersaturated solution at 25°C (g/L)	
	25°C	50°C	60°C	Conventional <sup>a</sup>	Water-mediated <sup>b</sup>
<i>tert</i> -Butanol <sup>c</sup>	0.3	— <sup>d</sup>	—	—	—
2-Methyl 2-butanol <sup>c</sup>	—	—	2.6	9.0 <sup>f</sup>	—
[Bmim][dca] <sup>c</sup>	145.0	—	—	—	—
[Emim][MS]	89.6	—	133.2	114.9	>500
[Emim][TfO]	6.1	—	27.8	22.9	113.4
[Bmim][TfO]	4.8	14.2	18.1	17.2	46.3
[Emim][BF <sub>4</sub> ]	1.1	—	4.8	2.6	11.3
[Bmim][BF <sub>4</sub> ]	0.9	2.7	3.5	2.1	6.9
[Omim][BF <sub>4</sub> ]	0.7	—	1.5	1.2	3.7
[Bmim][PF <sub>6</sub> ] <sup>c</sup>	< 0.5	—	—	—	—

<sup>a</sup>Slowly cooled from 60 to 25°C. The supernatant was then carefully obtained after centrifugation.

<sup>b</sup>Evaporated to remove water at 60°C, slowly cooled to 25°C. The supernatant was then carefully obtained.

<sup>c</sup>From Reference Liu et al. (2005).

<sup>d</sup>Not available.

<sup>e</sup>From Reference Flores et al. (2002).

<sup>f</sup>Slowly cooled from 102 to 60°C.

hydrogen bond acceptor properties of ILs (Anderson et al., 2002). ILs containing [dca]<sup>−</sup> have been reported to have the greatest capacity to dissolve glucose, although the stability of lipase was too low in these ILs. The glucose solubilities in [dca] ILs ranged from 66 to 145 g/L at 25°C (Liu et al., 2005). The glucose solubility in [Emim][MS] at 25°C was similar to those in [dca] ILs. The solubilities of glucose in ILs containing [TfO]<sup>−</sup> and [BF<sub>4</sub>]<sup>−</sup> were greatly influenced by temperature. The solubility of glucose in these ILs increased by a factor of 2–5 when the temperature was increased from 25 to 60°C.

The solubilities of fructose and sucrose in various ILs at 25 and 60°C are shown in Tables II and III. The solubilities of fructose in ILs were higher than those of glucose, while the solubilities of sucrose in ILs were much lower than those of glucose. A strong temperature dependency of solubility in ILs was also shown. After 12 h incubation of excess sucrose (50 g/L) in [Emim][MS] at 60°C, the solution changed to a viscous brown liquid, although no sucrose crystal was

**Table II.** Solubility and supersaturated concentration of fructose in ionic liquids.

Solvent	Solubility (g/L)		Concentration in supersaturated solution at 25°C (g/L)	
	25°C	60°C	Conventional <sup>a</sup>	Water-mediated <sup>b</sup>
[Emim][TfO]	32.8	123.9	98.0	233.0
[Bmim][TfO]	27.0	87.5	81.6	132.0
[Emim][BF <sub>4</sub> ]	7.7	25.7	20.3	28.4
[Bmim][BF <sub>4</sub> ]	3.3	15.9	15.3	17.9

<sup>a</sup>Slowly cooled from 60 to 25°C. The supernatant was then carefully obtained after centrifugation.

<sup>b</sup>Evaporated to remove water at 60°C, slowly cooled to 25°C. The supernatant was then carefully obtained.

**Table III.** Solubility and supersaturated concentration of sucrose in ionic liquids.

Solvent	Solubility (g/L)		Concentration in supersaturated solution at 25°C (g/L)	
	25°C	60°C	Conventional <sup>a</sup>	Water-mediated <sup>b</sup>
[Emim][MS]	12.4	ND <sup>c</sup>	ND	ND
[Emim][TfO]	3.1	7.1	3.2	15.3
[Bmim][TfO]	2.0	5.3	2.1	10.2
[Emim][BF <sub>4</sub> ]	0.6	0.6	0.6	5.1
[Bmim][BF <sub>4</sub> ]	0.5	0.6	0.6	2.9

<sup>a</sup>Slowly cooled from 60 to 25°C. The supernatant was then carefully obtained after centrifugation.

