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IONIC LIQUID DRIVEN ESTERIFICATION OF AQUEOUS FERMENTATION PRODUCTS

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Preface

Writing a preface gives a peculiar feeling of appeasement. After almost eleven months of inspiration and transpiration, this project comes to an end. Time to write the final lines, implement the concluding remarks and then entrust this work to you, the reader. Precious time, that is, for in the light of the approaching deadline, there is always another paragraph that needs rephrasing, a table to be adjusted or another idea to be discussed. This precious time could however not be invested more fruitful than by thanking the people that made this possible.

There are a lot of people that deserve my gratitude for inspiring me, influencing me, shaping me and bringing me to where I am today, and I hope I have expressed my gratitude enough along the way. I would however like to grasp this occasion to thank the people that were involved in this project, the concluding piece of my educational career.

I would like to thank professor Stevens and professor Rabaey for the opportunity to conduct this research. Their enthusiasm, keen perception and support made me always look forward to subject my work to their judgement and be inspired by their ideas. They moreover made me feel really involved in the project, and not just completing this as a task to graduate.

My tutors deserve to be honoured for their help, their patience, their ideas,… I'm particularly grateful for the way they initiated me to conducting a thesis in all its aspects, yet gave me the freedom and responsibility to bring up my own ideas and set my own course. Jan, your guidance and availability in the lab, your patience, your high standards for written and presented work and optimism during my seemingly everlasting NMR quest are very much appreciated and were of vital importance for this thesis. Stephen, I always looked forward to a meeting with you, to be enlightened by your strong conceptual thinking, to see the broader picture, the occasional quaint quote and your ability to motivate me yet keep me with my feet on the ground. You taught me there is always another point of view.

I would furthermore like to thank all the people of SynBioC and LabMET. It was an enrichment to conduct my thesis at two different research groups with different people, different visions, different inputs. Special thanks to Marta for the help with analytical and practical issues and giving feedback on presentations, to Sylvia for the MES extractant and to Wouter and Sigrid for their help and patience with my NMR analysis. Obviously thanks to the thesis buddies, to adorn my days with pleasant conversations, the support when things didn't go quite as expected, and the fun activities in and out the lab. I hope many reunions and unexpected encounters may follow.

Finally I want to thank my friends, for their understanding when I once more had to '*thesissen*', their support and the delightful moments we had this year, previous years and the many years to come. Special thanks to my family for their support and commitment over the years.

Abstract

Volatile fatty acids (VFAs) are sustainable platform chemicals that are ubiquitous in nature. They are appealing intermediates for valorisation in a resource recovery oriented industry, as they can be produced from bio-industrial waste streams through mixed culture fermentation and even from CO_2 through microbial electrosynthesis. Development of these emerging production platforms into solid technologies can drive the transition of disposal of the organic waste streams to valorisation and exploitation as sustainable sources with simultaneous recovery of water and nutrients. Their occurrence in low titre aqueous solutions as water-miscible species is however a challenging case for extraction, for which conventional methods such as distillation or liquid-liquid extraction are unattractive. The aim of this thesis is to perform a reactive extraction of VFAs from a low titre aqueous layer through biphasic esterification, i.e. extraction into a hydrophobic layer with subsequent conversion to the corresponding ester upon addition of an alcohol (e.g. bio-ethanol). This conversion results in an upgrade of low value intermediates to higher value products, that display a reduced water solubility and an increased volatility. The biphasic esterification is performed with ionic liquids (ILs) as hydrophobic layer. ILs are regarded as green solvents due to their good solvating properties, high stability and non-volatility. They are therefore suitable vectors to perform the biphasic esterification, as they pertain to the sustainability of the process and removal of the product is facilitated by the volatility difference between the product and the IL. Tetra-alkylphosphonium ILs were selected as the most suitable for this application, due to their hydrophobicity and low density. Physical data and applied research on these novel compounds is limited up-to-date, so this thesis is a pioneering study aiming to unravel the properties and behaviour of ILs in the biphasic esterification and give an indication on the potential of this process.

In the first phase, a proof of concept was performed on acetic acid as model VFA and trihexyl(tetradecyl)phosphonium dicyanamide as IL. The presence of ethyl acetate in the condensate after evaporation of the IL layer and a decrease in acetic acid concentration in the aqueous layer indicated the proof of concept was successful. On the other hand, it stressed the lack of a practical analysis method for the IL layer as a bottleneck for further experiments. A quantitative analysis method through ^{1}H NMR and ^{13}C NMR spectroscopy with an internal standard was developed and successfully implemented. The biphasic esterification was split into three separate steps in subsequent experiments: extraction of acetic acid with the IL, esterification of acetic acid in the IL and evaporation of ethyl acetate from the IL. These experiments were performed with two phosphonium ILs with different anions. The experiments indicated that the influence of the anion on the extraction (20% vs 5%) and the esterification (minimal conversion vs 85% conversion) is substantial. The evaporation experiments showed an almost quantitative removal of ethyl acetate at mild conditions (p=40mbar, T=55 °C, t=5 min.). Preliminary experiments demonstrated that exchange of the IL anion for aqueous layer anions is possible depending on the type of anion, thus altering the IL composition and properties.

The preliminary experiments in the first phase shaped a framework for a more structured study of the relationship between the IL anion and the extraction and esterification capacity, as well as the possible anion exchange. This was conducted in the second phase: five trihexyl(tetradecyl)phosphonium ILs were compared on the level of extraction and esterification of acetic acid, evaporation of ethyl acetate and anion exchange. In this way, we aimed to gain insight in the different steps and select a suitable IL for the biphasic esterification. Extraction showed to be the rate limiting step, with extraction capacities ranging from 4% to 23% for a 10:1 ratio aqueous to IL layer. The esterification performance was either

minimal or high and fast with conversions of 75% or 85% in the first 15 minutes. None of the five ILs showed a high extraction capacity and a high esterification capacity at the same time. The evaporation efficiency ranged between 75% and 95%, and showed to be inversely related to the viscosity of the IL. Trihexyl(tetradecyl)phosphonium bis(trifluoromethylsulfonyl)imide ($P_{666,14}$ Tf_2N) was selected as most suitable, due to a high and fast conversion (85% in 15 minutes) into the ester. It was moreover the only of the five ILs of which the anion did not exchange. The extraction capacity was 4% for a 10:1 ratio aqueous layer to IL layer. 89% removal of ethyl acetate was possible through evaporation in only 5 minutes.

In the third phase, experiments were conducted to unravel suitable reaction conditions with $P_{666,14}$ Tf_2N as IL. The effect of temperature and ethanol concentration on the esterification was studied. For milder conditions, even a 1:1 molar ratio of ethanol to acetic acid, the same conversion was reached, but at a lower rate. The presence of water constrains the rate and conversion of the esterification. It is preferable not only to remove the aqueous layer after extraction and before esterification, but even centrifuge the IL layer to remove residual water droplets. We furthermore showed that the extraction can be performed at room temperature with the same efficiency, which results in a decreased energy input. The most suitable reaction set-up was thus identified as a room-temperature extraction, decantation of the IL layer, centrifugation of the IL layer to remove small water droplets, heating and addition of ethanol to thrive the esterification and finally application of a vacuum pressure on the heated IL layer to remove the formed ester. The IL layer can then be recycled for the next extraction.

This reaction set-up was applied on microbial electrosynthesis extractant, containing 14 g/L acetic acid produced from CO_2, with $P_{666,14}$ Tf_2N as IL. The extraction efficiency was 3% after 2 hours, and dropped to 1% after centrifugation of the IL layer. 90% of this acetic acid was converted to ethyl acetate in 30 minutes, of which 78% could be removed by evaporation. The anion composition of the aqueous layer was unchanged, indicating no anion exchange had taken place. This experiment was repeated, but with a 1:1 ratio of aqueous layer to IL layer during extraction and for five consecutive cycles with the same IL layer and the same microbial electrosynthesis extractant as aqueous layer. There was no drop in performance of the process during the five cycles, and overall 28% of the acetic acid could be removed as ethyl acetate. The results from the possible anion exchange were inconclusive.

In the last phase, a preliminary experiment on the extraction of propionic, butyric and valeric acid was performed. Higher extraction capacities were obtained due to the higher hydrophobicity with increasing alkyl chain length. An extraction capacity of 25% was observed for valeric acid with a 10:1 ratio aqueous to IL layer. Esterification of valeric acid with ethanol in $P_{666,14}$ Tf_2N showed a threefold slower rate compared to acetic acid esterification, but the same conversion was reached.

This study demonstrates that esters can be produced at mild conditions from sustainable building blocks, i.e. bio-ethanol and VFAs from bio-waste or CO_2, using ILs both as extractant and medium for esterification. The volatility difference between the esters and the IL enables a fast and quantitative product separation. Further research is needed for optimisation and long-term stabiblity, to overcome the limited extraction capacity of the ILs and their high cost in developing a viable process.

Samenvatting

Vluchtige vetzuren zijn duurzame chemische bouwstenen die wijdverspreid zijn in de natuur. Het zijn aantrekkelijke intermediairen voor valorisatie in een *resource recovery* georiënteerde industrie, daar ze uit agro-industriële afvalstromen kunnen geproduceerd worden door mengcultuur fermentaties en zelfs uit CO_2 door microbiële electrosynthese. Ontwikkeling van deze opkomende productieplatformen tot solide technologieën kan een *driver* zijn voor de transitie van afvalzuivering tot valorisatie en exploitatie van het afval als duurzame grondstof. Het voorkomen van de wateroplosbare vluchtige vetzuren in lage concentraties in waterige oplossingen bemoeilijkt het afscheiden. Conventionele scheidingstechnieken zoals distillatie of vloeistof-vloeistofextractie zijn hierdoor geen aantrekkelijke opties. Het doel van deze thesis is het uitvoeren van een reactieve extractie van vluchtige vetzuren uit een waterige stroom door een tweefase verestering, i.e. extractie in een hydrofobe laag en omzetting tot het overeenkomstige ester door het toevoegen van een alcohol, bijvoorbeeld bio-ethanol. Door de verestering worden de laagwaardige vetzuren omgezet in producten met een hogere waarde, die bovendien minder goed oplosbaar zijn in water en een hogere vluchtigheid hebben. De tweefase verestering wordt uitgevoerd in een ionische vloeistof (IL) als de hydrofobe laag. ILs worden als groene solventen gezien door hun goede solventeigenschappen, hun hoge stabiliteit en het feit dat ze absoluut niet vluchtig zijn. Ze zijn daarom geschikt als tweede fase in de tweefase verestering, omdat ze bijdragen aan het duurzaamheidsaspect van het proces en ze het afscheiden van de gevormde esters mogelijk maken door het verschil in vluchtigheid tussen de esters en de IL. Tetra-alkylfosfonium ILs werden geselecteerd als de meest geschikte ionische vloeistoffen voor dit proces, vanwege hun hydrofoob karakter en hun lage dichtheid. Door het gebrek aan fysische data en toegepast onderzoek over deze nieuwe klasse van verbindingen is deze thesis vooral een verkennende studie, met als doel de eigenschappen en het gedrag van ILs in de tweefase verestering te ontrafelen en een indicatie te geven van het potentieel van dit proces.

In de eerste fase van het onderzoek werd een *proof of concept* uitgevoerd met azijnzuur als model korte-keten vetzuur en trihexyl(tetradecyl)phosphonium dicyanamide als IL. De aanwezigheid van ethylacetaat in het condensaat na evaporatie van de IL laag en een daling in de azijnzuurconcentratie in de waterige laag duidden op het welslagen van dit experiment. Het toonde echter ook meteen aan dat een praktische analysemethode van de IL laag cruciaal is voor het verder bestuderen van het tweefase veresteringsproces. Daarom werd een kwantitatieve analysemethode op basis van ^{1}H NMR en ^{13}C NMR spectroscopie met een interne standaard ontwikkeld en succesvol geïmplementeerd. De tweefase verestering werd in drie verschillende stappen opgesplitst die afzonderlijk bestudeerd werden: extractie van azijnzuur met een IL, verestering van azijnzuur in een IL en evaporatie van ethylacetaat uit een IL. Deze stappen werden met twee fosfonium ILs uitgevoerd met verschillende anionen. De resultaten toonden aan dat het anion een belangrijke invloed heeft op de extractie (20% tegenover 5%) en verestering (minimale conversie tegenover 85% conversie. De evaporatie-experimenten toonden aan dat het mogelijk is om ethylacetaat zo goed als kwantitatief uit de IL laag te verwijderen onder milde condities (p=40 mbar, T=55 °C, t=5 min.). Ten slotte werd getest of uitwisseling van het IL anion met anionen uit de waterige laag mogelijk is. Dit bleek het geval, afhankelijk van het type anion.

Na de verkennende experimenten in de eerste fase was het mogelijk om op een meer gestructureerde manier de relatie tussen het anion van de IL en de extractie, verestering en mogelijke anionuitwisseling te bestuderen. Daarvoor werden vijf trihexyll(tetradecyl)phosphonium ILs vergeleken op het niveau

van extractie en verestering van azijnzuur, evaporatie van ethylacetaat en anionuitwisseling. Op die manier werd er gepoogd inzicht te verwerven in de verschillende stappen en een geschikte IL te selecteren voor dit proces. Er werd aangetoond dat extractie de beperkende stap is: de extractiecapaciteit varieerde van 4% tot 23% voor een 10:1 verhouding waterige laag tot IL laag. De verestering ging voor drie van de vijf ILs beperkt door, terwijl voor de andere twee conversies van 75% of 85% bekomen werden in de eerste 15 minuten. Geen enkele van de geteste ILs toonde echter een hoge extractie- én een hoge veresteringscapaciteit. De efficiëntie van de evaporatiestap varieerde van 75% tot 95% en bleek invers gerelateerd te zijn aan de viscositeit van de IL. Trihexyl(tetradecyl)-phosphonium bis(trifluoromethylsulfonyl)imide ($P_{666,14}Tf_2N$) werd geselecteerd als de meest geschikte IL, vanwege de hoge (85%) en snelle (15 min) conversie tijdens de veresteringsreactie en het niet uitwisselen van het anion met anionen uit de waterige laag. De extractiecapaciteit bedroeg 4% als een 10:1 verhouding waterige laag tot IL laag gebruikt werd. Het was mogelijk om 89% van het ethylacetaat uit de IL laag te verwijderen in slechts 5 minuten tijdens de evaporatiestap.

In de derde fase werden de meest geschikte reactieomstandigheden onderzocht met $P_{666,14}Tf_2N$ als IL. Uit experimenten waarin het effect van de temperatuur en de ethanolconcentratie op de verestering werd bestudeerd, bleek dat voor mildere condities, zelfs voor een 1:1 verhouding ethanol tot azijnzuur, dezelfde conversie werd bekomen, maar pas na een langere tijd. De aanwezigheid van water heeft een sterke limiterende werking op de snelheid en omzetting van de verestering. Er werd verder aangetoond dat de extractie kan uitgevoerd worden bij kamertemperatuur met een even hoge efficiëntie.

Deze opstelling werd vervolgens toegepast op extractant uit een microbieel electrosyntheseproces, dat 14 g/L azijnzuur bevat. 3% van het azijnzuur was na 2 uur geëxtraheerd in de IL laag. Er bleef echter slechts 1% over na centrifugeren, waarvan 90% omgezet werd in ethylacetaat in 30 minuten. Hiervan werd 78% afgescheiden door evaporatie. De anionsamenstelling van de waterige laag bleef onveranderd, wat erop wijst dat er geen anionuitwisseling plaatsvond. Dit experiment werd herhaald met een 1:1 verhouding waterige laag tot IL laag en voor vijf opeenvolgende cycli met dezelfde IL laag en dezelfde waterige laag. De performantie van het proces bleef op hetzelfde niveau gedurende de vijf cycli en er kon in totaal 28% van het azijnzuur opgevangen worden als ethylacetaat. De resultaten konden geen uitsluitsel geven over mogelijke anionuitwisseling.

Finaal werden nog twee experimenten op C_3-C_5 korte-keten vetzuren uitgevoerd, om het effect van de ketenlengte op de extractie en de verestering te bepalen. De extractiecapaciteit was hoger met toenemende ketenlengte, door de toenemende hydrofobiciteit. De extractiecapaciteit voor pentaanzuur bedroeg 25%, in een 10:1 verhouding waterige laag tot IL laag. De verestering van pentaanzuur met ethanol toonde dat eenzelfde finale conversie werd bereikt, maar aan een ongeveer drie keer lagere snelheid.

Deze studie toont aan dat esters onder milde omstandigheden kunnen geproduceerd worden uit duurzame bouwstenen, namelijk bio-ethanol en vluchtige vetzuren afkomstig van organisch afval of CO_2, met ILs zowel als extractant en als veresteringsmedium. Het verschil in vluchtigheid tussen de esters en de IL maakt een snelle en kwantitatieve afscheiding van de producten mogelijk. Verder onderzoek is nodig om het proces te optimaliseren en lange termijn stabiliteit na te streven, zodat de problemen van de beperkte extractiecapaciteit van de ILs en hun hoge kost opgelost kunnen worden in het ontwikkelen van een uitvoerbaar proces.

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List of abbreviations

^{13}C NMR	carbon-13 nuclear magnetic resonance
DCN	dicyanamide
DMSO	dimethyl sulfoxide
GC	gas chromatography
HMBC	heteronuclear multiple-bond correlation
^{1}H NMR	proton nuclear magnetic resonance
HPLC	high-performance liquid chromatography
IG ^{13}C NMR	inverse gated carbon-13 nuclear magnetic resonance
IL	ionic liquid
OAc	acetate
$P_{666,14}$	trihexyl(tetradecyl)phosphonium
RPLC	reversed phase liquid chromatography
Tf_2N	bis(trifluoromethylsulfonyl)imide
TMS	tetramethylsilane
USD	US dollar
VFA	volatile fatty acid

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Chapter 1: Literature review

1.Introduction

Since his very first steps to the dawn of the industrial era, man has relied on renewable raw materials from his natural environment to supply his material and energy needs. Since the invention of the steam engine, coal became a key raw material and led to the rapid expansion and establishment of a coal-based industry. In the 20th century, due to lower prices and better logistics, crude oil and natural gas became the top raw materials.[1] This industrial revolution gave rise to an enhanced life standard and helped boost the world population from 1 billion people to the present-day number of over 7 billion, over a time span of merely 200 years.[2] Already in 1798 , Malthus brought the inherent unsustainability of this exponential growth to the attention.[3] It gradually became clear that the industrial demand would sooner or later surpass the capacity of the finite fossil fuel reserves. By the end of the 1970's, alongside economic commotion and environmental concern, the unsustainability of the fossil fuel based industry was, however inconvenient, acknowledged as a truth. The quest for alternatives to sustain this high life standard in harmony with nature has troubled a great number of minds since.

Biomass, as the only widespread renewable carbon source, can be the solution.[1,4] The transformation of a fossil fuel to a biomass based raw material platform will not be facile. The petrochemical domination regarding platform chemicals production is a consequence of the low prices of the raw material and optimised fractionation and refining routes. The fractionation in petrochemistry is based upon volatility, whereas biomass is less volatile and thus new processing alternatives have to be developed, generally based on extraction.[5] Furthermore, the raw material is more expensive and the production thereof can be under ethical challenge due to deforestation and the competition with food production.[6] A sustainable biomass production and subsequent processing into bioproducts will require an intense and concerted cooperation between several actors, ranging from farmers to companies, scientists and politicians. There are however substantial merits to tackling these issues on the way to a sustainable future, from an environmental point of view, from a strategic point of view, i.e. decreased dependency on oil-producing countries, and from an economic point of view, by meeting the increasing trend of consumer demand for greener products. Development of a large scale bioindustry can provide a boost to rural areas, creating opportunities for farmers and foresters and processing, distribution and service industries, which all have to be located in rural communities.[7]

Today approximately 50 million tonnes of bio-based chemicals and polymers are produced per year.[8] The most important bio-based chemicals are non-food starch, cellulose derivatives, fatty acids, tall oils, fermentation products such as ethanol and citric acid and polymers such as polylactic acid and polyhydroxyalkanoates. The range of applications of industrial bioproducts is very diverse, ranging from pharmaceuticals, cosmetics and surfactants to solvents, paints and building materials.[7,8] The majority of chemicals is however fossil fuel based, with a global annual production of approximately 330 million tonnes. Apart from that, another 50 million tonnes of 1st generation biofuels are produced annually and industrial scale implementation of 2nd generation biofuel producing plants is impending.[9] Whereas the renewable chemical production was worth $57.5 billion in 2012, it is estimated to grow to $83.4 billion by 2018, with an annual growth rate of 7.7%.[10] It however has to be taken into account that also a growth in the fossil fuel based chemicals is expected. In the case of bio-based polymers, the

produced volume is estimated to reach a maximum of 1.75 million tonnes by 2020 in a scenario with stimulating policies and measures. Compared to an estimated increase of petrochemical based polymers of 25 million tonnes from 2005 to 2020, this is only a small fraction. Due to this small market share of bio-based polymers, environmental benefits and job creation potential are rather low. However, the efforts of stimulating a bio-based economy result in positive societal impacts for education, for green chemistry, for the image of the participating companies and ultimately even for innovation.[11] For a further future, the BREW project estimated that the production of bulk chemicals from renewable resources could reach 17.5% to even 38% of all organic chemical production by 2050, in the case of conservative or favourable market conditions respectively. In other words, there are founded expectations for a firm future market share with substantial environmental and socio-economic benefits.[12]

Table 1.1: projected value (in billion US dollar) of world chemical production[123]

	2005			2010			2025		
Chemical sector	Total value	Biobased value	Biobased share (%)	Total value	Biobased value	Biobased share (%)	Total value	Biobased value	Biobased share (%)
Commodity	475	0.9	0.2	550	5-11	0.9-2.0	875	50-86	50-86
Speciality	375	5.0	1.3	435	87-110	20.0-25.3	679	300-340	44.2-40.1
Fine	100	15.0	15.0	125	25-32	20.0-25.6	195	88-98	45.1-50.3
Polymer	250	0.3	0.1	290	15-30	5.2-10.3	452	45-90	10.0-19.9
All chemicals	1,200	21.2	1.8	1,400	132-183	9.4-13.1	2,183	483-614	22.1-28.1

In analogy to a petrochemical refinery plant, biomass is processed in a biorefinery, which is defined as *'a facility that integrates conversion processes and equipment to produce fuels, power and chemicals from biomass'*.[13] Biomass can be converted into products, i.e. materials and energy, via two major strategies: thermochemical or biotechnological. In the thermochemical strategy, biomass is converted by submission to high temperature and pressure, whereas enzymes or whole cell organisms are used in the biotechnological strategy.[4,5] Which of the two strategies will economically and ecologically gain more importance remains still open, but it is highly likely they will be combined in future integrated biorefineries.[5]

The extensive processing steps within biorefineries, such as the biomass pretreatment, fermentation and extraction, give rise to large volumes of organic waste streams. These volumes will increase when crude lignocellulosic and algal biomass will find their way into biorefineries.[14] Since maximization of the value of each stream is a quintessential element of the next generation biorefinery concept, the use of these waste streams as a resource to generate bioproducts will play a significant role in an integrated biorefinery.[15] The so-called carboxylate platform provides the technology to convert the organic matter in waste into valuable bioproducts, while simultaneously recovering nutrients and water within the biorefinery. The carboxylate platform comprises biological pathways in an undefined mixed culture and the subsequent chemical pathways to form the end product.[14]

The use of a mixed culture enables conversion of the complex and wide range of substrates in the waste stream. The aptness of the mixed culture to deal with this, springs from its species-

rich composition, giving rise to a high metabolic flexibility.[16] In pure culture fermentations, single strains of microorganisms convert a pure and therefore relatively expensive substrate into a product, through a pathway which is enhanced or newly introduced by means of genetic or metabolic engineering. Because of the specificity of substrate, microorganism and end product, the conversion takes place under well-controlled, optimal conditions and is thus relatively fast, but also cost-intensive, limiting the viability of pure culture fermentations to products with high added value.[17] In mixed culture fermentations, a community of microorganisms converts a more complex substrate to a product, through a sequence of pathways stimulated or inhibited by environmental conditions. Mixed culture fermentations have some advantages compared to pure culture fermentations[14,16,18]:

1. They are open systems with a high microbial stability, avoiding the use of energy-consuming sterilization or antibiotic additions
2. They are anaerobic systems, avoiding energy-consuming aeration
3. They can handle more complex substrates, reducing pretreatment costs and making them apt for bioproduction on waste streams
4. They can be employed in a (semi-)continuous process for years

The most important products made by mixed culture fermentation are short-chain carboxylic acids and methane. The major downside of mixed culture fermentation as opposed to pure culture fermentation is the relatively low value of the products. Due to the easy separation of methane from the fermentation broth, the application of mixed cultures to produce biogas is extensively used in anaerobic digestion. Methane is however of low value and is only an intermediate in the conversion of organic waste to carbon dioxide, resulting from the combustion of methane to produce energy. Directing the outcome of the carboxylate platform to bioproducts, by recovery of the carboxylic acids from the fermentation broth and subsequent chemical conversion, is a superior alternative in terms of valorisation, since the carbon in the waste stream is embedded in high-value chemicals.

