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# **ISOAMYL ACETATE SYNTHESIS**

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# **ACKNOWLEDGEMENTS**

This study was carried out in the facilities of the "University College Leuven-Limburg" (UCLL), during an Erasmus mobility program, with the supervision of Herman Faes, organic chemist, also member of the group "Technology Department of Sustainable Chemical Processes" at KUL.

First of all, I want to thank my supervisor, professor Herman Faes, for accepting me on this Erasmus semester and helping me in everything I have need, and also for teaching me and allowing me to learn a lot about Organic Chemistry, as well as Belgium. His energy and sense of work are an example to be followed.

I had a great support and advice throughout this study and I would like to acknowledge to the UCLL for their contributions.

Furthermore, I would like to acknowledge to the "Universitat Rovira i Virgili" for offering me the opportunity to do an Erasmus mobility program and also to my mobility coordinator, Elena Fernández, for helping me from a distance in my adaptation to the new university and environment.

And last, gratefulness to my family and friends who, even thousands of kilometres away, they have always supported me.

For all of you, just say it has been an honour staying at Belgium with you in my final path of the chemistry bachelor.

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# 1. SUMMARY

The synthesis of esters has played a most important role in organic synthesis from its start. Esters constitute functional groups of chemical significance, in numerous natural and synthetic compounds, and also they have utility in diverse fields both in the industry and in laboratory. The essential feature of esterification, that particularly distinguishes it from other reactions, lies in its broad utilization in industry. Just a brief look reminds us of aspirin (acetyl salicylic acid), fatty acid esters, polyesters...

A variety of routes to arrive at esters are possible, and numerous methods have been reported. Since esterification has such a long history and the reaction itself is simple, many people take it for granted that little room is left for further scientific improvements. On the other hand, in industry, esterification still has permanent significance and so many new technologies remain undisclosed. Esterification has in fact been, and is still undergoing, extensive innovations.

The biggest problem frequently encountered in esterification technology arises from equilibration. The condensation between an alcohol and an acid is slow and reversible, thus the equilibrium can be shifted to the desired product by adding excess of one of the reagents or by the removal of products.

On the other hand, the rate of the reaction can be influenced by adding a catalyst such as an acid. Mineral liquid acids, Lewis acids and Bronsted acids are widely used in industry. However, their corrosive and hazardous nature, the subsequent need for neutralization generating large quantities of aqueous waste, and the difficulty to separate them from the reaction medium restrict their industrial applicability.

Innovations to further improve the efficiency and sustainability of the esterification process are highly desirable. To accomplish a green esterification protocol, recoverable heterogeneous solid catalysts are used.

Solid acid catalysts have received much attention as potential replacements for mineral liquid acids. The advantages of heterogeneous solid acid catalysts include reduction of corrosion, ease of separation from the reaction medium, good recyclability and enhanced product selectivity.

In addition to being essential molecular components in their own right, ester groups also play versatile temporary roles in organic synthesis for protection of carboxylic acids and hydroxyl groups. The synthesis of natural products, especially macrolides, sugars, and peptides, depends heavily on acylation technology. Furthermore, the low-molecular-weight esters have very pleasant odours and indeed are the major components of the flavour and odour aspects of a number of fruits. Although the natural flavour may contain nearly a hundred different compounds, single esters approximate the natural odours and are often used in the food industry for artificial flavours and fragrances.

# 2. LIST OF ABBREVIATIONS AND SYMBOLS

TsOH	p-Toluenesulfonic acid
DCC	Dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
DHU	Diclyclohexylurea
DCM	Dichloromethane

# **3. OBJECTIVES**

The ester group is an important functional group that can be synthesized in a number of different ways. Fischer-Speier esterification [1] is a good method of synthesis in the laboratory when carboxylic acid or alcohol to be used is an economic product and available in large quantities.

The aim of this project is the synthesis of a specific ester, the isoamyl acetate, which is obtained by the condensation of isoamyl acohol with acetic acid through a Fischer-Speier esterification. This ester is the main constituent of the essential banana oil.

The synthesis of esters directly from alcohols and carboxylic acids plays an important role in organic synthesis because of its utility in diverse fields both in academia and in industry. For this reason, the main objective of this final thesis is to develop new methods to obtain the maximum conversion of the isoamyl acetate synthesis. In this way, the best reaction conditions for this shynthesis will be known. To achieve this goal, different pathways will be followed.

- Different acid catalysts can be used to increase the rate of the reaction. In this study are used sulfuric acid, p-Toluenesulfonic acid (TsOH) and Amberlyst-15. The last one is a solid acid catalyst, which has received much attention as potential replacement for mineral liquid acids, due to its mild and highly selective properties combined with its environmentally benign character.
- 2) The use of the Chatelier's Principle to shift the equilibrium towards products, by increasing the concentration of the reactants.
- 3) The use of the Chatelier's Principle to shift the equilibrium towards products, by decreasing the concentration of a product by removing it from the equilibrium. In this case, the water will be absorbed and removed from the reaction mixture through two techniques.
  - By adding to the reaction mixture molecular sieves, an artificial zeolite, which preferentially adsorbs water. Two types of molecular sieves (3A and 4A) are used either in the reaction mixture and also placed above the reaction mixture.
  - b. By an azeotropic distillation with toluene. Water can form an azeotrope with certain alcohols at specific concentrations. A special apparatus, called a Dean Stark apparatus or trap, can be used to distil off the water/toluene azeotrope while the ester being is formed. Removing water from the reaction in this way will favour ester formation.

4) The use of carbodiimide activators through the Steglich Esterification [2]. It is an esterification variation with dicyclohexylcarbodiimide (DCC) as a coupling reagent and 4-N,N-dimethyilaminopyridine (DMAP) as a catalyst. The Steglich Esterification has been used to facilitate the esterification of acids and alcohols and does not require high temperatures.

# 4. INTRODUCTION

Esters are a group of compounds of general formula R-CO-O-R', which are widely distributed in nature. Simple esters usually have a pleasant smell. In many cases, although not all, the characteristic aroma and taste of fruits and some flowers are due to compounds that have the structure of the ester group (Table 1). The organoleptic properties of many aromas are due to a single ester which predominates as is the case with essential banana oil in which the main constituent is isoamyl acetate. In most cases however, the aroma is due to a complex mixture of esters and other compounds such as terpenes, norisoprenoids (e.g. damascenone), volatile phenols and other benzene derivatives (raspberry ketone), monoterpenes (e.g. linalool, nerol, geraniol...)



Table 1. Fragances and boiling point of esters [3]



For instance, esters are the class of volatile compounds that are responsible for a fruity smell in wines. Wine is commonly referred to as a "complex matrix" and this is certainly true. However, by breaking wine down into its fundamental components, it is possible to understand how to better manage the vineyards and wineries to attain the wine styles that the market desire. Esters could be considered as a whole of aromas that are very valuable to the wine industry, but only the aliphatic monocarboxylic esters make a significant impact in it [4].

Most of the esters in wine are formed during fermentation or during wine ageing. In particular, esters are critical in the production of Pinotage wine. If uncontrolled, this variety develops a pungent banana aroma from isoamyl acetate, which is not only produced during fermentation, but also found within the grape itself. This ester is formed from acetic acid and alcohol. The mechanism by which yeasts form esters is believed to be a reaction catalysed by an enzyme called alcohol acetyltransferase (AAT). This reaction uses alcohol as a substrate, co-enzyme A and ATP to form an ester. Esters may also be formed through simple hydrogen-ion-catalysed reactions [5].

Furthermore, the triglyceride ester group is the main functional group of lipids and their derivatives (fats, oils, waxes...). It is also widely represented among substances with therapeutic use such as local anaesthetics derived from structural cocaine natural alkaloids (Table 2) and in some food and cosmetics preservatives.