<sup>b</sup>Evaporated to remove water at 60°C, slowly cooled to 25°C. The supernatant was then carefully obtained.

<sup>c</sup>Sucrose was degraded at 60°C and the color of the solution changed to brown.

observed. It thus appears that sucrose can be degraded or reacted in [Emim][MS] at high temperature.

### Supersaturation of Sugars in ILs

If a saturated solution is prepared at an elevated temperature and the undissolved solute is removed, the solution can sometimes be cooled without crystallization of the solute. The solution contains more dissolved solute than the solubility limit, forming a supersaturated solution (Holtzclaw and Robinson, 1988). Sugars are known to produce supersaturated solutions in aqueous or organic systems, and metastable supersaturated solutions of sugars have been used for biotransformation. Millqvist-Fureby et al. (1998) produced a supersaturated solution containing glucose with a concentration roughly 150% higher than its solubility by preparing a saturated glucose solution at 95°C and cooling it at 37°C. More recently, Flores et al. (2002) made a supersaturated glucose solution with a concentration 300% higher than its solubility in 2-methyl 2-butanol by heating at 102°C and cooling to 60°C.

Supersaturated solutions are usually prepared by heating at high temperature to dissolve excess solute and then slowly cooling to low temperature. This process is hereafter referred to as the “conventional method.” After heating the excess sugars at 60°C and cooling them to 25°C without removing undissolved sugars, metastable supersaturated solutions in ILs were successfully obtained for the first time (Tables I–III). In order to obtain supersaturated solution in conventional solvents, the undissolved solute should be removed after heating. However, supersaturated solutions in ILs can be prepared without separation of undissolved solute. The dissolved glucose concentrations of the supersaturated solutions in [Emim][TfO] and [Bmim][TfO] were about 380% higher than their glucose solubilities. The degrees of supersaturation in the ILs were higher than those in an aqueous system (150% in ethylene glycol-citrate buffer) and an organic solvent system (300% in 2-methyl 2-butanol).

The supersaturated glucose solution and fructose solutions in ILs prepared by the conventional method was stable in the presence of solid glucose or under shaking of the solution. The crystallization process of the supersaturated sugar solutions in water is known to involve at least two steps. First, sugars diffuse from the bulk solution to the thin layer at the crystal/solution interface. Sugar molecules in the crystal then incorporate after the release of their hydration water. The dissociation of hydration water is considered to be a potential major hurdle in this process (Mathlouthi and Genotelle, 1998). However, among the energy barriers in the crystallization process of the supersaturated solution in ILs, the viscosity of ILs seems to be the most critical obstacle. The high viscosity of ILs can decrease the diffusion rate from the bulk solution at the crystal/solution interface and decrease the collision probability of sugar molecules. Therefore, the crystallization rate of a supersaturated solution in ILs may be very low. On the other hand, the supersaturated sucrose solution produced via the conventional method was unstable and easily crystallized. The higher stability of the supersaturated glucose and fructose solution relative to the supersaturated sucrose solution requires further study.

### Water-Mediated Supersaturation of Sugars in ILs

The dissolution of glucose in ILs is simply understood by the detachment of glucose molecules from the solid surface at the solid–liquid interface and the transport of glucose molecules from the solid–liquid interface to the bulk ILs solution (Flores et al., 2005). However, considerable time was required to dissolve glucose in ILs, and thus an alternative dissolution process that uses water as a mediator was developed to overcome this drawback. As the solubility of glucose in water (1,207 g/L at 30°C, Kajiwarra and Franks, 2004) and dissolution rate of glucose in water are much higher than those in ILs, the dissolution rate of glucose in ILs can be substantially increased. As ILs are non-volatile, the water contained in ILs can be easily removed by evaporation after the dissolution process. To make a supersaturated glucose solution in ILs, glucose was first dissolved in water. This solution was then mixed with ILs. The water in this mixture was then removed by vacuum evaporation. The supersaturated glucose solution was obtained after centrifugation and was fairly stable in the presence of excess glucose and under shaking of the solution for several hours. This process is hereafter referred to as the “water-mediated method.”