There are three barriers that interfere with the large-scale implementation of this bioproduct-directed carboxylate platform as a key technology in waste stream valorisation[14]:

1. *The separation barrier: efficient separation of carboxylates from fermentation broth*
   Bringing the carboxylates from a low titer broth to a concentrated stream that meets end-use quality standards is a major bottleneck. Due to the complexity of the substrate a variety of co-products can be formed, and therefore a recovery method with high specificity for the targeted compounds is needed when a specific product is targeted. In general about 60% of the process costs can be attributed to separation.[5,19] Separation and recovery processes with enhanced selectivity and performance can strongly benefit the economic case of biorefineries, even for the production of high-value added products with pure cultures.

2. *The methanogen barrier: economic inhibition of methanogens*
   For a maximal production of carboxylates, loss of carbon in the form of methane has to be avoided. Therefore, the methanogens have to be inhibited. This is generally done by anti-methanogen additives, by applying a heat-shock or by lowering the pH to around 5.5. The use of additives and applying a heat-shock is however too expensive for a large-scale reactor.

Lowering the pH can be incompatible with the conditions needed for an optimal microbiome (cf. point 3).

3. *The ecology barrier: directing the microbial process to generate the target carboxylates at sufficient rates*
   The environmental conditions drive the selection of microbes in the mixed culture and determine which conversions are thermodynamically feasible.[20] These environmental conditions change due to the complexity and the variability of the organic waste in an operational system. Therefore knowledge of the effect of these changing conditions on the outcome of the fermentation process is crucial.

The aim of this thesis is to address the separation barrier by elaborating and optimizing the extraction and subsequent esterification of carboxylates produced by a mixed culture fermentation. This is part of a pipeline developed to produce esters from an organic waste stream, thus upgrading the value from waste to carboxylates and from carboxylates to esters.[17] In the next paragraphs, the reactor set-up is presented, as well as the major characteristics of the compartments.

2. Presentation of the reactor set-up

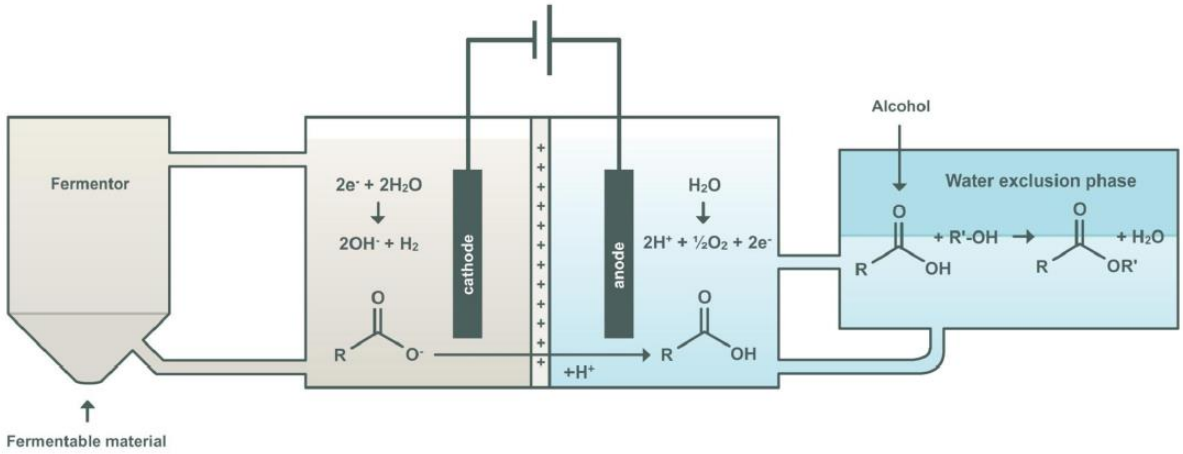

Figure 1.1: schematic presentation of the reactor set-up[17]

The developed three-step set-up connects an anaerobic fermentation to a membrane electrolysis reactor and a biphasic esterification reactor (Figure 1.1). Short-chain carboxylates are produced through anaerobic mixed culture fermentation of a biological waste stream. The carboxylate-rich fermentor broth is sent to a membrane electrolysis unit, in which the anions are driven through an anion exchange membrane, while the solids and biomass are retained. Protons, generated by the oxidation of water, convert the carboxylates to their acid forms. Subsequently the aqueous carboxylic acid concentrate is extracted into a hydrophobic phase, in which ethanol and sulphuric acid is added to yield the corresponding esters. It is paramount that the esterification takes place in a water exclusion phase, since water counteracts the formation of esters. In this way the acids are converted to a product which is more volatile, and thus easier to separate, and which has a higher value.

In sections 3, 4 and 5 a more in-depth discussion of the fermentation, the membrane electrolysis and the biphasic esterification will be displayed.

3. Fermentation

The anaerobic biological conversion of complex organic material comprises three major steps: hydrolysis, primary fermentation and secondary fermentation (Figure 1.2). In the first step, polymers are hydrolysed to oligomers and monomers by extracellular enzymes.

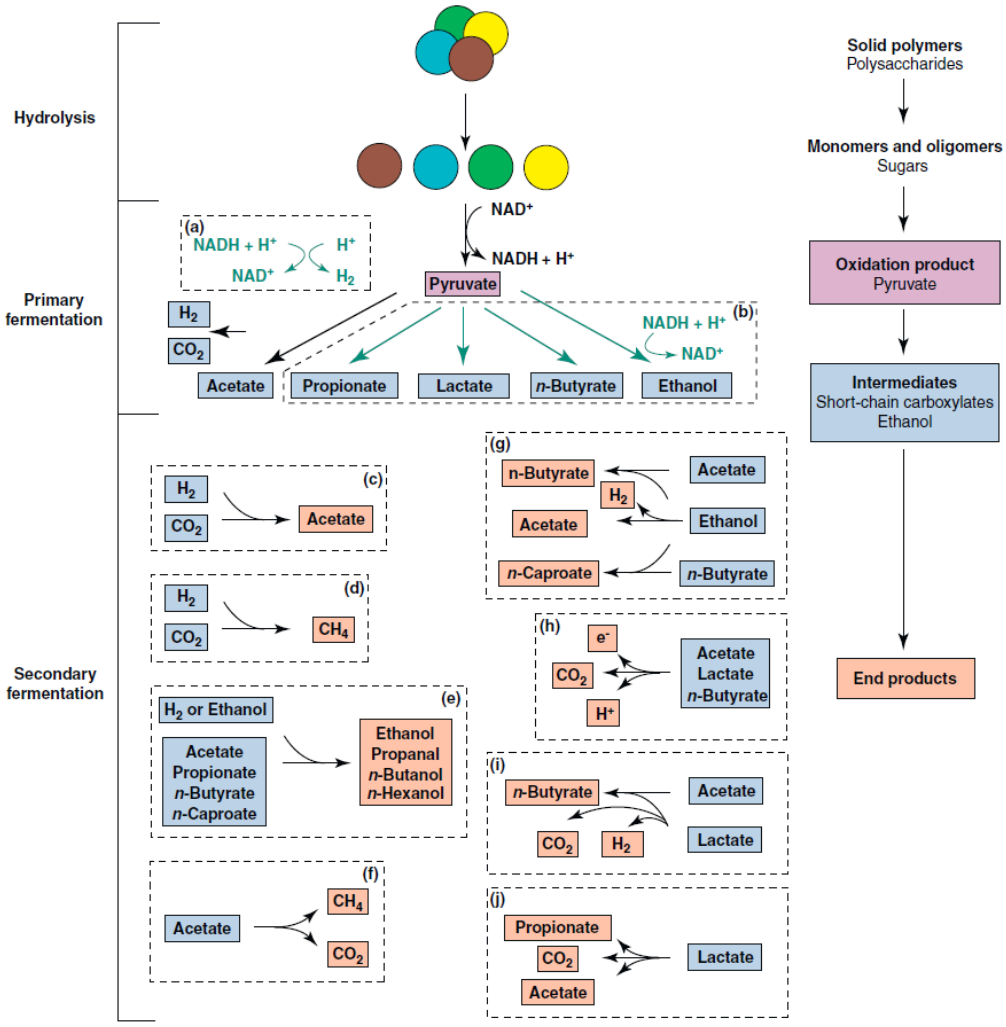

Figure 1.2: conversions in an anaerobic mixed culture fermentation[14]

These monomers are fermented to short chain organic acids and alcohols in the primary fermentation step. These intermediates are subsequently converted to end products in the secondary fermentation. An important compound in this process is acetate. It is formed in the primary fermentation, also referred to as acidogenesis, as well as in the secondary fermentation via the so-called acetogenesis step.[18] This can be performed by hydrogenotrophs, which form acetic acid from H_2 and CO_2 as is depicted in Figure 1.2 (c). If acetate is accumulated, these hydrogenotrophs are inhibited, giving rise to higher hydrogen partial pressures and a shift towards more reduced fermentation products.

Another important compound is methane. This is the organic compound with the lowest free energy content per electron upon oxidation to CO_2, which means that in a thermodynamically closed system all substrates will eventually be converted to CH_4 and CO_2.[16] It can be formed in the secondary fermentation from H_2 and CO_2 in the so-called hydrogenotrophic methanogenesis as can be seen in Figure 1.2 (d), from of acetate in the aceticlastic methanogenesis (f). The latter takes care of excess acetate out of acido- or acetogenesis, thus preventing an acetate accumulation.

If acetate is not removed from the fermentation broth, it can, apart from conversion to CH_4 by aceticlastic methanogens, be converted in 3 alternative reactions in secondary fermentation:

1. BES: bio-electrochemical systems (Figure 1.2 (h))
   In bio-electrochemical systems, micro-organisms utilize an electrode as an electron donor or acceptor to drive an added value process, for example in microbial fuel cells in which organic substrates are oxidized to generate electrons.[21] Several organic substrates have been tested, but only short-chain carboxylates have been found appropriate for scale-up.[22] More recently the conversion of organic substrates and bio-production of platform chemicals by applying an electric potential to give reductive power to micro-organisms has been found to be potentially more viable than the generation of an electric current.[23]

2. Biological reduction of carboxylates to the corresponding alcohols (Figure 1.2 (e))
   At elevated hydrogen partial pressures, the carboxylates can theoretically be reduced to the corresponding alcohols. The experimentally observed conversion rates were however very low.[24] Since very low partial hydrogen pressures are required to obtain high acetate production rates and reduction to ethanol can only proceed under high partial pressures, it is impossible to perform this reaction at a high rate.[14]

3. Biological elongation of short-chain carboxylates to longer chain products (Figure 1.2 (g))
   Ethanol, either as reduced acetate or added, can combine with acetate to yield *n*-butyrate. Although the acetate reduction is slow, this reaction can proceed, because the formed ethanol is rapidly consumed in the chain elongation reaction, thus enhancing the reduction reaction. This reaction is in competition with the methanogenesis reaction, which means methanogens should be inhibited, either by applying a heat-shock, by lowering the pH or by adding an inhibitor. Further chain elongation to *n*-caproate is also possible. In this case separation is easier, since *n*-caproate has a low solubility in water.[25] A dilute ethanol stream can be added to the reactor to make the chain elongation reaction economically more feasible.[26]

In this reaction set-up, the acetate is removed from the fermentation broth, as well as other short-chain carboxylates. These carboxylates in a low pH broth would effect in a toxicity towards the reactor microbiome. It is therefore of vital importance to continuously extract these products from the fermentation broth.[27,28]

A common separation step is solvent extraction. The carboxylates hereby migrate from the aqueous phase to the water-immiscible extractant layer. This extractant should have a high specificity towards the carboxylates and a low specificity towards the substrate, as well as compatibility towards the biomass. Long chain tertiary amines, for example trioctylamine, have been found suitable extractants for the recovery of carboxylates from an aqueous solution.[29,30] A variation upon this is membrane-

based solvent extraction, in which the feed and the extractant are separated by a membrane which is permeable for the compound of interest.[31] This has some advantages compared to solvent extraction: there is no fear of backmixing, no need for agitation, no direct exposure of microorganisms to extraction agents and a potentially high efficiency, depending on the selectivity of the membrane.[32] In both cases the solvent has to be regenerated and the solute re-extracted into a stripping solution, or crystallized after distillation of the solvent. A similar process is pertraction through a liquid membrane, combining both extraction and stripping of the solute.[31,33,34]

Another common option for carboxylate removal is by sorption to solid resins that are selective for carboxylic acids. The viability of this adsorptive recovery depends upon a high selectivity and capacity for the target carboxylates as well as the ability to elute the carboxylates and regenerate the resins.[35] In this reactor set-up, an in-situ recovery by sorption is not possible, because filtration of the resins from the broth is not possible in a continuous reaction mode. An external recycle loop could be used to overcome this problem. Still then this is not a beneficial separation method, since different carboxylates are formed and the performance of the adsorption depends upon the selectivity for the product. Furthermore, this efficiency depends upon the feed pH, which cannot be adjusted in order not to shift the outcome of the fermentation reactions.

A third common option is a membrane mediated separation in which the carboxylates flux through the membrane as a consequence of a driving force. This is applied in this reaction set-up, in the membrane electrolysis step, which is discussed in the next section.

4. Membrane electrolysis

In the membrane electrolysis step, an electrical potential is applied to the cathode-anode couple in a two-chamber system, separated by a single polymeric anion exchange membrane. Hydrogen gas and hydroxide ions are formed in the diluate stream due to cathodic electrolysis, whereas protons and oxygen gas are formed in the concentrate stream due to anodic electrolysis. The short chain carboxylates generated in the fermentor are driven across the membrane and thus extracted from the fermentation broth. The membrane retains all molecules other than negatively charged ones smaller than the effective pore size of the membrane. In this way a solid- and biomass-free anolyte with a high salinity and a low pH is obtained.[17] Because of this low pH, the carboxylates are protonated to yield the conjugated acids. Membrane electrolysis is based on the same principles as the conventional electrodialysis and bipolar electrodialysis, however only one membrane is used, compared to multiple membranes in the electrodialysis methods. Furthermore, in the electrodialysis methods, the treated streams do not engage in the electrochemical reactions, whereas in membrane electrolysis water from the catholyte and anolyte compartments is electrolised.[36]

The membrane electrolysis step has already been tested with synthetic anolyte containing short-chain carboxylic acids and with thin stillage. Almost complete extraction is possible in a batch experiment in 48 hours. In an experiment where the catholyte in continuously fed with acetate, acetic acid accumulated up to 14 g L^{-1}, but the extraction rate dropped when the anolyte concentration was higher than 12 g L^{-1}, indicating that removal of acetic acid from the anolyte is needed. Coulombic efficiencies are lower in the case of thin stillage, due to the presence of other anions, which compete with the acetate and protons flux.[17]

5. Biphasic esterification

A carboxylic acid is converted to an ester upon reaction with an alcohol in acidic conditions, as is depicted in Figure 1.3.

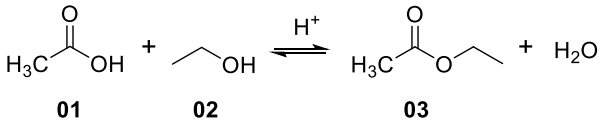

Figure 1.3: Fischer esterification of acetic acid with ethanol

5.1. Carboxylic acids vs. esters

In the following section, the major properties, production routes and applications of organic acids and the corresponding esters will be discussed, with acetic acid and ethyl acetate as model compounds for both classes.

5.1.1. Carboxylic acids

Carboxylic acids are colourless compounds containing a carboxyl group. Short-chain carboxylic acids are liquid, whereas medium- and long-chained carboxylic acids are solid. Because of the carboxyl group, the acids contain a polar side, explaining the complete water solubility of the short-chain carboxylic acids and the decreasing water solubility with increasing chain length due to increasing hydrophobicity. Carboxylic acids behave as weak acids in water; their pK_a is approximately 5. Compared to their corresponding alcohols, carboxylic acids have relatively high boiling points. This is because they form dimers, involving 2 hydrogen bonds.[37,38]

5.1.1.1. Production routes

The main synthesis routes of the model compound acetic acid are methanol carbonylation or liquid-phase oxidation of butane, naphta or acetaldehyde. The oxidation routes are historically important, but have been gradually replaced by the less expensive low-pressure methanol carbonylation technology. In 2012 circa 90% of all acetic acid was produced in this way.[37]

In the methanol carbonylation process, reaction of methanol with carbon monoxide yields acetic acid:

The two major advantages of this process are the favourable raw material and energy costs, as well as the use of synthesis gas as a starting product, which can be obtained from natural gas, coal or biomass.[39] The oxidation routes via aliphatic hydrocarbons or acetaldehyde are manganese or cobalt catalyzed radical chain reactions. Apart from these, there are also some minor production alternatives, such as acetic acid from ethane, ethylene or microorganisms. In the latter, the cost of the substrate, as well as the limited concentration of acetic acid in solution due to the inability of microorganisms to thrive in low-pH solutions, are bottlenecks to widespread implementation.

5.1.1.2. Applications

Global acetic acid demand rose from 6.1 million ton in 2000 to 10.2 million ton in 2011 and is estimated to reach as much as 15.5 million ton in 2020 because of expected growth and the Asia-Pacific region. The main applications of acetic acid are depicted in Figure 1.4. The market price of acetic acid is mainly driven by the prices of the feedstock and the downstream demand for its derivatives.[37,40] There are few direct uses for the short-chain, more or less malodorous carboxylic acids outside of the food and feed industry, where they are used for their physiological effects. However, derivatives such as metal salts, esters, acid anhydrides and acid chlorides are very important as end products or as intermediates in the chemical and pharmaceutical industry.

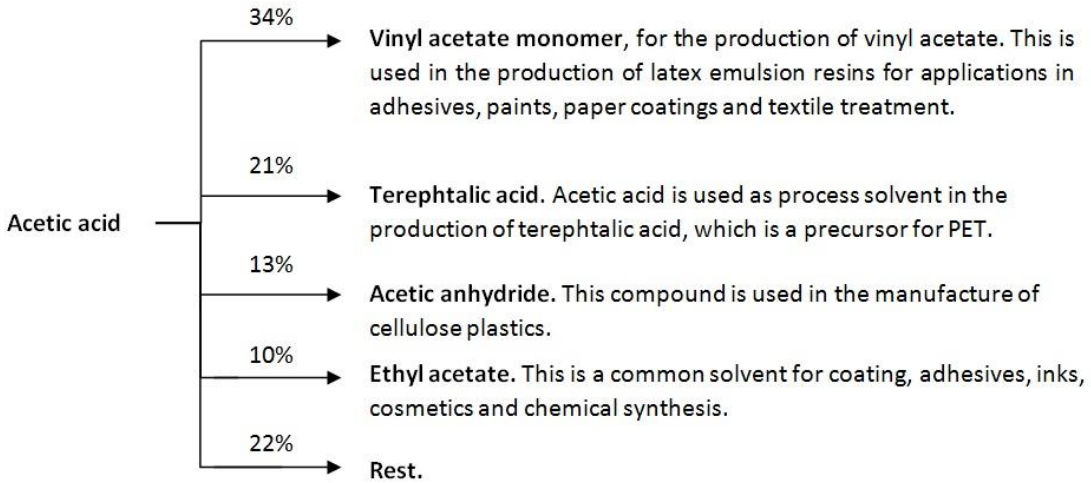

Figure 1.4: end-uses of acetic acid[37,40]

5.1.2. Esters

Esters are derivatives from a carboxylic acid, where the hydroxylgroup is replaced by an alkoxygroup. This makes them more hydrophobic compared to the corresponding acids, resulting in a lower water solubility. Low molecular weight esters often have a typical scent, making them interesting compounds for the food and fragrance industry. Most naturally occurring fats and oils are esters from glycerol and fatty acids.

5.1.2.1. Production routes

The most common method to produce esters is by reaction of an alcohol with a carboxylic acid with the elimination of water. This reaction is an equilibrium reaction, which means using an excess of the reagents or removing the ester or water is required to obtain a high conversion. This reaction is discussed more in detail in section 5.2. Other synthesis routes to esters are alkylation of metal carboxylates and acylation of alcohols with carboxylic acid halides or with carboxylic anhydrides. These reactions are irreversible and thus high conversions can be obtained. They are however more expensive and only used for rare or expensive esters.[37] Apart from these general synthesis routes, there are some more specific routes, for example the Reppe synthesis for the production of methyl and ethyl propionate, the Tishchenko reaction for the production of ethyl acetate and the acylation of ethene for the production of vinyl acetate. The world consumption of alkyl acetates grew at an average

rate of over 6% each year between 2009 and 2013 and is expected to grow with an average rate of 3% up to 2018.[41]

5.1.2.2. Applications

Esters are widely used as solvents, extractants and diluents due to their hydrophobicity and low polarity. The major examples are the use of ethyl acetate as solvent, the use of esters of short chain alcohols and acetate as solvents for cellulose derivatives and the use of esters as diluents in paints and coatings. Ethyl acetate is one of the most essential solvents in the chemical industry. Large amounts of ethyl acetate are used as coating material and for the production of chemicals. It is increasingly used as a replacement of hazardous solvents.[42] Furthermore, large quantities of phthalate, adipate and fatty acid esters are produced as plasticizers. Another important application of esters is the production of polyesters, from monomers such as acrylates, terephtalates and vinyl acetate. Polyvinyl acetate is used in plastics, adhesives, coatings and laminates. A more niche application of esters is in the flavour and fragrance industry where they are used because of their pleasant odour. [37]

5.1.3. Comparison carboxylic acids and esters

Upon conversion of a carboxylic acid to the corresponding ester, the boiling point is generally lowered. This means that a more volatile compound is formed, facilitating evaporation of the end product from the reaction mixture. Depending on the chain length of the alcohol, the boiling point may eventually exceed that of the acid. Furthermore, the corresponding ester of an acid is less soluble in water and even insoluble when longer chain carboxylic acids or alcohols are used, thus preventing the formed ester to leach back into the aqueous phase. The most important reason for esterification is however from a valorisation perspective. The economic values of the carboxylic acids are lower than of their corresponding esters.

