

Microwave assisted conversion of fructose and glucose to 5-hydroxymethylfurfural in a continuous flow reactor

FINAL BACHELOR THESIS IN CHEMISTRY

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1 Summary

English

This thesis deals with the acid-catalysed dehydration of carbohydrates to obtain 5-hydroxymethylfurfural (HMF). Conversion will be accomplished using a microwave assisted flow reactor.

During the last few decades, HMF has been widely studied as it is considered as one of the top value-added bio-based chemicals. It can be converted into a broad range of interesting chemicals applied in many different applications such as fuels and solvents but most importantly polyethylene 2,5-furandicarboxylate. This is a bio-based plastic which can replace PET in a lot of applications.

Objective

The main goal in this thesis is to optimize the reaction parameters such as HCl/AlCl₃ ratio (catalyst), temperature and reaction time using γ -valerolactone (GVL) as solvent. The starting reactant is glucose. Ideally it is obtained from renewable resources, more precisely procured from chocolate waste from Belgian industries.

Català

Aquesta tesi tracta de la deshidratació d'hidrats de carboni catalitzada amb àcid per obtenir 5-hidroxiimetilfurfural (HMF). La conversió es farà mitjançant un reactor de flux assistit per microones.

Durant les últimes dècades, l'HMF ha estat àmpliament estudiat, ja que es considera un dels productes químics basats en biotecnologia de gran valor afegit. Es pot convertir en una àmplia gamma de productes químics interessants aplicats en moltes aplicacions diferents, com ara combustibles i dissolvents, però sobretot, polietilè de 2,5-furandicarboxilat. Es tracta d'un plàstic basat en productes bio que pot reemplaçar el PET en moltes aplicacions.

Objectiu

L'objectiu principal d'aquesta tesi és optimitzar els paràmetres de reacció com la relació HCl/AlCl₃ (catalitzador), la temperatura i el temps de reacció utilitzant γ -

valerolactona (GVL) com a solvent. El reactiu inicial és glucosa. Idealment, s'obté a partir de recursos renovables. En aquest projecte, precisament es vol obtenir a partir de residus de xocolata procedents de les indústries belgues.

2 List of abbreviations and symbols

HMF 5-Hydroxymethylfurfural

GVL γ -Valerolactone

HCl Hydrochloric acid

AlCl₃ Aluminium chloride

CO₂ Carbon dioxide

HPLC High performance liquid chromatography

SMF 5-Sulfoxymethylfurfural

EM Electromagnetic

MW Microwave

3 Introduction

The introduction is divided into four parts. The first part gives general information about the importance of using renewable resources in order to synthesize HMF and the leading applications of this compound in the industry. The second part points out the structure, properties and dehydration reaction parameters of HMF. Next a brief introduction on how flow technology works is given as it is an upcoming technology, last a description of microwave synthesis is discussed and how can it help to improve reaction rates.

3.1 Importance of finding renewable resources for HMF

In past years, the interest in renewable resources has been growing fast as the world has been continuously dependant of petroleum-derived chemical components. Nowadays, rising CO₂ emissions, environmental pollution, diminishing petroleum reserves and global warming led to the investigation on new chemical processes which can be more suitable for the environment.

One of the potential resources is industrial waste. Waste and side products can be converted into valuable products. Carbohydrates are a really interesting material as starting material because they form by far the largest natural source of carbon, along with fatty acids, lipids, proteins, lignin and others. However, using this kind of biomass has a limitation, which is that the structure of these molecules has overabundance of oxygen so, a way of lowering the oxygen content of carbohydrates is doing a complete oxidation into CO₂, formic acid and formaldehyde. Another option is doing an hydrogenolysis which releases oxygen from the molecule by forming water at the expense of one hydrogen for each oxygen. Finally, the third option is dehydration of the carbohydrates into furans (for instance the HMF) and levulinic acid.

In this thesis, this last option will be used to convert carbohydrates into a valuable material. The starting material can be fructose or glucose obtained from a waste stream, in this case chocolate waste, and the target molecule to synthesize is HMF through an acid catalysed dehydration.

3.1.1 Why synthesize HMF?

HMF has been an excellent platform molecule because it is not only used to obtain highly energetic products such as 5-ethoxymethylfurfural, but also monomers like 2,5-furandicarboxylic acid and 2,5-hydroxymethylfuran which can be used to make high value polymers. HMF can also be used to make valuable intermediates for fine chemistry. Even levulinic acid which is an undesirable side-product in the synthesis of HMF, is a fine chemical, with high value and a wide range of small-scale applications. Therefore, many efforts have been performed to accomplish acid-catalysed dehydration of carbohydrates into HMF.

3.2 HMF

3.2.1 What is HMF?

5-Hydroxymethylfurfural, *Figure 1*, is an organic compound, with chemical formula $C_6H_6O_3$, which is highly soluble in both water and organic solvents. It consists of a furan ring with both an aldehyde and an alcohol as functional groups.

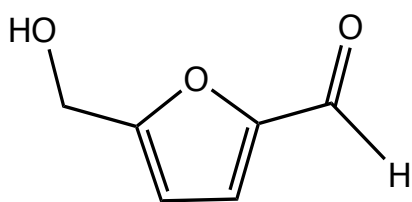


Figure 1. HMF

This compound is a yellow solid with caramel odor. Its melting point is between 30-34 °C and the boiling point between 114-116 °C at 1 mbar. Moreover, HMF absorbs UV-vis ($\lambda_{\max}$) at 284 nm, which is an important property for the quantification of HMF with HPLC.

3.2.2 HMF: Natural formation in honey and during baking process

HMF is present in nature. It can be found in natural honey as a consequence of the natural acidity of honey reducing the sugars usually at room temperature. This is a very slow process, only small quantities of HMF are formed when temperature is increased. For example during thermal treatment or improper storage, the quantity of HMF increases remarkably. So for honey HMF can be used as a quality marker of deterioration caused by age, improper storage or excessive heating.

Similarly, in the baking industry, during the baking process the rise in temperature leads to the evaporation of water from the dough and consequently forming the compounds,

such as HMF that contribute to flavour and browning. However, it is also observed the degradation of the sugar into HMF, for instance during the heating of the milk, which has high concentrations of lactose, a small amount of HMF is formed.¹ So, a lot of products which contain sugar can also contain small amounts of HMF. Although HMF is also present in nature it has some toxicity issues.

Several experiments with rats and mice have been performed in order to see the possible carcinogenic effects of HMF.² Itself, HMF is not mutagenic. However, its metabolite 5-sulfoxymethylfurfural (SMF) has a mutagenic potential. After the administration in mice's drinking water, the SMF was detected in vivo in the plasma of the mice. The cytotoxic effect of SMF is due to the interference with the transport of organic anions into renal proximal tubular cells, leading to kidney damage. Also, it was observed an increasing number of adenomas in small intestine, but they had no effect on the mice size and thus were classified as weak intestinal carcinogens. So, there's no evidence either the HMF is a substance with high toxic potential. The maximum exposure resulting from sources other than caramel colours and dried plums beverages (which have a high presence of this compound) is expected to be lower than 500 µg/kg per day. So, there is a margin of safety of more than 100. However, the data on exposure is still incomplete and more experiments have to be done.

3.2.3 HMF synthesis

The aim of this section is taking into account the reactions that occur. By analysing the reaction, different conditions can be evaluated. Variation in solvents, catalysts and reaction/residence time can be used to check the influence on the formation of HMF and the avoidance of side product formation. *Figure 2* illustrates the general pattern of acid-based reaction of glucose to HMF. This compound is mainly synthesized by the dehydration of carbohydrates as mentioned before. Main problem of this reaction is that it is a complex process due to the possibility of side-reaction, further explained in *Figure 4*.

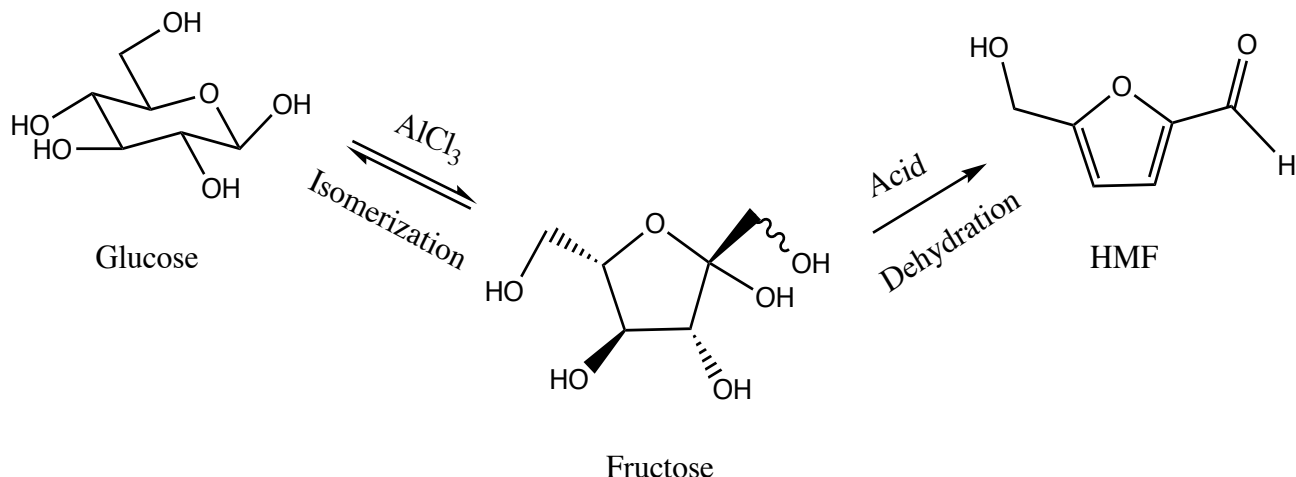


Figure 2. Acid-based dehydration of glucose to 5-hydroxymethylfurfural

In *Figure 3* can be seen the mechanism for the conversion of glucose and fructose to HMF. This conversion requires the loss of three water molecules.

