

## Kinetics of the Esterification Reaction Catalyzed by Lipase in W/O Microemulsions of Alkyl Polyglucoside

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**Abstract:** A novel kinetic mechanism of esterification reaction of 1-hexanoic acid with 1-butanol, catalyzed by lipase, was studied in water-in-oil microemulsions. The microemulsions were formed by alkyl polyglucoside C<sub>10</sub>G<sub>1.54</sub>/1-butanol / cyclohexane/phosphate buffer solution. The result shows that when the ratio of mol concentration of 1-butanol to 1-hexanoic acid is about 3.0, the initial rate  $V_0$  get the maximum values. This phenomenon was explained by the modified fishlike phase diagrams.

**Keywords:** Alkyl polyglucoside, W/O microemulsion, lipase catalysis, kinetics.

Lipase-catalyzed reactions have received much attention in recent years for their increasingly biotechnological applications in the preparation of fine chemicals, food and pharmaceutical industries<sup>1-3</sup>. The W/O microemulsions as the reaction media can provide an adequate environment to the lipase, and therefore protect it against denaturation by the organic solvent. Additionally, microemulsions can supply an extremely large interfacial area between the oil-continuous phase and the dispersed aqueous phase for the water-insoluble reactants to contact with the entrapped lipase. So the lipase-catalyzed reaction conducted in W/O microemulsion is one of the most intensively studied subjects.

The more commonly used surfactants to produce microemulsions for the lipase-catalyzed reaction media are anionic AOT, cationic CTAB and nonionic C<sub>12</sub>E<sub>8</sub>, etc. It is found that cationic surfactants may form aggregates with lipase<sup>4</sup>, anionic surfactants are known to promote denaturation of the protein, and unbranched nonionic alkyl polyglycol ethers C<sub>12</sub>E<sub>8</sub> may interfere with vital domains of the lipase<sup>5</sup>. Alkyl polyglucosides<sup>6,7</sup> (APG) are synthesized from fatty alcohols and sugars, display dermatological safety, a good biodegradability, and excellent surface-active properties. Since alkyl polyglucosides are also known as a mild, nondenaturing solubilizer<sup>8</sup> and is used in the extraction of membrane proteins, it is predicted that APG-based microemulsions are potential drug carriers for encapsulating water-soluble drugs. Therefore, research on the kinetics of esterification reaction catalyzed by lipase in W/O microemulsions of alkyl polyglucoside

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is of great importance.

In the present paper, the kinetics of esterification reaction of 1-hexanoic acid with 1-butanol, catalyzed by *Novozym 100T* lipase, was studied in water-in-oil microemulsions formed by alkyl polyglucoside  $C_{10}G_{1.54}$ /1-butanol / cyclohexane/water buffer solution.

### Experimental

Alkyl polyglucoside was synthesized<sup>9</sup> from the corresponding long-chain alcohol by our research group, its composition was determined by the chromatograms to be  $C_{10}G_{1.54}$  (monoglucoside 68.8%, diglucoside 17.20%, triglucoside 5.50%, tetraglucoside 8.50%). *Novozym 100T* lipase was supplied by *Novozym* company (China), LTD. The other materials used in this study are all A. R. grade and are used without further purification. Water was doubly distilled.

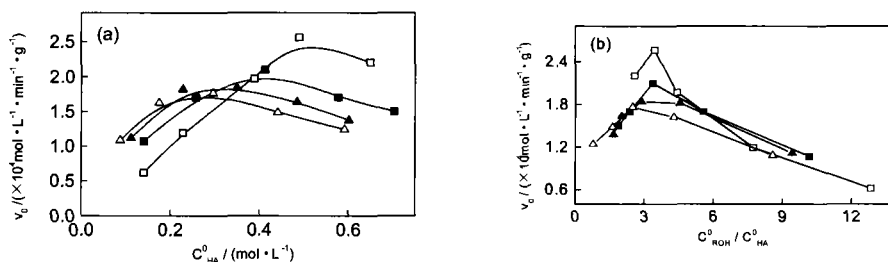
W/O microemulsions were prepared by adding appropriate phosphate solution (pH 6.864) to 0.05 g/mL  $C_{10}G_{1.54}$  / 1-butanol / cyclohexane solution (system A) and 0.05 g/mL  $C_{10}G_{1.54}$  / 1- hexanoic acid / cyclohexane solution (system B), in a capped conical flask. Then 1.5 mg/mL *Novozym 100T* lipase was solubilized into the two microemulsions. The two microemulsions were mixed into one solution and placed in a thermostatic and shaky water bath at 37°C and 150 r.p.m. One milliliter of the reaction mixture was withdrawn at selected time intervals and then added to 2 mL ethanol: acetone = 1:1 (V/V) solutions. The depletion of 1- hexanoic acid was assayed by titration with NaOH solution. Curves of depletion in 1- hexanoic acid concentration as a function of time were plotted. The initial rate of reaction equals the initial slope obtained by linear-regression calculation. A sample without lipase was used as a blank.