Table 1.2: basic properties of acetic and butyric acid and their corresponding ethyl esters[124]

	Acetic acid	Butyric acid	Ethyl acetate	Ethyl butyrate
boiling point (°C)	117.9	163.8	77.1	121
water solubility (g/L)	completely soluble		83	6.3
market price (€/ton)	600	1070	1085	1200

5.2. Esterification reaction

There are four major production methods for the model ester ethyl acetate: esterification of acetic acid with ethanol, addition of ethylene to acetic acid, dimerization of acetaldehyde and one-pot synthesis from ethanol. Among these, the esterification is industrially the most important one.

The reaction of a carboxylic acid with an alcohol in the presence of an acid catalyst has first been described by Fischer and Speier in 1895, and has since been referred to as the Fischer esterification.[43] It is an equilibrium reaction, which means it can be inhibited or stimulated by Le Chatelier's principle. An effective method to overcome equilibrium limitations is by separating the formed water from the reaction mixture, or by using a large excess of the alcohol or the acid. Moreover, as the esterification is thermodynamically controlled, a high temperature is favourable for the reaction.[44] The rate of the esterification reaction depends on the carboxylic acid and the alcohol that is used. The longer the alkyl

chains, the slower the reaction. The reaction proceeds faster with primary alcohols compared to secondary and tertiary alcohols. Straight-chain carboxylic acids react faster than branched ones; the rate is particularly lower when the branching is in the α-position.[37]

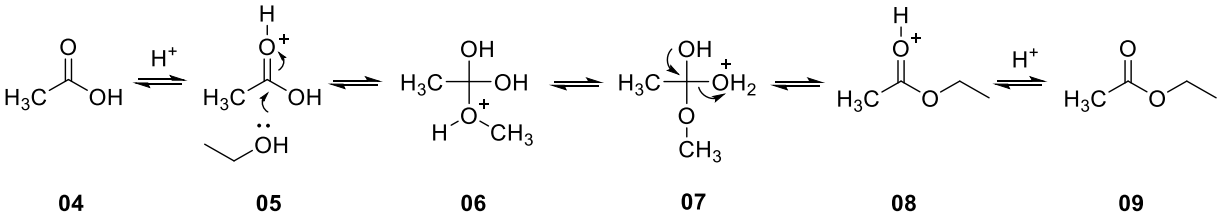

Figure 1.5: mechanism of the Fischer esterification of acetic acid and ethanol

The reaction mechanism of the Fischer esterification of acetic and ethanol is depicted in Figure 1.5. The reaction rate determining step in this reaction is the nucleophilic attack of the alcohol on the carboxylic acid. In this way a tetrahedral intermediate is formed, which yields the ester and water by decomposition.[45] An acid catalyst is needed to increase the electrophilicity of the carbonyl carbon of the acid by protonation of the oxygen, and thus make it more prone to nucleophilic attack by the oxygen of the alcohol. Due to the excellent contact between reactants and catalyst, homogeneous catalysts often show high catalytic efficiency. The most important catalysts in industry are still today inorganic acids: H_2SO_4, HCl and HI. They are cheap, they have a high activity and display a stable performance.[45] It is however hard to separate them from the products. Moreover they cannot be reused, they cause large volumes of salt waste after neutralization and they can cause corrosion.[46] These drawbacks could be overcome by the use of heterogeneous catalysts; such as resins, heteropolyacids and molecular sieves. There are however also some factors standing in the way of a widespread implementation of these catalysts, namely deactivation, high mass transfer resistance and difficult installation in a reactive distillation column.[47] Ion exchange resins in which the acid groups are chemically bound to a polymeric material, e.g. sulfonated polystyrene, are heterogeneous catalysts which are used in industry. The use of this type of catalysts also results in a more pure end product, since side reactions such as etherification, dehydration and rearrangement are suppressed.[37]

For the esterification reaction to proceed, the alcohol has to be added to the carboxylic acid in presence of an acid catalyst. In this reaction set-up, the carboxylic acid is present in the anolyte of the electrochemical extraction. Furthermore, protons are generated at the anode. This means that at this stage, both the carboxylic acid and the protons to catalyze the reaction are present in an aqueous solution. However, despite the economic and environmental preference for water as a solvent, it has to be avoided to get a favourable equilibrium in the esterification reaction. Attempts have been made to perform the reaction in aqueous media, but the obtained conversions were very poor and addition of a hydrophobic phase was a necessity to get a decent yield (> 75%).[48] In this reaction set-up, it is therefore inevitable to use a hydrophobic solvent as an extractant for the carboxylic acids and as water-exclusion layer for the esterification reaction.

This biphasic, i.e. with the anolyte from the electrochemical extraction as aqueous layer and an added hydrophobic layer, esterification was tested on synthetic and real anolyte with xylene as water-exclusion phase. For the synthetic anolyte, the acetic acid concentration in the aqueous phase dropped

at 26 ± 5% for methyl acetate and 10 ± 3% for ethyl acetate, whereas it dropped at 58 ± 3% for ethyl acetate and 65 ± 2% for methyl acetate in case of the real anolyte. This is due to the lower pH in the real anolyte, resulting in an improved catalysis. A maximum esterification rate was calculated at 4.14 g ethyl acetate per liter of anolyte per hour, assuming complete stoichiometric conversion.[17] The aim of this thesis is to perform the extraction and esterification of carboxylic acids with an ionic liquid as hydrophobic phase. An introduction to ionic liquids and motivation for their use in this application is provided in section 6.

For the production of ethyl acetate, normally ethanol as a product of ethylene hydration and acetic acid as a product of methanol carbonylation are used as feedstocks. The ethylene is derived from fossil fuels, whereas for the methanol carbonylation syngas is used, which can be derived both from fossil fuels and from biomass. However, ethanol can also be produced in a biorefinery, with increasing productivity and at reduced cost due to development of production technology. It promises more benefits when it is used as a feedstock to produce chemicals, compared to use as biofuel.[49,50] With the acetic acid which is produced by the mixed culture fermentation, it is possible to make ethyl acetate entirely from renewable building blocks. Furthermore, within the biorefinery, there is a lot of heat generated by microbial activity. As the esterification proceeds better at higher temperatures, this heat is a useful stream and by efficiently applying it for the manufacture of bioproducts, the value of this stream is maximized. The presence of the starting materials and heat make a strong case for the implementation of the esterification pipeline as a step towards an integrated biorefinery.

6. Ionic liquids

6.1. Introduction

Ionic liquids (ILs) are commonly defined as salts which possess melting points below 100 °C, and can be designed to be liquid at room temperature.[51] They exist of an organic cation and an organic or inorganic anion, of which some common examples are depicted in Figure 1.6.

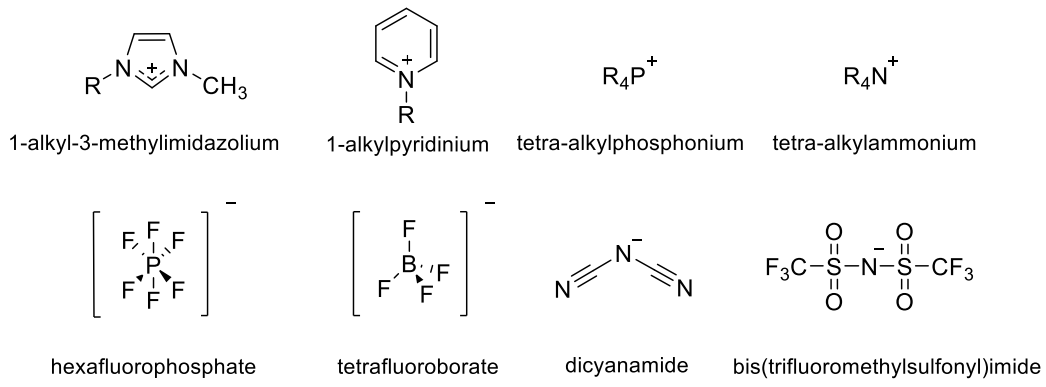

Figure 1.6: common cations and anions in ionic liquids[57]

Ionic liquids display an interesting combination of properties making them suitable as solvents: they are non-volatile, polar, non-coordinating and physically and chemically stable.[52] Furthermore they have a low melting point and a wide liquid range (-200 to 300°C).[53] Because there are a lot of different ions which can be combined to form an ionic liquid, it is theoretically possible to select an ionic liquid with the appropriate properties for a wide variety of applications. For this reason ionic liquids are often referred to as 'designer solvents'.[54,55]

The most important features of ionic liquids are:

1. Non-volatility
   The non-volatility is one of the most advantageous properties of ILs. When an IL is used as a solvent in a reaction, the products can be easily separated by distillation, without contamination by the solvent. The non-volatility also makes the manipulation, purification and recycling of the IL easier.[52] Recycling is a financial necessity for ILs to be industrially applicable.

   Furthermore the non-volatility means that no volatile organic compounds are emitted. This decreases the loss of solvent by evaporation and the associated risks of worker exposure.[56] It also means that they are in most cases non-flammable and there is no risk of damaging atmospheric photochemistry.[57]

2. Polarity
   Since ILs consist of ions, they are generally thought of as polar solvents. Experiments to determine the polarity of common ILs however demonstrated that they have a considerably lower polarity than expected.[58] It has been found that they can dissolve polar solutes as well as various apolar compounds and even polymeric materials.[52,59] This has led to the belief that the classic rule of thumb 'like dissolves like' does not apply to ILs. Further research on solute-solvent interactions has to be performed in order to be able to design ILs as specific solvents for certain compounds or applications.[52]

3. Non-coordinating
   ILs were originally described as being non-coordinating towards metals. They were tested and successfully applied as solvents for transition metal catalysed reactions.[60] More recent research however demonstrated that some anions (Cl^- and Br^-) do coordinate and some cations coordinate weakly.[52] Applications based on this coordinating capacity of some ionic liquids are already used commercially.[61,62]

In addition to these properties, ILs are also regarded as being more sustainable than classical organic solvents and therefore labeled 'green solvents'. This stems from their non-volatility, which means risks for pollution and harm to people and planet are reduced. It has however to be stated that this does not satisfy all the principles of Green Chemistry. Nevertheless this has led to a broader belief and misconception that they are nontoxic and biodegradable.[63] So far no study has demonstrated a thorough and broad research on toxicity. The toxicity has been tested on some micro-organisms and some higher forms of organisms[52,64]. Imidazolium ILs are often found to be more toxic to micro-organisms than conventional organic solvents[65]. Although there is no risk for inhalation, transdermal exposure remains a risk. Longer alkyl chains of the cation means greater lipophilicity, which means a bigger ability to interfere with biological membranes.[66]

ILs are not biodegradable, but they can be made from biodegradable and renewable resources.[67–69] Given the huge number of possibilities in designing ILs, it might be possible in the future to design non-toxic, readily degradable and even edible ILs.[52]

A drawback of the use of ILs as solvents is their high viscosity. The viscosity of ionic liquids is generally in the range of 10 to 2000 mPa s, whereas conventional solvents have a viscosity of 0.4 to 1.2 mPa s at room temperature. This is a limiting factor regarding the rate of mass transport and the ease of manipulation. The viscosity is related to the anion, with smaller and more symmetric anions leading to higher viscosity.[59] Also their ability to form hydrogen bonds is related to viscosity: the bigger this ability, the higher the viscosity. Cations have an effect on the viscosity, be it on a lesser extent. The longer the alkyl chains, the higher the viscosity, because van der Waals interactions are stronger between larger cations.[70]

This problem related to viscosity is in practice reduced by working at elevated temperatures, since temperature has a strong influence on viscosity. Furthermore, diluents such as nonanol can be used. The presence of impurities also strongly influences the viscosity, so data upon viscosity should be used with caution.

6.2. Selection procedure

The ionic liquid best suited for this application was selected on basis of the following criteria:

1. density: in the biphasic system, the IL layer should preferably be the upper layer to enable evaporation of the esters without forcing it to travel across the aqueous layer. The density of the selected IL should therefore be smaller than the density of water. This is a very constraining criterium, because the vast majority of ionic liquids are more dense than water.[71] Because of this restriction only phosphonium ILs and some ammonium ILs can be considered.

2. hydrophobicity: the IL should act as an extractant for the VFA's in the aqueous solution. Therefore the IL has to be as hydrophobic as possible so that the IL forms a separate layer. Moreover, a hydrophobic IL should result in an improved esterification as it will shift the equilibrium to the right. A more hydrophobic IL layer also means a faster phase separation after mixing and a reduced loss of IL in the aqueous layer. Phosphonium and ammonium ILs are typically more hydrophobic than their imidazolium (and pyridinium) counterparts, making them very suited as extractants on an aqueous phase.[72]

3. melting point: the melting point of the IL should be low enough that it is liquid at working conditions.

4. viscosity: the viscosity of the ionic liquid should be as low as possible to ease manipulation.

5. extraction capacities: the ionic liquid should be a good solvent for the VFA's, the alcohols and the catalyst, so that these compounds can migrate into the IL layer and react to form esters. Research on extraction of carboxylic acids from an aqueous phase with ILs has shown that the best performance in extraction has been obtained with phosphonium ILs (better than the classical extractant trioctylamine). Ammonium ILs showed weaker extractive properties and imidazolium ILs were not suited as extractants for carboxylic acids.[73,74] Phosphonium ILs have

been investigated on their extraction capacities towards alcohols and have shown to retain most of the alcohol in a biphasic system with water.[75]

On basis of the density, the hydrophobicity and the extraction capacity, only phosphonium and ammonium ILs were considered as appropriate for this application. These types of ILs have only recently gained more attention for both research and industrially applied purposes compared to imidazolium ILs.[76] Much more information on the fundamental properties of these types of ILs is needed to gain momentum for the development of applications.[63] Reliable data on the physico-chemical properties of phosphonium ILs are still limited[77] and moreover should be interpreted carefully, since the purity of the IL has a big influence on these properties.[63] Also further research on the recovery and recycling of the ILs, as well as the degradation in time, needs to be conducted.

Phosphonium ionic liquids are more stable to degradation than their ammonium counterparts. A typical decomposition reaction by internal dealkylation at higher temperatures is:

$$[R_4E]^+X^- \rightarrow R_3E + RX$$

in which E is nitrogen or phosphorus. This reaction happens faster for ammonium ionic liquids.[78] These are also more prone to Hoffmann- or β-elimination in alkaline conditions, whereas phosphonium ionic liquids decompose to a tertiary phosphine oxide and an alkane, or a phosphorane, depending on the nature of R and R'.[79]

$$[R_3P - CH_2 - R']^+ + OH^- \rightarrow R_3P{=}O + CH_3 - R'$$

$$[R_3P - CH_2 - R']^+ + OH^- \rightarrow R_3P{=}CHR' + H_2O$$

Thermogravimetric analysis has shown that phosphonium ionic liquids are generally stable up to 300°C. The exact decomposition point varies with the anion. [76]

Table 1.3: physical properties of some common phosphonium ionic liquids[115]

	ρ (g/cm³) (25°C)	μ (mPa.s) (25°C)	T decomp (°C)	Physical state at room temperature	Maximum water capacity
$P_{666,14}$ Cl	0.822	1824	350	liquid	8%
$P_{666,14}$ Br	0.955	2094	320	liquid	4.5%
$P_{666,14}$ decanoaat	0.888	319	380	liquid	21.2%
$P_{666,14}$ dicyanamide	**0.898**	**256**	**395**	**liquid**	**3.1%**
$P_{666,14}$ bistriflimide	1.064	312	400	liquid	0.7%
$P_{666,14}$ PF_6	1.03	\	340	solid (Tm=50°C)	2.2%
$P_{666,14}$ BF_4	0.938	787	380	liquid	1.8%
P_{8888} Br	0.938	\	290	solid (Tm=42°C)	ND
$P_{666,14}$ dodecylsulfonaat	\	\	\	liquid	ND
$P_{666,14}$ diisobutylmonothiofosfaat	0.92	624	260	liquid	ND
$P_{666,14}$ diisobutyldithiofosfaat	0.93	616	279	liquid	ND
$P_{666,14}$ bis(2-ethylhexyl)fosfaat	0.91	916	283	liquid	ND
$P_{666,14}$ 2-ethylhexanoaat	0.89	706	256	liquid	ND

Phosphonium ionic liquids generally have viscosities that are higher than their ammonium counterparts, but the viscosity strongly decreases on heating to higher temperatures and by adding solutes.[76,80,81]

On basis of the physical properties in Table 1.3**,** $P_{666,14}$ dicyanamide has been selected as the most appropriate IL for this application. It is one of the most hydrophobic phosphonium ILs, has a lower density than water, it is liquid at room temperature and it has a high termal stability. Furthermore the viscosity is relatively low.

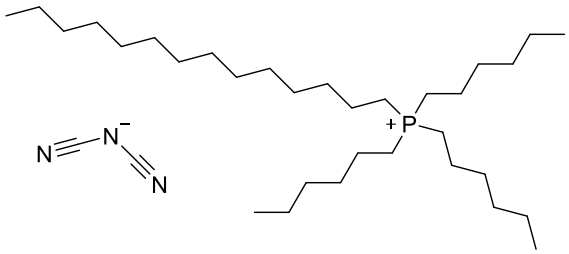

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Figure 1.7: structure of $P_{666,14}$ DCN

6.3. Synthesis of ionic liquids

When synthesizing ionic liquids, a sequence of two steps is commonly followed: the first step is the alkylation of a compound (a tertiary amine, a tertiary phosphine, an alkylimidazole etc.) **11** in Figure 1.8 to form the halide salt **13**, after which the halide is exchanged for the wanted anion in the so-called metathesis step. This anion exchange is necessary, because the preparation is out of an alkylhalide with the halide anion as a leaving group, and anions that are desired in ionic liquids are not good leaving groups. The starting materials are commercially available compounds.

The alkylation (step (1) in Figure 1.8) is a nucleophilic substitution reaction which is highly exothermic, so the temperature has to be controlled to avoid runaway reactions and the formation of elimination products.[82] The heating of the reaction mixture is generally done by microwave heating. In this way however, it is harder to control the temperature and hotspots could be formed, leading to a variable quality in the product.[83] Alternatively, ultrasound heating has been used, giving better yields and a faster reaction. This is probably due to the more efficient mixing.[84] During this reaction, air and moisture should be excluded to prevent formation of byproducts by substitution of the haloalkane by water.

The second step is an anion exchange step to yield the ionic liquid **16**. The anion exchange step is performed in an aqueous solution if the synthesized ionic liquid is hydrophobic; if it is hydrophilic, metathesis is performed in a water-immiscible organic solvent. In this way the product forms a separate layer, which is washed and dried.[85] The residual organic solvent is removed by distillation.[57]

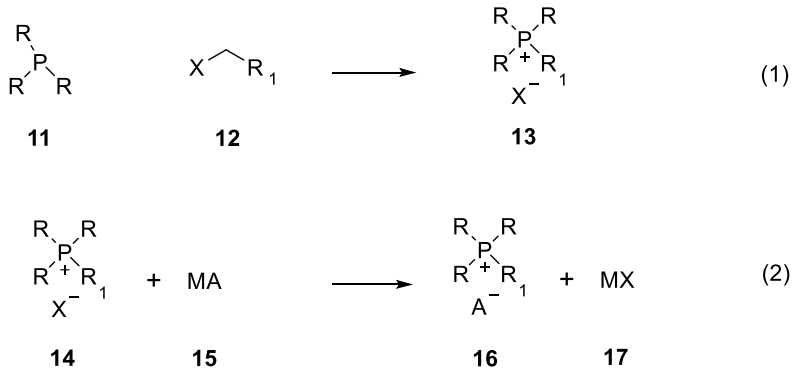


Figure 1.8: synthesis of an IL (in this case a phosphonium IL) by alkylation and metathesis

6.3.1. Synthesis of phosphonium ionic liquids

6.3.1.1.Synthesis of phosphonium halide and anion exchange

As is general the case in synthesizing ionic liquids, phosphonium ionic liquids are commonly made as the tetra-alkylphosphonium halide **13**, followed by anion exchange to give the desired ionic liquid (Figure 1.8). The initial phosphonium halide ionic liquid **13** is made by quaternisation of a trialkylphosphine **11** with a haloalkane (generally a chloroalkane) **12**.[86] The anion exchange reaction is accomplished by mixing the sodium salt of the anion with the tetra-alkylphosphonium halide in a mixture of water and acetone, which yields the desired ionic liquid after extraction and evaporation of the solvent.[87]

The trihexyl(tetradecyl)phosphonium ion is the most common cation for this type of ionic liquids. Trihexyl(tetradecyl)phosphonium chloride is synthesized by nucleophilic substitution (S_N2) of 1-chlorotetradecane by trihexylphosphine.[76] The progress of this reaction can be monitored with ^{31}P NMR spectroscopy. After 16 hours of reaction the mixture is vacuum stripped to remove unreacted reagents. Trihexyl(tetradeyl)phosphonium chloride is nowadays produced on ton-scale and is therefore one of the relatively cheaper ILs.[63] To obtain the trihexyl(tetradecyl)phosphonium dicyanamide, sodium dicyanamide is added to $P_{666,14}Cl$ in a mixture of water and acetone. After 6 hours of stirring at ambient temperature, the acetone was removed by evaporation and the ionic liquid was obtained after extractive work-up.[87]

The trialkylphosphine starting material is made by free radical addition of α-olefins to phosphine gas.[88] This is a gas which, apart from being able to ignite spontaneously to the air, is highly toxic. Trialkylphosphines also have these properties, albeit to a lesser extent. [76] They are commercially available. The most common phosphonium cations have three identical alkyl groups, coming from the tertiary phosphine, and a fourth different group, coming from the haloalkane. However, the synthesis of asymmetric tertiary phosphines is possible. Hence primary and secondary phosphines are converted into asymmetric tertiary phosphines by the free radical addition to olefins.[88] In this way it is possible to better tune the properties of phosphonium ionic liquids.

6.3.1.2.Halide-free synthesis

When ionic liquids are made through metathesis of a phosphonium halide, they still contain a residual amount of halide ions. This limits the use of these ionic liquids, since these halides can decrease the activity of metal catalysts or contaminate reaction products.[89] They are also more corrosive and affect the physical properties, which has to be taken into account in the engineering of the reaction.[63,80] Furthermore anion exchange processes generally have a high economic and ecological cost, since they generate a lot of waste (from extraction and washing) and often hazardous solvents, such as CH_2Cl_2, are used. A halide-free synthesis route in which a tertiary phosphine reacts with an alkylating agent is described in literature (Figure 1.9). It makes use of reagents such as benzenesulfonate, alkyltosylates, dialkylsulfates, trialkylphosphates and dialkylphosphates. The leaving group of the alkylating reagent is a suitable counterion in the IL.

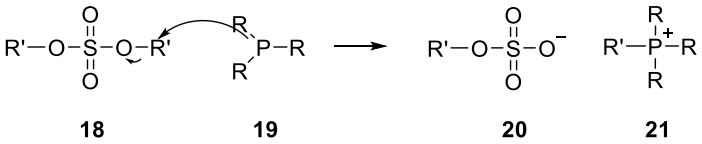


Figure 1.9: Halide-free synthesis of phosphonium ionic liquids[76]

6.4. Market

Ionic liquids are relatively expensive compounds. They are generally 5 to 20 times more expensive than conventional organic solvents, but there are also cheaper and much more expensive ones. It has however to be taken into account that loss during reaction is smaller because of their non-volatility, that they are reused in most applications and that a higher industrial demand may lead to lower prices.[51] The same cost per cycle as conventional organic solvents is obtained after approximately 15 recycles for the cheaper ionic liquids and a significantly reduced cost is obtained after more than 50 recycles.

