Study of the Influence of Alcohols in the Synthesis of Short Chain Esters

Maria Dolores Romero*, Jose María Gómez, Beatriz Díaz-Suelto, Alicia García-Sanz

Catalysis and Separation Processes Group, Universidad Complutense Madrid
Av. Complutense s/n, 28040, Madrid, Spain. mdolores@quim.ucm.es

The effect of the type of alcohol and the alcohol/acyl donor was explored on the synthesis of short chain esters by enzymatic catalysis in n hexane. First, the effect of the alcohol chain length was investigated. Among several alcohols, those with a larger hydrocarbon chain gave the best yields. Then, the position of the –OH group was analyzed, concluding that the steric hindrance of the 3-hexanol leads to a worse esterification extent. To impel reaction yield, excess alcohol was used. The results obtained validate this assumption.

1. Introduction

Esterification is the most widely used reaction in the organic chemistry industry (Otera, 2003), because it presents lot of applications ranging from natural products synthesis at lab scale to industrial scale production.

According to the report made by Van Beuzekom and Arundel (2009), biotechnology plays a very important role in industry, health and agriculture. The main industrial applications are the production of bulk and specialty chemicals, enzymes, plastics, biofuels, and bioremediation. Also, it is used in the development, production, and prescribing of therapeutics, in vivo diagnostics, and vaccines for humans. These applications are complemented with those in the agricultural sector, which include diagnostics, vaccines and therapeutics for animal health and genetic modification to develop improved plant and animal varieties.

1.1 Esterification in biotechnology

Esters of different alcohols and acids are used for a wide variety of applications in industry as flavors, emulsifiers, lubricants, and additives in cosmetics (Chand et al., 1997). Traditionally, this kind of compounds have been isolated from natural sources or produced by chemical synthesis (Karra-Chaabouni et al., 2006). The chemical route often suffers from poor reaction selectivity leading to undesirable side reactions and low yields. However, commodity chemicals and specialty chemicals can be produced by biotechnological processes, in which the same chemicals can be produced in a more economical and environmentally friendly way. Besides the advantages of mild operating conditions, availability of enzymes from different microbial sources possessing specificity of action and the fact that their catalytic activity can be easily regulated are some of the profound virtues that most chemical catalysts do not possess.
1.2 Enzymes as biocatalysts
The importance of lipase-catalyzed synthesis of such esters in presence of several solvents has been reported in many publications (An et al., 2010; Chiang et al., 2003; Habulin and Knez, 2009; Leblanc et al., 1998). Esters from a variety of alcohols and acids can be synthesized by various lipases (EC 3.1.1.3), according to their specificity in aqueous medium (De Barros et al., 2010). However, there are many problems in establishing such a reaction system, the main problem being that an excess of water in the reaction mixture tends to favour hydrolysis rather than esterification (Tewari and Bunk, 2001). So, the interest to non-aqueous solvents, as organic solvents, SC-CO₂ and ionic liquids, is growing due to its capability of overcoming the difficulty of dissolving hydrophobic substances and shifting the equilibrium to the formation of the desired products (Ghanem, 2007; Kragl et al., 2002; Romero et al., 2005a).
Candida antarctica lipase B is one of the most commonly used lipases. It is commercially available in both free and immobilized form. The application of lipase to synthesize esters has been studied for many years since the work of Iwai (1964).

2. Materials and Methods

2.1 Reagents and Materials
Hexanoic acid, 2-butanol, n-hexane and n-decane were from Fluka with GC purity (> 99%). Butyric acid, 2-hexanol and 3-hexanol were from Sigma-Aldrich with at least 98% purity. Finally, 1-propanol and 1-butanol were from Panreac, in these cases the purity was 99.5%. The enzyme was Novozym 435® from Candida Antarctica hydrolase B, immobilized on a macro porous acrylic resin with a water content of 1-2 % w/w, and kindly provided by Novo Nordisk, Denmark.

2.2 Method for esters synthesis
Reactions in n-hexane were carried out in a shaker, equipped with a temperature controller. In all the synthesis remained constant not only the agitation rate (200 rpm) but also the atmospheric pressure. The amount of enzyme used was 13.8 g/mol substrate in defect. All the experiments were carried out at equimolar concentrations of reactants except when the opposite is indicated. Reaction mixture volume was 20 ml. n-Decane was used as internal standard (1% w/w). After different reaction times, 200 μl of sample were taken in order to follow the reaction. Samples were analyzed by GC.