 Table 2. Chemical structures of cocaine and some local anaesthetics derived from cocaine.

Esters are used as protecting groups in synthesis to obtain chemoselectivity in a subsequent chemical reaction. The protecting group is introduced into a molecule by chemical modification of a functional group, to temporally mask the characteristic chemistry of the functional group because it interferes with another reaction. The most reactive functional groups commonly requiring protection are alcohols, aldehydes, ketones, carboxylic acids and amines. Esters are the common protecting groups for carboxylic acids, which have an acidic proton that can be abstracted by bases including organometallic reagents.

A good protecting group should be readily, but selectively introduced to the desired functional group in a poly-functional molecule. It should be stable to the reagents employed in subsequent reactions steps in which the group being masked is desired to remain protected. Moreover, it should be capable of being selectively removed under mild conditions when its protection is no longer required.

For instance, in peptide synthesis due to the possibility of unintended reactions, protecting groups are usually necessary. Peptide synthesis is the production of peptides, which are organic compounds in which multiple amino acids are linked via amide bonds, also known as peptide bonds. Peptides can be synthesized by coupling the carboxyl group of one amino acid to the amino group of another amino acid molecule (Scheme 1).

The use of protecting groups is essential during peptide synthesis to avoid side reactions such as self-coupling of the activated amino acid leading to polymerization. This would compete with the intended peptide coupling reaction, resulting in low yield or even complete failure to synthesize the desired peptide. When protecting groups are used, the unprotected amine of one reacts with the unprotected carboxylic acid group of the other to form a peptide bond.



Scheme 1. Formation of a peptide bond using protecting groups

Esters are usually prepared by reaction between an acid derivative and an alcohol. An organic base such as pyridine is usually added to react with the hydrogen chloride formed. The reaction of an alcohol with an acid chloride or acid anhydride is produced quickly and quantitatively due to the large electrophilicity of the carbonyl group of these compounds so that it can work almost stoichiometric conditions (Scheme 2).



Scheme 2. Conversion of acid derivative to ester.

Although this is a widely used method in the laboratory, in the industry the reaction of alcohols with carboxylic acids is preferred. Acid chlorides form hydrogen chloride upon contact with atmospheric moisture, are corrosive and react often dangerously with nucleophiles.

The reaction of an alcohol with a carboxylic acid is known by the name of Fischer-Speier esterification and is a reaction of equilibrium that occurs very slowly because of the little electrophilicity of the carboxyl group (Scheme 3). If a catalytic amount of a mineral acid such as sulfuric, hydrochloric or phosphoric acid is added, the two components react faster to form an ester and water. This transformation is not very exothermic and the ester forms up to a balance concentration.



#### Scheme 3. Conversion of carboxylic acid to ester.

This transformation is exothermic or endothermic depending on the esters that are formed. Hess's law can be used to determine the overall energy required or released for a chemical reaction. Allows the enthalpy change for a reaction to be calculated even when it cannot be measured directly, using previously determined values for the enthalpies of formation (Table 3). The esterification of isoamyl alcohol and acetic acid to form isoamyl acetate will be used as an example.

Tahle	<b>3</b> Standard	enthalnies	of formation	of the	compounds in	isoamyl	acetate	synthesis	[6]
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Compound	Δ <sub>f</sub> H <sup>0</sup> (kJ/mol)
Isoamyl alcohol	-356
Acetic acid	-484
Isoamyl acetate	-438
Water	-286

For Hess's Law

$$\begin{aligned} \Delta_r H^0 &= \sum_{products} \Delta_f H^0 - \sum_{reactants} \Delta_f H^0 = \\ &= \left( -438 \frac{kJ}{mol} + \left( -286 \frac{kJ}{mol} \right) \right) - \left( -356 \frac{kJ}{mol} + \left( -484 \frac{kJ}{mol} \right) \right) = \\ &= 116 \frac{kJ}{mol} \end{aligned}$$

As the enthalpy change is positive, this reaction is endothermic.

Fischer-Speier esterification is also a good method of synthesis in the laboratory when carboxylic acid or alcohol to be used is an economic product and available in large quantities.

Different acid catalysts can be used to increase the rate of the reaction. In this project sulfuric acid, p-Toluenesulfonic acid (TsOH) and Amberlyst-15 are used. In the first place, sulfuric acid is a strong mineral acid ( $pK_a$  of -3.0) widely used in chemical industry.

In the second place, TsOH is an organic compound soluble in water, alcohols and polar organic solvents. It is a strong organic acid ( $pK_a$  of -2.8) and unlike some strong mineral acids as sulfuric acid, TsOH is non-oxidizing. These two catalysts are widely used in industry, however their corrosive and hazardous nature, the subsequent need for neutralization generating large quantities of aqueous waste, and the difficulty to separate them from the reaction medium restrict their industrial applicability.

Solid acid catalysts have received much attention as potential replacements for mineral liquid acids. The advantages of heterogeneous solid acid catalysts include ease of separation from the reaction medium, good recyclability, reduction of corrosion and enhanced product selectivity. The isolation and reuse of this catalyst is an example of "Green Chemistry", reducing consumption of non-renewable resources.

An example of heterogeneous solid acid catalyst is Amberlyst-15, a powerful and selective acid catalyst for esterification reactions. It has played an important role in organic synthesis, with versatile synthetic applications in different chemical transformations as esterification. The growing interest in this catalyst is mainly due to its mild and highly selective properties combined with its environmentally benign character and commercial availability.

Additionally, this catalyst can be regenerated and recycled and is used in organic synthesis as heterogeneous reusable acid catalyst. The maximum operating temperature is  $120^{\circ}C$  and the capacity is 4.7 mmol H<sup>+</sup>/g [7].

Amberlyst-15 is a macro reticular polystyrene based ion exchange resin with strongly acidic sulfonic group (Table 4). It serves as an excellent source of strong acid and is used in acid catalysed reactions as esterification. It is easy to measure, safe to use and readily removed at the end of the reaction. [8].

### Table 4. Different catalysts structures.



### **5. LITERATURE REVIEW**

The aim of this project is the synthesis of the isoamyl acetate by direct Fischer-Speier esterification of acetic acid and commercial isoamyl alcohol (Scheme 4). The isoamyl acetate, in addition to being the main component of the banana aroma, it also constitutes the bee alarm pheromone, the release of which is a defensive reaction to alert nearby bees. Its uses are as fruits (banana, pear, apple, berry) flavour for foods, candy, butter, coconut, cola and rum; and also flavour for paint and insecticide.



Scheme 4. Synthesis reaction of isopentyl acetate.

Fischer-Speier esterification proceeds according to the following general mechanism (Scheme 5). Initially, the protonation of carboxyl oxygen is produced by generating a more reactive species that is stabilized by resonance. The carboxyl carbon develops a full positive charge that is attacked by the nucleophilic alcohol group, giving a tetrahedral intermediate. This tetrahedral intermediate with shorter bonds can either lead to the formation of the ester or go backwards to the starting products. The protonation of either of the two hydroxyls causes the removal of water followed by loss of the catalysing proton and the formation of the ester.





Scheme 5. Reaction mechanism of the synthesis of isoamyl acetate

Because each of these steps is completely reversible, this process is also, in reverse, the mechanism for the hydrolysis of an ester. The acid hydrolysis reaction of esters takes place when heating an ester with excess water and acid medium. In this case, the equilibrium is shifted to the left. This reaction of hydrolysis of esters, which can also be catalysed by bases, also has a great synthetic utility and serves, for example, to obtain the fatty acids from the triglycerides.