At the moment, there are about 300 different ionic liquids commercially available and there are much more in the pipeline for commercial production, giving an annual production for a value of 300 million US dollars in 2010 and an estimated value of 3,4 billion US dollars in 2020. Companies from Germany and the United States lead the market, but are closely followed by China and Japan.[90] Furthermore, research into ionic liquids is marked by a '*steeper than exponential growth*', which may lead to a diverse range of ionic liquids that can be used in a vast array of applications, thus driving development of cost-effective production processes that benefit from economies of scale.[91]

6.5. Ionic liquids as medium for esterification

Ionic liquids are of special interest for the biphasic esterification due to their non-volatility. A hydrophobic ionic liquid can be used as extractant of short-chain carboxylic acids from an aqueous layer, which are subsequently converted to the corresponding esters upon addition of an alcohol to the IL layer. The protons to catalyse the esterification are expected to come from the acidic anolyte layer, in which protons are generated during the membrane electrolysis step. The esterification can be performed at higher temperatures without evaporation and subsequent loss of the solvent. It is

moreover expected that the formed esters can be separated from the IL layer by evaporation, since the esters are more volatile compared to the VFAs and to the non-volatile IL.

To the author's knowledge, there has been no research on the classic Fischer esterification with addition of an acid catalyst in an ionic liquid as reaction medium. There are publications on the use of imidazolium and pyridinium ionic liquids with HSO_4^- as anion, which served as catalyst for the esterification.[92,93] Conversions of 71% to 91% were reported, in 2 to 4 hours of reaction, with a highest reported value of 75% conversion in 30 minutes, for the esterification of acetic acid with butanol at 85 °C.[94] This strategy will not be investigated in this thesis, since it is expected that the protons in the anolyte will be sufficient to catalyse the esterification, as previously mentioned.

7. Objectives

The aim of this thesis is to perform a proof of concept and study the influence of reaction parameters on the performance of the biphasic esterification and the properties of the IL. This will be studied for the IL that was selected based on literature data: trihexyl(tetradecyl)phosphonium dicyanamide. No prior experimental screening of ILs to select the most suitable for the biphasic esterification was performed, since it is too time- and cost-intensive. The results will therefore not be for an optimised system, but will allow to study the influence of the reaction parameters and the core relationship between the properties of the ionic liquid and the aqueous reactant.

In the first phase, the development of an analytical method for the challenging IL matrix will be crucial. A proof of concept will be performed on acetic acid as model carboxylic acid. In this way, an indication of the migration and conversion of acetic acid in the IL layer will be given, but will be further explored in preliminary experiments in which the extraction of acetic acid with an IL layer and the esterification in an IL layer will be studied. These will be performed under the most extreme conditions, i.e. a temperature of 75 °C and an fivefold excess of ethanol, to see what the maximum possible rates are and if there are possible bottlenecks that require another approach in further experiments. Preliminary experiments on the evaporation will be performed to see if the removal of the formed esters from the IL layer is possible through evaporation. The biphasic esterification will be compared to extraction and subsequent esterification in absence of the water layer. This first part of the thesis aims for an exploratory study of the potential and the bottlenecks of the process.

Depending on the outcome of the preliminary experiments, the most suitable reaction set-up will be performed under various permutations of temperature, ethanol concentration and acetic acid concentration. This will give us insight in the influence of these parameters on the performance of the biphasic esterification and will allow for selection of the mildest possible conditions with a sufficiently high yield. Longer chain carboxylic acids and alcohols will be tested, to determine the effect of alkyl chain length on extraction, esterification and evaporation.

The biphasic esterification with the selected reaction conditions will be performed on two types of real fermentation extractant with increasing complexity: microbial electrosynthesis extractant containing only acetic acid and mixed culture fermentation extractant containing C_2-C_6 carboxylic acids. In this way we will explore if the same rates and conversions can be achieved if the process is performed with real fermentation extractant, or if there are limiting factors. The use of extractant containing a mixture of carboxylic acids will allow to assess if selectivity can be obtained, either in the extraction, esterification, evaporation or a combination of these steps. Finally, the process will be run for multiple cycles with the same IL layer to assess the performance of the process and the stability of the IL upon recycling, which is a necessity due to the high costs of ILs.

Chapter 2: Results and discussion

1. Proof of concept and preliminary tests

In the first phase, a proof of concept was conducted on acetic acid as model VFA to assess the feasibility of the biphasic esterification with trihexyl(tetradecyl)phosphonium dicyanamide ($P_{666,14}$ DCN) as IL. The development of a practical and reliable analysis method was crucial and was successfully implemented. Follow-up experiments were conducted to explore the behaviour and properties of the IL during extraction, esterification and evaporation and its stability towards the aqueous layer. In this way, the basic properties were unravelled and a framework was established for a further, more structured study, which is presented in section 2.

1.1. Proof of concept

The proof of concept of the biphasic esterification was tested with $P_{666,14}$ DCN as IL, because of its hydrophobicity, low viscosity and promising extraction capacities (chapter 1, section 6.2.). 1 g of the IL was added to 10 mL of a 0.33 M acetic acid solution (i.e. 20 g/L, a representative VFA concentration in the anolyte), to which respectively 5 and 1 equivalents ethanol and sulfuric acid were added. A control experiment without ethanol was also run. GC analysis of the aqueous layer showed a drop in acetic acid concentration, from 0.33 M to 0.24 M in 90 minutes, after which the acetic acid concentration remained unchanged (Figure 2.1). This means that 27% of the acetic acid in the 10 mL aqueous layer was transferred to the 1 g IL layer, in which a calculated acetic acid concentration of 0.90 M was reached. Minor amounts of ethyl acetate were detected in the aqueous layer, at a concentration of 0.03 M after 90 minutes and remaining unchanged up to 10 hours. There was no ethyl acetate detected in the control reaction; the acetic acid concentration decreased similarly to 0.28 M after 90 minutes and a further decrease to the same level as the biphasic esterification reaction. From the aqueous layer concentrations, it can only be derived that a portion of the acetic acid was transferred to the IL layer and transformed to ethyl acetate in the presence of ethanol. There is however no evidence on what part of the transferred acetic acid was converted. The ethyl acetate concentration in the IL layer could not be detected due to the incompatibility of the non-volatile IL and the GC analysis.

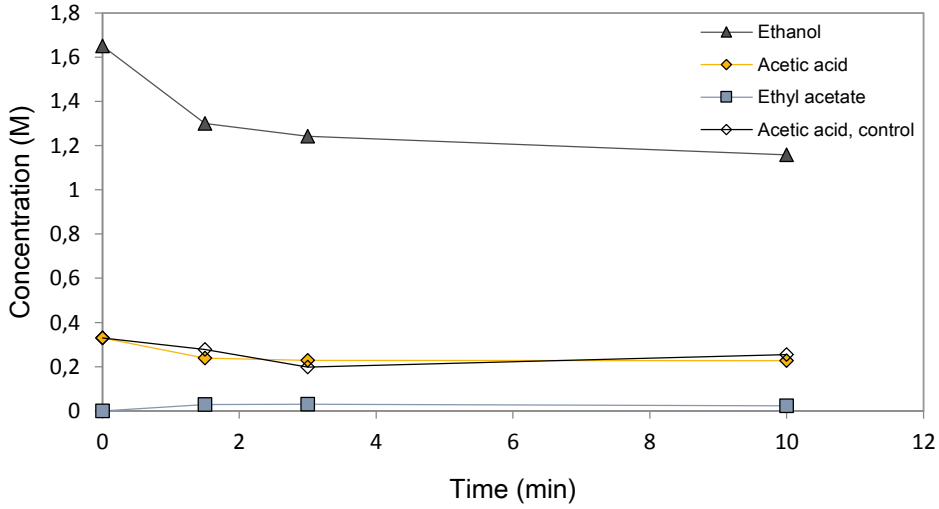


Figure 2.1: aqueous layer concentrations of ethanol, acetic acid and ethyl acetate during biphasic esterification and control reaction, determined through GC analysis

The IL layer in the biphasic esterification was separated from the aqueous layer after 10 hours, to remove the ethyl acetate by evaporation. A vacuum pressure of 40 mbar was applied to the IL layer, from which the produced vapours were condensed in a liquid nitrogen cooled cold trap. Qualitative ^{1}H NMR analysis of the condensate showed the presence of ethyl acetate, next to water and ethanol.

This preliminary experiment shows that extraction from the aqueous phase and subsequent esterification is possible, but at the same time highlights the need for an appropriate quantitative analytical technique. To gain a better insight in the kinetics of the acetic acid transfer and the conversion to ethyl acetate, it was of paramount importance to find a practical method to analyse the composition of the IL layer.

1.2.Towards a practical analysis method for the IL layer

The IL layer is a challenging matrix for analysis due to its physical properties. GC analysis is not feasible because the IL is non-volatile. LC analysis would be detrimental to the column due to the high viscosity of the IL and the presence of acids in the samples. The IL matrix could be avoided by separation of the analytes from the IL layer. This was however not attempted, as the aim is to develop a fast and practical analysis method and a quantitative detection implies quantitative removal. Therefore, NMR spectroscopy was selected as the most suitable analysis method. The challenging physical properties of the sample are avoided, since there is no contact between the sample and the NMR spectrometer. Furthermore, it is a practical method, as the sample preparation and the analysis time are limited.

The development of a quantitative NMR method is described in the addendum (chapter 6), but a brief overview is given here. The use of NMR for IL layer samples is not straightforward due to the presence of a multitude of CH_2- and CH_3-peaks of the IL cation and the narrow chemical shift ranges in which the VFA and ester peaks are present. Only acetic acid and ethyl acetate can be quantified through ^{1}H NMR, or higher esters if no other esters are present. A quantitative ^{13}C NMR method (inverse-gated ^{13}C NMR or IG ^{13}C NMR) was developed by disabling the NOE effect. Different internal standards were tested for a stable peak area to enable accurate quantification of the analytes, but eventually the introduction of a response factor was necessary. 1,4-dioxane was selected as the most suitable internal standard. The optimal conditions for a practical IG ^{13}C NMR experiment (i.e. run time of 10 minutes) have been determined as 58 scans and 8 seconds relaxation delay. The IL layer samples are prepared by diluting a 300 mg IL layer mixture with 300 mg $CDCl_3$.

A quantitative ^{1}H NMR method was developed to be used during the first phase of the experiments, in which acetic acid and ethyl acetate will be studied (from 1.5 on). The standard, 1,4-dioxane, is used in a coaxial insert, to enable reuse and eliminate the error that comes from inaccurate addition of the standard. The samples are prepared by diluting a 25 mg IL layer mixture with 400 mg DMSO-d_6.

1.3. Biphasic esterification with $P_{666,14}$ DCN

At this time we gained insight into esterification in the IL layer through application of inverse gated ^{13}C NMR analysis. Two unexpected phenomena occurred when performing the biphasic esterification to determine the rate of acetic acid migration and the rate of conversion to ethyl acetate:

- The chemical shift of the $\underline{C}H_3$-peak of acetic acid moved downfield as a function of time, from 21.3 to 22.0 ppm in the most extreme case (Figure 2.2). The reason for this unusual behaviour remains speculative, but a possible explanation is that part of the acetic acid exchanges for the

dicyanamide anion of the IL in the form of acetate. The interaction of the acetate with the phosphonium ion results in a change of the electronic environment and thus in a changed chemical shift. The reason why there is only one peak and not one for acetic acid and one for phosphonium-linked acetate can be explained by the tendency of carboxylic acids to form dimers or a fast exchange between free acetic acid and phosphonium-linked acetate. In the former, the free acetic acid experiences the same electronic environment as the acetate, while in the latter one peak is showing the average between the two forms.

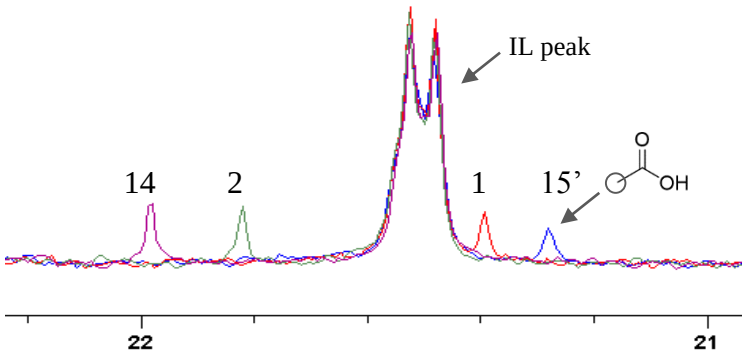
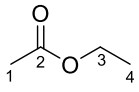


Figure 2.2: downfield shift of $\underline{C}H_3$-peak of acetic acid in ^{13}C NMR as a function of time

- The ethoxy peaks, presumably of ethyl acetate (3 and 4 in figure 2.3), were observed in the IG ^{13}C NMR spectrum. However, the $\underline{C}H_3CO$-peak is not observed (1 in figure 2.3). It could be that this peak is slightly shifted and is hidden under the broad IL peak around 21.5 ppm (figure 2.2), as is presumably the case in ^{1}H NMR where it is hidden under a broad IL peak around 2.1 ppm. However, an HMBC spectrum revealed no coupling between 1 and 2 (figure 2.3). Moreover, the carbonyl peak should be at 170 ppm, while there is only a peak at 156 ppm and one at 164 ppm. We reasoned that the latter could be the ethyl acetate carbonyl peak that has shifted due to the high ionic strength of the samples. However, when spiking with extra ethyl acetate, a carbonyl peak appeared at the appropriate shift and 2 more ethoxy peaks appeared close to the ones previously assigned to ethyl acetate. All of this indicates that a product that structurally resembles ethyl acetate is formed, due to the presence of an ethoxy and a carbonyl group, but not ethyl acetate itself. Further efforts were put in the identification of the ethyl acetate resembling product and possible byproducts of which smaller peaks were found.



	1	2	3	4
^{1}H NMR	1.99	/	4.03	1.17
^{13}C NMR	19.83	170.96	60.56	14.50

Figure 2.3: structure and chemical shifts (ppm) of ethyl acetate

1.4. Identification of the product

Since ethanol and acetic acid did not react to form ethyl acetate and ^{31}P NMR did not show any changes on the phosphonium ion, the only possible reaction would involve a transformation of the dicyanamide anion. Hydrolysis, nucleophilic addition of ethanol or a combination of the two reactions seem possible in acidic conditions leading to the product called diethyl imidodicarbonate **26** or the 1,3-diethylester of imidodicarbonimidic acid **25**.

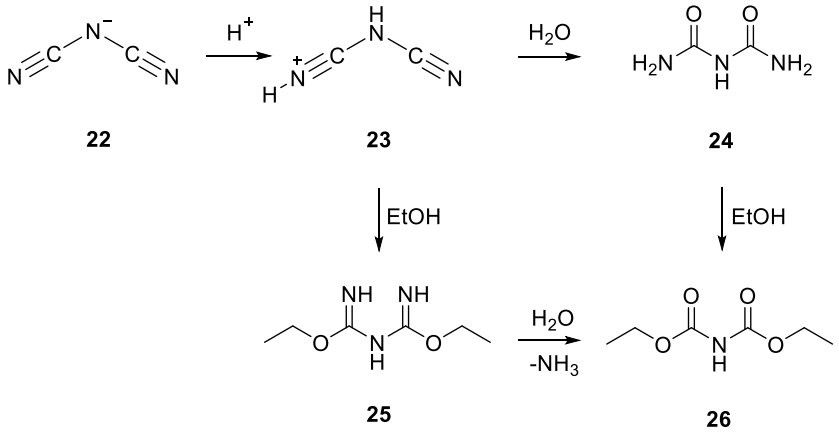


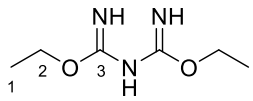
Figure 2.4: proposed reaction of the dicyanamide anion of $P_{666,14}$ DCN with water and ethanol in acidic conditions

This reaction does not involve acetic acid, so the same ethoxy and carbonyl peaks should appear on ^{13}C NMR when performing the biphasic esterification in the same conditions, but without acetic acid. This was the case, confirming acetic acid is not involved. We unsuccessfully attempted to extract these compounds from the IL layer and identify with LC-MS. Commercially available diethyl imidodicarbonate was ordered to compare the peaks in ^{13}C NMR and to see if it further reacts to form the smaller unidentified byproduct peaks. Additionally, attempts were made to perform the proposed reaction with sodium dicyanamide as starting product, in conditions similar to the biphasic esterification, as there were no literature data available on this reaction.

The reaction of sodium dicyanamide in water, ethanol and sulfuric acid at 75 °C gave rise to two products. According to ^{13}C NMR, one is the target product from the $P_{666,14}$ DCN reaction, consisting of the ethoxy peaks and the carbonyl peak at 164 ppm, although the product has not been isolated. LC-MS analysis revealed an m/z value (i.e. an indication for the molecular weight of the compound through mass spectrometry) of 160 for the formed product, indicating that the 1,3-diethyl ester of imidodicarbonimidic acid **25** is formed instead of diethyl imidodicarbonate **26**. Based on the spectral information, the other product seems to be isopropanol. Isopropanol had also been detected in a very small amount in the aqueous layer through GC analysis during the proof of concept experiment. This is unlikely from a reaction mechanism point of view. There is no logical reaction pathway for any of the reagents to give rise to isopropanol. There has been no elucidation on the identity or possible formation of this product so far.

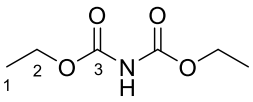
The spectral information of the commercially available diethyl imidodicarbonate **26** shows close resemblance to the target product. The shifts in ^{13}C NMR are however not completely the same: the -

O<u>C</u>H$_2$- peak and the carbonyl peak from the target product have a higher shift than from the diethyl imidodicarbonate **26**. This again points to the formation of the 1,3-diethyl ester of imidodicarbonimidic acid **25** instead of diethyl imidodicarbonate **26**. A reaction of diethyl imidodicarbonate **26** with ethanol, water and sulfuric acid was performed at the same conditions to see if by-products can be formed from this compound. Again isopropanol was detected, and 2 smaller ethoxy peaks of which the identity and the formation is not clear.



25

	1	2	3
^{1}H NMR	1.20	4.11	/
^{13}C NMR	14.0	65.0	164.0



26

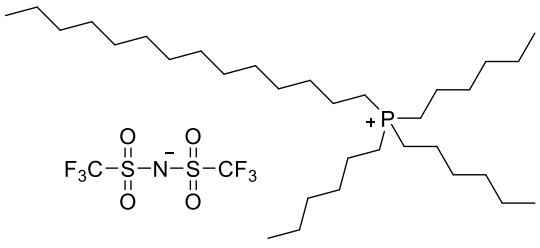
	1	2	3
^{1}H NMR	1.17	4.06	/
^{13}C NMR	14.0	60.7	151.2

Figure 2.5: structure and chemical shifts of 1,3-diethyl ester of imido-dicarbonimidic acid **25** and diethyl imidodicarbonate **26**

From the previous experiments it is clear that the dicyanamide anion is not stable in the biphasic esterification reaction conditions. The negligible esterification yields may be due to outcompetition of the esterification reaction by the anion breakdown reaction. It is likely that a small amount of ethyl acetate was formed since it was detected through GC analysis of the aqueous layer, but it was not detected in the IL layer through IG ^{13}C NMR. It is clear that due to this minimal yield and instability of the anion, the biphasic esterification with $P_{666,14}$ DCN is not suitable.

1.5. Preliminary tests with $P_{666,14}$ Tf_2N

The previous sections showed that the use of $P_{666,14}$ DCN for this application is not suited due to the breakdown of the anion. The use of a phosphonium IL remains however attractive due to the high acetic acid transference into the IL layer. It was therefore decided to test an IL with the same phosphonium cation, but with an anion that is expected to be stable under these conditions. A commercially available IL that fits these requirements is trihexyl(tetradecyl)-phosphonium bis(trifluoromethylsulfonyl)imide ($P_{666,14}$ Tf_2N, Figure 2.6). A preliminary esterification experiment in $P_{666,14}$ Tf_2N showed promising results: a conversion of approximately 90% after 2 hours. Moreover, the C<u>H</u>$_3$CO-peaks of ethyl acetate and acetic acid were well resolved from one another and from the neighbouring IL peak in ^{1}H NMR with DMSO-d_6 (Figure 2.7), which was not the case when $P_{666,14}$ DCN was used. This means a faster analysis method is possible. Quantification was done by adding a coaxial insert containing a reference compound.



27

Figure 2.6: structure of $P_{666,14}$ Tf_2N

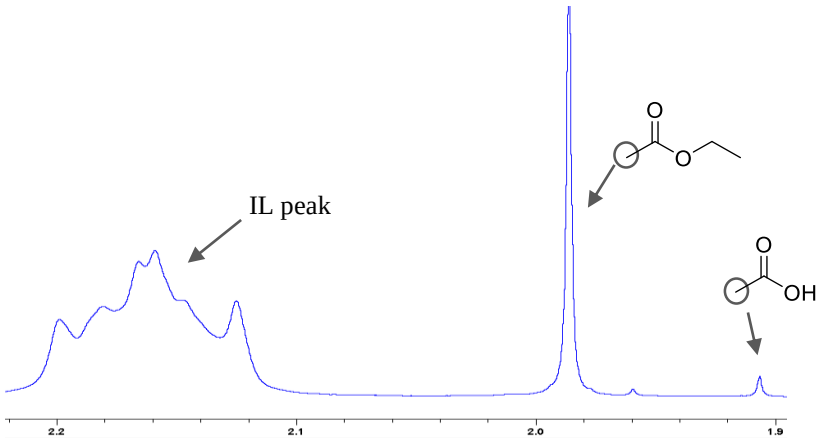


Figure 2.7: ^{1}H NMR spectrum in DMSO-d_6 from a preliminary esterification experiment in $P_{666,14}$ Tf_2N shows well resolved CH_3CO-peaks from ethyl acetate and acetic acid and a conversion of approx. 90% after 2 hours reaction

Extraction was performed identically: 1 g $P_{666,14}$ Tf_2N was added to 10 mL of a 0.33 M acetic acid solution and mixed to emulsification. Analysis of the IL layer showed that 5.6 ± 2.0% of the acetic acid was transferred to the IL layer after 2 hours. The esterification reaction was performed by adding acetic acid, ethanol and sulfuric acid in a 1:3:0.1 ratio to the IL layer and mixing it at 75 °C. It showed a conversion of 85.3 ± 4.4% after 30 minutes.

Table 2.1: overview of extraction and esterification efficiency for $P_{666,14}$ DCN and $P_{666,14}$ Tf_2N in the preliminary experiments

	$P_{666,14}$ DCN	$P_{666,14}$ Tf_2N
Extraction	20%	5.6 ± 2.0%
Esterification	Minimal	85.3 ± 4.4%
Analysis	aqueous layer: GC analysis	IL layer: ^{1}H NMR with internal standard

The overview in Table 2.1 demonstrates a big difference in extraction and esterification capacities by changing the anion of the IL: the extracted portion of acetic acid is comparatively lower with $P_{666,14}$ Tf_2N than with $P_{666,14}$ DCN, whereas the conversion of the esterification is much higher for $P_{666,14}$ Tf_2N. It was therefore decided to elaborate on the type of anion of the IL, to gain insight into its relationship with the observed extraction efficiency and esterification rate. In this way we hoped to shape a framework for the selection of a trihexyl(tetradecyl)phosphonium IL or at least identify one that can be used for this application, as literature data and applications on this class of ILs are very limited. The biphasic esterification was split into 3 steps: extraction, esterification and evaporation, which will further be investigated separately for 5 selected $P_{666,14}$ ILs.