The glucose concentration of the supersaturated solution in ILs was much higher than the solubility at 25°C. In particular, the supersaturated glucose solutions in [Emim][TfO] and [Bmim][TfO] had 19 and 10 times higher concentrations than solubility at 25°C, respectively (Table I). As residual water of less than 0.1% can increase the solubility of glucose, it was investigated whether this high supersaturation was induced by the remaining water. However, the glucose solubility in [Bmim][TfO] increased



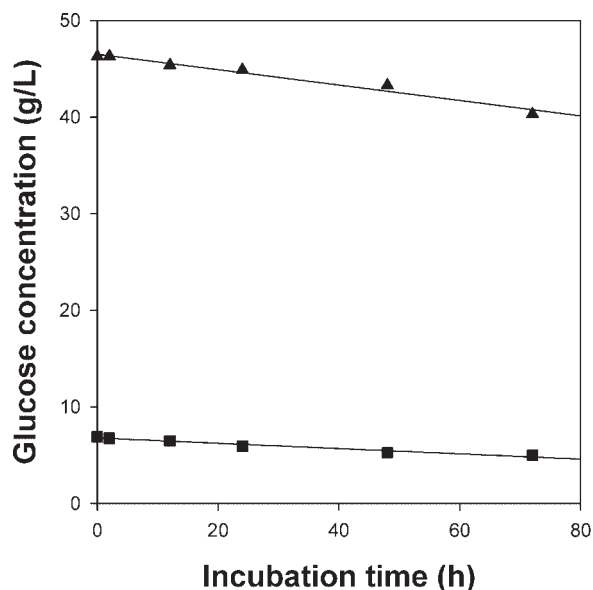
from 4.8 to 8.2 g/L at 25°C by adding 1% water, which was much lower than the glucose content of 46.3 g/L in the supersaturated solution. As for the stability of the supersaturated glucose solution in [Bmim][TfO] at 25°C (Fig. 1), the solution was stable for 1 day and the glucose concentration was 87% of the initial content even after 3 days.

Supersaturated fructose solutions and sucrose solutions were also prepared using the water-mediated method. These solutions were fairly stable under shaking of the solution for several hours, while the supersaturated sucrose solutions produced by the conventional method were fully crystallized after 2 h. The supersaturated fructose solutions in all ILs prepared by the water-mediated method had four to seven times higher concentrations than their solubilities at 25°C. The supersaturated sucrose solutions in all ILs had concentrations that were approximately five times higher than their solubilities.

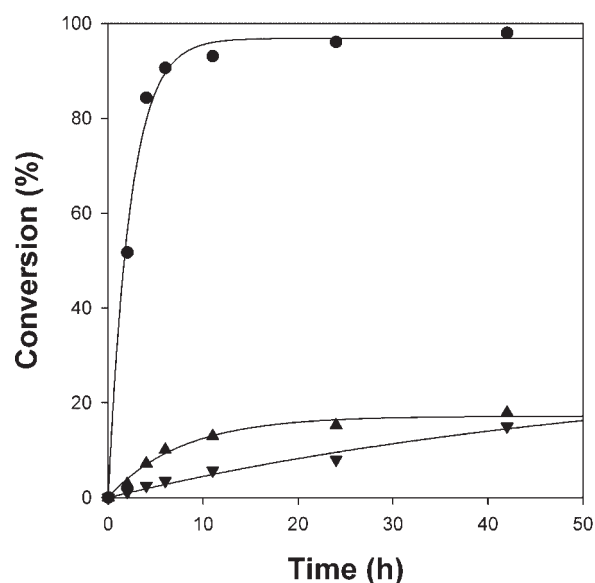
### Transesterification of Glucose With Fatty Acid Vinyl Ester in IL