First of all, there must be a fast isomerization from glucose to fructose using, in this case, AlCl_3 as a Lewis acid as catalyst. The formation of HMF easily occurs from fructose by cyclic intermediate enol, 2,5-anhydro-D-mannose, formed in tautomerization step. In the mechanism dehydration of fructose is initiated by the protonation of the most basic hydroxyl group of the molecule that is directly attached to the ring in a position alpha to the oxygen. The protonated form leading to spontaneous dehydration generates the intermediate enol that rearranges and then loses another water molecule, followed by deprotonation regenerating the catalyst and forming HMF.³

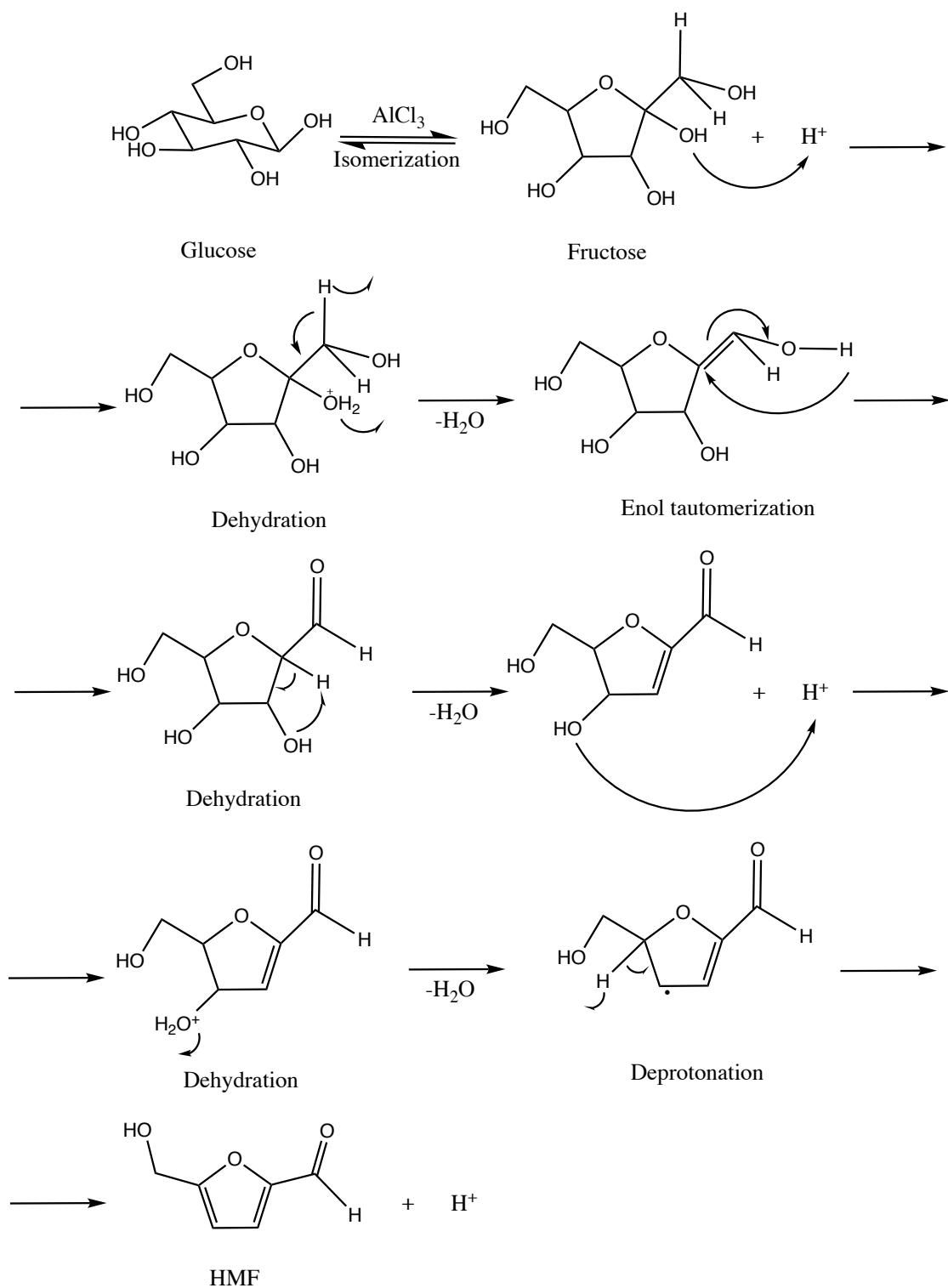


Figure 3. Mechanism acid-based dehydration of glucose to 5-hydroxymethylfurfural

3.2.4 Glucose vs fructose

Numerous articles have been reported in the literature using fructose as starting material. Many of them reach high yields for HMF conversion. Many efforts have been done to reach the same high yields when starting from glucose because it can be found in a lot of products like fruit, pasta, bread, vegetables, plants, paper and wood (in the form of cellulose).

One of the hypothesis why the yield is lower using the latter is because glucose reactivity is a lot lower than fructose because it is present in lower quantities of acyclic form compared to acyclic fructose. That means that glucose can form really stable ring structures compared to fructose, so the enolisation rate in solutions is lower in the former. This fact leads us to deduce that the rate-determining step for HMF formation, fructose will react faster than glucose. On the other hand, fructose forms by-products due to the equilibrium mixtures of difructose and dianhydrides where the most reactive groups are internally blocked. Glucose, for its part, forms oligosaccharides which still contain less reacting groups, resulting in a greater risk to obtain undesired products and lower reaction yield.

3.2.5 Reaction parameters

The HMF formation will depend on acid-based catalytic system combined with an adequate reaction medium and process. Down below the main parameters are listed which should be taken in to account during the reaction.

Side products

The low selectivity obtained when the reaction is carried out in pure water is due to the rehydration of the HMF into levulinic and formic acid forms. Furthermore, intermediates or the HMF itself can also polymerize to produce oligomers, called humins, that are not soluble in water. Also, there can be aldol polymerisation which give soluble polymers and the formation of furfurals. All the side products of the reaction are schematized in *Figure 4*. The formation of this secondary reaction is the main disadvantage, burdening the process by the loss of material and the necessity of purifying the product.

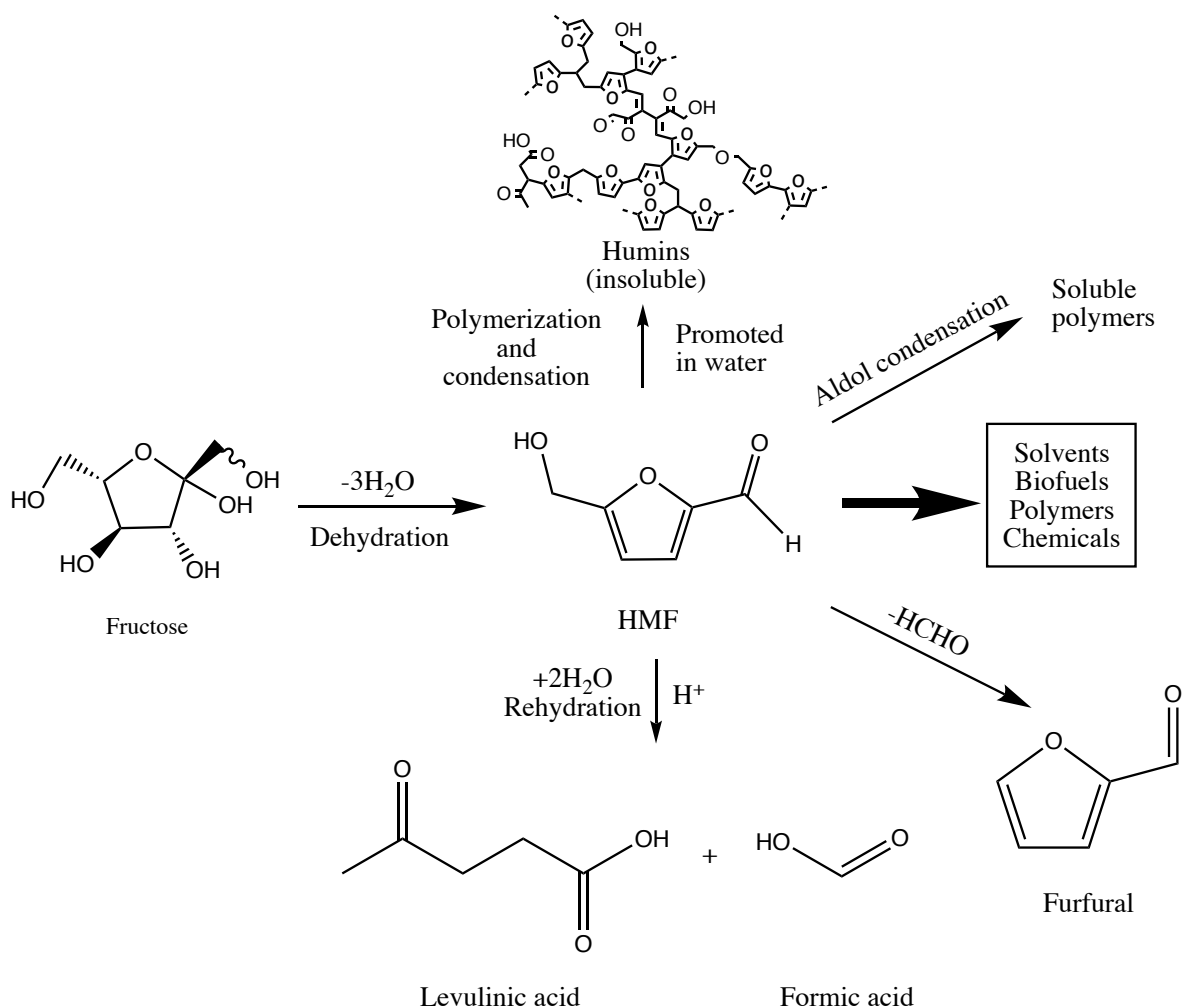


Figure 4. Side products HMF synthesis reaction

Solvent

Solvents are expected to play an important role in HMF formation. Water is an ideal green solvent for transformation of sugars, but the poor stability and high solubility of HMF in water results in low HMF selectivity and difficulties in HMF isolation.

According to literature, in order to solve the problem of this low selectivity of HMF in water as medium, the solvent should be able to draw water away from the reaction so that it cannot rehydrate the HMF to levulinic acid and formic acid. Therefore, different solvents and reaction methods have been tested.¹

The first method uses a biphasic system to avoid the successive transformation of HMF into side products by doing an extraction with a solvent immiscible in water.

The main drawback of this first option is the use of great amounts of solvent. This can be a problem for further applications in industry because it will be necessary to recycle this solvent. This recycle step will add an extra cost to the process. However, point out that the use of salts such as NaCl or KCl might be helpful during the reaction as the ions in solution alter the intermolecular forces between the liquids in equilibrium, resulting in an increase in immiscibility which enhances the extraction of HMF from the aqueous phase.

The second one is to perform the reaction in homogeneous organic/aqueous mixture or in pure organic solvent. Most widely used is DMSO reaching higher yields (42%) compared with water but also other types of solvents, like THF, can be used. New lines of research are pointing that gamma-valerolactone, which is a bio-based solvent, can also be an interesting solvent to use as it is produced from levulinic acid, a side product of HMF synthesis. In fact, this means that GVL can be made by converting carbohydrates into HMF which will then further be reacted to levulinic acid. GVL has like DMSO the possibility to mix with water and keep it away from the reaction. GVL has quite a similar structure to HMF which helps to extract the HMF out of the mixture of water and solvent.