The equilibrium expression for this reaction is the following:



For primary alcohols reacting with unhindered carboxylic acids, the  $K_{eq}$  is approximately of 4%. If equal quantities of the alcohol and acetic acid are allowed to react, the theoretical yield of ester is only 67% at equilibrium. [3]

As a matter of fact, the Fischer-Speier esterification reaction takes advantage of Le Chatelier's principle to increase the amount of alcohol that is esterified. Chatelier's Principle is when one side of an equilibrium is altered by changing concentration or pressure and the equilibrium shifts to counteract this change and restore the equilibrium.

The equilibrium is shifted towards products by two different techniques:

- 1) Using a large excess of one of the reactants. The equilibrium shifts to use up the increased amount of reactant, thereby producing more products.
- Decreasing the concentration of a product by removing it from the equilibrium. This causes more reactants to be consumed to restore the product concentration.

In the case of isoamyl acetate synthesis, is used an excess of acetic acid because it is cheaper and easier to separate. Additionally, the water formed as a byproduct, is continuously removed and the equilibrium will continuously shift producing more product until all of the reactants are consumed. The absorption of water by molecular sieves or an azeotropic distillation with toluene could be both good options to remove the water from the reaction mixture.

One the one hand, molecular sieve adsorbents are crystalline alumina-silicates, known as zeolites. Their unique structure allows the water of crystallization to be removed, leaving a porous crystalline structure. These pores or "cages" have a high affinity to readsorb water or other polar molecules. Aided by strong ionic forces due to the presence of cations such as sodium, calcium and potassium and by the absolutely enormous internal surface area of close to 1000 m<sup>2</sup>/g, molecular sieves will adsorb a considerable amount of water or other fluids. If the fluid to be adsorbed is a polar compound, it can be adsorbed with high loading, even at very low concentrations of the fluid. Molecular sieves will therefore remove many gas or liquid impurities to very low levels. Another feature of molecular sieve adsorbents is their ability to separate gases or liquids by molecular size or polarity.

These crystals are characterised by a three-dimensional pore system, with identical pores of precisely defined diameter. This structure is formed by tetrahedras of  $AIO_4$  and  $SiO_4$ . These tetrahedras are the basic building blocks for various zeolite structures, such as zeolites A, the most common commercial adsorbent.

Due to the presence of alumina, zeolites exhibit a negatively charged framework, which is counterbalanced by positive cations resulting in a strong electrostatic field on the internal surface. These cations can be exchanged to adjust the pore size or the adsorption characteristics. For instance, the sodium form of zeolite A has a pore opening of approximately 4 Angstrom ( $4 \times 10^{-10}$  m), called 4A molecular sieve. If the sodium ion is exchanged with the larger potassium ion, the pore opening is reduced to

approximately 3 Angstrom, called 3A molecular sieve (Table 5 and 6). The ability to adjust the pores to precisely determined uniform openings allows for molecules smaller than its pore diameter to be adsorbed whilst excluding larger molecules, hence the name "molecular sieve". The different pore sizes of synthetic zeolites open up a wide range of possibilities in terms of "sieving" molecules of different size or shape from gases and liquids.

Туре	3 A						
Composition	0.6 K <sub>2</sub> O: 0.40 Na <sub>2</sub> O : 1 Al <sub>2</sub> O <sub>3</sub> : 2.0 SiO <sub>2</sub> : x H <sub>2</sub> O						
Description	The 3A form is made by substituting potassium cations for the						
	inherent sodium ions of the 4A structure, reducing the effective pore						
	size to 3 Å, excluding diameter > 3 Å						
Properties	3 Å pore diameter, 8-12 mesh (common in liquid phase applications),						
	adsorbed water 19-20 % (w/w), regeneration temperature 175-260 ºC.						
Major	Commercial dehydration of unsaturated hydrocarbon streams,						
applications	including cracked gas, propylene, butadiene, acetylene; drying polar						
	liquids such as methanol and ethanol. Adsorption of molecules such as						
	$NH_3$ and $H_2O$ from a $N_2/H_2$ flow. Considered a general-purpose drying						
	agent in polar and nonpolar media.						

#### Table 5. Properties and characteristics of 3A molecular sieves [9].

#### Table 6. Properties and characteristics of 4A molecular sieves [9].

Туре	4 A
Composition	1 Na <sub>2</sub> O : 1 Al <sub>2</sub> O <sub>3</sub> : 2.0 SiO <sub>2</sub> : x H <sub>2</sub> O
Description	This sodium form represents the type A family of molecular sieves.
	Effective pore opening is 4 Å, thus excluding molecules of effective
	diameter >4 Å.
Properties	4 Å pore diameter, 8-12 mesh, adsorbed water 20-21 % (w/w),
	regeneration temperature 200-315 ºC.
Major	Preferred for static dehydration in closed liquid or gas systems, water
applications	scavenging in printing and plastics systems and drying saturated
	hydrocarbon streams. Adsorbed species include SO <sub>2</sub> , CO <sub>2</sub> , H <sub>2</sub> S, C <sub>2</sub> H <sub>4</sub> ,
	$C_2H_6$ , and $C_3H_6$ . Generally considered a universal drying agent in polar
	and nonpolar media.

Molecular sieves (zeolites of types 3A and 4A) are widely used to dry laboratory solvents. Dry organic solvents are important for many industrial sectors. Adsorptive water removal is one technique to obtain highly pure solvents. It is widely used in the drying, refining and purification of liquids and gases [10].

On the other hand, the equilibrium can be shifted towards products by removing the water as an azeotrope. Water can form an azeotrope with certain alcohols at specific concentrations. A special apparatus, called a Dean Stark apparatus or trap, can be used

to distil off the water as an azeotrope while the ester being is formed. Removing water from the reaction in this way will favour ester formation.

Furthermore, another variation of an esterification is used to obtain the maximum possible yield of the reaction, the Steglich Esterification. It is a variation of an esterification with dicyclohexylcarbodiimide (DCC) as a coupling reagent and 4-N,N-dimethyilaminopyridine (DMAP) as a catalyst. This reaction takes place at room temperature and a suitable solvent could be acetonitrile.

RCOOH + R'OH  $\xrightarrow{\text{DCC} / \text{DMAP}}$  RCOOR'

#### Scheme 6. Steglich Esterification.

It is a mild reaction, which allows the conversion of sterically demanding and acid labile substrates. It's one of the convenient methods for the formation of *tert*-butyl esters because *t*-BuOH tends to form carbocations and isobutene after a subsequent elimination under the conditions employed in the Fischer-Speier Esterification.

The application of the DCC method in pure organic synthesis dates back to 1967. The Steglich Esterification proceeds according to the following general mechanism (Scheme 7). Initially, the DCC and the carboxylic acid are able to form an O-acylisourea intermediate, which offers reactivity similar to the corresponding carboxylic acid anhydride. The alcohol may now add to the activated carboxylic acid to form the stable diclyclohexylurea (DHU) and the ester [11].





Scheme 7. Reaction mechanism of the Steglich Esterification.

In peptide synthesis, the reaction with carboxylic acids, DCC and amines leads to amides without problems (Scheme 8). This is because amines are more nucleophilic than alcohols. Strong nucleophiles such as amines react readily with the *O*-acylisourea and therefore need no additives such as DMAP.



Scheme 8. Fast reaction between amines and O-acylisourea.

In the case of alcohols, the esterification is slow (Scheme 9) and a side-reaction occurs, diminishing the final yield or complicating purification of the product. This side-reaction is a 1,3-rearrangement of the O-acyl intermediate to an N-acylurea which is unable to further react with the alcohol (Scheme 10).



Scheme 9. Slow reaction between alcohols and O-acylisourea.



Scheme 10. Formation of N-acylurea through an acyl migration.