The choice of solvent for the esterifications of glucose is very difficult, because one reactant is polar (glucose), the other is non-polar (fatty acid vinyl ester or fatty acid), and the product is amphiphilic (glucose ester). Most ILs possess both a hydrophilic ionic head and a hydrophobic organic chain, comprising one category of surfactants. Therefore, ILs may be good solvents for the esterifications of glucose. The lipase-catalyzed transesterification of glucose with vinyl

laurate was performed in ILs. Although [Emim][MS] is the most suitable IL to dissolve glucose, no enzymatic reaction could be detected with this IL. The acidic condition of this IL may inactivate the enzyme. In an effort to dissolve high concentrations of glucose and perform an enzyme reaction, [Bmim][TfO] was used as a reaction media for lipase-catalyzed transesterification. The [Bmim][TfO] has special properties: [Bmim][TfO] is fully miscible with water ( $\delta_H = 47.8$ ) but the Hildebrand solubility parameter of this IL ( $\delta_H = 25.4$ ) is similar to that of [Omim][Tf<sub>2</sub>N] ( $\delta_H = 25.0$ ), which is known as a very hydrophobic and water immiscible IL (Lee and Lee, 2005). The Hildebrand solubility parameter has been widely used for predicting the solubilities of various chemicals in organic solvents. The maximum solubility is observed when the Hildebrand solubility parameters of the solute and solvent are identical. This implies that [Bmim][TfO] can dissolve not only hydrophilic substrates but also hydrophobic organic compounds. In addition, [Bmim][TfO] was reported to be a good reaction media for lipase-catalyzed reactions (Itoh et al., 2001). The transesterification of glucose could be performed in [Bmim][TfO] with high conversion. To investigate the influence of the dissolved glucose concentration on the reaction rate and conversion, reaction was carried out with partially dissolved glucose solution and supersaturated glucose solutions containing the same content of glucose (40 g/L) (Fig. 2). Dissolved concentrations of glucose in the saturated solution and supersaturated solution prepared by the conventional and water-mediated



**Figure 1.** Stability of supersaturated glucose solution in ILs prepared by water-mediated method. After incubation at 25°C, the supernatant was periodically obtained and diluted with deionized water to analyze the glucose content ((▲) [Bmim][TfO], (■) [Bmim][BF<sub>4</sub>]).



**Figure 2.** Lipase-catalyzed transesterification of glucose with vinyl laurate in IL. Reaction conditions: 0.11 mmol glucose, 0.22 mmol vinyl laurate, 0.5 mL [Bmim][TfO], 50 mg Novozym 435, 40°C. The water contents in ILs were similar and less than 0.1% ((●) reaction with supersaturated glucose solution prepared by water-mediated method, (▲) reaction with supersaturated glucose solution prepared by the conventional method, (▼) reaction with saturated solution in the presence of crystalline glucose).