1.6. Evaporation experiments

The removal of ethyl acetate from the IL through evaporation was tested by adding a quantitative amount of ethyl acetate to $P_{666,14}$ DCN. This was connected to a vacuum pump with a liquid nitrogen cooled cold trap in between to condensate the formed vapours. The removal efficiency was

determined through mass difference of the flask containing the IL and ethyl acetate before and after evaporation. Control samples without ethyl acetate were run, in which no mass difference was observed. The method was verified through quantitative ^{1}H NMR. The evaporation efficiencies at different time and temperature combinations and with our without stirring of the IL layer are depicted in Table 2.2.

Table 2.2: evaporation efficiency as function of temperature, time and mixing of the IL layer, for evaporation at 40 mbar

temperature (°C)	time (min)	stirring	% evaporation
25	30	no	13.1
25	30	yes	72.4
25	60	yes	72.6
55	30	no	39.5
55	30	yes	91.3
75	30	no	63.1
75	30	yes	95.5
75	15	yes	94.2
75	5	yes	92.2

The results demonstrate that an increased efficiency is reached at higher temperatures. This is due to a viscosity decrease of the IL at increased temperatures, which enables a higher mass transfer rate. Stirring of the IL layer during evaporation leads to a higher release of ethyl acetate. From the experiments that were conducted at 75 °C, it can be seen that almost all ethyl acetate was removed in the first 5 minutes. Furthermore, the difference in evaporation efficiency for a 30 minutes experiment with a stirred IL layer at 75 °C and at 55 °C is small. It was therefore decided to run a 5 minute evaporation experiment with a stirred IL layer, both at 55 °C and at 75 °C in triplicates, to make a comparison between these conditions. The efficiency at 55 °C was 94.9 ± 1.3%, while it was 95.9 ± 1.4% at 75 °C. Thus the optimal conditions were identified as a 5 minute evaporation of a mixed IL layer at 55 °C, at a pressure of 40 mbar. This experiment shows that almost quantitative removal of ethyl acetate is possible, in a short amount of time and at a mild temperature.

1.7. Preliminary experiments on anion exchange

Another aspect that needs to be elucidated in the first phase is if the ions from the ionic liquid can be replaced by ions that are present in the aqueous layer. This ion exchange would alter the composition and properties of the IL. A transfer of the cation to the water layer is not expected, since the cation is very hydrophobic. The anion is small and water-soluble, so an exchange for anions in the water layer is possible (Figure 2.8). The concentration of anions in the water is relatively high, since it is a high salinity anolyte. This anion exchange would result in the anion composition of the IL layer reflecting the anion composition of the aqueous layer, or the anions of the IL would be of one type that has the highest attraction to the phosphonium ion and cannot be replaced by other anions. Either way this would mean that the IL would go through a lag phase in which the initial anions are replaced, to a phase of stable operation once the anion composition is set. We previously demonstrated the anion of the IL affects extraction and esterification, so the final anion composition of the IL should be beneficial for extraction and esterification. Continuous replenishing of the IL to make up for this anion loss is not an option due to the high IL cost.

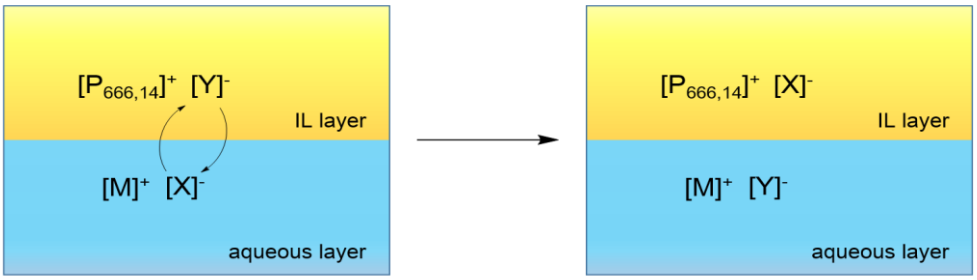


Figure 2.8: schematic representation of anion exchange in the water – IL biphasic system

Anion exchange was tested by adding 1 g $P_{666,14}$ Cl to 10 mL of a solution containing 2 g/L chloride, nitrate, phosphate and sulfate. These anions were selected because they are the most abundant anions in water and are easily detected through anion IC. This was mixed to emulsification at 75 °C. An IL with chloride as anion was chosen because the chloride concentration in the aqueous phase can easily be measured through anion IC. In this way there can be differentiation between anion concentration decrease due to possible uptake of ion pairs by the IL on the one hand and anion concentration decrease due to anion exchange on the other hand. In the latter the chloride concentration in the water should rise, while it is not the case in the former. The results of this test are depicted in Figure 2.9, (A). The phosphate concentration remains constant throughout the experiment. There is a 99% and a 50% decrease for respectively the nitrate and sulfate concentration during the first hour, whereas the chloride concentration increased with 80%. This indicates that the chloride in the IL is indeed replaced with nitrate and sulfate from the aqueous layer. Assuming charge neutral exchange (i.e. two chloride anions exchange for one sulfate anion and one chloride anion for one nitrate anion), 0.527 mmol chloride is released in the water, which is close to the experimentally determined 0.482 mmol chloride increase in the water. This experiment demonstrates that anion exchange does take place and that there is a sequence in likeliness to exchange: chloride is readily replaced by nitrate, to a lesser extent by sulfate and is not replaced by phosphate.

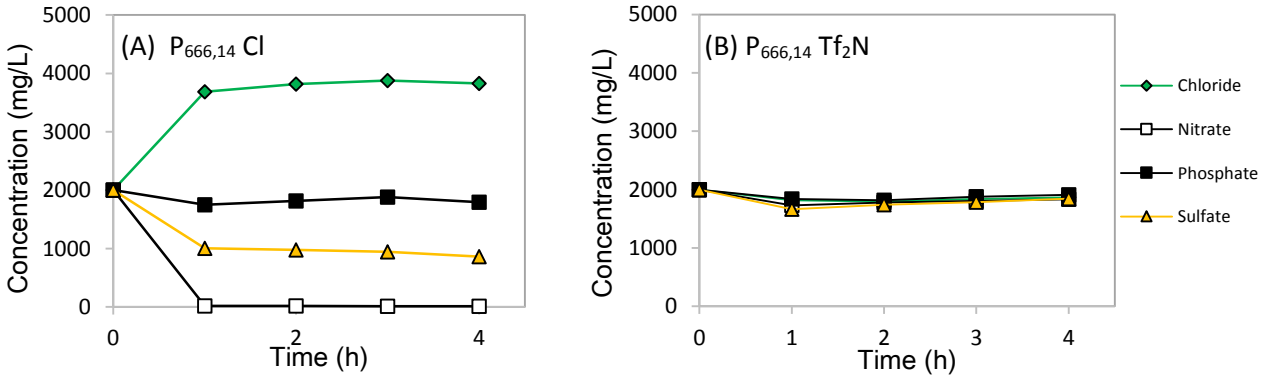


Figure 2.9: preliminary test on anion exchange showing the concentration of Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-} in the aqueous layer (10mL) when mixing with IL (1 g): $P_{666,14}$ Cl (A) and $P_{666,14}$ Tf_2N (B)

The same experiment was performed with $P_{666,14}$ Tf_2N (Figure 2.9, (B)). There is no change in the concentration of any of the anions up to 4 hours. This indicates that there is no anion exchange when Tf_2N is used as anion for the IL, or that it is a much slower process.

These preliminary experiments indicate that anion exchange is possible, depending on the anion of the IL. Furthermore, not all anions exchange to an equal extent. Preferably an IL of which the anion does not exchange should be used in the biphasic esterification process, or the final anion composition should be beneficial for extraction and esterification.

Based on the characteristics of $P_{666,14}$ ILs in biphasic systems that have been explored in the previous experiments, it was decided to compare five $P_{666,14}$ ILs with different anions on the level of:

1. Extraction of acetic acid: the efficiency and rate of the extraction of acetic acid from a 10 mL 0.33 M acetic acid solution with 1 g IL
2. Esterification with ethanol: the efficiency and rate for the sulfuric acid catalysed esterification of acetic acid with ethanol in the IL
3. Evaporation of ethyl acetate: the efficiency of the evaporation of ethyl acetate from the IL
4. Anion exchange: the stability of the IL towards chloride, nitrate, sulfate and phosphate in the water layer

The five ILs were selected based on Table 1.3:

1. Trihexyl(tetradecyl)phosponium dicyanamide ($P_{666,14}$ DCN) because it has a relatively low water capacity (3.1%) and is available in the lab. Although the anion was shown to be instable, further experiments are useful to compare to ILs with different anions
2. Trihexyl(tetradecyl)phosponium bis(trifluoromethylsulfonyl)imide or bistriflimide ($P_{666,14}$ Tf_2N) because it has a relatively low water capacity (0.7%) and is commercially available
3. Trihexyl(tetradecyl)phosponium chloride ($P_{666,14}$ Cl) because it has a relatively low water capacity (8%) and is commercially available
4. Trihexyl(tetradecyl)phosponium tetrafluoroborate ($P_{666,14}$ BF_4) because it has a relatively low water capacity (1.8%) and can easily be synthesised from $P_{666,14}$ Cl
5. Trihexyl(tetradecyl)phosponium acetate ($P_{666,14}$ OAc) because it is expected to show a higher extraction capacity towards acetic acid by the formation of hydrogen bonds between the acetate anion and acetic acid. It is hydrophobic and less dense than water. It can be synthesised from $P_{666,14}$ Cl

2. Comparison of ILs

2.1. Extraction

The extraction capacity of the ILs towards acetic acid has been tested by adding 1 g of IL to 10 mL of a 0.33 M acetic acid stock solution and mixing for emulsification at 75 °C. The acetic acid concentration in the IL layer was determined through quantitative ^{1}H NMR and is plotted in Figure 2.10. Most of the transfer from the aqueous layer to the IL layer takes place in the first 30 minutes, with a subsequent slow rise in concentration up to 2 hours, after which no significant changes are noticed up to 4 hours. Note that the case of $P_{666,14}$ DCN is an exception, as the dicyanamide anion breaks down already in the extraction stage and is gradually replaced by acetate, explaining the steady rise up to 240 minutes. $P_{666,14}$ DCN and $P_{666,14}$ Cl are the only cases in which the concentration in the IL layer exceeds the initial concentration in the aqueous layer. The final concentration IL layer concentration for $P_{666,14}$ Tf_2N, $P_{666,14}$ OAc and $P_{666,14}$ BF_4 is approx. 0.13 M, corresponding to a 4% extraction efficiency.

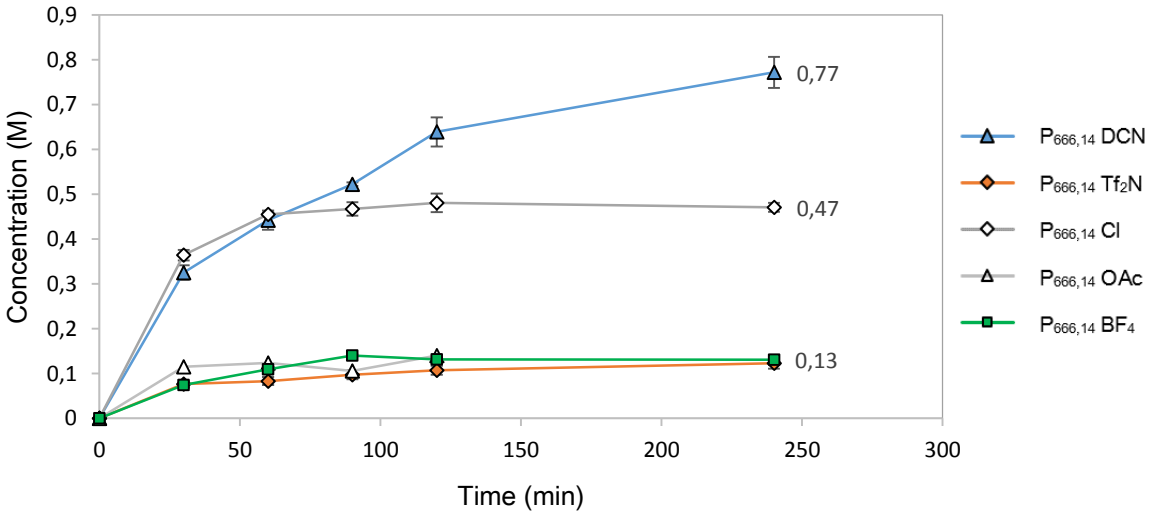


Figure 2.10: comparison of the IL layer acetic acid concentration during extraction, for the five tested ILs. Average values and standard deviations of three measurments are given, determined through ^{1}H NMR

In this way, an indication for the relative extraction capacity was obtained. No efforts for optimisation were conducted at this stage, so the efficiency is not representative for comparison to conventional liquid-liquid extraction. These conditions were selected for practical testing: only a small IL volume was used due to the high IL cost. A higher extraction capacity may be obtained through optimisation of the conditions. Furthermore, other ILs may show a bigger affinity for acetic acid. McFarlane et al. tested the distribution of organic compounds between an aqueous layer and nine ILs with imidazolium, pyrolidinium and phosphonium cations. The partitioning of acetic acid in the IL layer was not significant (< 1%), but a considerably higher distribution was found for hexanoic acid and other more hydrophobic compounds.[95] Oliveira et al. studied the extraction of L-lactic, L-malic and succinic acid with $P_{666,14}$ ILs. They found maximal partition coefficients of 20.9, 7.3 and 10.6 for lactic, malic and succinic acid, respectively. The partition coefficients were found to be related to the hydrophilicity of the solute and the ability to form hydrogen bonds with the anion of the IL.[96] Martak et al. demonstrated a partition coefficient of 40 for the extraction of lactic acid with a $P_{666,14}$ IL, due to hydrogen bonding between the

IL anion and lactic acid.[73] This is shown to have a big impact on the extraction, as lactic acid is a hydrophilic compound and a partition coefficient of around 1 was reported for the conventional trialkylamines. We tested $P_{666,14}$ OAc for this reason: carboxylic acids form dimers through hydrogen bonding, which might result in a higher extraction efficiency through linking of the aqueous acetic acid to the IL acetate anion. This could not be concluded from the results. Further research might designate other anion(s) that extract more acetic due to hydrogen bonding. It is not clear to what extent the cation has an influence on the extraction. The alkyl chains have to be of sufficient length to provide the hydrophobicity. Care has to be taken when replacing the alkyl chains by other side groups, for instance aromatic substituents, since this strongly affects the physical properties. Going for a total different cation might increase the extraction efficiency, however up to date the phosphonium ILs have shown to be the best extractants.[63]

The previously discussed papers highlight the challenge in extracting hydrophilic compounds, especially acetic acid, with ILs from an aqueous layer. Further research to improve the extraction efficiency will need to focus on the use of an IL with a high affinity for the compounds, for instance through the formation of hydrogen bonds.

2.2. Esterification

The performance of the esterification reaction in the ionic liquid was evaluated by adding the reagents to 1g of ionic liquid and running the reaction at 75 °C in a stirred one-phase system. The conversion as a function of time is depicted in Figure 2.11. The esterification does not proceed at all in $P_{666,14}$ DCN and $P_{666,14}$ OAc and only in a very limited amount in $P_{666,14}$ Cl. A higher conversion was observed in the cases of $P_{666,14}$ BF_4 and $P_{666,14}$ Tf_2N: respectively 73.9 ± 0.9% and 84.5 ± 6.2% after 15 minutes. These values can be considered as an equilibrium as the conversion at further time points was not significantly different.

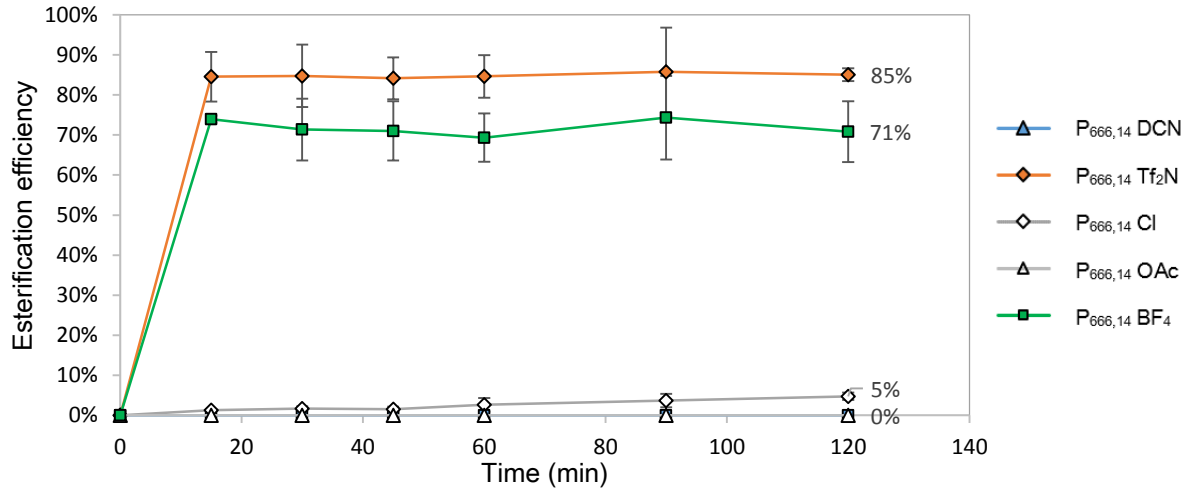


Figure 2.11: comparison of the conversion for the esterification of acetic acid in five ILs. Average values and standard deviations of three measurments are given, determined through ^{1}H NMR

2.3. Evaporation

An accurately weighed quantity of ethyl acetate was added to 1g IL and the evaporation was performed at the conditions previously determined for $P_{666,14}$ DCN: 5 minutes evaporation at 40 mbar for a mixed IL layer at 55 °C. The efficiency was determined by mass difference and verified with quantitative ^{1}H NMR analysis. The efficiencies for the other tested ILs are lower compared to the previously determined efficiency for $P_{666,14}$ DCN. The results indicate an inverse relationship between the efficiency and the viscosity of the IL, for which literature data at 25°C are given (Table 2.3). Higher efficiencies for the high viscosity ILs could be obtained by raising the temperature as the viscosity is strongly temperature dependent.[97] This effect was not observed for $P_{666,14}$ DCN where the efficiency was 95.9 ± 1.4% at 75 °C, and was not significant for $P_{666,14}$ Tf_2N where the efficiency was 89.1 ± 3.0% at 75 °C. This is because these ILs have relatively low viscosities and the biggest effect of temperature is observed at temperatures below 55 °C.[97] A higher effect by raising the temperature can be expected for $P_{666,14}$ Cl and $P_{666,14}$ BF_4, but should be verified experimentally.

Table 2.3: efficiencies for evaporation of ethyl acetate at 55 °C for five different ILs and literature data for their viscosities at 25 °C. Averages and standard deviations of three measurements are given

	Evaporation efficiency (%)	viscosity (mPa.s)
$P_{666,14}$ DCN	94.9 ± 1.3	256
$P_{666,14}$ Tf_2N	86.7 ± 5.4	312
$P_{666,14}$ Cl	75.3 ± 1.9	1824
$P_{666,14}$ OAc	85.7 ± 2.2	/
$P_{666,14}$ BF_4	77.3 ± 4.2	787

To the best of our knowledge, there is only one study in which the separation of the extracted compounds from the IL layer is assessed through other techniques than back-extraction or spontaneous phase separation of the product. Two strategies are compared for the removal of lactic, malic and succinic acid from $P_{666,14}$ ILs. In the first strategy, NaOH is added to the IL layer, upon which the corresponding sodium salts are formed in a small water layer that can be decanted. This resulted in a 36%, 73% and 46% recovery of lactic, malic and succinic acid respectively. This strategy can however not be applied to the esters, as these cannot form sodium salts. In the second strategy, a vacuum pressure is applied to evaporate the compounds. A vacuum of 6 mbar and a temperature of 130 °C were applied for the evaporation of lactic acid (T_b=122 °C at 1 atm). No significant amounts of lactic acid could be removed. For the less volatile malic and succinic acid, a vacuum of 0.5 atm and respective temperatures of 120 °C and 150 °C were applied, but again no acid was removed.[96] This demonstrates that removal of the acid through evaporation is not a suitable strategy. Esters are however more volatile than the corresponding VFAs, which mitigates removal by evaporation. These preliminary experiments demonstrate that a major to almost quantitative removal from sometimes viscous media is possible, in a limited amount of time and at low temperatures.

2.4. Anion exchange

Anion exchange was tested by mixing 1 g IL with 10 mL of a 1 g/L anion solution (i.e. 1 g Cl^-/L, 1 g NO_3^-/L, 1 g SO_4^{2-}/L and 1 g PO_4^{3-}/L). Samples of the aqueous phase were analysed with ion chromatography to monitor the concentration of the anions.

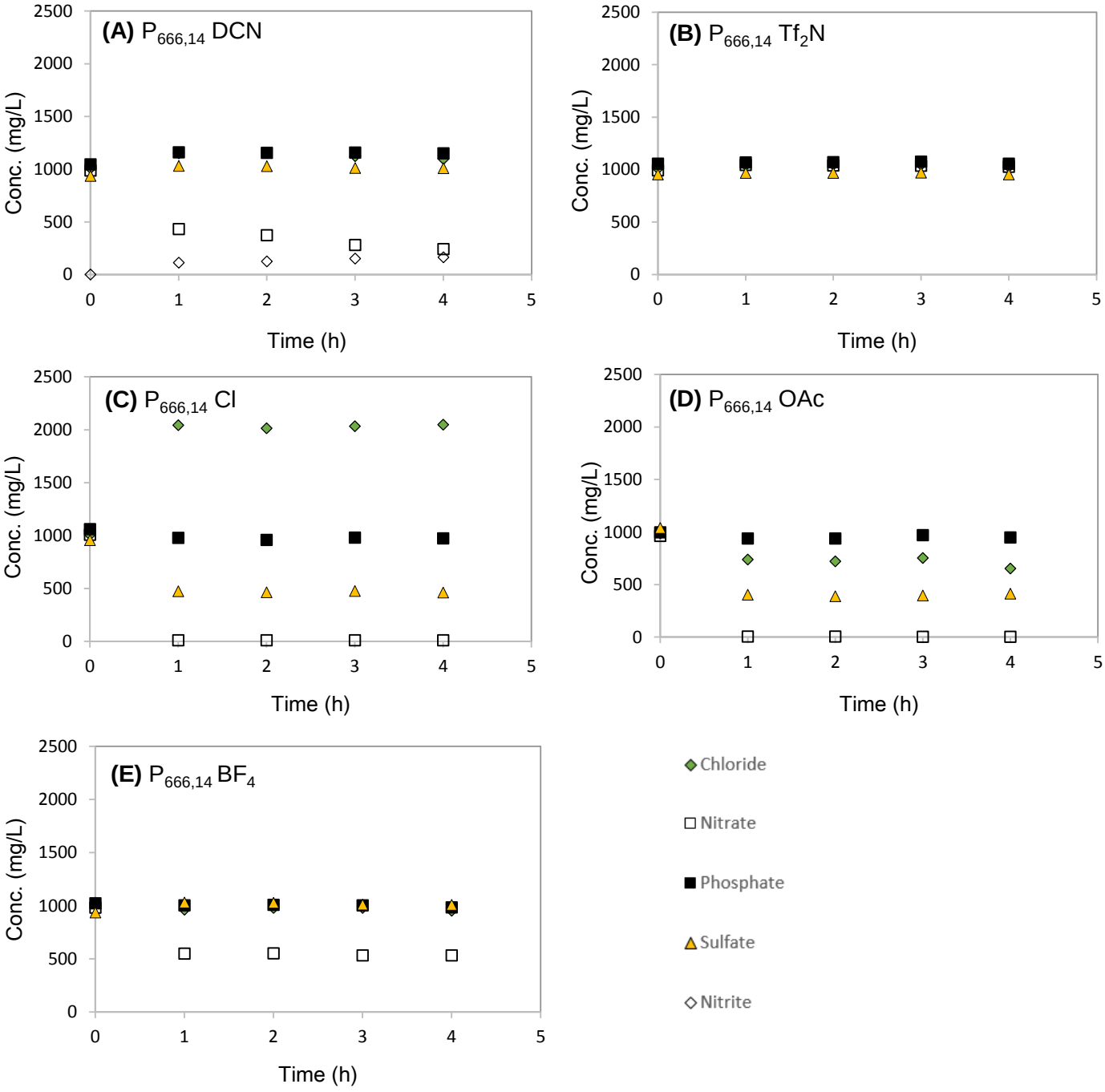


Figure 2.12: concentrations of chloride, nitrate, phosphate, sulfate and nitrite in the aqueous layer (10 mL) upon mixing with IL layer (1 g), with $P_{666,14}$ DCN **(A)**, $P_{666,14}$ Tf_2N **(B)**, $P_{666,14}$ Cl **(C)**, $P_{666,14}$ OAc **(D)**, $P_{666,14}$ BF_4 **(E)** as ionic liquids. The values are averages of three measurements

In the case of $P_{666,14}$ DCN (Figure 2.12, (A)), a drop of 74.4% for the nitrate concentration in the water was observed, whereas the concentration differences for the other anions remained within 10.5% over 4 hours (Table 2.4). The concentration decrease continues up to 4 hours, by which still 25.6% of the initial amount of nitrate is left. This indicates that the dicyanamide anion from the IL exchanges for nitrate in the water, corresponding to a 6.60% exchange of the dicyanamide, if 1 dicyanamide anion exchanges for 1 nitrate anion (ref. to calculations in appendix). Furthermore, there appears to be nitrite formed, with a concentration increase from 0 mg/L to 162.7 ± 2.2 mg/L after 4 hours. It is the only IL in which this formation of nitrite has been observed.