These drawbacks can be overcome by addition of catalytic amounts of *p*-aminopyridines. For the efficient formation of esters, the addition of approximately 5 mol % DMAP is crucial. DMAP accelerates the reaction because, as a stronger nucleophile than the alcohol, reacts with the *O*-acylisourea leading to a reactive amide (active ester).

This intermediate cannot form intramolecular side products but reacts rapidly with alcohols. DMAP suppresses the side reaction, acting as an acyl transfer reagent in this way, and subsequent reaction with the alcohol gives the ester (Scheme 11).



Scheme 11. Reaction mechanism when the catalyst DMAP is used.

# 6. MATERIALS AND METHODOLOGY

# 6.1. CHEMICAL PRODUCTS

The physical properties, hazards and safety of all chemical products used in this study appear in Table 7.

Reactive	Purity	Вр	MW	Density	Hazards	Safety
Acetic acid	<b>(%)</b> 99.9	(ºC) 118	(g/mol) 60	(g/mL) 1.05	H226: Flammable liquid and vapour H312: Harmful in contact with skin H314: Causes severe skin burns and eye damage H318: Causes serious eye damage H335: May cause respiratory irritation	Use a fumehood
Isopentyl alcohol	99	129	88.2	0.81	H225: Highly Flammable liquid and vapour H226: Flammable liquid and vapour H315: Causes skin irritation H318: Causes serious eye damage H332: Harmful if inhaled H335: May cause respiratory irritation H336:May cause drowsiness or dizziness	Avoid contact with skin, eyes and clothing
Sulfuric acid	96.5	337	98.1	1.84	H290: May be corrosive to metals H314: Causes severe skin burns and eye damage H318: Causes serious eye damage H335: May cause respiratory irritation	Use a fumehood

 Table 7. Physical properties, hazards and safety of the chemical products used [12].

p-toluene sulfonic acid monohydrate	99	38 (m.p.)	190.22	1.24	H290: May be corrosive to metals H302: Harmful if swallowed H314: Causes severe skin burns and eye damage H315: Causes skin irritation H318: Causes serious eye damage H319: Serious eye irritation H335: May cause respiratory irritation	Use a fumehood
Sodium bicarbonate	Not known	50 (m.p.)	84	2.1	H319: Serious eye irritation	Avoid contact with eyes
Sodium chloride	98	801 (m.p.)	58.44	2.17	H319: Serious eye irritation	Avoid contact with eyes
Anhydrous calcium chloride	Not known	772 (m.p.)	110.98	2.2	H319: Serious eye irritation	Avoid contact with eyes
Isopentyl acetate	Not known	142	130.2	0.867	H226: Flammable liquid and vapour H315: Causes skin irritation H319: Causes serious eye irritation H335: May cause respiratory irritation H336: May cause drowsiness or dizziness H372: Causes damage to organs through prolonged or repeated exposure	Avoid contact with skin, eyes and clothing
Molecular sieves	Not known	-	202.1	-	H315: Causes skin irritation H319: Causes serious eye	Avoid contact

					irritation H335: May cause respiratory irritation	with skin and eyes
Toluene	99.5	110.6	92.14	0.86	H225: Highly Flammable liquid and vapour H304: May be fatal if swallowed and enters airways H315: Causes skin irritation H336: May cause drowsiness or dizziness H361: Suspected of damaging fertility or the unborn child H373: Causes damage to organs through prolonged or repeated exposure	Avoid contact with skin, eyes and clothing
Amberlyst-15	-	-	314.4	-	H319: Causes serious eye irritation	Avoid contact with eyes
DMAP	98	110 (m.p.)	122.2	-	H301+H331: Toxic if swallowed or if inhaled H301: Toxic if swallowed H310: Fatal in contact with skin H311: Toxic in contact with skin H314: Causes severe skin burns and eye damage H315: Causes skin irritation H318: Causes serious eye damage H319: Causes serious eye damage H319: Causes serious eye irritation H331: Toxic if inhaled H335: May cause respiratory irritation H411: Toxic to aquatic life with long lasting effects	Avoid contact with skin, eyes and clothing. Wear nitrile gloves and use fumehood.

DCC	99	34 (m.p.)	206.3	-	H302: Harmful if swallowed H311: Toxic in contact with skin H314: Causes severe skin burns and eye damage H317: May cause an allergic skin reaction H318: Causes serious eye damage H330: Fatal if inhaled	Avoid contact with skin, eyes and clothing. Wear nitrile gloves and use fumehood.
DCM	99.8	39.8	84.9	1.33	H302: Harmful if swallowed H315: Causes skin irritation H319: Causes serious eye irritation H335: May cause respiratory irritation H336: May cause drowsiness or dizziness H341: Suspected of causing genetic defects H351: Suspected of causing cancer H373: Causes damage to organs through prolonged or repeated exposure	Avoid contact with eyes and skin.
DHU	Not known	230 (m.p.)	224.3	-	H302: Harmful if swallowed	Use a fumehood
Acetonitrile	99.9	82	41.1	0.78	H225: Highly Flammable liquid and vapour H302: Harmful if swallowed H312: Harmful in contact with skin H319: Causes serious eye irritation H332: Harmful if inhaled	Use a fumehood

## 6.2. MATERIAL

Different laboratory set-ups are used in order to do the different variations of the reaction. First of all, a laboratory reflux apparatus for heating the chemical reaction is used (Table 8).

Then, when the molecular sieves are used to absorb the water from the reaction mixture, a cylindrical pressure-equalizing dropping funnel is used. The molecular sieves are placed inside this glassware, in order to avoid the contact of these with the reaction mixture and only have contact with the water. The side arm of the glassware is isolated, so the vapours of water go up by this, then condense in the cooling system above and condensing fluid gets into contact with the sieves.

Also a Dean-Stark trap is employed for the removal of water from the reaction mixture in order to alter equilibrium concentrations. The reaction is carried out in a solvent that forms an azeotrope with water, in this case toluene. The reaction mixture is refluxed and the distillate condenses and drips into the trap, whereupon it separates into two layers. The volume of condensate increases until the organic solvent drips back into the reaction vessel, while the water remains in the trap. Thus, continual azeotropic removal of water/toluene is effected with only a relatively small volume of the organic solvent being required.

Finally, a Claisen fractional distillation head is used in order to separate a mixture of components with similar boiling points.



Table 8. Differents laboratory set-ups with special glassware.

# 6.3. CHARACTERIZATION EQUIPMENT

Two techniques are used to confirm the presence of isoamyl acetate, nuclear magnetic resonance spectroscopy (NMR) and infrared spectroscopy (IR).

• Spinsolve Education NMR-spectrometer (Table 9):

### Table 9. NMR system data

Spectrometer type	Spinsolve Education
Protocol	1D Proton
Spectral range	63 to -54 ppm
Scan type (number of scans)	Standardscan (4 scans)
Aquisition time	1 minut
Linewidth at 50% peak height	<0,7 Hz (for standard calibration 10% v/v
	$H_2O/D_2O$ sample)
Linewidth at 55% peak height	<20 Hz (for standard calibration 10% v/v
	$H_2O/D_2O$ sample)
Field strength	1 Tesla
Proton Larmor frequency	43 Mhz

• Thermo Fisher IR200 IR-spectrometer, Serienummer AFN0600591

# 6.4. METHODOLOGY

The project consists in the synthesis of isoamyl acetate, a colourless liquid with banana-like odour, by direct Fischer-Speier esterification of acetic acid and isoamyl alcohol. The aim of the project is to make variations of the reaction to obtain the maximum possible yield. In this way, the best reaction conditions for the synthesis of isoamyl acetate will be known.