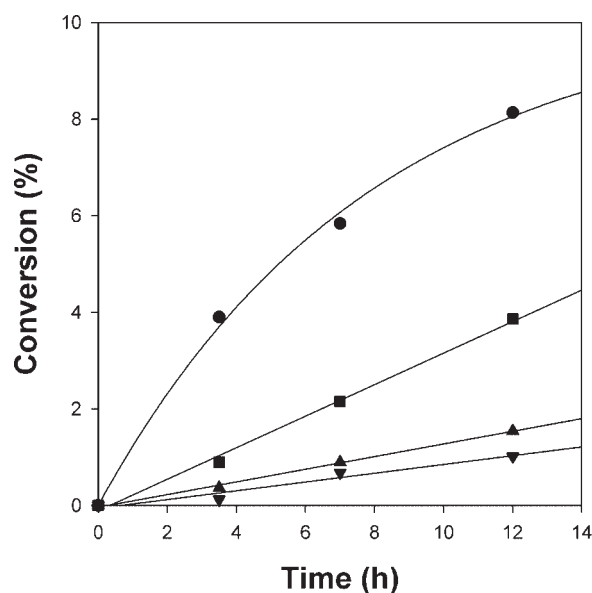
method were initially 9.9, 17.2, and 40.0 g/L, respectively. Only the supersaturated solution prepared by our method could fully dissolve glucose. Water contents in these reaction media were similar. The initial reaction rate (15.0  $\mu\text{mol}/\text{min}/\text{g}$ ) for the reaction with supersaturated glucose solution prepared by the water-mediated method was much higher than those of the reaction with the supersaturated solution in the presence of solid crystals prepared by the conventional method (0.83  $\mu\text{mol}/\text{min}/\text{g}$ ) and partially dissolved glucose solution (0.17  $\mu\text{mol}/\text{min}/\text{g}$ ). The obtained conversion (i.e., 96%) at 1 day of reaction was also significantly higher than those of other reactions. The higher dissolved concentration of glucose in the supersaturated solution led to an increase in the reaction rate and conversion. These results indicate that homogeneously dissolved glucose in a supersaturated solution can be easily transported to the active site of lipase, while the low dissolution rate of partially dissolved glucose in [Bmim][TfO] may limit the productivity of the enzyme. In addition, the increase of dissolved glucose concentration yielded better conversion because the equilibrium was forced toward synthesis. On the other hand, only the monoester, 6-*O*-lauroyl-D-glucose, was formed, as confirmed by  $^1\text{H}$ -NMR and LC-MS. As the solubility of monoester in [Bmim][TfO] is lower than 0.01 g/L at 25°C, precipitation of monoester as it is formed may restrain the formation of diester and increase the conversion of glucose.

Scale-up of the reaction system was investigated by utilizing 10 times higher volume of IL. In the reaction using 1.1 mmol glucose with the same conditions, 84% conversion and 70% yield were obtained after 1 day. In comparison with conventional methods using a saturated solution in the presence of solid crystal or solid-phase, this process may be relatively easy to scale up.

The influence of water content in ILs was also studied in order to clarify whether the increased initial rate and conversion were induced by residual water in the supersaturated solution. Figure 3 shows the effect of water content in the saturated glucose solution containing undissolved glucose crystals on the lipase-catalyzed reaction. The increase of water content in ILs drastically decreased the reaction rate. This indicates that the water content in reaction media should be lowered in order to increase the activity of Novozym 435. Therefore, it can be concluded that the increased reaction rate and conversion obtained using the supersaturated solution was induced by the increase of dissolved glucose content in ILs.

### Direct Esterification of Glucose With Fatty Acid in ILs

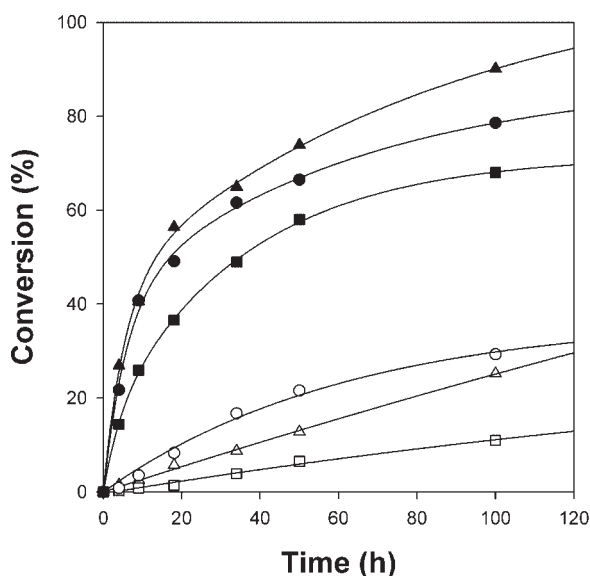
The direct esterifications of glucose with fatty acids are usually preferred to transesterifications of glucose with fatty acid vinyl esters, because water as a by-product is non-toxic and can be easily removed during the reaction. Ganske and Bornscheuer (2005a,b) carried out the direct esterification of