Finally, other alternatives like using ionic liquids, for instance 1-H,3-methyl imidazolium chloride. These solvents have a low steam pressure, are not flammable and can be recycled many times as they are stable. Literature articles reported the efficient dehydration of fructose to HMF reaching 85% yield when using this kind of compounds. As in the first option, it is not viable for further applications in industry because of the high cost.

Catalyst

Some articles give insights on the catalyst properties which will suit the selective formation of HMF. Acidic pH range between 2,7-3,9 could prevent the formation of levulinic and formic acid. So far, catalysts efficient for production of HMF from fructose are rarely active for glucose and therefore low HMF is expected. Generally, best performances for the conversion of glucose to HMF are reported when a combination of both Brønsted and Lewis acids is used.⁴

For fructose, the Brønsted acid-catalysed dehydration improve HMF yield. However, for glucose, this kind of acidic compound does not enhance the reaction then, in order to use glucose as starting material, the isomerization to fructose has to be done.

Moreover, it is an equilibrium-limited reaction and Lewis acids have been shown to be active for glucose-fructose isomerization and can also tolerate high temperature and Brønsted acidity thus providing a promising alternative for the production of HMF.⁵

Selecting an efficient catalyst for glucose isomerization has a significant impact on glucose conversion to HMF. In industry, isomerase and Brønsted acids are catalyst commonly used to produce fructose from glucose. However, the limitations about using enzymes were the high cost of enzymes and the high sensitivity of enzymatic reaction under the operating conditions.⁶ Other studies investigated other tandem catalyst including aluminosilicate zeolites⁷, activated carbons⁸ and homogeneous metal chlorides⁹, such as CrCl_3 and AlCl_3 . The heterogeneous catalysts have been widely employed because of the easy separation.

The main drawback is that solid acid catalyst present additional challenges such as the formation of humins (insoluble polymers) during the reaction, which deactivate the catalyst due to the deposition of these side products on the surface.

New lines in investigation show that homogeneous metal chlorides can also be efficient. Both CrCl_3 and AlCl_3 work on the conversion of carbohydrates to HMF. Comparison of CrCl_3 with AlCl_3 shows that the latter is a safer choice although trivalent chromium is far less poisonous than hexavalent, chromium salts are generally considered toxic.

The object of this work is using the co-catalyst metal chloride AlCl_3 (Lewis acid) combined with Brønsted acid HCl to achieve the high efficiency production of HMF directly from glucose.

3.3 Flow technology

In the past few years, the continuous flow reactors have gained popularity in organic synthesis in both academic research and industrial industry. The concept of flow chemistry consists of a widely range of chemical processes that occur in a continuous flowing stream. Moreover, running a reaction under flow conditions requires the knowledge of the stoichiometry, residence time/ reaction time, flow rates and temperature control.

The set-up can be seen in *Figure 5*.¹⁰ First of all, the pumps which are used to deliver reproducible quantities of solvents and reagents. Next, the loops that introduce small quantities of reagents and the mixing point where the reagents streams are combined. Then there is the reactor which provides the residence time for the reaction. After it, the heat exchanger in order to cool down and the back-pressure regulator in order to control the pressure of the system and finally the downstream unit.

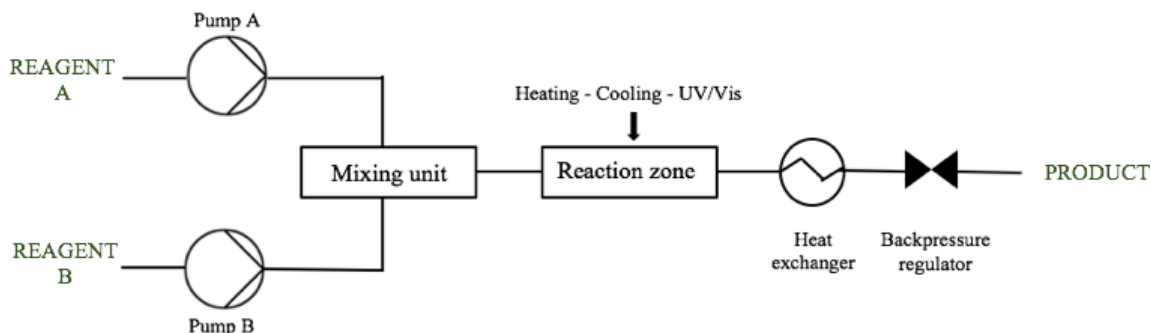


Figure 5. General concept of flow chemistry using microreactors. Reagents can be combined at precisely specified points along the reactor (residence time), heated, cooled, and quenched. The pressure resistance and high heat exchange efficiency allows high-temperature operation in super- heated solvents.

3.3.1 Flow technology versus batch

The characteristics and properties of these reactors offers many benefits for different reactions over traditional batch reactions.¹¹

- **Mixing in batch versus flow reactors**
In a chemical reaction, two important parameters must be carefully controlled: the mass and heat transfer. In batch systems, convection processes are mainly used. Stirring in classical reactors is limited by the inhomogeneity of the flow fields created by the stirring mechanism, resulting in turbulences and a chaotic mix. This leads to the formation of hot spots, which are inefficient, in the mixing dead points lowering the production. On the other hand, continuously reactors allow for rapid and homogeneous mixing because of their small dimensions.
- **Thermal control**
The temperature of the reaction is an important variable, leading to the desired product between either kinetic or thermodynamic pathway. In batch, the undesired side reaction can occur because of the broad temperature

distribution. Different from the flow reactor that can achieve such efficient input or removal of heat and nearly constant target temperature during the reaction.

- Increased rates of reaction, yields and selectivity
Because of the improvement of heat and mass transfer, reactions can be carried out significantly faster than those in batch, typically with the increase of both yield and selectivity.
- Wide range of reactions
Reactions that conventionally are defined as dangerous like, runaway or explosive reactions and cannot be done for safety reasons or are not possible to do in batch, are thus manageable with relatively low risk. For example, hazardous or sensitive molecules can be generated and immediately consumed in a subsequent synthesis step. This eliminates the need to handle or store excessive amounts of these potentially toxic, reactive or explosive intermediates.

As clearly pictured on the previous sections, the continuous flow comprises the following advantages. The high surface to volume ratios because of the small dimensions, enhanced mass and heat transfer, high volumetric productivity and laminar flow conditions.¹² Furthermore, in terms of the industrial application it offers low manufacturing, operating and maintenance cost, low power consumption and increased safety.

3.3.2 Outlook and perspectives

Although it is a promising technique, it is also an emerging one. Some issues are still significant and there are some limitations such as the obstruction of back pressure regulators and tubing when solids are formed.

There is already an impressive progress made in continuous flow technologies. Next goal is to set up a complete manufacturing process. Moreover, another challenge is to accomplish efficient synthesis of enantiomerically pure products under the flow conditions.¹²

3.4 Microwave synthesis

Using microwave as a heating method is over six decades old. Nowadays, fire is rarely used in order to heat a reaction. In 1855, a Bunsen burner was the heating source mostly used. It was applied to the reaction vessel in a focused manner. After short time, it was replaced by heating mantles, oil bath or hot plates where the solution heat was distributed more homogeneously. Finally, in the seventies the first microwave laboratory instrument was developed to analyse moisture in solids, but it was only in 1990 that microwave chemistry emerged and was developed as a field of study for its applications in chemical reactions.¹³

Microwave heating for chemical reactions has revolutionized organic synthesis. In certain chemical reactions, microwave heating produces higher yields compared to conventional heating methods. Moreover, this fact opens up new opportunities to the synthetic chemistry, in the form of new reactions that are not possible using conventional heating.

The fundamentals of this technique are that microwaves are a form of electromagnetic energy which lies in the EM spectrum between infrared and radio frequencies. Wavelengths are between 1 cm and 1 m and frequencies go from 300 to 300.000 MHz. The main difference comparing microwave to other forms of radiation, for instance X-rays or γ -rays, is that microwave is less energetic. Thus, it is non-ionizing and don't alter the molecular structure of the compounds being heated. It only provides thermal activation.

The mechanism of microwave heating involves agitation of polar molecules or ions that oscillate under the effect of an oscillating electric or magnetic field. In the presence of this kind of field, particles try to orient themselves to be in phase with it. The rapidly changing field affects the molecules constantly and consequently the molecule attempts to align itself with it by absorbing energy. Owing to intermolecular forces, polar molecules experience inertia and are unable to follow the field. This results in the random motion of particles and this random interaction generates heat.

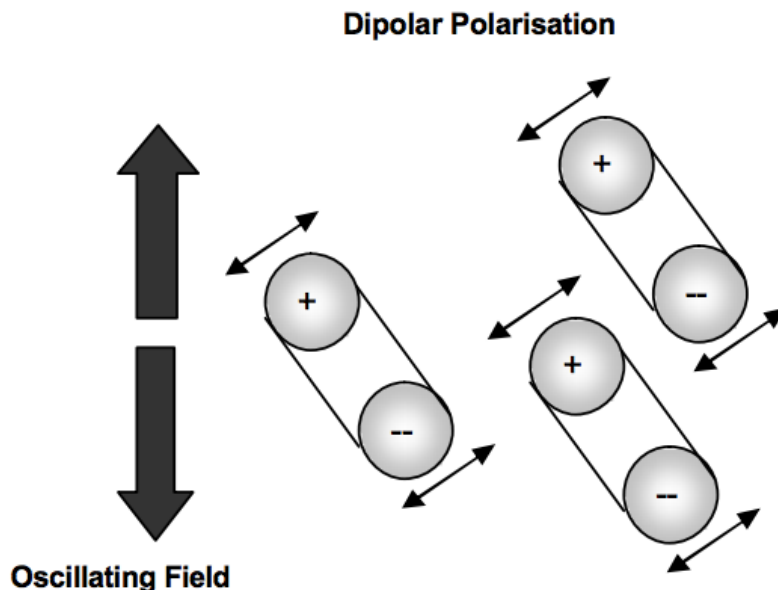


Figure 6. Heating method by dipolar polarisation.

The main requirement for dipolar polarisation is that the frequency range of the oscillating field should be appropriate to enable adequate inter-particle interaction. If the frequency range is very high, the intermolecular forces will stop the motion of a polar molecule before it tries to follow the field, resulting in inadequate interparticle interaction. Otherwise, if the interaction is too low, the polar molecule gets sufficient time to align itself in phase with the field. Therefore, no random interactions occur between the molecules.