## 6.4.1. Sulfuric Acid Catalyst

In order to synthesize isoamyl acetate, using sulfuric acid as catalyst, the procedure explained below is followed [13]. A flowchart can be found in Appendix A.

## MODUS:

16 mL of isopentyl alcohol (0.147 mol) and 22 mL of glacial acetic (0.385 mol) are added into a 100 mL Erlenmeyer. Carefully with swirling, 1 mL of concentrated sulfuric acid (0.018 mol) is added to the contents of the flask, and also boiling stones or a stirring bar. A reflux apparatus is assembled (Figure 1) and the reaction mixture is heated at 100°C reflux for 1 hour. Then the heat source is removed and the mixture cooled to room temperature using a water bath of room-temperature water.



Figure 1. Reflux apparatus.

The cooled mixture is poured into a separatory funnel and 50 mL of cold distilled water are added carefully. The reaction flask is rinsed with 10 mL of cold water and this rinse is also added to the separatory funnel. The funnel is stoppered and inverted several times before separating the lower aqueous layer from the upper organic layer. The aqueous phase is set aside.

25 mL of a 0.5M sodium bicarbonate solution are poured into the separatory funnel. The two phases are stirred until the evolution of  $CO_2$  gas nearly stops. Then the funnel is stopped, carefully is turned it upside down, and immediately the  $CO_2$  gas that forms is vented. The funnel is shaken and vented frequently until no more gas evolved when the funnel is vented. The lower aqueous layer is

removed and the extraction is repeated with another 25 mL of 0.5M sodium bicarbonate solution. NaHCO<sub>3</sub> extractions are continuously done until the lower layer remains basic, using a litmus paper after the extraction. The organic layer is washed a last time with 20 mL of 4M sodium chloride solution. The aqueous layer is removed again and the ester is poured into a dry 50 mL Erlenmeyer flask.

The product is dried with anhydrous calcium chloride, allowing it to stand over the drying agent for 10 minutes. The product is filtered from the CaCl<sub>2</sub> through a small plug of glass wool into a 50 mL round-bottomed flask. A simple distillation apparatus is assembled using an oil-bath to heat. The fraction boiling between 134 and 145°C is collected in a tared 25 mL round-bottomed flask.

### 6.4.2. p-Toluenesulfonic Acid Catalyst

The first variation is the use of a different catalyst. Instead of sulfuric acid, ptoluenesulfonic acid is used. This catalyst is a solid does not contain water, so by using this catalyst, no water will be add to the reaction mixture that could influence the equilibrium.

The procedure used to synthesize isoamyl acetate using p-toluenesulfonic acid as catalyst is the same as using sulfuric acid. The only difference is that instead of adding 1 mL of sulfuric acid, a certain amount of p-toluenesulfonic acid is added and dissolved. The amount of catalyst needed is known by calculating the concentration of the sulfuric acid.

$$\frac{96.5 \ g \ H_2 SO_4}{100 \ g} \times \frac{mol \ H_2 SO_4}{98 \ g} \times \frac{1.84 \ g}{mL} \times \frac{1000 \ mL}{L} = 18.1 \ M$$

If 1 mL of sulfuric acid is used and the concentration is 18.1 M:

$$1 mL \times \frac{L}{1000 mL} \times \frac{18.1 mol}{L} = 0.018 mol$$

 $\begin{array}{l} 0.018 \ mol \ H_2SO_4 \times \displaystyle \frac{mol \ p-toluene sulfonic \ acid \ monohydrate}{mol \ H^+} \times \displaystyle \frac{190.22 \ g}{mol} \times \\ \\ \displaystyle \times \displaystyle \frac{100}{99} = 3.46 \ g \ p-toluene sulfonic \ acid \ monohydrate \end{array}$ 

### 6.4.3. Amberlyst-15 Catalyst

The second variation is the use of a solid catalyst instead of the previous. The capacity of Amberlyst-15 is 4.7 mmol  $H^*/g$  by dry weight. The sulfuric acid (-3; 1.96 pK<sub>a</sub>) is considered as a mono-protic acid because only the first proton is acidic enough to acidify acetic acid (4.76 pK<sub>a</sub>). Compared to the 1 mL of acid sulfuric:

$$0.018 \ mol \times \frac{mol \ acidic \ H^+}{mol \ H_2SO_4} = 0.018 \ mol \ H^+ = 18 \ mmol \ H^+$$

$$18 \ mmol \ H^+ \times \frac{g \ Amberlyst - 15}{4.7 \ mmol \ H^+} = 3.83 \ g \ Amberlyst - 15$$

This amount of Amberlyst-15 is added to the reaction mixture, not exceeding 120°C. It is removed at the end of the reaction through a filtration [17].

#### 6.4.4. Molecular sieves

The next variation is the use of molecular sieves to absorb the water of the reaction mixture, keeping other parameters as the sulfuric acid catalyst constant. As well as trying two types of molecular sieves (3A and 4A), is also studied two ways of placing them, either in contact with the reaction mixture or without contact.

For both types of molecular sieves (3A and 4A) is used the same procedure. In the first case, using the "reflux set-up" (first set-up of Table 8 and Figure 2), the molecular sieves would be placed within the Erlenmeyer with the reaction mixture. After the reaction, a filtration is needed to remove the molecular sieves.



Figure 2. Set-up with molecular sieves in contact with the reaction mixture.



In the second case, the molecular sieves would be placed without contact with the reaction mixture, at a point between the Erlenmeyer and the reflux, inside the cylindrical pressure-equalizing dropping funnel (second set-up of Table 8 and Figure 3). In this case, the filtration is not necessary.

In order to know the amount of molecular sieves needed, it is calculated the amount of water formed by the reaction.

Figure 3. Set-up with molecular sieves inside the cylindrical pressure-equalizing dropping funnel.

$$16 mL isoamylic alcohol \times \frac{0.81 g}{mL} \times \frac{99 g}{100 g} \times \frac{mol}{88.2 g} \times \frac{mol H_2 0}{mol alcohol} \times \frac{18 g}{mol} \times \frac{1 mL}{1 g}$$
$$= 2.6 mL H_2 0$$

Molecular sieves 3A and 4A have an adsorption water capacity of 20 % (w/w), and with this information it can be known the amount of molecular sieves needed.

$$2.6 g H_2O \times \frac{100 g adsorbent}{20 g water adsorbed} = 13 g adsorbent$$

Good results are obtained in drying organic solvents with 20% (w/v) molecular sieves [15], so the amount of molecular sieves is also calculated and studied by this way.

16 mL alcohol isoamylic + 22 mL acetic acid + 1 mL sulfuric acid = 39 mL starting liquid

$$39 mL \times \frac{20 g adsorbent}{100 mL} = 7.8 g adsorbent$$

### 6.4.5. Azeotropic distillation

Another variation is the use of a Dean-Stark trap for the removal of water from the reaction mixture in order to alter equilibrium concentrations (third set-up of Table 8 and Figure 4). The reaction is carried out in a solvent that forms an azeotrope with water, therefore in the reaction mixture is added 15 mL of toluene.

The boiling point of the azeotropic mixture of water and toluene is 84 °C (b.p. toluene 110 °C, b.p. water 100 °C). The reaction mixture is refluxed and the distillate condenses and drips into the trap, whereupon it separates into two layers. The volume of condensate increases until the organic solvent drips back into the reaction vessel,

while the water remains in the trap. It can be observed approximately 2.6 mL of water remained in the trap (Figure 5).



Figure 4. Dean-Stark set-up.



Figure 5. Dean Stark trap with 2.6 mL of water removed.

All the procedure is the same, except that in the distillation a Claisen fractional distillation head is used (last set-up of Table 8). Not only to separate the mixture of components with similar boiling points, but also to be sure that all the toluene is gone.