**Figure 3.** Influence of water content on the lipase-catalyzed transesterification of glucose with vinyl laurate by using saturated glucose solution in ILs in the presence of crystalline glucose. Reaction conditions: 0.11 mmol glucose, 0.22 mmol vinyl laurate, 0.5 mL [Bmim][TfO], 50 mg Novozym 435, 40°C ((●) 0.1%, (■) 0.4%, (▲) 1%, (▼) 2%).

glucose with fatty acid in mixtures of ILs ([Bmim][BF<sub>4</sub>] or [Bmim][PF<sub>6</sub>]) and *t*-butanol, but no sugar ester synthesis took place in pure ILs. This may be a result of the low reaction rate in these ILs. In this work, lipase-catalyzed direct esterification of glucose with lauric acid was successfully achieved with high concentrations (40 g/L) of glucose in a pure IL without organic solvent (Fig. 4). However, the reaction rate and conversion were too low, because the solubility of glucose at 50°C is still low (14.2 g/L). To increase the dissolved concentration of glucose in [Bmim][TfO], we used a supersaturated glucose solution prepared by the water-mediated method. The lipase-catalyzed direct esterification of glucose with lauric acid was efficiently carried out with the supersaturated glucose solution in [Bmim][TfO] (Fig. 4). The initial reaction rate (2.03  $\mu\text{mol}/\text{min}/\text{g}$ ) for the reaction with the supersaturated glucose solution in [Bmim][TfO] was 20 times higher than that of the reaction with the saturated glucose solution in the presence of glucose crystals (0.10  $\mu\text{mol}/\text{min}/\text{g}$ ). The conversion level (i.e., 91%) at 100 h of reaction with supersaturated solution was also significantly higher than that (i.e., 27%) of the reaction with the saturated solution.

The same reaction was carried out in [Emim][TfO] and [Bmim][BF<sub>4</sub>]. Regarding the reaction with the saturated glucose solution, the highest reaction rate and conversion was obtained in [Emim][TfO]. This may be attributed to the increase of dissolved glucose concentration in ILs, because the solubility of glucose in [Emim][TfO] is higher than those in other ILs. Regarding the reactions with the



**Figure 4.** Lipase-catalyzed direct esterification of glucose with lauric acid in ILs. Reaction conditions: 0.11 mmol glucose, 0.11 mmol lauric acid, 0.5 mL ILs, 50 mg Novozym 435, molecular sieve (4 Å, 15% w/v), 50°C. Filled symbols represent the reaction with supersaturated glucose solution in ILs prepared by the water-mediated method. Empty symbols represent the reaction with saturated glucose solution in the presence of crystalline glucose (circle, [Emim][TfO]; triangle, [Bmim][TfO]; square, [Bmim][BF<sub>4</sub>]).

supersaturated solution are compared, the highest reaction rate and conversion appeared in the reaction using [Bmim][TfO] as reaction media, although [Emim][TfO] and [Bmim][TfO] showed the same dissolved glucose concentrations. This means that the more hydrophobic nature of [Bmim][TfO] may be suitable to increase activity of lipase. Specifically, the use of a supersaturated glucose solution in ILs drastically increased the reaction rate of Novozym 435, compared with the method of using a saturated solution in the presence of glucose crystals. Therefore, it is concluded that water-mediated supersaturation is a useful method to increase the dissolved concentration of glucose in ILs.

## Conclusions

In this study, we made supersaturated sugar solutions in ILs through conventional and water-mediated methods. The water-mediated supersaturation method can be used to dissolve excess hydrophilic substrates such as sugars, peptides, and water miscible vitamin without their degradation by heat. We successfully achieved lipase-catalyzed esterifications of glucose in pure ILs. [Bmim][TfO] was found to be a very useful reaction media for reactions involving both hydrophilic and hydrophobic substrates.

The initial rate and conversion can be significantly improved by using a supersaturated glucose solution with concentration higher than its solubility. Therefore, lipase-catalyzed esterification in the supersaturated solution using a water-mediated method can be employed to overcome the problem of restricted glucose solubility in various reactions.

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