2.3 Analytical Methods
Substrates and products concentrations were determined by Gas Chromatography with a Varian Gas Chromatograph equipped with a hydrogen flame ionization detector and a ChiralDEX™ B-PM column (30 m length x 0.25 mm i.d.). Helium was used as carrier gas at a flow rate of 1 ml·min⁻¹. In all cases the initial temperature of the column oven was 40°C with a heating rate of 2°C·min⁻¹ to 54°C and after one minute of stabilization the temperature was increased up to 150°C (at 15°C·min⁻¹). Injector temperature was 200°C and detector temperature was 250°C.
All the samples were analyzed three times to determine the experimental error, which was less than 4% in all cases.
3. Results and Discussion

The purpose of the present study was to investigate the effect of the type of alcohol, as well as the alcohol/acyl donor ratio. The effect of the alcohol chain length was studied for primary and secondary alcohols. Also, the position of the –OH group was analyzed. Esterification extent here was defined as the amount of ester produced to initial substrate in defect (acyl donor). The most significant findings are summarized below.

3.1 Effect of alcohol chain length: primary and secondary alcohols

In order to study the effect of the alcohol chain length esterification reactions using butyric acid as acyl donor were developed. The equimolar substrates concentration was 0.5 M.

Figure 1 shows the results obtained when primary alcohols are tried, while Figure 2 depicts those obtained when secondary alcohols were tried.

The results depicted in Figure 1 do not show any noticeable influence on the esterification extent when 1-propanol, 1-butanol, 1-hexanol and 1-octanol are used. There is only a slight difference between the slopes of the largest chain alcohols (1-hexanol and 1-octanol) and those of the shortest (1-propanol and 1-butanol).

![Graph showing esterification extent over time for different alcohols](image)

*Figure 1. Esterification of butyric acid using short primary alcohols*

Romero et al. (2002) studied the esterification of acetic anhydride using several alcohols (from ethanol to 1-dodecanol), one of the main conclusions obtained was that esterification rate increased when the carbon number of the alcohol grew. Butyric acid and acetic acid (acetic anhydride can split up to give two acetic acid molecules) are both short chain carboxylic acids, but the inhibiting properties of acetic acid (Romero et al., 2005b) do not benefit the reaction, therefore the differences between the extents obtained in that case are clearly more marked than those obtained with butyric acid.

On the other hand, when secondary alcohols are used, the esterification extent obtained with 2-hexanol is much higher than with 2-butanol. So, this means that large chain alcohols lead to get a more productive esterification, with less reactive consumption.
3.2 Effect of the position of –OH group
In order to study the effect of the position of the –OH group, three different alcohols (1-hexanol, 2-hexanol and 3-hexanol) were employed for the butyric acid esterification. The equimolar substrates concentration was 0.5 M.

The results are showed in Figure 3. As expected, the esterification extent obtained is much higher when 1-hexanol is used, due to the less steric hindrance. The worst extent is obtained with 3-hexanol because of the high steric resistance that the hydrocarbon chains, which surrounds the –OH group, create.

3.3 Effect of alcohol/acyl donor ratio
The effect of alcohol/acyl donor ratio was also studied. Hexanoic acid was used as acyl donor and 2-butanol as alcohol. A set of experiments were made using a fixed concentration of acyl donor (0.5 M) and several alcohol/acyl donor ratios (1/1 - 1.6/1 –
2/1 – 3/1 – 8/1). The results are depicted in Figure 4 and it shows that an equimolar alcohol/acyl donor ratio leads to obtain worse results.

\[ \text{Figure 4. Esterification of hexanoic acid and 2-butanol at several alcohol/acyl donor ratios} \]

The effect of excess alcohol on ester yield is shown in Table 1. As alcohol to acyl donor ratio increased so did ester yield, because the equilibrium of the reaction was pushed toward the product formation as the nucleophile (alcohol) concentration raised.

\[ \text{Table 1: Results obtained at a fixed time of 90 minutes for the esterification of hexanoic acid with 2-butanol. Different alcohol/acyl donor were used.} \]

<table>
<thead>
<tr>
<th>Alcohol/Acy1 donor ratio</th>
<th>[2-butyl caproate] (mol·l⁻¹)</th>
<th>Esterification extent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 / 1</td>
<td>0,25</td>
<td>50</td>
</tr>
<tr>
<td>1,6 / 1</td>
<td>0,26</td>
<td>52</td>
</tr>
<tr>
<td>2 / 1</td>
<td>0,29</td>
<td>58</td>
</tr>
<tr>
<td>3 / 1</td>
<td>0,3</td>
<td>60</td>
</tr>
<tr>
<td>8 / 1</td>
<td>0,33</td>
<td>66</td>
</tr>
</tbody>
</table>

\[ \text{4. Conclusions} \]

The synthesis of short chain esters using lipases as biocatalysts has been developed. The use of larger chain alcohols brings higher esterification extent. This effect is more marked when secondary alcohols are used. The optimal alcohol/acyl donor ratio is the one that fulfills with the requirements of a high esterification yield and less chemical consumption. According to that, a higher alcohol/acyl donor ratio benefits the esterification yield but does not meet the saving approach.
References


Tewari Y.B. and Bunk D.M., 2001, Thermodynamics of the lipase-catalyzed esterification of glycerol and n-octanoic acid in organic solvents and in the neat reaction mixture, Journal of Molecular Catalysis B: Enzymatic, 15, 135–145.