## 6.4.6. Steglich reaction

The last variation is the Steglich Esterification, used to obtain the maximum possible yield of the reaction. This reaction is a variation of an esterification with dicyclohexylcarbodiimide (DCC) as a coupling reagent and 4-N,N-dimethyilaminopyridine (DMAP) as a catalyst.

In this esterification variation is followed a protocol with carboxylic acid as the limiting reagent. In order to compare the yields, the amounts are adjusted as follows:

limiting reagent: 0,147 mol isopentyl alcohol  $\rightarrow$  0,147 mol acetic acid

The other reagents' amounts are calculated using the molar ratio of the reaction protocol followed (0.14 mmol of DMAP, 1.35 mmol of acid, 1.76 mmol of alcohol and 1.76 mmol of DCC) [16].

• 0.147 mol acetic acid 
$$\times \frac{1.76 \text{ mmol alcohol}}{1.35 \text{ mmol acid}} = 0.192 \text{ mol isopentyl alcohol}$$

 $0.192 \ mol \ isopentyl \ alcohol \times \frac{88.2 \ g}{mol} \times \frac{100}{99} \times \frac{mL}{0.81 \ g} = 21 \ mL \ isopentyl \ alcohol$ 

• 0.147 mol acetic acid  $\times \frac{1.76 \text{ mmol DCC}}{1.35 \text{ mmol acid}} = 0.192 \text{ mol DCC}$ 0.192 mol DCC  $\times \frac{206.3 \text{ g}}{\text{mol}} \times \frac{100}{99} = 40 \text{ g DCC}$ 

• 0.147 mol acetic acid  $\times \frac{0.14 \text{ mmol DMAP}}{1.35 \text{ mmol acid}} = 0.015 \text{ mol DMAP}$ 

$$0.015 \ mol \ DMAP \times \frac{122.2 \ g}{mol} \times \frac{100}{98} = 1.9 \ g$$

The procedure followed for this synthesis is explained below [16]:

### MODUS:

To a solution of DMAP (0.015 mol) and acetic acid (0.147 mol) in acetonitrile (30 mL), a solution of isopentyl alcohol (0.192 mol) in acetonitrile (80 mL) is added. Then, a solution of DCC (0.192 mol) in acetonitrile (90 mL) is added dropwise to the reaction mixture at 0°C. The reaction mixture is stirred during 3 hours at room temperature (Figure 6), after which the acetonitrile is evaporated *in vacuo*.

The residue is taken up in dichloromethane (DCM), resulting in the formation of a white precipitate of dicyclohexylurea. The suspension is filtered and the solvent is evaporated *in vacuo* (Figure 7).



Figure 4. Reaction mixture stirring at room temperature.



Figure 5. Evaporation of solvent in vacuo.

Then, as in the previous procedures, the ester is isolated by distillation, collecting the fraction boiling between 134 and 145°C.

The DCC and DMAP are highly toxic compounds, therefore, it has to be worked with caution and very safely. It is strictly important to work under fumehood and using nitrile gloves. The nitrile gloves show excellent resistance to most chemicals and especially strong solvents, in addition the nitrile film is highly resistant to punctures and tears.

# 7. RESULTS AND DISCUSSION

The percentage yield of the reaction variations is compared in order to decide which one gets the maximum amount of product possible.

Amounts of products calculated from the complete reaction of the limiting reagent is called theoretical yield, whereas the amount actually produced of a product is the actual yield. The ratio of actual yield to theoretical yield expressed in percentage is the percentage yield.

$$Percentage Yield = \frac{actual yield}{theoretical yield} \times 100$$

The theoretical yield is calculated from the limiting reagent:

$$\begin{array}{l} 16 \ mL \ isopentyl \ alcohol \ \times \displaystyle \frac{0.81 \ g}{mL} \times \displaystyle \frac{99}{100} \times \displaystyle \frac{mol}{88.2 \ g} \times \displaystyle \frac{mol \ isopentyl \ acetate}{mol \ isopentyl \ alcohol} \times \\ \\ \times \displaystyle \frac{130.2 \ g}{mol} = 18.9 \ g \end{array}$$

 $Percentage \ Yield = \frac{actual \ yield}{theoretical \ yield} \times 100 = \frac{actual \ yield}{18.9} \times 100 = Yield \ \%$ 

The yield Y is a function of reaction temperature, reaction time, type of catalyst, reaction set-up, removal of water...

When comparing different variations, it is strictly spoken necessary to change only one parameter while keeping all other parameters constant. In this study, the reaction temperature was set at 100°C and the reaction time at 1 hour. Higher conversion would be achieved by extending the reaction time, but 1 hour is enough to compare the results.

In order to provide evidence that the results are reproducible, the whole experiment independently was done twice. Therefore, the yield result is an average of 2 experiments. In all the experiments, the repetitions gave almost equal results.

# 7.1. CATALYST

First of all, three different acid catalysts were used to increase the rate of the reaction and overcome the slow kinetics of the reaction. These catalysts were sulfuric acid, ptoluenesulfonic acid and Amberlyst-15.

To compare the potential of Amberlyst-15 as acid catalyst with conventional catalysts, the reaction yields are evaluated. The results are shown in Table 10. It can be observed

that the reaction with sulfuric acid catalyst ends up with a higher yield than both p-toluenesulfonic acid (4% less) and Amberlyst-15 (13% less).

Table 10. Yield results using different catalysts.

Catalyst	Percentage Yield
Sulfuric Acid Catalyst	79%
p-Toluenesulfonic Acid Catalyst	75%
Amberlyst-15 Catalyst	66%

A convenient way to express the relative strength of an acid is by the value of its pKa.

$$pK_a = -\log K_a$$

Small values of  $pK_a$  equal large values of  $K_a$  (dissociation constant) and, as the  $pK_a$  decreases, the strength of the acid increases.

Certainly is that both sulfuric acid and p-toluenesulfonic acid are strong acids, but there is a small difference in  $pK_a$  between sulfuric acid ( $pK_a$  of -3.0) and TsOH ( $pK_a$  of -2.8). So when comparing the results, it is possible that the lower acidity strength of TsOH is the responsible of decreasing the conversion.

When comparing sulfuric acid with Amberlyst-15, a conventional heterogeneous catalyst, the higher conversion can be explained by the initial homogeneous nature of the hydrophilic sulfuric acid which reacts faster than heterogeneous hydrophobic Amberlyst-15.

Heterogeneous acid catalysts suffer from severely decreased or even lost activity in the presence of moisture due to water adsorption on the active sites. Also lower activity may arise due to interaction of acid group with the support or due to more restricted conformations of intermediate complexes adsorbed onto the solid surface in comparison with homogeneous catalysts. [17] Moreover, the Amberlyst-15 was added to the reaction mixture and in the end of the reaction it had to be removed through a filtration. The filtration, in addition to that a part of the product was stuck to the solid catalyst, decrease the yield.

Although higher conversion is achieved with sulfuric acid and p-toluenesulfonic acid catalysts, their corrosive and hazardous nature and the subsequent need for neutralization generating large quantities of aqueous waste restrict their industrial applicability.

Due to these conventional catalysts' disadvantages, solid acid catalysts have received much attention as potential replacements for mineral liquid acids. The advantages of heterogeneous solid acid catalysts include ease of separation from the reaction medium, good recyclability, reduction of corrosion and enhanced product selectivity. The isolation and reuse of this catalyst is an example of "Green Chemistry", reducing consumption of non-renewable resources.

Given these points, Amberlyst-15 is selected as the optimal catalyst since a high conversion and high turnover frequency is highly desired for industrial applications.