The ability of a material to convert EM energy to thermal energy is related to the dielectric constant of each molecule. The larger the dielectric constant, the greater is the coupling with microwaves. Hence, polar solvents such as water, methanol, DMF, acetone, etc. are all heated rapidly when irradiated with microwaves. However, non-polar solvents like hexane, toluene, carbon tetrachloride, etc. do not heat in the microwave.

3.4.1 Microwave versus general heating methods

As it has been seen, microwave heating has some advantages over traditional batch chemical reactors. First of all, microwave heating is instantly controllable, the energy input responds immediately to the control input and it is easily scalable. Therefore, there is convenient programming, data management and automatic data recording.

Moreover, it heats the reaction mixture directly, not through heated surfaces and conduction or convection so, no hot or cold spots are formed. While with conventional heating, the heat comes from the outside and goes into the reaction mixture by convection currents (resulting in a very hot vessel wall), microwaves go through the microwave-transparent vessel wall and directly heat the reaction mixture on a molecular basis.¹⁴ This comparison between both methods is graphically illustrated in *Image 1*.

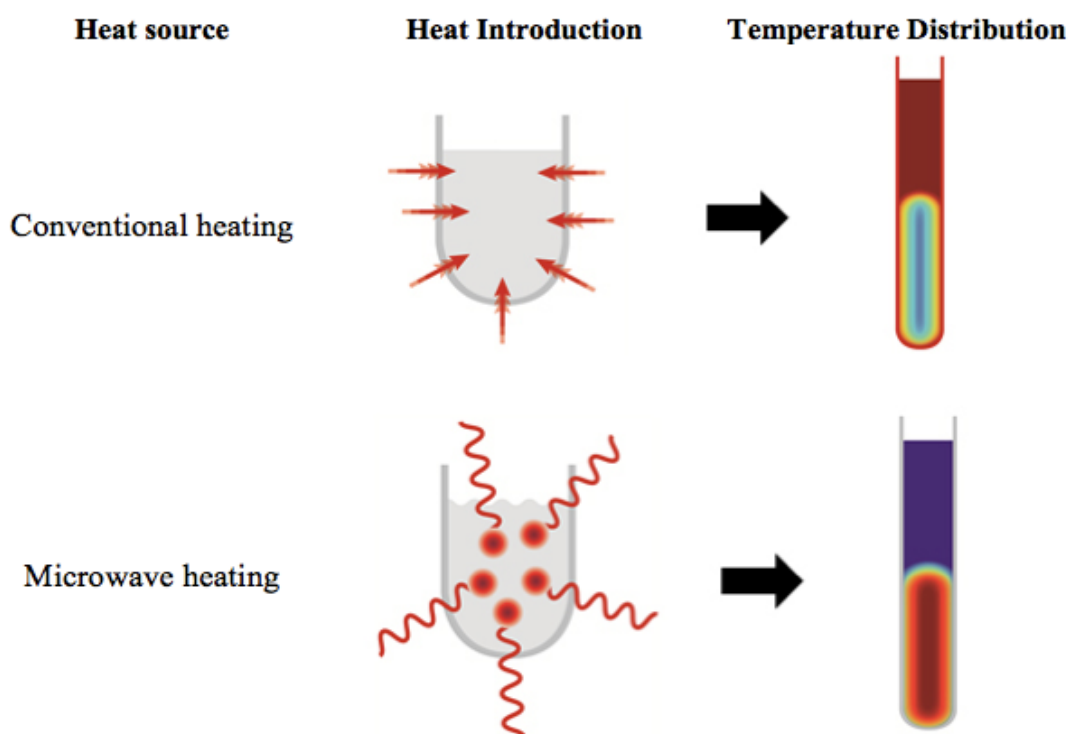


Image 1. Illustration of heat introduction and temperature into a reaction mixture for a conventional heating and microwave heating.

Eventually, it is cleaner because there is no oil or no fouling of hot surfaces. Furthermore, the microwave gives faster reactions with better yields and fewer by-products and finally, it supposes the reduction of energy use up to 90% versus conventionally heated batch reactions.

4 Experimental

4.1 Materials

D(+)-Glucose monohydrate, fructose, gamma-valerolactone (GVL), 5-hydroxymethylfurfural (HMF) and hydrochloric acid (HCl, 35%) were purchased in Sigma Aldrich. ZORBAX Eclipse XDB-C18 column (4,6 x 150 mm 5-Micron) was purchased in Agilent Technologies.

All the chemicals in this work were commercially available and were used without further purification.

4.2 Methodology of the conversion of fructose and glucose to HMF

The catalytic conversion of fructose to HMF was performed in a batch system consisting of an oil bath heated by a stirring and heating plate with temperature control. In a typical experiment, the adequate quantity of glucose and HCl was put in the solvent 9:1 GVL/water in a total volume of 5 ml. The reactor was electrically heated to the target temperature and after the reaction time of 20 minutes the vial was removed from the oil bath and quenched in tap water. Samples were diluted in 9:1 GVL/water solvent and filtered with 0,2 μ l PTFE syringe filter, prior to HPLC analysis.

The catalytic conversion of glucose to HMF was performed in 2 different batch systems. The first system is the same system which is used for fructose, the second system is a batch system which uses microwaves for heating. In this microwave system a reaction vial was introduced in the cavity of the microwave system (CEM Discover) and irradiated at different temperatures. After cooling the tub to room temperature, the reaction mixture was diluted in 9:1 GVL/water solvent and filtered with 0,2 μ l PTFE syringe filter, prior to HPLC analysis.

4.3 Analysis methods

The quantities of the product were determined using a high-performance liquid chromatography (HPLC) system (Waters e2695 Module). Specifically, HMF was determined using Agilent ZORBAX Eclipse XDB-C18 column (4,6 x 150 mm 5-Micron) and an Ultraviolet Detector (Waters 2998) at 284 nm. The temperature of the

column oven was set at 35°C, and the mobile phase was chosen to be acetonitrile/water (5:95, v/v), with a flowrate of 1 ml min⁻¹ and an injection volume of 20 µl.

4.3.1 Calibration curve

A calibration curve for HMF is done. Quantification range between 10 and 80 µg/ml was performed following an external calibration curve, at concentrations 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml. All the calibration standards were prepared from a 2000 µg/ml stock solution of HMF and GVL was used as the solvent.

The standards are stable during a long period of time, without the need of storing them in the fridge. Every day, the calibration curve is calculated by measuring the calibration standards. A calibration curve with a $R^2 \geq 0,9995$ is always obtained.

5 Results and discussion

Many efforts to achieve higher yields have been done in order to optimize the reaction from fructose to HMF using different methods such as ionic liquids, salts or HCl. In this work, the acid will be the catalyst to carry on the reaction. New tendencies are moving to use glucose as starting material instead of fructose as it is a more abundant reagent, but the main problem is the isomerization from glucose to fructose, which can be solved using the right AlCl₃ concentration. So, optimizing the ratio HCl/AlCl₃, high yields of HMF can be obtained. Furthermore, other parameters that are going to be optimized are reaction time and temperature.

5.1 Optimization fructose in batch

In this section, the optimization of the conversion to HMF from fructose is going to be done using the catalyst hydrochloric acid. Afterwards, different concentrations of fructose in the optimum acid concentration was performed. The reaction carried out can be seen in Figure 6.

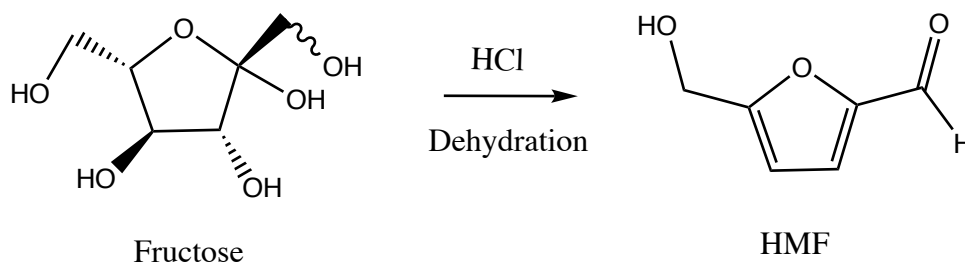
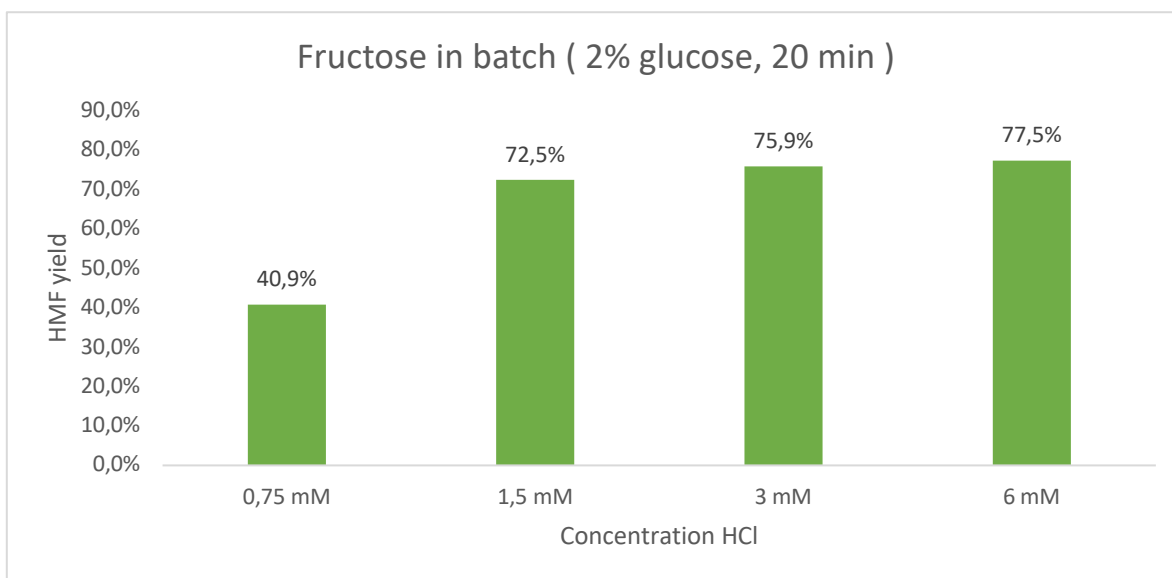


Figure 7. Conversion of fructose to HMF

5.1.1 HCl concentration

According to literature ¹⁵ the initial conditions were established as 2% of fructose, 3mM HCl, solvent ratio 9:1 GVL/H₂O, 170°C and reaction time of 20 minutes. Then, different HCl concentrations were tested: 6, 1,5 and 0,75 mM HCl. The results of HMF yield can be seen in *Graph 1*.