Table 2.4: conc. (mg/L) at 0 and 4 h and conc. difference (%) of the anions in the aqueous layer for $P_{666,14}$ DCN

	t=0	t=4 hours	Δ mol%
Cl^-	1008 ± 14	1096 ± 12	8,8%
NO_3^-	985 ± 11	241 ± 9	-74,4%
PO_4^{3-}	1044 ± 14	1149 ± 11	10,5%
SO_4^{2-}	935 ± 13	1008 ± 32	7,3%

There is no significant change in anion composition of the aqueous layer upon mixing with $P_{666,14}$ Tf_2N (Figure 2.12, (B)). This demonstrates that this IL is stable with regard to exchange with the most common anions.

In the case of $P_{666,14}$ Cl (Figure 2.12, (C)), only the phosphate concentration remains at the same level throughout the experiment. The sulfate and nitrate concentration decrease with 49.3% and 99.9% respectively (Table 2.5). Assuming that 1 nitrate anion exchanges for 1 chloride anion from the IL and 1 sulfate anion for 2 chloride anions, 14.5% of the chloride from the IL is exchanged for nitrate and sulfate. A 104.0% increase in chloride concentration was observed. This means that based upon this chloride concentration increase in the aqueous phase, 15.7% of the chloride from the IL is replaced. This is approximately the same value as calculated based upon the nitrate and sulfate drop, indicating that it is indeed an exchange of 1 nitrate anion for 1 chloride anion and 1 sulfate anion for 2 chloride anions, and not an uptake of these anions with their cations in the bulk phase of the IL. Furthermore still the largest portion of the anions in the IL layer remains chloride and approximately half of the sulfate remains in the aqueous phase, which indicates this anion exchange is an equilibrium reaction and does not proceed until complete replacement.

Table 2.5: conc. (mg/L) at 0 and 4 h and conc. difference (%) of the anions in the aqueous layer for $P_{666,14}$ Cl

	t=0	t=4 hours	Δ mol%
Cl^-	1007 ± 56	2048 ± 9	104,0%
NO_3^-	1007 ± 41	8 ± 0	-99,9%
PO_4^{3-}	1059 ± 54	972 ± 6	-13,3%
SO_4^{2-}	955 ± 47	462 ± 5	-49,3%

Also in the case of $P_{666,14}$ OAc (Figure 2.12, (D)), only the phosphate concentration remains unchanged upon mixing with the IL. The concentration from the other anions decreases to values that fluctuate around an equilibrium value obtained after 1 hour. There is a 33.6% decrease in chloride concentration, a 95.9% decrease in nitrate concentration and a 62.5% decrease in sulfate concentration (Table 2.6). 20.65 % of the acetate in the IL is replaced. The leftover acetate in the IL layer and the leftover sulfate and chloride in the water again indicate this anion exchange is an equilibrium reaction.

Table 2.6: conc. (mg/L) at 0 and 4 h and conc. difference (%) of the anions in the aqueous layer for $P_{666,14}$ OAc

	t=0	t=4 hours	Δ mol%
Cl^-	989 ± 13	653 ± 6	-33,6%
NO_3^-	963 ± 16	4 ± 0	-95,9%
PO_4^{3-}	998 ± 12	949 ± 10	-4,9%
SO_4^{2-}	1038 ± 66	414 ± 2	-62,5%

For $P_{666,14}$ BF_4 (Figure 2.12, (E)), only the nitrate concentration changes significantly, with a 44.8% drop after 4 hours (Table 2.7). This corresponds to a calculated amount of 4.12 % of the tetrafluoroborate that is replaced by nitrate. There is nitrate left in the aqueous layer and tetrafluoroborate in the water layer, indicating it is an equilibrium reaction.

Table 2.7: conc. (mg/L) at 0 and 4 h and conc. difference (%) of the anions in the aqueous layer for $P_{666,14}$ BF_4

	t=0	t=4 hours	Δ mol%
Cl^-	1027 ± 73	952 ± 18	
NO_3^-	981 ± 78	532 ± 7	-44,8%
PO_4^{3-}	1022 ± 70	984 ± 13	
SO_4^{2-}	930 ± 50	994 ± 90	

The previous experiments demonstrate that the anion exchange is a fast reaction: an equilibrium value is reached within the first hour. Only in the case of $P_{666,14}$ DCN, a gradual decrease of the nitrate concentration is observed. We speculate this is because the nitrate only takes the place of the dicyanamide anion when the latter gets protonated in the breakdown reaction (chapter 2, section 1.4), which is the rate limiting step for exchange. The results indicate that the exchange does not proceed until completion, but an equilibrium between the anions in the IL layer and in the water layer is reached. Furthermore, the anion exchange is a selective process, as not all anions do exchange to the same extent.

$P_{666,14}$ Tf_2N is the only IL in which the anion composition remains unaltered upon mixing with an aqueous layer containing chloride, nitrate, phosphate and sulfate. It is in other words the only one of the five tested ILs that can be used with a stable IL composition in a process in which an aqueous stream containing salts is involved. It is however not clear from this simple test if the possibility to exchange is dependent on the pH and if there are other anions that can replace the Tf_2N anion. The effect of renewed exposure is not clear: will the portion of replaced anions increase when contacting the IL layer with a second batch of anion solution, or is the final equilibrium already reached and thus ensure a stable operation. Further research is needed to clarify this anion exchange process to enable the prediction of the IL needed for a certain application. It has to be noted that in this application also $P_{666,14}$ BF_4 could be used, since the nitrate concentration in the anolyte is negligible due to the presence of denitrifying micro-organisms in the mixed culture.

2.5. Discussion on extraction, esterification and anion exchange

The overview in Table 2.8 shows that none of the 5 tested ILs display a high efficiency both at extraction and at esterification. $P_{666,14}$ OAc, $P_{666,14}$ BF_4 and $P_{666,14}$ Tf_2N show an extraction efficiency of approx. 4%. $P_{666,14}$ DCN and $P_{666,14}$ Cl show a relatively high extraction efficiency, leading to acetic acid concentrations in the IL layer that are higher than in the aqueous layer. A higher acetic acid uptake in the case of $P_{666,14}$ DCN has previously been contributed to hydrolysis of the anion and subsequent replacement by acetate. We speculated that the higher extraction efficiency was not only for $P_{666,14}$ DCN related to anion exchange, but also for $P_{666,14}$ Cl as it shows a high level of anion exchange in Table 2.8. Therefore, the extraction of acetic acid might be a combined effect of acetic acid solubility in the IL layer and of uptake as acetate anion for the IL. The extraction capacity of acetic acid in the IL layer due to solubility would consequentially be approx. 4%, as in the case of $P_{666,14}$ Tf_2N and $P_{666,14}$ BF_4, which are not prone to anion exchange. The extra uptake in the case of $P_{666,14}$ DCN and $P_{666,14}$ Cl might be due to exchange of these anions for acetate. This was confirmed in a preliminary experiment in

which all of the acetic acid was washed out of the IL layer with Milli-Q water in the case of $P_{666,14}$ Tf_2N, whereas a part was retained in the case of $P_{666,14}$ Cl, even after three washing steps.

Table 2.8: overview of extraction and esterification efficiencies (left) and anion exchange (right) for the 5 tested ILs

	Extraction	Esterification
$P_{666,14}$ DCN	23.4 ± 3.4%	0%
$P_{666,14}$ Tf_2N	3.7 ± 1.2%	84.5 ± 6.2%
$P_{666,14}$ Cl	14.3 ± 1.0%	4.8 ± 0.9%
$P_{666,14}$ OAc	4%	0%
$P_{666,14}$ BF_4	4.0 ± 0.2%	70.8 ± 7.6%

	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
$P_{666,14}$ DCN		-74.4%		
$P_{666,14}$ Tf_2N				
$P_{666,14}$ Cl	104.0%	-99.9%		-49.3%
$P_{666,14}$ OAc	-33.6%	95.9%		-62.5%
$P_{666,14}$ BF_4		-44.8%		

It is possible to propose from these data a sequence of anion affinity from the anion for the IL cation or for the water. This series is depicted in Figure 2.13, with anions ranging from a weak affinity for the IL cation and a strong affinity for water as is the case for PO_4^{3-} (left), to the opposite for Tf_2N^- (right).

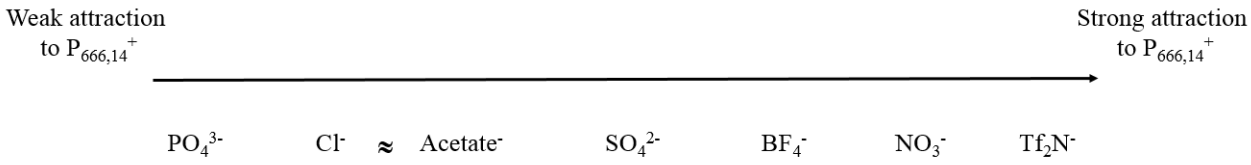


Figure 2.13: experimentally determined series of anion affinity for the IL cation

The first speculation that the anion composition of the IL after mixing with the aqueous layer might reflect the anion composition of the latter is hereby disproved, since there is a clear difference for the different anions. The question remains what mechanism lies on the basis of this sequence. It does not seem to reflect the pKa nor the solubility of the individual anions. It needs a framework explaining the tendency of the anion to stay in the aqueous phase or to migrate to the hydrophobic layer. This information is provided by the Hofmeister series, originally established to explain the salting out of proteins with inorganic salts.[98] The Hofmeister series is depicted in Figure 2.14 (A). On the left side of the series are the most hydrophilic ions, the so-called kosmotropes. These induce salting-out behaviour of solutes (macromolecules) by what was originally thought of as structuring of the bulk water, since they are strongly hydrated. The ions of the right side are referred to as chaotropes, or water structure breakers. They induce salting-in behaviour. More recent research has however shown that these anions are not able to affect the properties of the bulk water, only the water in the immediate environment is restructured, with a dense monolayer for strongly hydrated ions and a loose monolayer for chaotropic ions.[99]

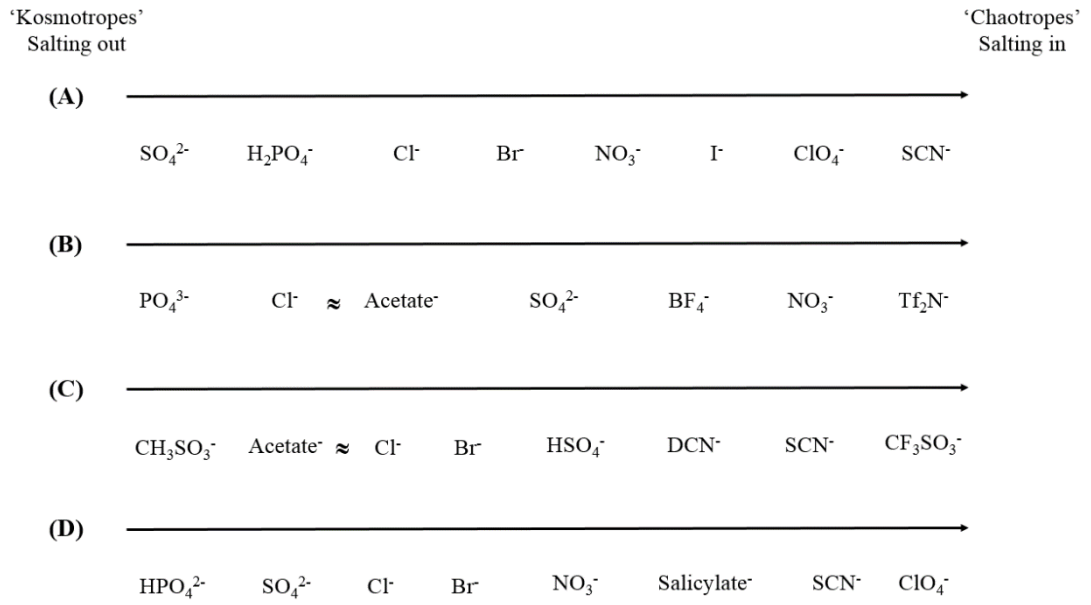


Figure 2.14: Hofmeister series[98] **(A)**, experimentally determined anion sequence in this thesis **(B)**, anion sequence from Ventura et al.[100,101] **(C)** and anion sequence from Gourishetty et al.[105] **(D)**

Research on the Hofmeister series and ILs has mainly been conducted on the effect of inorganic salts on the salting out of hydrophilic imidazolium ILs aiming for creation of aqueous biphasic systems. When inorganic salts with kosmotropic anions are added, the IL-water mixture starts to phase separate. When chaotropic anions are added, they link up to the hydrophobic moiety of the IL, giving it a higher polarity and thus increasing the water solubility. The effect of anions is higher than that of cations since they are typically more polarizable due to their more diffuse valence electronic configuration. Ventura et al. demonstrated that the lower the ability of the anions of the IL to form H-bonds, the bigger their ability to form aqueous biphasic systems. A sequence was drawn ranging from anions that form easily salting out ILs to anions that form very water-miscible ILs (Figure 2.14, (C)). This sequence is similar to the Hofmeister series.[100,101] It is important to note that in both previous articles the behaviour of anions in an IL-water system has been shown to follow the Hofmeister series, demonstrating the effect of the water layer anions and the IL layer anions on the water-miscibility of imidazolium ILs separately, but no exchange between these two types of anions has been described.

Berthod et al. demonstrated that ILs are just salts when used in dilute aqueous solutions, by demonstrating the presence of ion-pairing, ion-exchange and hydrophobic interactions.[102] They tested the adsorption of ILs on a C_{18} stationary phase and showed that the adsorption is dependent on the anion, according to the Hofmeister series. ILs with chaotropic anions showed more adsorption than kosmotropic anions. The same authors demonstrated that when running a mixture of 1-hexyl-3-methyl imidazolium chloride and sodium hexafluorophosphate or a mixture of 1-hexyl-3-methyl imidazolium hexafluorophosphate and sodium chloride for RPLC, the chromatograms were the same. The IL cation eluted together with hexafluorophosphate in both cases, showing there is exchange of chloride for hexafluorophosphate in the first mixture. There was a much smaller peak from the cation associated with chloride, demonstrating that the exchange is not complete, but an equilibrium.[103]

Anion exchange in the case of phosphonium-ILs has been shown by Peng et al. The author demonstrated the use of $P_{666,14}$ Cl as both plasticizer and anion exchanger in polymeric membranes, forming an ion-selective membrane for the detection of anions with an electrode. The hydrophilic chloride anion of the IL can be exchanged for a more lipophilic anion when the ion-selective electrode is conditioned in an aqueous solution. It was again found to relate to the Hofmeister series: the more chaotropic anions could exchange for chloride and thus be detected with the electrode. The more kosmotropic anions stayed in the water phase and cannot be detected. A hexafluorophosphate imidazolium IL was also tested, but did not show any response to common anions in the water phase, because it is on the chaotropic end of the series and is not exchanged. It only showed response to even more lipophilic anions such as ClO_4^-, salicylate and dodecylsulfate. The authors also observed non-Hofmeister conforming behaviour of the sulfate anion: in the case of $P_{666,14}$ Cl and 1-octyl-3-methyl imidazolium chloride, there was detection of SO_4^{2-}, meaning the chloride did exchange for SO_4^{2-}.[104] Gourishetty et al. demonstrated that the selectivity of anion-selective electrodes with $P_{666,14}$ ILs as anion exchanger and plasticizer is in line with the Hofmeister series: if the IL anions are more hydrophobic than the anions in the water, they will be replaced by the latter and be detected. The experimentally derived sequence is given in Figure 2.14, (D).[105]

Furthermore, Freire et al. demonstrated the effect of inorganic salts in the aqueous phase on the extraction of alkaloids with different phosphonium ionic liquids.[106] They used triheyltetradecylphosphonium ILs with different anions to study the relationship between the anion and the extracting capacity towards the relatively hydrophilic caffeine and the relatively hydrophobic nicotine. The authors demonstrated that partition coefficients were higher when ILs with more hydrophobic anions were used for the more hydrophobic nicotine, whereas the opposite was observed for caffeine. Next, they tested the influence of salts in the aqueous phase on the partition coefficients of caffeine, with $P_{666,14}$ Cl and $P_{666,14}$ Br as ILs. These anions are both in the middle of the Hofmeister series. The authors describe a salting out effect of the solutes by addition of kosmotropic anions to the aqueous phase, but also a salting in effect by addition of chaotropic anions. They do not consider anion exchange. The anion exchange however fits into their results: they describe a salting in effect (or lower partition coefficient of the solute) by addition of hexafluorophosphate to the water layer. This can still be explained by anion exchange, since exchange of the bromide or chloride for hexafluorophosphate would give rise to $P_{666,14}$ PF_6, which is more hydrophobic and displays a lower partition coefficient for caffeine as previously demonstrated.

It is clear from previous research that anion exchange is possible, but is not widespread recognised. It is shown in some niche applications, e.g. ion-selective electrodes, but has not been included in studies on the extraction of solutes in an aqueous biphasic system. As our results show, it is however an important parameter for the selection of an IL for a certain application. More in-depth research on this is required to fully understand and apply this mechanism. It can be increasingly important when ILs are used as reactive extractants in a fermentation broth, as certain anions can be depleted and thus influence the viability or growth of the reactor microbiome.

We attempted to explain the difference in esterification performance in different ILs from an anion exchange mechanism. The overview in Table 2.8 shows that a high conversion is reached for $P_{666,14}$ Tf_2N and $P_{666,14}$ BF_4, where there is no exchange of the IL anion for sulfate. The esterification does not proceed, or only to a limited extent, in the case of $P_{666,14}$ OAc and $P_{666,14}$ Cl, where the IL anion does exchange for sulfate. It also does not proceed for $P_{666,14}$ DCN where there is no exchange for sulfate,

but this is due to the anion breakdown reaction as previously discussed. Therefore it was initially speculated that due to the exchange of sulfate for the anion of the IL, which is a weaker acid in the case of acetate, the protons of the added sulfuric acid are linked to acetic acid that is now released in the aqueous layer, and therefore are no longer available for catalysis of the esterification. This is not the case for $P_{666,14}$ BF_4 and $P_{666,14}$ Tf_2N, where the sulfate remains in the aqueous layer and thus the protons of sulfuric acid remain available for catalysis. The results in Table 2.8 however already disprove this speculation, because the esterification in $P_{666,14}$ Cl should proceed since HCl is also a strong acid. A preliminary experiment was conducted to further explore the relationship between the IL anion and the esterification performance by testing four different strong acids as catalysts for the esterification in three different ILs. Table 2.9 shows that the conversions are different when different acids are used, despite the fact that each acid was dosed to obtain an equal amount of protons. This indicates that the anion of the acid has an impact on the conversion, but the underlying mechanism is not clear. Further research is needed to elucidate this mechanism and enable selection of the most suitable catalyst.

Table 2.9: approx. conversion of the esterification catalysed by four different strong acids, in three ILs

	HCl	HNO_3	H_3PO_4	H_2SO_4
$P_{666,14}$ Cl	30%	30%	25%	5%
$P_{666,14}$ OAc	30%	0%	2%	0%
$P_{666,14}$ BF_4	-	-	18%	70%

3. Determination of process parameters

3.1. Influence of heat and ethanol concentration on esterification performance

The conversion of the esterification can be increased by using an excess of ethanol or by removing the formed water or ethyl acetate, since it is an equilibrium reaction. Moreover, the reaction rate can be increased by increasing the temperature, as it is an endothermic reaction. These strategies are applied in the industrial esterification process in the so-called reactive distillation set-up: the carboxylic acid is mixed with an excess of alcohol under acid catalysis at high temperatures. The reaction mixture is refluxed in the reactive distillation reactor, while there is continuous removal of either water or the ester, depending on the boiling points of the reagents and products.[107] The aim of the biphasic esterification is however to be a sustainable process, in which the principles of green chemistry are applied; a high conversion should be reached in a low impact process. Therefore, the impact of the ethanol concentration and temperature on the esterification conversion and rate was studied, to determine if it is possible to perform the reaction at mild conditions with a high conversion in a limited amount of time. The one-phase esterification was performed at three (temperature, [ethanol]) sets: (75 °C, 3 eq. EtOH), (75 °C, 1 eq. EtOH) and (55 °C, 1 eq. EtOH). The effect on reaction rate and conversion is given in Figure 2.15.