# 7.2. REMOVAL OF WATER

Fischer-Speier esterification reaction takes advantage of Le Chatelier's principle to increase the amount of alcohol that is esterified. The equilibrium is shifted towards products by decreasing the concentration of a product by removing it from the equilibrium. This causes more reactants to be consumed to restore the product concentration. Therefore, the water formed as a byproduct was continuously removed by molecular sieves or by an azeotropic distillation with toluene.

In the first place, molecular sieves were added to the reaction mixture to adsorb water. Two types of molecular sieves (3A and 4A) were used either in the reaction mixture and also placed above the reaction mixture. Sulfuric acid and Amberlyst-15 were used as catalysts, but not TsOH since the result in the previous study was very similar to sulfuric acid.

By using 13 g of adsorbent, amount calculated by the 20% (w/w) adsorption water capacity, the yield obtained is 46%. As this result is very low and good results are obtained in drying organic solvents with 20% (w/v) molecular sieves [15], was used 7.8 g of adsorbent instead of 13 g. The results are shown in Table 11.

Set-up	Catalyst	Percentage Yield	
		3A	4A
In contact with reaction	Sulfuric Acid	72%	71%
mixture	Amberlyst-15	49%	48%
Without contact with	Sulfuric Acid	74%	72%
reaction mixture	Amberlyst-15	65%	64%

First of all, it can be observed in all cases that the reaction with sulfuric acid catalyst ends up with a higher yield than with Amberlyst-15, as expected from the previous study.

To continue, there is almost no difference between the two types of molecular sieves, but it is worth noting that in all cases 3A molecular sieves achieve between 1-2% higher yield. 4A molecular sieve has a pore opening of approximately 4 Å ( $4 \times 10^{-10}$  m), but if the sodium ion is exchanged with the larger potassium ion, the pore opening is reduced to approximately 3 Å (3A molecular sieve). Both molecular sieves adsorb

water (2.6 Å) because is a molecule smaller than its pore diameters, excluding larger molecules. All other reactants and products are larger than 4 Å. The only one that could have adsorption problems is the acetic acid, since its size is just over 4 Å. This could explain the small improvement in the experiments with 3A molecular sieves.

In reference to the set-up, the reaction with molecular sieves in contact with the reaction mixture ends up with a lower yield. This may be due to the fact that in this case, a filtration after the reaction was needed to remove the molecular sieves from the product. Also a lot of product was stuck to the solid, therefore lowering the yield. This problem is observed when working in small quantities, at the laboratory level. But on a large scale it is not so important, and for this reason at industrial level it can work well.

Placing the molecular sieves above the reaction mixture ends up with a higher yield, but it does not reach an improvement compared to the previous study, in which it was not carried out the removal of water.

To further evaluate if the removal of water favour the ester formation, the water was removed by an azeotropic distillation with toluene. The three different acid catalysts were used. The results are shown in Table 12.

Catalyst	Percentage Yield
Sulfuric Acid	85%
p-Toluenesulfonic Acid	79%
Amberlyst-15	71%

 Table 12. Yield results with azeotropic distillation and different catalysts.

It can be observed, as expected, that the reaction with sulfuric acid catalyst ends up with a higher yield than both p-toluenesulfonic acid (6% less) and Amberlyst-15 (14% less).

In reference to the removal of water, a great improvement is observed comparing with the first study. Higher conversion is achieved in all cases; a 6% higher yield with sulfuric acid, 4% with TsOH and finally 5% with Amberlyst-15. Therefore, the use of a Dean-Stark trap for the removal of water from the reaction mixture, in order to alter equilibrium concentrations, is a good option to get the maximum amount of product possible.

All things considered, it can be affirmed that the removal of water is a good option to shift the equilibrium producing more product. The absorption of water by molecular sieves or by an azeotropic distillation with toluene are both good options for the removal of water. On the one hand, molecular sieves are widely used in the drying, refining and purification of liquids and gases. Also to obtain dry organic solvents which are important for many industrial sectors. Most other drying agents, such as anhydrous sodium sulphate or calcium chloride, would not remove water at the higher temperatures used to make esters.

On the other hand, the azeotropic distillation with toluene is a technique often used for the removal of water and the results obtained confirm it. Gaining 6% more conversion in a large-scale reaction can greatly reduce economic costs and the environmental impact.

# 7.3. STEGLICH ESTERIFICATION

The last variation is the Steglich Esterification, using carbodiimide activators to obtain the maximum possible yield of the reaction. The yield obtained was the highest for the normal set-up, 83%.

The use of DCC as a promoter represents one of the most versatile esterification methods. Although this reagent is irritant to skin and a stoichiometric dosage or more is necessary, this procedure enjoys various advantages. The reaction usually proceeds at room temperature, and the reaction conditions are so mild that substrates with various functional groups can be employed. The reaction is not sensitive to the steric bulk of the reactants, allowing production of esters of tertiary alcohols. As such, a wide range of applications has been achieved in the fields of natural products, peptides, nucleotides, etc [14].

The favourable catalytic action of DMAP proves to be a useful agent in the study on the esterification of carboxylic acids. Indeed, the addition of DMAP accelerates the DCC-activated esterification of carboxylic acids with alcohols, such an extent that formation of side products is suppressed and even sterically demanding esters are formed in good yields at room temperature.

Steglich esterification has been used to facilitate the esterification of acids and alcohols, allowing the conversion of sterically demanding substrates. The fact that this reaction is not sensitive to the steric bulk of the reactants can explain the high conversion obtained. Although the isoamyl alcohol is primary, it has an isopropyl group that could present steric impediments.

It was no necessary to use the Chatelier's Principle to shift the equilibrium towards products, by increasing the concentration of the reactants or by decreasing the concentration of a product. Therefore, the protocol allows high conversion without the need of removing the water and with almost equimolar amounts of alcohol and acid. Moreover, it is a mild reaction which allows the conversion of substrates without requiring high temperatures.

### 7.4. ISOAMYL ACETATE CHARACTERIZATION

The characterization of the isoamyl acetate was done by <sup>1</sup>H NMR spectra. NMR results obtained are exposed under Figure 8.



Figure 6. Isoamyl acetate structure.

<sup>1</sup>H NMR (43 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.55 (6H, d, <sup>5</sup>CH<sub>3</sub>),  $\delta$  = 0.98 (3H, m, <sup>3</sup>CH<sub>2</sub> and <sup>4</sup>CH(CH<sub>3</sub>)<sub>3</sub>),  $\delta$  = 1.48 (3H, s, <sup>1</sup>CH<sub>3</sub>CO),  $\delta$  = 3.61 (2H, t, <sup>2</sup>CH<sub>2</sub>O).



Figure 7. Isoamyl acetate <sup>1</sup>H NMR spectra.

In figure 9 is shown the isoamyl acetate <sup>1</sup>H NMR spectra. It can be observed that 0.55 ppm signal is a doublet that integrates six, so it fits very well with the six protons bonded to <sup>5'</sup>C due to the fact that they are coupled with the single proton in <sup>4'</sup>C. These protons are the furthest from the electronegative acetate group, so they are shielded to a higher degree by a higher electron density of its surrounding molecular orbital. Therefore, its NMR frequency will be shifted "upfield" with a lower chemical shift.

About the signal at 0.98 ppm, it can be said that is a multiplet that integrates three. This is due to the fact that the proton bonded to <sup>4</sup>C and the two protons of <sup>3</sup>C have very close signals and therefore overlap in the same peak, integrating three. It is a complex signal (multiplet) because of the coupling with neighbouring protons.

The signal at 1.48 ppm is a singlet that integrates three, so it fits perfectly with the three protons of the acyl group ( $^{1'}CH_3CO$ ), that can not be coupled with any proton.