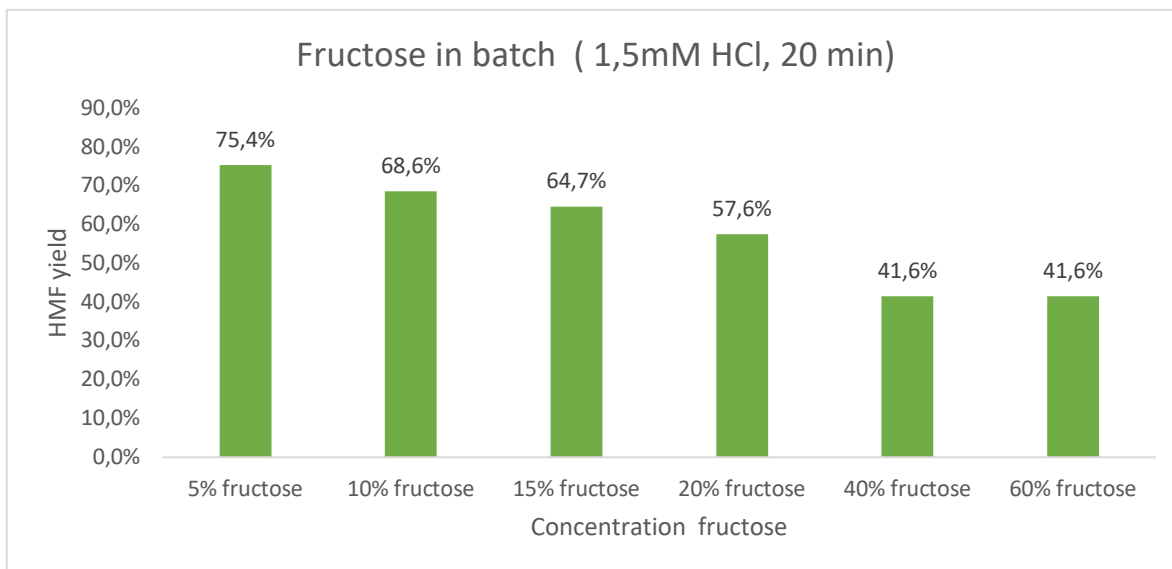


Graph 1. HMF yield for batch reaction. Conditions: 2% fructose, solvent 9:1 GVL/H₂O, reaction time 20 minutes.

The higher yield is obtained using 6 mM of HCl but it can be seen that using 1,5 mM of HCl gives barely the same yield as 3 mM although half of the concentration of the catalyst is used. The lower concentration of acid, the better for the industry process. Therefore, 1,5 mM HCl will be fixed from now on.

5.1.2 Initial fructose concentration

Next step is, with fixed 1,5mM HCl, determinate the yield for different initial fructose concentrations. From 5 to 60% concentrations were performed and the results can be seen in *Graph 2*.



Graph 2. HMF yield for batch reaction. Conditions: 2% fructose, 1,5mM HCl, solvent 9:1 GVL/H₂O, reaction time 20 minutes.

According to the results, the fructose concentration can be increased to 5% achieving a yield of 75,4%, which is a little bit higher compared to the 72,5% using 2% of fructose. The higher the concentration of fructose, the lower the yield. This fact can be explained because more humin formation is possible if there is more quantity of fructose and therefore, HMF production is hindered.

5.2 Optimization glucose in batch

The determining step from glucose to HMF is the isomerization of glucose to fructose, *Figure 7*. In order to improve this step, AlCl₃ is used as catalyst. Therefore, different concentrations of AlCl₃ and different reaction times are tested.

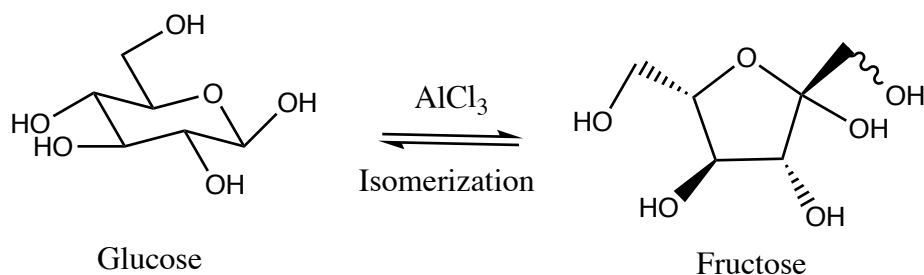
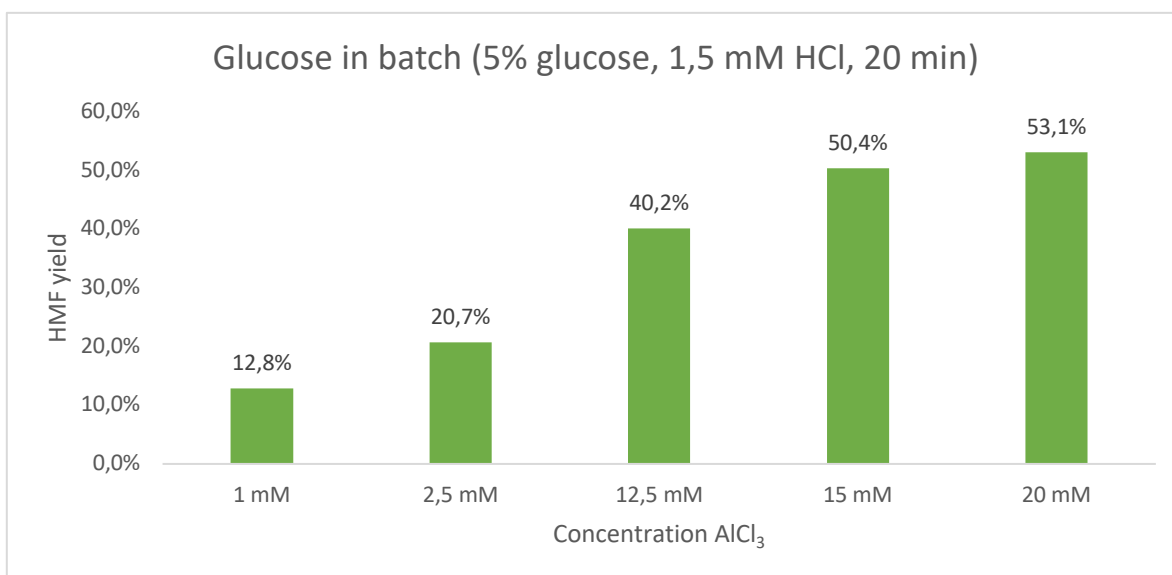


Figure 8. Isomerization of glucose to fructose.

5.2.1 AlCl_3 concentration

Reaction conditions were fixed according to the results of fructose in batch: 5% glucose and 1,5 mM HCl. Different concentrations of AlCl_3 were tested from 1 mM to 20 mM AlCl_3 . The results are shown in *Graph 3*.



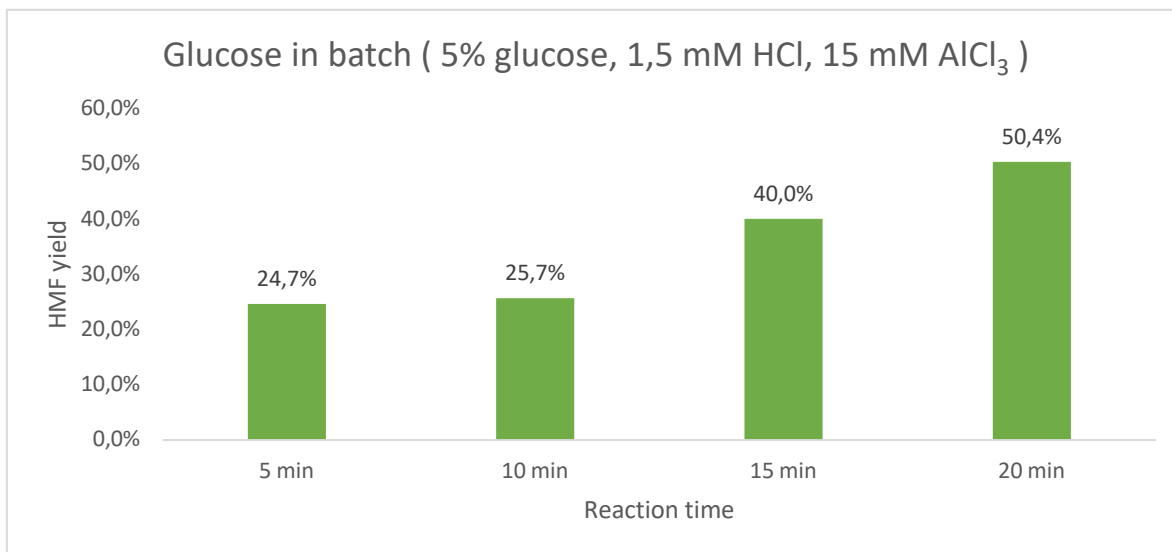
Graph 3. HMF yield for batch reaction. Conditions: 5% glucose, 1,5mM HCl, solvent 9:1 GVL/ H_2O , reaction time 20 minutes.

As it can be seen in *Graph 3*, the higher AlCl_3 concentration, the higher HMF yield. Main explication is that more quantity of catalyst allows a faster isomerization from glucose to fructose and so, the reaction to form HMF is enhanced.

Between 15 mM and 20 mM there is not a great difference. Therefore, in order to keep going on the optimization, only 3% of difference, so it is decided to use 15 mM AlCl_3 achieving 50,4% of yield.

5.2.2 Reaction time

In this section, the reaction time is optimized with the optimal ratio of HCl/AlCl₃. Different reaction times were performed in batch system. *Graph 4* illustrates the values obtained.



Graph 4. HMF yield for batch reaction. Conditions: 5% fructose, 1,5mM HCl, 15 mM AlCl₃, solvent 9:1 GVL/H₂O.

Chemical reactions vary greatly in the speed at which they occur. Some are essentially instantaneous, while others may take years to reach the equilibrium. The reaction rate for the given reaction is the measure of the change in concentration of the products per unit of time. As it was expected, lower reaction times aren't enough to reach a comparable yield of 20 minutes reaction.

The increase in yield can also be noticed with the naked eye because the color of the solution increases from the initial colorless to light yellow after 5 minutes, light orange after 10 minutes, brown after 15 minutes to finally reach dark brown after 20 minutes. Therefore, the formation of humis, non-desired side products, can also be seen in the walls of the vial as *Image 2* shows.