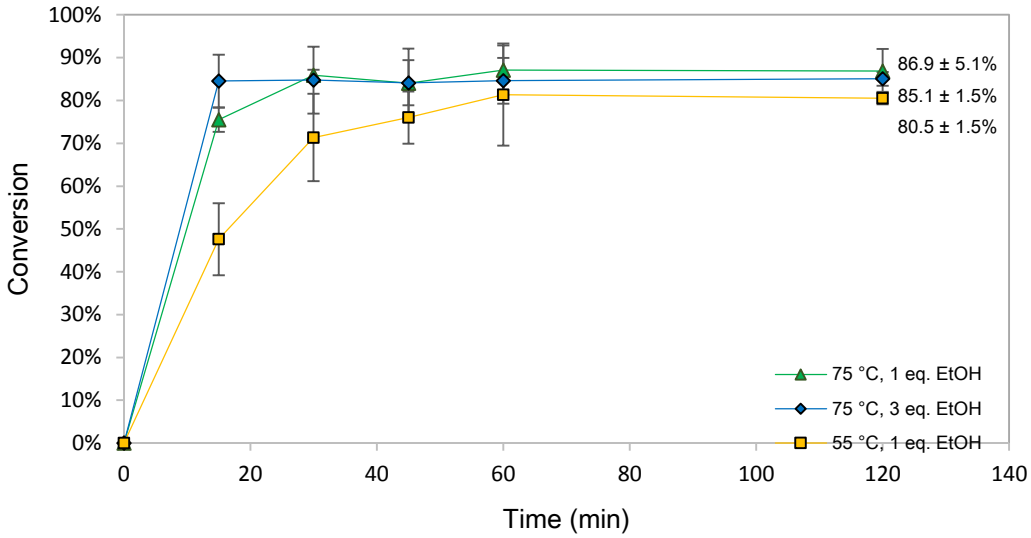


Figure 2.15: effect of temperature and heat on reaction rate and conversion of the esterification reaction of acetic acid with ethanol. Averages and standard deviations of three measurements are given, determined through quantitative ^{1}H NMR

The results demonstrate that the different conditions affect the reaction rate, but not the conversion. An equilibrium value is reached in 15 minutes in the case of (75 °C, 3 eq. EtOH), in 30 minutes in the case of (75 °C, 1 eq. EtOH) and in 60 minutes in the case of (55 °C, 1 eq. EtOH). This means that lowering the temperature or the ethanol concentration results in a slower conversion, but the same end level is still reached within 60 minutes. It is possible to perform the esterification reaction with a 1:1 molar ratio of ethanol to acetic acid with the same conversion as when a 3:1 excess is used, which is an advantage for product purification. Since the boiling points of ethanol and ethyl acetate are very close (78.4 °C and 77.1 °C respectively) they will come off simultaneously during the evaporation step. It is therefore important to minimise the ethanol content after esterification to obtain a higher ethyl acetate purity in the condensate.

This experiment demonstrates that the esterification can be performed to the same conversion at milder conditions if the process time can be long enough. Again, this is important for industrial reasons, as the net energy input can be lowered in this way, contributing to the sustainability and the lower cost of the process.

3.2. Influence of water on esterification performance

The initial aim was to perform the esterification in a biphasic system consisting of an aqueous and an IL layer. The IL layer provides a hydrophobic environment in which acetic acid can be converted into ethyl acetate, creating a driving force for acetic acid transfer from the aqueous layer. The ethyl acetate yield in the proof of concept of this set up with $P_{666,14}$ DCN was minimal (cf. section 1.1.), but we speculate this is because the esterification is outcompeted by the anion breakdown reaction. So far, $P_{666,14}$ Tf_2N has been demonstrated to be the most suitable of the 5 tested ILs for this application and the extraction and esterification have been tested separately, but the outcome in the biphasic system still has to be determined. Therefore, a biphasic reaction was set up in a 10:1 ratio of water to $P_{666,14}$ Tf_2N layer. After 2 hours of mixing at 75 °C for extraction, 0.1 equivalent sulfuric acid and 3

equivalents ethanol were added to perform the esterification. The progress of the reaction is slower than the studied one-phase esterification, and reaches a comparatively lower conversion of 44.2 ± 2.9% after 120 minutes (Figure 2.16). This is due to the presence of the large water volume. Water constrains the conversion of the esterification reaction and the formed ethyl acetate can leach into the aqueous layer.

Then the esterification in absence of the water layer was tested, by removing the IL layer after 2 hours of extraction and adding 0.1 equivalent sulfuric acid and 1 equivalent ethanol. Again a lower conversion and rate than the one-phase esterification was observed (Figure 2.16). A maximum conversion of 54.1 ± 6.3% was reached after 60 minutes. This can be explained by the presence of small water droplets that are present in the IL layer after extraction, which constrain the conversion of the esterification.

To study the effect of the water droplets in the IL layer after extraction, they were removed by centrifugation in the next step. This resulted in an esterification rate and conversion similar to the previously studied 1-phase esterification. The conversion was 80.2 ± 6.5% after 15 minutes and remained at the same level throughout the reaction with a conversion of 83.2 ± 7.2% after 120 minutes. This clearly demonstrates the impact of water on the esterification reaction: even a small amount of water present in the form of droplets considerably delays the reaction and lowers the maximal conversion from 83% to 55%. The presence of a 10-fold volume of water lowers the conversion even more, but the impact is lower.

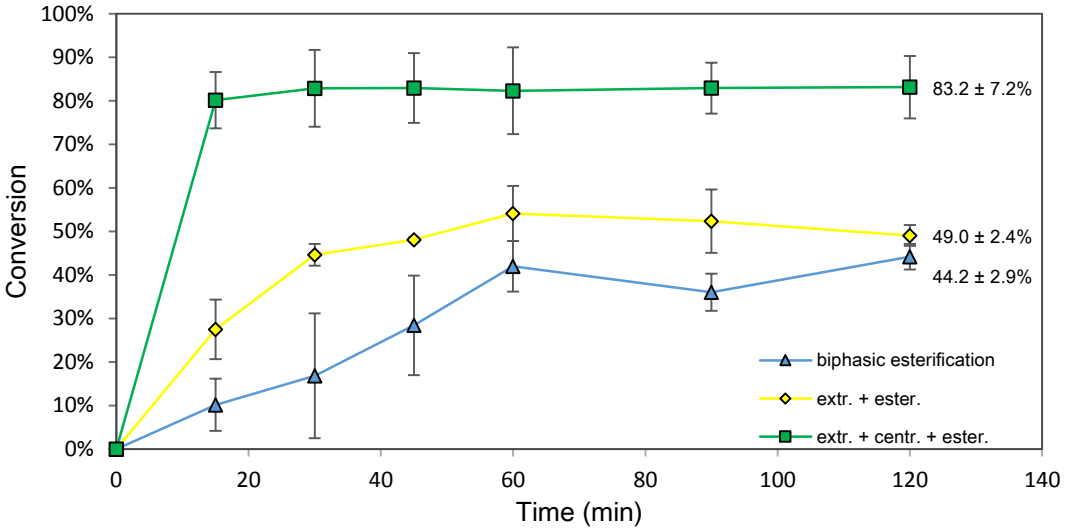


Figure 2.16: effect of water on esterification performance. Conversion for biphasic esterification, esterification after separation of the IL layer and esterification after separation and centrifugation of the IL layer. Averages and standard deviations of three measurements are given, determined through quantitative ^{1}H NMR

There is however a downside to the use of centrifugation: the acetic acid content in the IL layer, relative to the initial amount of acetic acid in the water, decreases from 4.8 ± 3.6% after extraction to 1.5 ± 0.1% after centrifugation. This is due to acetic acid in the water droplets. The acetic acid concentration in this water is higher compared to in the IL, so removing this water by centrifugation results in a lower

measured concentration in the IL layer. This is also reflected in the standard deviation: the same quantification method is used, yet the standard deviation in the samples after extraction is 100 times higher than in the samples after centrifugation. This is contributed to the varying number and size of water droplets in the samples.

The implementation of a centrifugation step gives rise to a higher conversion of the esterification, but a 70% decrease in acetic acid concentration in the IL layer. The net conversion is depicted in Figure 2.17, alongside the net conversions for the biphasic esterification and the extraction and esterification process. After 2 hours, the net conversions are within the same range around 1.4%. A rate difference is observed: the set-up with centrifugation goes faster to an equilibrium conversion. It has however to be noted that the time for IL layer separation and centrifugation is not included, so the total process time might be significantly longer and undo the time profit by the faster conversion. Still the set-up with centrifugation is preferred, as it leads to a lower water content in the IL layer which is advantageous in the final evaporation step.

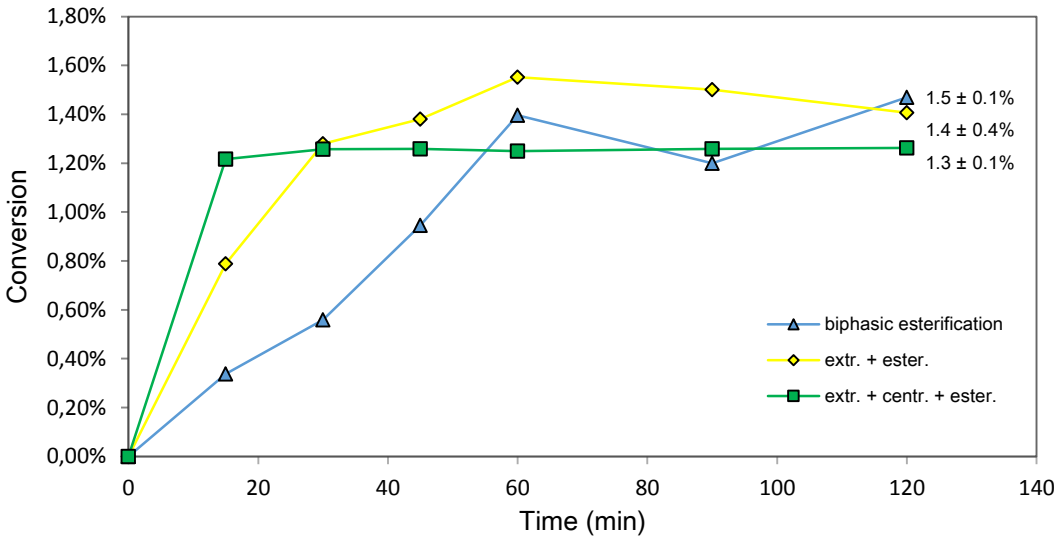


Figure 2.17: net conversions for the 'biphasic esterification' set-up, the 'extraction, separation of IL layer and esterification' set-up and the 'extraction, centrifugation of IL layer and esterification' set-up. Averages and standard deviations of three measurements are given, determined through ^{1}H NMR

3.3. Influence of temperature on extraction

The extraction of acetic acid was up to now performed at 75 °C, because a high temperature was needed for the esterification in the biphasic esterification set-up. Other extraction experiments were performed at the same conditions for comparison. However, since removal of the aqueous layer and centrifugation of the IL layer prior to esterification is the preferred set-up, it is important to study the effect of temperature on the extraction. The net energy input and associated cost of the

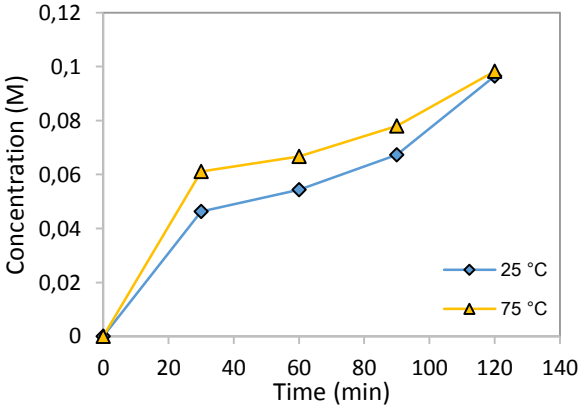


Figure 2.18: acetic acid concentration in the IL layer during extraction at 25 °C and 75 °C

process can be reduced if the extraction can be performed at a lower temperature with the same efficiency. The acetic acid concentration in the IL layer during extraction at 25 °C and at 75 °C is depicted in Figure 2.18. The results show that the same concentration is reached after 120 minutes, but at a slower rate when performed at 25 °C. This is due to a strong decrease in viscosity of the IL between 25 and 75 °C, leading to a higher mass transfer rate.[73]

The important consequence of this result is that not the entire biphasic system needs to be heated to the temperature required for esterification. Instead, the extraction can be performed at room temperature, and only the IL layer needs to be heated for esterification. In this way the energy-input can be significantly reduced.

4. Performing the process on microbial electrosynthesis extractant

4.1. Proof of concept

The preferred reaction set-up, consisting of extraction at room temperature, separation and centrifugation of the IL layer, subsequent esterification in the IL layer at 75 °C and evaporation, was tested for microbial electrosynthesis extractant.[108] The extraction was performed at 25 °C with a 1:10 ratio $P_{666,14}Tf_2N$ to aqueous layer. An IL layer acetic acid concentration of 0.079 ± 0.029 M was reached in 2 hours, corresponding to an extraction efficiency of 3.0 ± 1.1% (Table 2.10). The IL layer was separated from the aqueous layer and centrifuged. 68.1 ± 12.6% of the acetic acid was lost in the supernatant water layer. The IL layer was transferred to a reaction vial and heated to 75 °C. Esterification was initiated by addition of 3 equivalents of ethanol and 0.1 equivalent sulfuric acid. The progress of the reaction is depicted in Figure 2.19. The progress is similar to the previously determined 'extraction, centrifugation and esterification set-up' in 3.2, but the equilibrium is reached after 30 minutes. 77.5 ± 14.7% of the ethyl acetate was removed during evaporation. There was no anion exchange during this process, since there were no substantial changes in the anion concentration of the aqueous layer, as can be seen from Table 2.11.

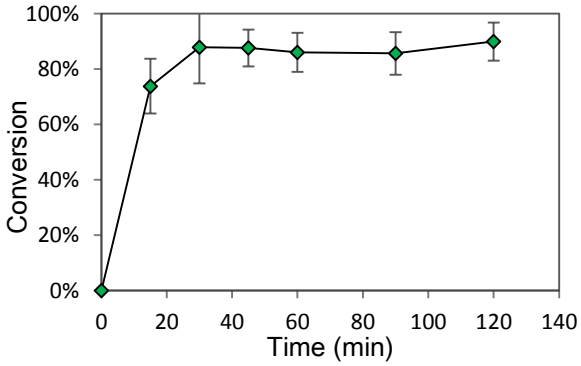


Figure 2.19: rate of the esterification, after extraction of microbial electrosynthesis extractant and centrifugation of the IL layer

Table 2.10: percentage of acetic acid (for extraction and centrifugation) or ethyl acetate (for esterification and evaporation) relative to the initial amount of acetic acid after the respective steps, and corresponding efficiencies

	% target compound	Efficiency	
Extraction	3.0 ± 1.1%	3.0 ± 1.1%	of aqueous acetic acid is extracted into IL
Centrifugation	1.0 ± 0.1%	-68.1 ± 12.6%	of IL layer acetic acid is lost in supernatant
Esterification	0.9 ± 0.1%	90.0 ± 6.9%	of IL layer acetic acid is converted
Evaporation	0.2 ± 0.1%	77.5 ± 14.7%	of ethyl acetate is removed

Table 2.11: anion composition of the aqueous layer before and after extraction. Average values and standard deviations are given, based on triplicates

	Cl^- (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	SO_4^{2-} (mg/L)
before	6821.8 ± 99.0	6.3 ± 0.2	419.7 ± 14.5	944.0 ± 18.6
after	6643.9 ± 57.9	5.8 ± 0.1	422.0 ± 4.6	921.4 ± 8.4

4.2. Multiple cycles experiment

The previous experiment demonstrates the potential of this process for extraction of acetic acid from microbial electrosynthesis extractant and subsequent conversion and separation as ethyl acetate. In the next phase, the performance of the process was evaluated during multiple cycles with the same IL layer and the same microbial electrosynthesis extractant. In this way the stability of the IL and the efficiency of extraction and esterification can be monitored upon recycling of the IL. The concentration of acetic acid and ethyl acetate in the IL layer was determined through quantitative ^{1}H NMR in each step. All steps except the centrifugation were performed in a cylindrical flask which was closed with a tap. The IL layer was transferred to an Eppendorf tube for centrifugation, and after removal of the supernatant to the aqueous layer, it was transferred back to the same flask. The same glassware was used throughout the experiment to reduce IL loss.

The ratio aqueous layer to IL layer was now 1:1, since the extraction has previously been identified as the rate limiting step. The extraction was performed at 25 °C for 2 hours, by mixing until emulsification. After extraction, the IL layer was separated from the aqueous layer and centrifuged. The IL layer was subsequently heated to 75 °C to perform the esterification for 30 minutes after addition of 3 equivalents ethanol and 0.1 equivalent sulfuric acid. The tap was connected to the vacuum set-up and opened after the pressure was set at 40 mbar. The conditions for the evaporation were different than the previously determined ones: the temperature was 75 °C instead of 55 °C for practical reasons. The evaporation time was doubled, because the IL layer volume was higher. The ongoing evaporation could visually be perceived up to 10 minutes through bubbling at the surface. The purity of the condensate in the cold trap was assessed through ^{1}H NMR. The aqueous layer from the previous cycle, i.e. the combined supernatant layers from extraction and centrifugation, was added to the IL layer after evaporation for extraction in the next cycle.

In this way, the process was performed for 5 cycles. The amount of acetic acid in the IL layer for the extraction and centrifugation and the amount of ethyl acetate for the esterification and evaporation are given in Table 2.12, as percentages relative to the initial amount of acetic acid in the microbial electrosynthesis extractant.

By raising the IL layer volume to a 1:1 ratio with the aqueous layer, 32.0 ± 5.8% of the acetic acid was extracted in the first cycle. The acetic acid concentration in the IL layer however dropped with 68.0 ± 12.6% upon centrifugation. The conversion of the esterification in the first cycle was 70.0 ± 14.1%, which is lower than in the proof of concept experiment. We speculate this may be due to a decreased heat and mass transfer in the larger IL volume. Intense bubbling was perceived during the evaporation, however the efficiency was not as high as previous evaporation experiments. This can be due to the uneven increase in process time compared to the increase in IL volume, but we speculate it may also

be contributed to the removal of volatile compounds that were present in the microbial electrosynthesis extractant, notably sulphur compounds that caused a strong odour before the experiment and were absent after the first evaporation. The lower efficiency of the evaporation in the first cycle means that there are still a lot of compounds retained in the IL layer for the second cycle. This is reflected in the extraction efficiency of the second cycle, which is only half the value of the first cycle. A progress similar to the first cycle was observed during the subsequent cycles. Note that from cycle 2 on, the percentage for esterification is higher than the percentage for centrifugation. This is because not all ethyl acetate in the esterification is formed from acetic acid in the centrifugation, but part of it is present from the previous cycle(s).

Table 2.12: percentage of acetic acid (for aqueous layer, extraction and centrifugation) or ethyl acetate (for esterification and evaporation) relative to the initial amount of acetic acid (aqueous layer, 1st cycle)

	1st cycle	2nd cycle	3rd cycle	4th cycle	5th cycle
Aqueous layer	100.0 ± 11.2%	95.1 ± 11.3%	89.2 ± 11.3%	82.8 ± 11.3%	78.5 ± 13.1%
Extraction	32.0 ± 5.8%	15.7 ± 0.2%	32.2 ± 2.1%	15.1 ± 6.9%	15.8 ± 5.8%
Centrifugation	9.3 ± 5.8%	5.5 ± 0.8%	7.1 ± 1.3%	4.2 ± 1.3%	7.8 ± 3.8%
Esterification	6.6 ± 0.7%	7.3 ± 1.0%	8.4 ± 0.7%	5.4 ± 2.3%	8.7 ± 2.4%
Evaporation	1.7 ± 0.6%	1.4 ± 1.3%	2.0 ± 1.2%	1.1 ± 0.8%	1.9 ± 0.3%
Ethyl acetate removed	**4.9 ± 0.9%**	**5.9 ± 1.7%**	**6.4 ± 1.4%**	**4.3 ± 2.4%**	**6.8 ± 2.4%**

The anion concentration of the aqueous layer before the experiment and after 5 cycles is given in Table 2.13. There is however not much information that can be derived from it. There is a 10% decrease in chloride and phosphate concentration. The nitrate concentration was initially very low and seemed to have raised a little bit throughout the cycles. It is not clear if these differences are due to anion exchange or just fluctuations throughout the 5 cycles. A measurement after every cycle to observe a trend was not possible due to the small aqueous layer volume that was used and the relatively high volume that is needed for analysis. The sulfate concentrations after the 5 cycles were bigger than the upper limit of the anion chromatography. This is due to the addition of sulfuric acid to perform the esterification. Furthermore, there seems to have nitrite formed, which was initially not present but after 5 cycles amounted to a concentration of 731.4 ± 126.3 mg/L. It is unlikely that nitrite is formed, or it would have to be from the anion of the IL which is the only compound containing nitrogen. It is not clear if this is the case, or if it is another anion with the same retention time on the anion chromatography column as nitrite.

Table 2.13: anion concentration of the microbial electrosynthesis extractant before and after 5 cycles. Averages of triplicates and standard deviations are given

	Cl^- (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	SO_4^{2-} (mg/L)	NO_2^- (mg/L)
before	6508.4 ± 51.5	5.1 ± 0.3	483.0 ± 12.1	1306.4 ± 382.0	0
after 5 cycles	5862.2 ± 411.0	14.0 ± 1.6	428.2 ± 56.4	UL	731.4 ± 126.3

Overall, this experiment demonstrates that the performance does not drop during the first 5 cycles. The removed amount of ethyl acetate, relative to the initial amount of acetic acid is 4.9 ± 0.9%, 5.9 ± 1.7%, 6.4 ± 1.4%, 4.3 ± 2.4% and 6.8 ± 2.4 % for the respective cycles. The results indicate that during the first cycle also some minor compounds are extracted, which affect the evaporation efficiency in the first cycle and the extraction in the second cycle. This would not pose a problem in a continuous process, as these compounds are released gradually, but may demand a more thorough evaporation step in the first cycle if a batch modus is applied. The extraction efficiency rises again in the 3rd cycle, and drops again to around 15% in cycle 4 and 5. It is not clear from this experiment if a further concentration decrease in the aqueous layer will result in a strong decrease in extraction efficiency in further cycles.

5. Preliminary tests on higher VFAs

So far, the process was tested for acetic acid as model VFA, both as a solution of pure acetic acid in water and as microbial electrosynthesis extractant. In the next section, some preliminary experiments on extraction and esterification of higher VFAs are presented, to get a perspective on the effect of alkyl chain length on the extraction and esterification efficiency.

The extraction of higher VFAs with $P_{666,14}$ Tf_2N was tested by mixing a 10 mL 0.33 M solution of propionic acid, butyric acid or valeric acid with 1 g of $P_{666,14}$ Tf_2N. The concentration of the carboxylic acid was determined after 2 hours of extraction, through quantitative IG ^{13}C NMR. Quantitative ^{1}H NMR was not possible because there were no resolved signals for any of the tested VFAs. The efficiencies are 1.5 ± 3.0%, 1.24 ± 0.5%, 7.8 ± 1.7% and 24.9 ± 3.4% for acetic acid, propionic acid, butyric acid and valeric acid respectively (Figure 2.20). There is no difference between acetic acid and propionic acid, except for the six fold smaller standard deviation in the latter. A clear trend can be observed for butyric and valeric acid: the longer the alkyl chain, the higher the extraction efficiency. This is due to the higher hydrophobicity of the higher VFAs.