Finally, the signal at 3.61 ppm is a triplet that integrates two and corresponds with the protons of  $^{2'}$ C. It is a triplet due to the fact that they are coupled with the two protons bonded to  $^{3'}$ C. These protons are less shielded by such surrounding electron density because they are next to an acetate group, an electronegative functional group that attracts the electronic cloud. Therefore, its NMR frequency will be shifted "downfield" with a higher chemical shift.

Appendix B contains an ampliation of the <sup>1</sup>H NMR spectra (Figure 9), including the spectra of isoamyl acetate analytical standard.

In addition, this characterization was supported by infrared spectroscopy. The most important wavenumbers are 1055 cm<sup>-1</sup> and 1210 cm<sup>-1</sup> ( $v_{stret}$  C-O), 1366 cm<sup>-1</sup> ( $v_{bend}$  CH<sub>3</sub>), 1466 cm<sup>-1</sup> ( $v_{bend}$  CH<sub>3</sub> & CH<sub>2</sub>), 1739 cm<sup>-1</sup> ( $v_{stret}$  C=O), 2956 cm<sup>-1</sup> ( $v_{stret}$  CH<sub>3</sub>, CH<sub>2</sub> & CH).



Figure 8. Isoamyl acetate infrared spectra.

Appendix C contains an ampliation of the infrared spectra (Figure 10), including also the spectra of isoamyl acetate analytical standard.

# 8. CONCLUSIONS

Esterification will continue to play an important role both in organic synthesis and in the chemical industry. However, further innovations need to be pursued in response to the demands emerging from the continuing progress in organic synthesis.

The mail goal of this final thesis was to develop new methods to obtain the maximum conversion of the isoamyl acetate synthesis. In this way, the best reaction conditions for this shynthesis would be known. First of all, Amberlyst-15 is selected as the optimal catalyst since a high conversion and high turnover frequency is highly desired for industrial applications. High conversion is reached and the catalyst can be recycled and reused. Special attention is given to the development of an efficient green esterification process, although higher conversion is achieved with sulfuric acid and p-toluenesulfonic acid catalysts.

Secondly, it is confirmed that the removal of water is a good option to shift the equilibrium producing more product. The absorption of water by molecular sieves or by an azeotropic distillation with toluene are both good options for the removal of water. Particularly, the azeotropic distillation allows achieving high conversion, and this fact, in a large-scale reaction can greatly reduce economic costs and the environmental impact.

Finally, the use of carbodiimide activators through the Steglich Esterification is one of the convenient methods for the formation of esters. This esterification variation allows high conversion without the need of removing the water and with almost equimolar amounts of alcohol and acid. Moreover, it is a mild reaction which allows the conversion of substrates without requiring high temperatures.

It should be remembered that pot-treatment processes such as separation, isolation and purification of the products and recovery of catalysts in practical processes often take more time and energy than the reaction itself. Achievement of 100% yields with equimolar amounts of reactants simplifies the separation steps and reduces excesses.

Not only esterification, but all chemical processes, should be as optimal as possible. Since the materials involved in esterification (esters, carboxylic acids and alcohols) are mostly non-toxic, attention has to be devoted to solvents. There are already some processes that do no not use solvent, and it would be highly desirable to develop these lines further. It can reasonably be said that esterification technology has the potential to contribute a great deal to green chemistry.

## 9. LIST OF REFERENCES

[1] Emil Fischer, Arthur Speier; "Darstellung der Ester"; *Chemische Berichte*, **1895**, *28*, 3252–3258.

[2] B. Neises, W. Steglich; Simple Method for the Esterification of Carboxylic Acids; *Angew. Chem. Int. Ed.*, **1978**, *17* (7), 522-524.

[3] Pavia, Donald L.; Lampman, Gary M.; Kriz, George S.; Engel, Randall G.; *Introduction to Organic Laboratory Techniques: A Microscale Approach*, 5th Edition; Brooks/Cole Laboratory Series for Organic Chemistry, 2007; 109-117.

[4] Plata, C.; Millán, C.; Mauricio, J. .; Ortega, J., Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts; *Food Microbiol.* **2003**, *20* (2), 217–224.

[5] American Society of Enologists., C. J. van; Augustyn, O. P. H.; Wet, P. de; Joubert, W. A. American journal of enology and viticulture; *American Society of Enologists*, **1979**; *Vol. 30*.

[6] NIST Chemistry Webbook <u>https://webbook.nist.gov/chemistry/</u> (Date consulted 27 May 2018)

 [7] Sigma-Aldrich; Amberlyst-15 hydrogen form, Technical Information Bulletin; https://www.sigmaaldrich.com/catalog/product/sial/216380?lang=es&region=ES
 (Date consulted 26 April 2018).

[8] Pal, Rammohan; Sarkar, Taradas; Khasnobis, Shampa; Amberlyst-15 in organic synthesis; *ARKIVOC*. **2012**, (i) 570-609.

[9] Sigma-Aldrich; Molecular Sieves, Technical Information Bulletin; https://www.sigmaaldrich.com/chemistry/chemical-synthesis/learningcenter/technical-bulletins/al-1430/molecular-sieves.html (Date consulted 13 March 2018).

[10] Pahl, C.; Pasel, C.; Luckas, M.; Bathen, D. *J.*, Adsorptive Water Removal from Primary Alcohols and Acetic Acid Esters in the ppm-Region; *Chem. Eng. Data* **2012**, *57* (9), 2465–2471.

[11] Organic Chemistry Portal; Steglich Esterification; <u>http://www.organic-chemistry.org/namedreactions/steglich-esterification.shtm</u> (Date consulted 17 April 2018)

[12] Pub Chem, Open Chemistry Database; *Chemical and Physical Properties*; <u>https://pubchem.ncbi.nlm.nih.gov/search/search.cgi</u> (Date consulted 3 March 2018)

[13] R. Mohrig, Jerry; Noring Hammond, Christina; et al; *Moderns Projects and Experiments in Organic Chemistry: Miniscale and Standard Taper Microscale*, 2nd edition; W.H. Freeman and Company: New York, 2003; 93-96.

[14] Otera, Junzo; *Esterification: Methods, Reactions and Applications,* Wiley-VCH: Weinheim, 2003, 15.

[15] Williams, D. B. G.; Lawton, M. *J.;* Drying of Organic Solvents: Quantitative Evaluation of the Efficiency of Several Desiccants; *Org. Chem.* **2010**, *75* (24), 8351–8354.

[16] B. Neises, W. Steglich; Simple Method for the Esterification of Carboxylic Acids; *Angew. Chem. Int. Ed.*, **1978**, *17* (7), 522-524.

[17] Verheyen, T.; Smet, M.; De Borggraeve, W. M.; Water Tolerant and Reusable Sulfonated Hyperbranched Poly(aryleneoxindole) Acid Catalyst for Solvent-Free Esterification; *ChemistrySelect*, **2017**, 2, 1-8.



## **APPENDIX B**

## ISOAMYL ACETATE ANALYTICAL STANDARD <sup>1</sup>H NMR SPECTRA



## ISOAMYL ACETATE <sup>1</sup>H NMR SPECTRA



#### 1D-1H-"StandardScan"



Parameter	Value	
1 Data File Name	c:/ projects/ data/ 2018/ 05/ 02/ 163849-2nd 14;3/ Enhanced/ data.1d	
2 Spectrometer	Magritek Spinsolve	
3 Number of Scans	4	
4 Acquisition Date	2018-05-02T16:38:50,512	
5 Total acquisition time (min)	0.88333333333333333	
6 Nucleus	1H	



# **APPENDIX C**

ISOAMYL ACETATE ANALYTICAL STANDARD INFRARED SPECTRA



### **ISOAMYL ACETATE INFRARED SPECTRA**