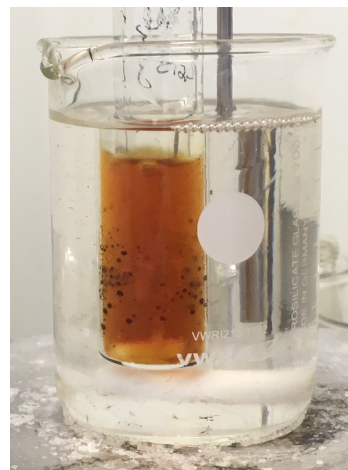


Image 2. Humins formed in vial's walls

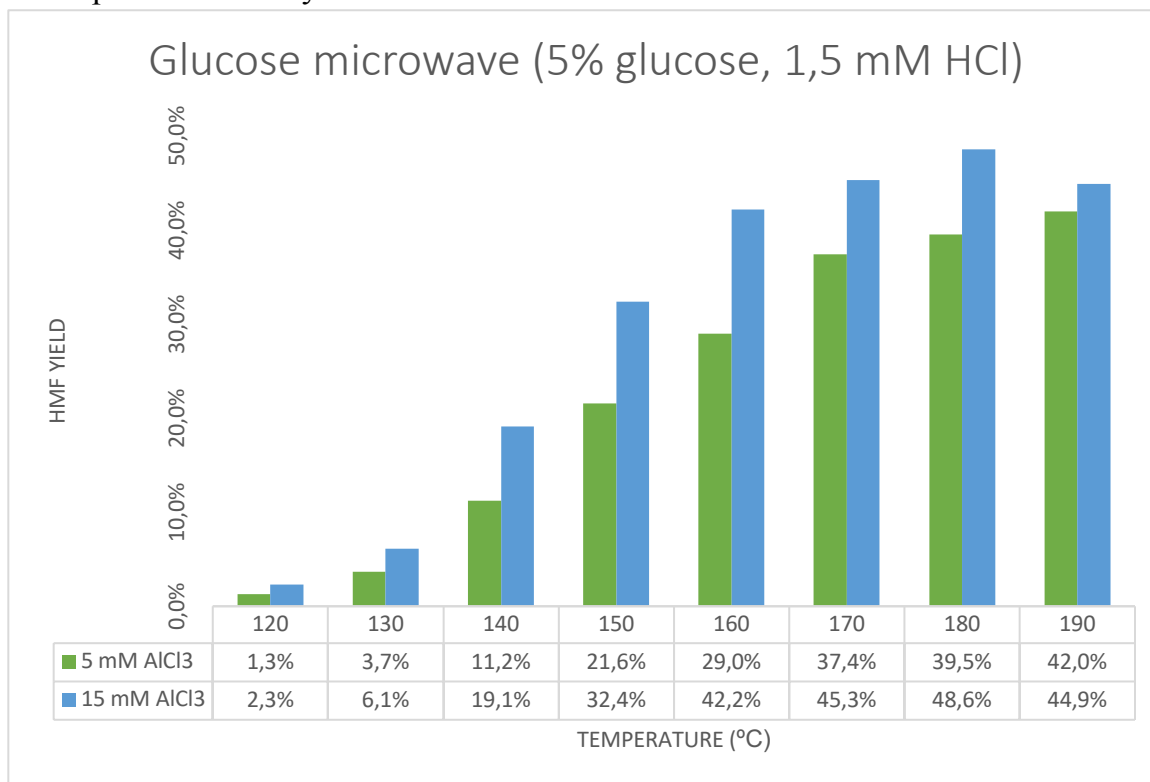
5.3 Optimization glucose in batch assisted by microwave

Once that it was optimized the catalyst concentration (HCl and AlCl_3) and the reaction time in batch for glucose, next step is optimizing the temperature using the microwave system because in the oil bath the temperature was set at 170 °C but it normally varied from 155 °C to 170 °C.

5.3.1 Temperature

For the moment, reaction conditions are 5% glucose, 1,5 mM HCl and reaction time of 2,5 minutes. Notice that using microwaves, the reaction time can be reduced by nearly 10 times. That is one of the main advantages when using this type of heating source.

For all the range of temperatures from 120°C to 190°C, 5mM and 15mM AlCl_3 reactions were tested, even if it was known that 15 mM AlCl_3 had a higher yield, it has to be taken in account that the lower concentration of acid, the better in order to scale up in the industry this reaction.



Graph 5. HMF yield for batch assisted with microwave. Conditions: 5% glucose, 1,5 mM HCl, 15 mM AlCl_3 , solvent 9:1 GVL/ H_2O , reaction time 2,5 minutes.

According to *Graph 5*, it can clearly be seen that the higher the temperature, the higher HMF yield. Reaching 48,6% in 180°C and 15 mM.

The tendency using 15 mM AlCl_3 seems to reach the maximum at 180°C and then going down for higher temperatures. By contrast, when 5 mM AlCl_3 is used, going to higher temperatures apparently should increase the HMF yield.

As said before, in this case can also be seen with the naked eye the different color of the solution. Solutions with higher yield are browner, see *Image 3*.

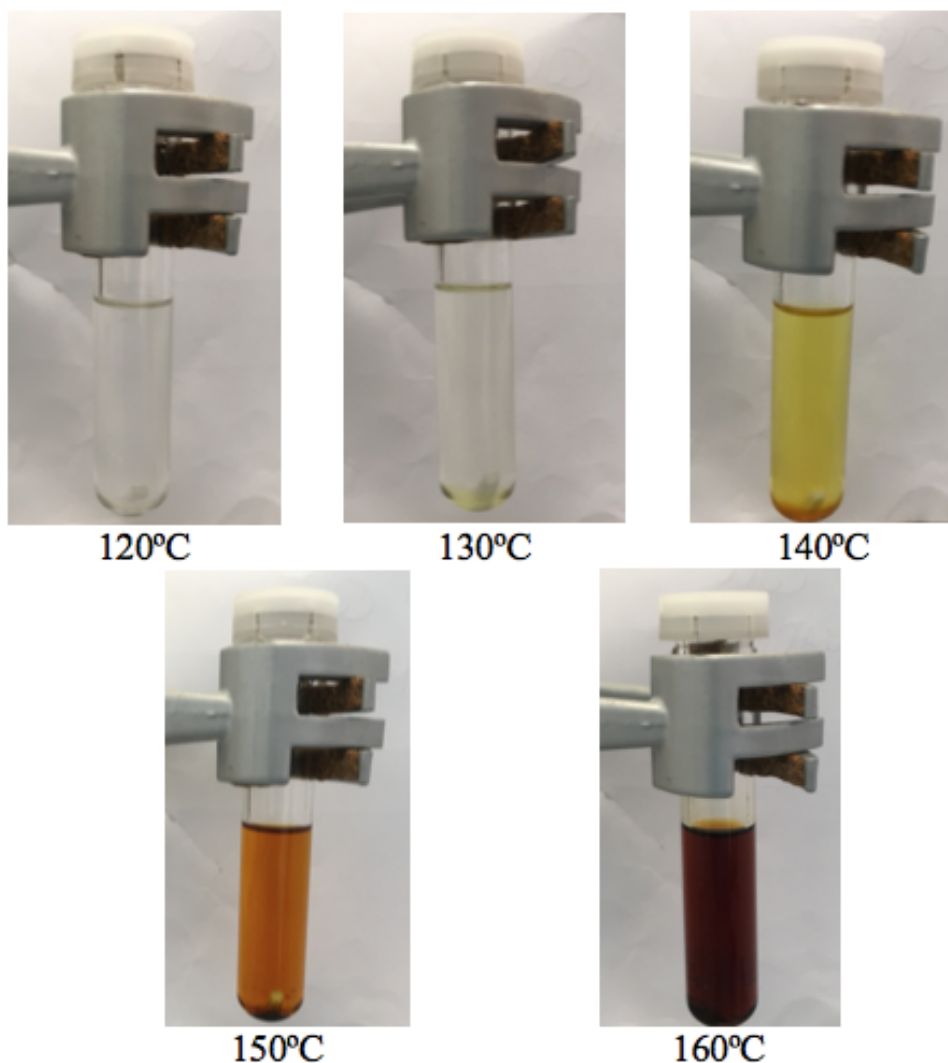
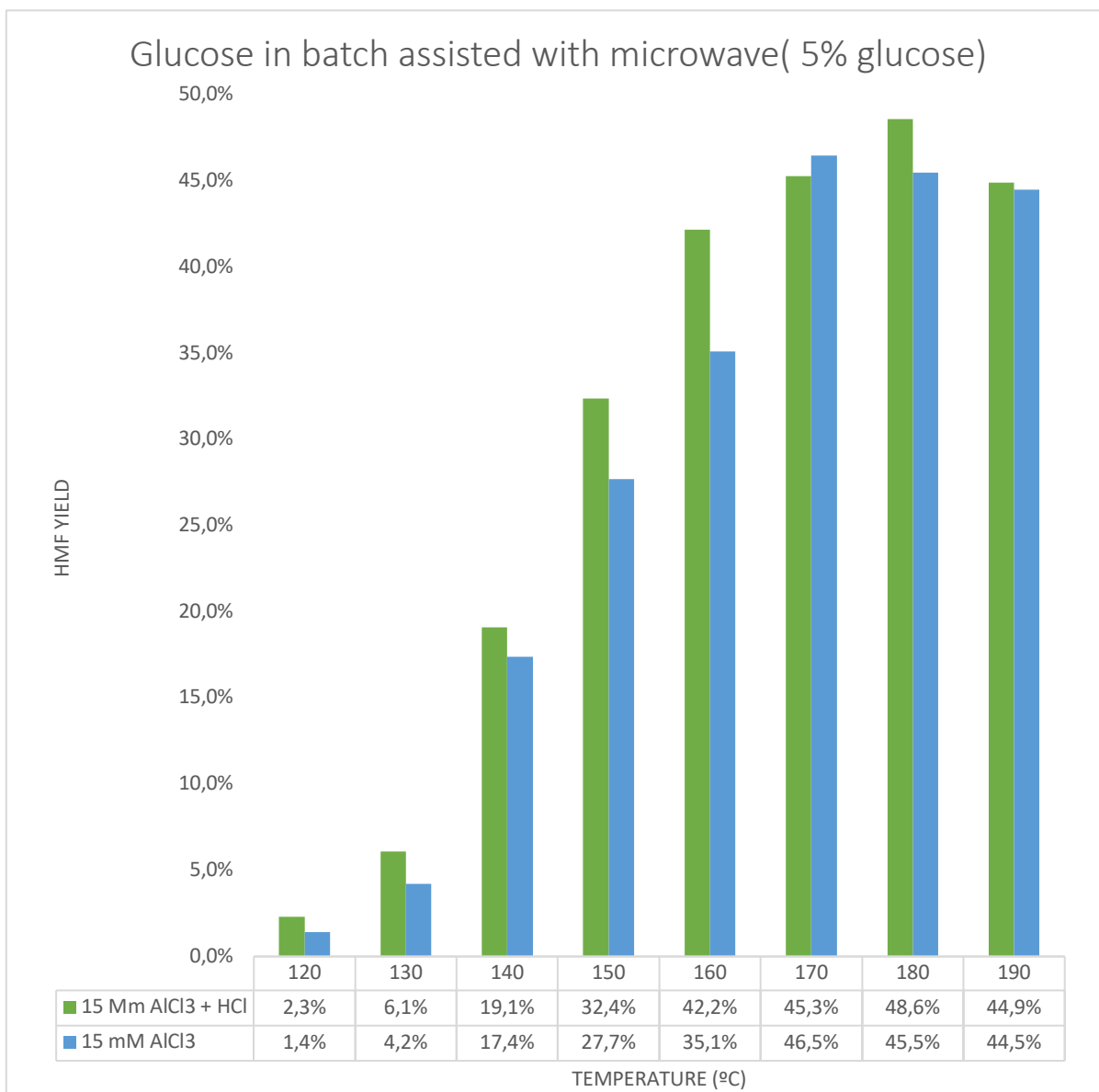