### Results and Discussion

The kinetic mechanism for enzymatic two-substrate reaction may be supposed by measuring the initial rates of reaction when the concentration of one substrate is held constant and the other is varied. The decays of 1- hexanoic acid concentration are shown in **Figure 1**. It can be seen from **Figure 1(a)** that when the initial concentration of 1-hexanoic acid is relatively small, the initial rate  $V_0$  of the esterification reaction increases as 1-hexanoic acid concentration increases or as 1-butanol concentration decreases; When the initial concentration of 1-hexanoic acid is relatively large, the initial rate  $V_0$  decreases as 1-hexanoic acid concentration increases or as 1-butanol concentration decreases. Furthermore, **Figure 1(b)** shows that when the ratio of mol concentration for 1-butanol to 1-hexanoic acid is about 3.0,  $V_0$  get the maximum value.

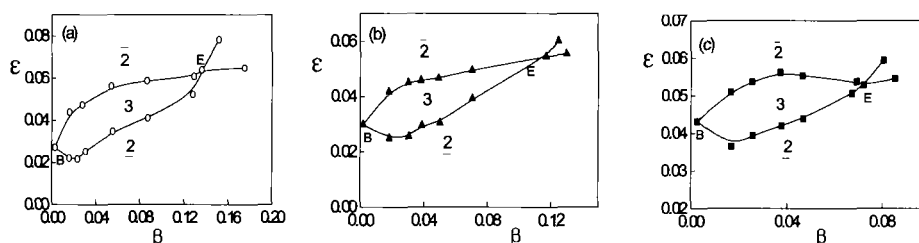
The modified fishlike phase diagrams<sup>10</sup> for systems  $C_{10}G_{1.54}$  / 1-butanol and/or 1-hexanoic acid / cyclohexane /phosphate buffer were shown in **Figure 2**. In which, the abscissa  $\beta$  is the mass fraction of  $C_{10}G_{1.54}$  in the system, the ordinate  $\varepsilon$  the mass fraction of cosurfactant (1-butanol and 1- hexanoic acid) in the system.

**Figure 1** Initial rate of esterification reaction as a function of initial 1-hexanoic acid concentration  $C_{HA}^0$ (a) and  $C_{ROH}^0/C_{HA}^0$ (b)



The concentration of initial 1-butanol: 0.74(▲); 1.00(△); 1.38(■); 1.71(□)mol · L<sup>-1</sup>

**Figure 2** The modified fishlike phase diagrams of the quaternary systems C<sub>10</sub>G<sub>1.54</sub>/ 1-butanol or (and) 1-hexanoic acid / cyclohexane / phosphate buffer system at 37 °C



1-butanol (a); 1-butanol+1-hexanoic acid (mol ratio 3:1)(b); 1-hexanoic acid (c)

**Figure 2** was plotted according to literatures<sup>10,11</sup>. The mass fraction of 1-butanol [from **Figure 2(a)**] of the mixture of 1-butanol and 1-hexanoic acid with a mol ratio 3:1 [from **Figure 2(b)**] and of 1-hexanoic acid [from **Figure 2(c)**], in the balanced interfacial layer  $A^S$ , were calculated from **Figure 2** to be 0.238, 0.200 and 0.158, respectively. Correspondingly, the mol ratios of 1-butanol and/or 1-hexanoic acid to C<sub>10</sub>G<sub>1.54</sub> are 1.72, 1.21 and 0.66, respectively. Apparently, three 1-butanol molecules have the same efficiency with one 1-hexanoic acid molecule in changing the curvature of the interfacial layer. Therefore when the mixture of 1-butanol and 1-hexanoic acid with a mol ratio 3:1 was incorporated into the interfacial layer, one half of the interfacial layer will be occupied by 1-butanol molecules, and the other half occupied by 1-hexanoic acid molecules. This can be verified by the corresponding mol ratio of the mixture of 1-butanol and 1-hexanoic acid to C<sub>10</sub>G<sub>1.54</sub> of 1.21, which is nearly the same as the calculated value 1.19 [(1.72+0.66)/2=1.19]. On this condition  $V_0$  can get the maximum value.

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