Image 3. Gradient of color for different reaction temperature

5.3.2 Comparison using AlCl_3/HCl or only AlCl_3 as catalyst

Although AlCl_3 can be used as a Lewis acid in water to carry on the isomerization of glucose to fructose, it is sensitive and can hydrolyse to form Brønsted acid (H_3O^+). Moreover, this process is more significant when increasing the temperature. For that reason, some experiments have been performed only using AlCl_3 as catalyst without HCl . It seemed that it was not necessary to add the 1,5 mM HCl because during the hydrolysis of AlCl_3 , HCl is formed.



Graph 6. HMF yield for batch assisted with microwave. Conditions: 5% glucose, 15 mM AlCl_3 , solvent 9:1 GVL/ H_2O , reaction time 2,5 minutes.

In order to test if there was a significant difference between using both 15 mM AlCl_3 with 1,5 mM HCl or only 15 mM AlCl_3 as catalyst, some experiments were carried out. For 5% glucose and the reaction in batch assisted with microwave, both reactions were done from 120°C to 190°C. The results can be seen in *Graph 6*.

On one hand, when using both acids as catalyst, the highest percentage of HMF yield is at 180°C reaching 48,6%.

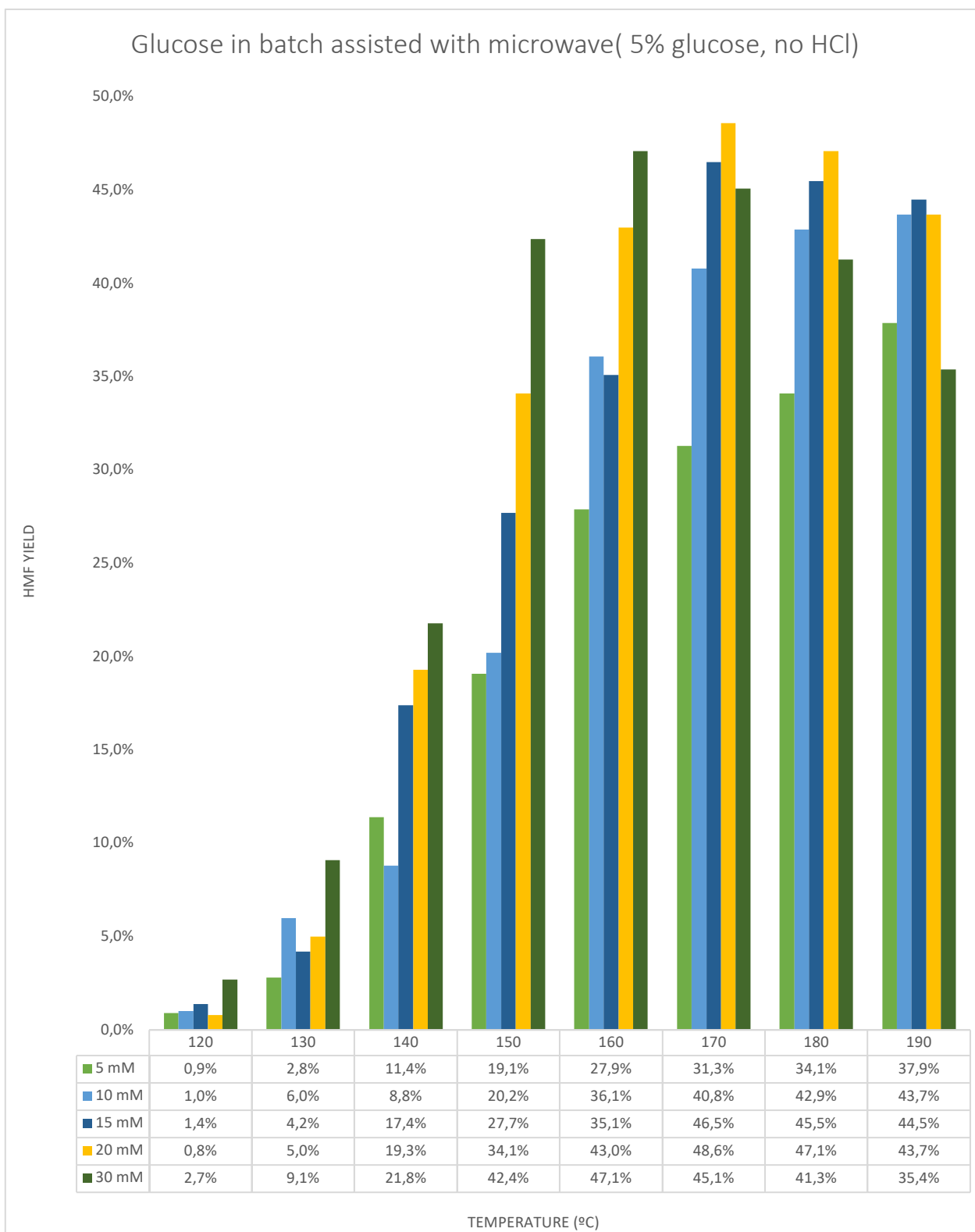
On the other hand, the highest yield for HMF when using only AlCl_3 (the same quantity) is at lower temperatures. 170°C are needed to get 45,3% of HMF. Therefore, there is a difference of less than 3% which is not that significant because there is no need of using HCl which is a corrosive acid and the reaction can be done at lower temperatures.

Also, it is important to point out that in both cases, after the maximum yield temperature, the tendency is to decrease again the yield of HMF. Consequently, no higher temperatures will be tested further than 190°C.

5.3.3 Only AlCl_3 as catalyst

After the results obtained in the previous section 5.3.2, it was found that the use of HCl does not make a huge difference in order to obtain a higher yield. It was subsequently decided to improve the reaction only using AlCl_3 and testing different concentrations and temperatures.

The concentrations went from 5 mM to 30 mM AlCl_3 and the range of temperatures was decided to be from 120°C to 190°C. Therefore, for each temperature were tested 5, 10, 15, 20 and 30 mM AlCl_3 .



Graph 7. HMF yield for batch assisted with microwave. Conditions: 5% glucose, 15 mM $AlCl_3$, no HCl, solvent 9:1 GVL/ H_2O , reaction time 2,5 minutes.

All the results are given in *Graph 7*. The chart outlines information about the main tendencies of HMF yield using different concentrations and temperatures.

According to the graph, there were upward trends on HMF yield when the concentration of AlCl_3 was increased and this pattern is repeated in each set of different temperature. This fact is due to the faster isomerisation from glucose to fructose, as the aluminium chloride is the catalyst and the main responsible to take forward the equilibrium between these two molecules. Therefore, there is more fructose available to react and so, the following step of dehydration and loss of three water molecules to finally convert into HMF is easier. As explained before, the hydrolysis of the Lewis acid provides the protons in the solution which are the catalyst of this second part of the reaction.

It is also worth mentioning the behaviour of the HMF yield in each concentration of AlCl_3 and how it progresses when different temperature conditions are set. First of all, if we focus on the lower concentration (5 mM AlCl_3), it has a linear growth when increasing the temperature until the maximum of 37,9% at 190°C, while leaving open the possibility for having higher yield to higher temperatures. Moreover, 10 mM AlCl_3 shows up the same tendency as the lowest concentration of catalyst but with the main difference that it has a slightly higher HMF yield, reaching its greatest concentration of 43,7% at 190°C.

Building up in this analysis, the behaviour of 15, 20 and 30 mM AlCl_3 has some differences compared to the two lowest catalyst concentrations. Henceforth, a pure linear growth cannot be seen. Once each concentration reaches its maximum, then immediately stops rising and starts falling moderately. The higher the concentration is, the lower temperature is needed to reach this point of highest HMF yield. While for 15 and 20 mM AlCl_3 the maximum is at 170°C 46,5% and 48,6%, respectively, for 30 mM AlCl_3 the highest HMF concentration is at 160°C and a final percentage of 47,1%. By decreasing 10°C in temperature and increasing the concentration of catalyst, the HMF yield dropped only 1% compared to the yields obtained at 170°C. This means that there is not a huge difference. However, it cannot be said that this difference is due to the conditions, because the HPLC system has a variation of about 2% for every result. This is experimentally determined by measuring the calibration curve every day.

Overall, this latest evidence leaves the possibility to decide whether it is better to work with higher temperature or higher concentration of aluminium chloride when scaling up this reaction to the industry.

6 Conclusion

English

As clearly pictured in the previous sections, considerable efforts have been made to improve the transformation of carbohydrates into HMF. Extensive research has been done for the conversion of fructose to HMF obtaining promising results, while the transformation of glucose remains difficult. The isomerization of glucose into HMF and the humin formation are the main drawbacks to achieve high yields of the desired product as it requires different conditions from the fructose dehydration step. Hence, in this thesis, the conversion of glucose has been explored and an overall process based on the combination of Brønsted and Lewis acid as catalyst was optimised. For the optimisation parameters like acid concentration, temperature and reaction time were tested. The future scope is to attain positive results and achieve higher yields with more efficient reaction conditions (lower temperature, higher carbohydrate initial concentration and lower concentration of acid) which provides an environmentally friendly process.

Overall, all the results obtained during the research can be differentiated in three blocks.

- Optimization fructose in batch

Batch reaction conditions established: reaction time 20 minutes and solvent 9:1 GVL/H₂O.

- When hydrochloric concentration is optimised in a range from 0,75 mM to 6 mM HCl and using 2% of initial concentration of fructose, the best HMF yield was chosen to be 1,5 mM HCl, reaching 72,5% and allowing to decrease the concentration mentioned in literature by half.
- When the initial fructose concentration is optimised in a range from 5 to 60% and fixed 1,5 mM HCl, the higher percentage is for 5% of fructose achieving 75,4%. Therefore, it permits increase from 2% to 5% the initial fructose concentration.

- Optimization glucose in batch

Batch reaction conditions established: 5% initial concentration of glucose, 1,5 mM HCl, reaction time 20 minutes and solvent 9:1 GVL/H₂O.

- When aluminium chloride concentration is optimised in a range from 1 mM to 20 mM AlCl₃, the highest yield is using 20 mM AlCl₃, but it is decided to use 15 mM AlCl₃ (50,4% of yield) as it is only 3% less of difference and it is better to use less quantity of acid.
- When reaction time is optimised in a range from 5 to 20 minutes and fixed conditions of catalyst at 1,5 mM HCl and 15 mM AlCl₃, a clear difference in HMF yield can be noticed with the naked eye because of the colour change from the initial colourless solution at 5 minutes to a dark brown final solution after 20 minutes. Therefore, the darker the solution, the higher HMF yield was achieved. Consequently, the reaction time is maintained at 20 minutes.

- Optimisation glucose in microwave assisted batch

Batch reaction conditions established: 5% initial concentration of glucose, 1,5 mM HCl, reaction time 2,5 minutes and solvent 9:1 GVL/H₂O.

- When temperature is optimised in a range from 120°C to 190°C, 5 mM and 15 mM AlCl₃ are tested. Reasonably, the higher concentration of acid, the higher HMF yield. Hence, the maximum reaction yield is using 15 mM AlCl₃ accomplishing 48,6% at 180°C.
- Because of the hydrolysis of aluminium chloride in water to form Brønsted acid, some experiments have been done in order to know if there is a significant difference between using both catalysts (AlCl₃ and HCl) or only aluminium chloride. The results show that the variation is less than 3% of HMF yield which is not significant. Further experiments were conducted to test only the Lewis catalyst.
- When only using aluminium chloride without using the hydrochloric acid in a range of temperatures from 120°C to 190°C, the tendency is to have the maximum of the yield at lower temperatures every time the concentration of catalyst is increased. For 20 mM the higher yield is 48,6% at 170°C and for 30 mM is 47,1% at 160°C.

This fact opens the possibility to make the process greener and cheaper. If it is possible to recycle the catalyst, the lower the temperature, the less power is needed to supply into the process. Otherwise, if it cannot be recycled the catalyst then has to be taken in account and balance whether it is better to use higher temperature or higher concentration of acid in order to get the higher yield.

It is also important to mention that one of the main objectives of this thesis was studying the reaction rate when using flow reaction technology. However, due to some problems that we had with the HPLC system at the beginning of the internship the experiments were delayed. Due to the lack of time no reactions using flow technology where performed so further research in this way has to be done from now on.

Català

Com es pot veure clarament en els apartats anteriors, s'han fet esforços considerables per millorar la transformació dels hidrats de carboni a HMF. S'ha fet una investigació exhaustiva per a la conversió de la fructosa a HMF obtenint resultats prometedors, mentre que la transformació de la glucosa continua sent difícil. La isomerització de la glucosa en HMF és el principal inconvenient per aconseguir alts rendiments del producte desitjat ja que requereix de diferents condicions que el pas de deshidratació de fructosa. Per tant, en aquesta tesi, s'ha explorat el procés combinant àcids de Brønsted i Lewis com a catalitzadors i també s'ha optimitzat altres paràmetres com la temperatura i el temps de reacció. L'objectiu principal és assolir resultats positius i obtenir rendiments més alts amb condicions de reacció més eficients (menor temperatura, major concentració d'hidrats de carboni inicial i menor concentració d'àcid) que proporciona un procés més respectuós amb el medi ambient.

En general, tots els resultats obtinguts durant la recerca, es poden diferenciar tres blocs.

- Optimització de la fructosa en batch

Es van establir les condicions de reacció en batch: temps de reacció 20 minuts i solvent 9:1 GVL / H₂O.

- Quan es va optimitzar la concentració d'àcid clorhídric en un interval de 0,75 mM a 6 mM HCl i amb un 2% de concentració inicial de fructosa, el millor rendiment de HMF va ser 1,5 mM HCl, arribant al

72,5% permetent disminuir a la meitat la concentració establerta inicialment que s'indicava en els articles científics.

- Quan es va optimitzar la concentració inicial de fructosa en un interval de 5% a 60% i es va fixar 1,5 mM HCl, el percentatge més alt és el de 5% de fructosa que arriba fins al 75,4%. Per tant, permet augmentar del 2% al 5% la concentració inicial de fructosa.
- Optimització de la glucosa en batch
Es van establir les condicions de reacció en batch: 5% concentració inicial de glucosa, 1,5 mM HCl, temps de reacció 20 minuts i dissolvent 9:1 GVL / H₂O.
 - Quan es va optimitzar la concentració de clorur d'alumini en un interval d' AlCl₃ de 1 mM a 20 mM, el major rendiment va ser 20 mM d' AlCl₃, però finalment, es decideix utilitzar 15 mM AlCl₃ (50,4% de rendiment) ja que la diferència és només del 3% i és millor utilitzar menys quantitat d'àcid.
 - Quan el temps de reacció es va optimitzar en un interval de 5 a 20 minuts i condicions fixes de catalitzador a 1,5 mM HCl i 15 mM AlCl₃, es pot notar una clara diferència a simple vista en el rendiment d' HMF a causa del canvi de color. S'observa el pas de solució incolora inicial a una solució final marró fosc. Per tant és proporcional, com més fosca és la solució, s'aconsegueix un major rendiment HMF. En conseqüència, el temps de reacció es manté a 20 minuts.
- Optimització de la glucosa en batch assistida amb microones
Es van establir condicions de reacció en batch: 5% concentració inicial de glucosa, 1,5 mM HCl, temps de reacció 2,5 minuts i dissolvent 9:1 GVL / H₂O.
 - Quan es va optimitzar la temperatura en un interval de 120°C a 190°C, es va provar 5 mM i 15 mM d' AlCl₃. Raonablement, a major concentració d'àcid, major rendiment d' HMF. Per tant, el màxim rendiment obtingut de la reacció és quan s' utilitza 15 mM AlCl₃ aconseguint el 48,6% a 180°C.
 - A causa de la hidròlisi del clorur d'alumini a l'aigua per formar l'àcid de Brønsted, s'han realitzat alguns experiments per saber si hi ha una diferència significativa entre utilitzar els dos catalitzadors (AlCl₃ i HCl)

o només clorur d'alumini. Els resultats mostren que la variació és inferior al 3% del rendiment HMF, no és molt significativa la diferència. Per tant, es van decidir provar altres experiments utilitzant solament el catalitzador de Lewis, AlCl_3 .

- Quan només es va emprar clorur d'alumini sense utilitzar l'àcid clorhídric en un interval de temperatures de 120°C a 190°C , la tendència és tenir el màxim rendiment a temperatures més baixes cada vegada que augmenta la concentració de catalitzador. Amb 20 mM AlCl_3 , el rendiment màxim és del 48,6% a 170°C i en canvi, en el cas de 30 mM és del 47,1% a 160°C .

Aquest fet obre la possibilitat de fer el procés més ecològic i econòmic. Si és possible reciclar el catalitzador, com més baixa sigui la temperatura, es necessita menys energia per alimentar el procés. En cas contrari, si no es pot reciclar el catalitzador, s'ha de tenir en compte i decidir si és millor utilitzar una temperatura més alta o una major concentració d'àcid per aconseguir un major rendiment.

També cal esmentar que un dels objectius principals d'aquesta tesi era estudiar la reacció quan s'utilitza la tecnologia de reactors de flux. Tanmateix, a causa d'alguns problemes que van haver-hi amb el sistema HPLC al començament de la pràctiques, es va veure retardat el començament dels experiments. Així doncs, finalment no s'ha tingut temps de fer les reaccions utilitzant la tecnologia de flux, per la qual cosa s'ha de continuar en aquesta direcció en la recerca d'ara endavant.

7 List of references

- (1) Rosatella, A. A.; Simeonov, S. P.; Frade, R. F. M.; Afonso, C. A. M. *Green Chem.* **2011**, *13*, 754.
- (2) Abraham, K.; Gürtler, R.; Berg, K.; Heinemeyer, G.; Lampen, A.; Appel, K. E. *Mol. Nutr. Food Res.* **2011**, *55*, 667–678.
- (3) Melo, F. C. de; Souza, R. F. de; Coutinho, P. L. A.; Souza, M. O. de; Melo, F. C. de; Souza, R. F. de; Coutinho, P. L. A.; Souza, M. O. de. *J. Braz. Chem. Soc.* **2014**, *25*, 2378–2384.

- (4) Essayem, N.; Rataboul, F.; Souza, R. L. de; Yu, H. *Challenges.*; MDPI AG, 2012; Vol. 3.
- (5) Dallas Swift, T.; Nguyen, H.; Anderko, A.; Nikolakis, V.; Vlachos, D. G. *Green Chem.* **2015**, *17*, 4725–4735.
- (6) Wang, C.; Zhang, L.; Zhou, T.; Chen, J.; Xu, F. *Sci. Rep.* **2017**, *7*, 40908.
- (7) Elsevier Scientific Publishing Company. *Applied catalysis. A, General.*; Elsevier, 1991.
- (8) Mazzotta, M. G.; Gupta, D.; Saha, B.; Patra, A. K.; Bhaumik, A.; Abu-Omar, M. M. *ChemSusChem* **2014**, *7*, 2342–2350.
- (9) Saha, B.; Abu-Omar, M. M. *Green Chem.* **2014**, *16*, 24–38.
- (10) Gutmann, B.; Cantillo, D.; Kappe, C. O. *Angew. Chemie - Int. Ed.* **2015**, *54*, 6688–6728.
- (11) Mason, B. P.; Price, K. E.; Steinbacher, J. L.; Bogdan, A. R.; McQuade, T. D. *Chem. Rev.* **2007**, *107*, 2300–2318.
- (12) Porta, R.; Benaglia, M.; Puglisi, A. *Org. Process Res. Dev.* **2016**, *20*, 2–25.
- (13) Taylor M; Atri B; Minhas S. *Evalueserve* **2005**, 1–50.
- (14) Hayes, B. L. *Microwave Synthesis. Chemistry at the Speed Light*; 2000.
- (15) Gallo, J. M. R.; Alonso, D. M.; Mellmer, M. A.; Dumesic, J. A. *Green Chem.* **2013**, *15*, 85–90.