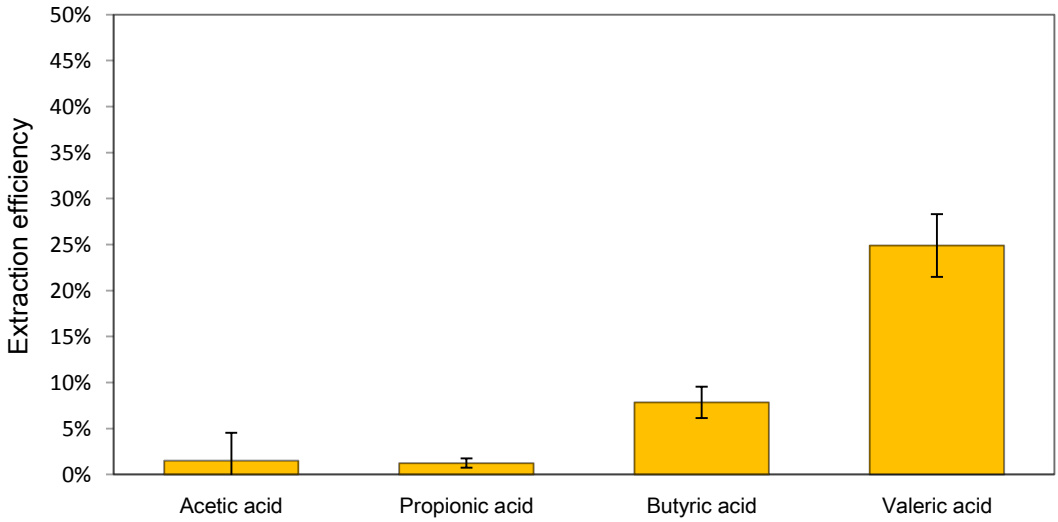


Figure 2.20: extraction efficiency for higher VFAs, for a 1:10 ratio IL to aqueous layer. Averages of three measurements are given, determined through IG ^{13}C NMR

The esterification reaction was only performed for valeric acid. The comparison with the esterification of acetic acid at the same conditions, shows a clear effect for increasing alkyl chain length (Figure 2.21). The same conversion value of approx. 85% is reached, but at a slower rate in the case of valeric acid. A similar result has been found in a previous study on the effect of alkyl chain length on the esterification: the reaction rate was similar for butyric and valeric acid, and was approx. 3 times lower compared to acetic acid esterification. Propionic acid displayed an intermediary reaction rate. This decrease in reaction rate with increasing alkyl chain length is contributed to increased steric and inductive effects. The steric effect decreases the availability of the carbonyl carbon, whereas the inductive effect decreases its electrophilicity, resulting in a slower nucleophilic attack by the alcohol.[109]

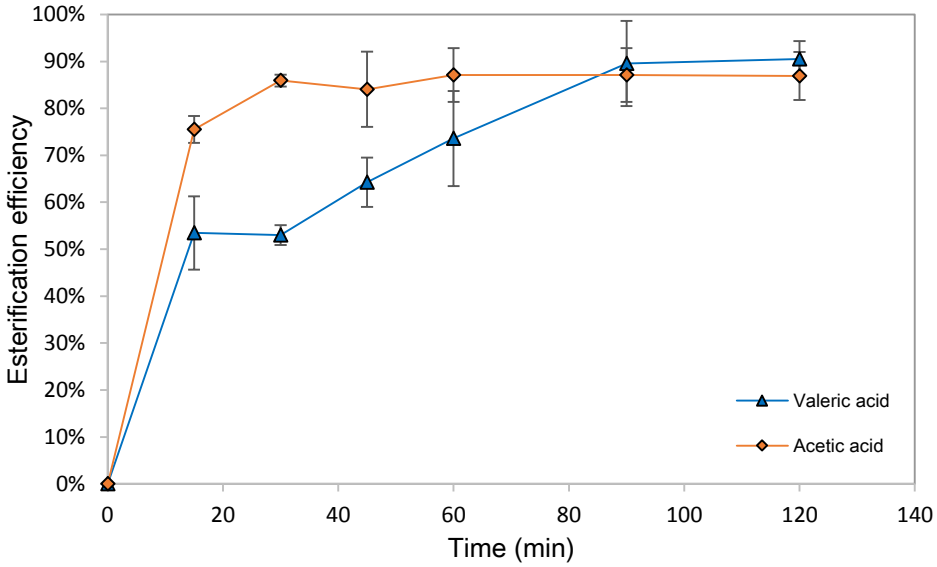


Figure 2.21: effect of alkyl chain length of the carboxylic acid on esterification rate: comparison between acetic acid and valeric acid esterification (75 °C, 1 eq. ethanol). Averages and standard deviations of three measurements are given, determined through quantitative ^{1}H NMR

The preliminary experiments on higher VFAs show a strong increase in extraction capacity with increasing alkyl chain length. Even at a 10:1 ratio of aqueous layer to IL layer, 25% of the valeric acid could be extracted into the IL layer. The subsequent esterification takes place at a lower but still reasonable rate, leading to a high conversion of 90.5 ± 3.8%. This demonstrates that the biphasic esterification can be performed with a higher turnover for butyric and valeric acid, compared to acetic and propionic acid. Valerate esters are attractive compounds as novel solvents and biofuels. They have appropriate volatility-ignition properties, high energy densities and lower polarities compared to bio-ethanol or butanol, which results in a lower water pickup. Valerate esters from C_1 until C_3 alcohols can be mixed up to 20% with gasoline, while C_5 and higher alcohol esters can be mixed in diesel. They were successfully tested in road trials with a cumulative distance of 250 000 km. The good performance, oil quality, durability for the engine and regulated emissions demonstrated these are promising alternatives for current candidate biofuels.[110] The value of biofuels is however relatively low, so a highly efficient and cost-effective process will be required to provide competitive edge in the (bio)fuel market.

6. Discussion

Apart from the specific discussions for the previous experiments, a broader discussion on the whole process, possible alternatives and improvements and an outlook for further research will be given in the next section. A very brief draft of an economic analysis is presented, since a more in-depth analysis is premature at this stage. Optimization of the process can substantially improve the economics, however a brief analysis is useful to identify the most critical points for improvement.

The esterification of acetic acid as model VFA is considered, with acetic acid and ethanol at indicative European market prices as starting material. The costs of these reagents to produce one tonne of ethyl acetate are given in Table 2.14, assuming a 1:1 ratio of ethanol to acetic acid in the reaction and a 100% conversion due to recycling of the unreacted starting material. This shows there is a margin of € 580/tonne ethyl acetate for the IL, operation costs and write-off.

Table 2.14: indicative European market prices of acetic acid, ethanol and ethyl acetate and costs for production of 1 tonne ethyl acetate

	price (€/tonne)[8]	kg / kg ethyl acetate	€/tonne ethyl acetate
Acetic acid	600	0.68	409
Ethanol	500	0.52	261
Ethyl acetate	1250	1	1250

In the next calculation, a process with a targeted production of 1000 kg ethyl acetate per day is presented, working at the efficiencies and rates that were determined in the five-cycle biphasic esterification experiment on the microbial electrosynthesis extractant (section 4.2). This means 2 hours of extraction resulting in 7% of the acetic acid that is in the IL layer after extraction and centrifugation, 85% conversion into ethyl acetate and complete evaporation of the ethyl acetate. It was assumed that all ethyl acetate eventually has to leave the system by evaporation, hence an efficiency of 100%. This means that per cycle:

83.3 kg or 947 mol ethyl acetate has to be produced

for which: 1114 mol acetic acid should be present in the IL layer after extraction and centrifugation, assuming a conversion of 85%

for which: 15 916 mol acetic acid should be present in the aqueous layer, assuming a 7% extraction

for which: the volume of the aqueous layer should be 48 229 L at an acetic acid concentration of 0.33 M

for which: the volume of the IL layer should be 48 229 L per cycle, to obtain the 7% extraction in a 1:1 ratio

Based on an IL cost of € 1000/L (the lowest market price at this point), a margin of € 580 per tonne ethyl acetate and 365 days of production per year; only 212 litres of IL could be purchased per year, if the entire margin is invested in IL. Provided an estimated 48 229 L IL per cycle and at least 2 alternate cycles are needed, this shows that this lab scale study stands a long way from possible economic feasibility.

Although the previous economic assessment is very superficial due to the limited data from an unoptimized system, it shows there are five crucial aspects of which improvement could alter the outcome of the assessment. Two of them are not in the scope of the process: the market price of the ionic liquid and the market price of ethyl acetate. Ethyl acetate has a substantially higher value than acetic acid (Table 2.14), however it is still relatively low to provide a feasible margin for sustainable production. Performing alternative reactions on the VFAs leading to products with a higher added value can improve the economic case of waste to bioproducts via VFA intermediates. Improvements in the production of ILs are expected to result in larger production quantities and a significant price drop, of which some authors believe future market prices of 50 USD per kg are possible.[111]

Three crucial aspects that pertain to the process itself are the VFA concentration in the aqueous layer, the extraction capacity and the length of the extraction stage. The acetic acid concentration in the experiments and the preceding assessment is 0.33 M, or 20 g/L. It is however possible to go to higher concentrations, up to 80 g/L, by applying a higher current in the membrane electrolysis, however at decreasing current efficiencies. Further developments in the fermentation and the membrane electrolysis step can lead to increased concentrations in the anolyte. Optimisation of the biphasic esterification conditions and selection of a possibly more suitable IL can increase the extraction capacity and decrease the required extraction time.

To give an indication of how all these improvements can affect the outcome of the assessment, it is recalculated for a future scenario. The targeted production remains at 1000 kg ethyl acetate per day. It is assumed that there are 2 alternate cycles of 60 minutes: 30 minutes of extraction and 30 minutes of esterification and evaporation. The extraction and evaporation efficiencies are identical to the previous calculation: 85% and 100% respectively. A 5% extraction capacity is assumed for a 10:1 ratio aqueous layer to IL layer. Furthermore, the acetic acid concentration in the aqueous layer is assumed to be 60g/L, or 1 M. This means that per cycle:

	20.8 kg or 237 mol ethyl acetate has to be produced
for which:	279 mol acetic acid should be present in the IL layer after extraction and centrifugation, assuming a conversion of 85%
for which:	5 570 mol acetic acid should be present in the aqueous layer, assuming a 5% extraction
for which:	the volume of the aqueous layer should be 5 570 L at an acetic acid concentration of 1 M
for which:	the volume of the IL layer should be 557 L per cycle, to obtain the 7% extraction in a 10:1 ratio aqueous layer to IL layer

Based on a future IL cost of € 200/L, a margin of € 580 per tonne ethyl acetate and 365 days of production per year; 2281 litres of IL could be purchased per year, if the entire margin is invested in IL. Provided an estimated 557 L IL per cycle and 2 alternate cycles are needed, this shows that there is still margin for operating costs, write-off and profit. If long-term stability and performance can be achieved to limit replenishment of the IL to once per year, the margin is approx. € 233 000 per year.

The preceding analysis identified the extraction capacity of the IL and its high cost as crucial factors. Selection of a suitable IL is therefore of vital importance. It has to display a high extraction capacity towards short-chain carboxylic acids, be a good medium for esterification and preferably have a relatively low viscosity to mitigate processability and evaporation of esters. It should be stable towards anion exchange or yield a stable anion composition that is beneficial for the aforementioned parameters. It should furthermore be commercially available at a low cost and be chemically stable, to avoid frequent replenishment. A simple improvement could be $P_{666,14}$ NO_3, as nitrate does not exchange for any of the common aqueous anions. It is more green than the fluorine containing bis(trifluoromethylsulfonyl)imide anion (Tf_2N^-) and it is a cheaper anion, so it could be produced at a market price that is closer to that of $P_{666,14}$ Cl compared to the fivefold more expensive $P_{666,14}$ Tf_2N. Apart from the unknown physical data, the extraction and esterification capacity should be determined to see if this is a viable alternative.

Another important aspect of the IL is that it should preferably be made from renewable resources, to pertain to the sustainability of the process. The phosphonium ILs that were used in this thesis are produced from phosphine, which is a highly toxic and hazardous gas. Ionic liquids derived from renewable resources have been described in literature, for example from fructose, amino acids, choline and nicotine. Most research is up to now focused on synthesis of these compounds, with limited applied research and physical data. To the author's knowledge, there is no renewable resource based IL up to now with the required hydrophobicity to be used as extractant. Future research might however define a suitable ionic liquid from renewable biomaterials.

The economic assessment furthermore stresses the need for an improved extraction. Our results show that the longer the alkyl chain length of the carboxylic acid, the better the extraction due to increased hydrophobicity. An easier extraction is however possible from hexanoic acid on due to the low water solubility of the medium- and long chain carboxylic acids. Once they are extracted from the fermentation broth through the anion exchange membrane and protonated to the acid form, they readily phase separate. Moreover, the esters of medium-chain carboxylic acids are more niche products compared to ethyl acetate. It is important to optimize a method to harvest the short-chain carboxylic acids, as they will always be present as a side stream apart from the easy separable longer chain carboxylic acids and the market for their products is attractive.

The question if ionic liquids are the method of choice can however not be answered from this exploratory research. Optimisation of the conditions can lead to higher extraction capacities for the phosphonium ILs that were used in this study. Selecting an anion that displays a specific affinity towards carboxylic acids, for instance through hydrogen bonding, might substantially increase the extraction. A phosphonium cation with shorter alkyl chains might improve the extraction of the relatively hydrophilic carboxylic acids, however care has to be taken that the hydrophobicity remains sufficiently high to be used as a biphasic system. Moreover, another class of ILs could be selected for which the extraction of carboxylic acids is higher. Phosphonium ILs were selected for their promising extraction capacities and their comparatively higher hydrophobicity than the other common classes of ILs. It is however not unthinkable that in the future better alternatives will be provided, due to the exponential increase in research on the synthesis, physical properties and applications of novel ILs.

Garcia et al. compared the economic and environmental incentives of separation of acetic acid from water through binary distillation or a combination of extraction with diethyl ether and solvent

recovery. They showed that for concentrations below 40% acetic acid, liquid-liquid extraction is the most appropriate separation method. The capital and operating costs for separation through binary distillation are excessive due to the large number of stages and the high reflux ratio that is required. It is more attractive to implement an extraction step with diethyl ether before the distillation so that only the organic layer needs to be separated into an acetic acid and a diethyl ether fraction. The latter is recycled for extraction. The authors showed that at least 34 to maximum 60 stages are required for the best economic potential. They showed that separation of acetic acid in this way in an optimised process can be profitable.[112]

Levy et al. described the extraction and subsequent processing of carboxylic acids from a mixed culture fermentation.[113] They showed that liquid-liquid extraction is desirable above anion exchange with quaternary ammonium resins. They suggest a process in which the medium-chain carboxylic acids are extracted with kerosene, while the short-chain carboxylic acids can be extracted with ethyl acetate or diethyl ether. These organic layers are subsequently back-extracted with an aqueous alkali layer, in which the acids dissolve as salts. At that point, the carboxylates are again present in an aqueous layer, but in higher concentrations. They can then be acidified by addition of a mineral acid, or converted to other compounds through aqueous electrolysis. A variety of products can be made by adjustment of the conditions: alkanes, alkenes, esters, alcohols etc. Depending on the nature of these compounds, they will phase separate, or an extra separation step is required. Although carboxylic acids can be extracted and upgraded in this process, it is clear there are some major downsides to it. The carboxylic acids are extracted from the fermentation broth, meaning large volumes of aqueous layer need to be extracted. The addition of a base and a mineral acid for the back-extraction and subsequent acidification of the salts means a high input of chemicals. Finally, an extra distillation or conversion step is required to remove the acids or a corresponding product respectively. In the pipeline developed by Andersen et al., there is already a pre-concentration and acidification of the carboxylates in the membrane electrolysis step.[17] When screening the preceding alternatives for this pipeline, it is clear each of them have some downsides.

Anion exchange resins for capture of the carboxylates are not compatible with the acidic effluent from the membrane electrolysis step. A distillation on the membrane electrolysis effluent to separate the carboxylic acid fraction from the water is again expected to be inferior to a set-up combining extraction and distillation. A biphasic esterification with diethyl ether is not possible due to the expected constrained esterification rate at temperatures below the boiling point of diethyl ether (34.6 °C). Diethyl ether can however still be used as mere extractant, followed by a distillation in which the carboxylic acids are separated from the diethyl ether, which can be recycled for further extraction. It might even be possible to separate the different carboxylic acids according to their boiling point in a well-designed distillation column, and thus achieve selective separation of carboxylic acids. This will require a large number of stages and large capital and operational costs. An in-depth economic analysis of this process is required to assess the viability. It is however expected that the main bottleneck will be in the low titres of the aqueous layer, so that an elaborate distillation and associated costs will not fit this process.

By combining the extraction with an esterification in the biphasic esterification process, the need for an extra back-extraction step is avoided, since the produced esters can be separated by evaporation. This is an important aspect that has to be kept in mind when alternative added value reactions are performed on carboxylic acids in ILs: the products should either be volatile or readily phase separate

upon formation. Electrochemical conversion of carboxylic acids, as performed in the set-up of Levy et al., can also be performed in the IL layer. ILs are promising media for electrochemical conversion due to their big electrochemical window and high stability. Pyrrolidinium and imidazolium ILs have been widely investigated as electrolytes in batteries and solar cells and as media in electrochemistry.[114] Phosphonium ILs show a higher electrochemical stability compared to these ILs, however their typically large cations result in a higher viscosity and a reduced conductivity. Triethylalkylphosphonium cations were tested as more conductive, lower viscosity alternatives.[115] This might however hamper extraction and phase separation, as the cation provides the hydrophobicity of the IL. It is not clear at this point if there exists an optimum in cation chain length that enables phase separation and use as medium for electrochemical conversion. A tributylalkylphosphonium cation can be a good intermediate, but no research on extraction or electrochemical use has been conducted to our knowledge.

Lopez et al. presented a process for separation of carboxylic acids from a dilute aqueous solution through ionic liquid assisted electrodialysis.[111] They used a 20 g/L sodium butyrate solution as diluate stream and water or 1-ethyl-3-methylimidazolium trifluoromethanesulfonate as concentrate stream. A batch electrodialysis experiment for water and the IL was compared to a batch bipolar electrodialysis experiment. In the electrodialysis set-up, the butyrate anion is transferred from the diluate stream into a concentrate stream through an anion exchange membrane by the application of an electric field. With an aqueous concentrate stream, a butyrate concentration increase at a rate of 2.2 g/L.h^{-1} was observed, for an average current efficiency of 82%. With an IL concentrate stream, the butyrate concentration increased at a rate of 0.98 g/L.h^{-1}, for an average current efficiency of 30%, which is significantly lower but still reasonable according to the authors. The major downside of the electrodialysis is however that butyrate is still present as a salt in the concentrate stream, which makes subsequent separation of the butyrate from the IL layer very challenging.

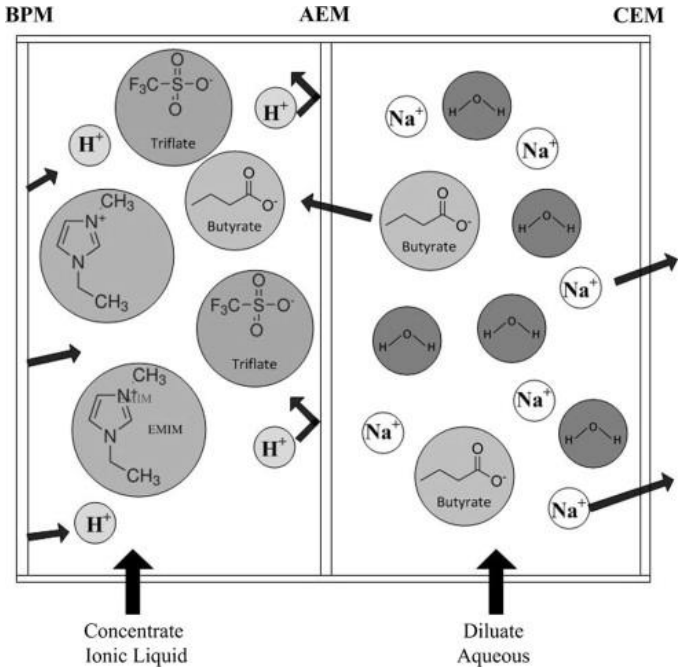


Figure 2.22: bipolar electrodialysis stack with an IL as concentrate stream, used for separation of butyric acid from water[111]

This problem is eliminated in bipolar electrodialysis, as salt concentration and acidification occur simultaneously in the concentrate stream (Figure 2.22). A distillation of the concentrate stream can then be performed to separate the butyric acid from the IL. They again started with 20 g/L butyric acid in the diluate stream and neat IL in the concentrate stream. After 4 hours of performing the bipolar electrodialysis, the butyric acid concentration in the concentrate stream had increased to 2 g/L and continued to rise to 3 g/L after 10 hours of operation. A big problem was in the transfer of water from the diluate stream through the membrane into the concentrate stream. This has a lower boiling point than butyric acid (100 °C vs 163 °C), resulting in only a 7 g/L butyric acid solution after distillation.

This study shows that, apart from a low current efficiency, the separation of carboxylic acids from a dilute aqueous stream through bipolar electrodialysis suffers from water transfer through the membrane and an energy-intensive distillation of the acids, that is again hindered by the presence of a large volume of lower boiling water. It is however an attractive alternative that could combine the biphasic esterification with the preceding membrane electrolysis step in the pipeline (chapter 1, section 2). Trihexyl(tetradecyl)phosphonium ILs were used in this study, which are much more hydrophobic compared to the water miscible imidazolium IL that was used by Lopez et al. This could limit water transfer into the concentrate stream to reasonable amounts. Transferred water would moreover phase separate and can easily be decanted. By performing an esterification of the carboxylic acids that are transferred into the IL layer, they become much more volatile, which facilitates distillation and thus tackles a second bottleneck of the system of Lopez et al. The big advantage of this system compared to the biphasic esterification is that the limited extraction capacity of the IL could be overcome by the electric field from the electrodes. In this way, a driving force for carboxylic acid uptake is applied, which could result in a carboxylic acid concentration in the IL layer that exceeds the partitioning of the carboxylic acid between water and the IL. Further research is needed to determine if the combined bipolar electrodialysis and esterification displays a higher efficiency compared to the membrane electrolysis and consecutive biphasic esterification. The biggest risk of this system is the relatively low conductivity and viscosity of the trihexyl(tetradecyl)phosphonium ILs, as previously discussed. This could result in unreasonably low current efficiencies. The viscosity can be increased by working at elevated temperatures, as the viscosity substantially drops from 30 °C on. A cation with shorter alkyl chain lengths could be used for increased conductivity, however care should be taken it remains sufficiently hydrophobic to avoid too much water transfer.

An even further step, that might seem attractive at first sight, is the use of ILs as reactive extractants in the fermentation broth itself. There are however some expected pitfalls in the combination of ILs with micro-organisms. The required hydrophobicity for use as a separate extractant layer, means it can interact with the cell membrane and thus display a toxicity towards the micro-organisms. This was demonstrated for phosphonium ILs.[116] An exploratory study has shown a 40% decrease in microbial activity in the presence of an imidazolium IL.[74] We furthermore expect that the anion exchange that was demonstrated in this study could result in depletion of certain anions in the broth. This could result in a limited growth or activity of the micro-organisms. A more detailed study on the effect of ILs on micro-organisms in a fermenter is needed, to assess the toxicity, the possible anion exchange and possible retention of organic compounds from the broth by the IL.

If the biphasic esterification can be optimised to a feasible process, a next important question for practical implementation is how to achieve a selective separation of the VFAs or their corresponding esters, when an aqueous layer with a mixed VFA profile is used. The results in this thesis demonstrate

that the extraction capacity is substantially higher for higher VFAs, while their conversion to the corresponding ester takes place at a lower rate. None of these two processes is however selective enough for separation of one type of VFA. It is theoretically possible to obtain a selective separation through a controlled evaporation, as consecutive esters from increasing carboxylic acid chain lengths display boiling point increments of approx. 20 °C. The evaporation could either be controlled by the temperature of the IL layer or the applied vacuum pressure. The possible selectivity of the evaporation should be verified experimentally, even though lab scale control of evaporation conditions is expected to be challenging. It is most likely that on an industrial scale process, selectivity will have to be pursued by a combination of the extraction, esterification and evaporation.

For a further future, the fully realized system could be integrated in a biorefinery, where on-site production of VFAs from a side stream, e.g. thin stillage, could be combined with bio-ethanol and a heat stream to convert the low value organics in the thin stillage into higher value bioproducts. This pertains to the biorefinery concept, in which maximization of the value of the streams is a quintessential element. The membrane electrolysis step generates protons that can be used for biomass processing within the biorefinery, and hydroxide anions that can be used for pH control. Furthermore, implementation of the pipeline is noninvasive, and chemical production capacity is present within the biorefinery. The pipeline could be attractive for processing the organics in agro-industrial waste streams and even at waste treatment facilities into valuable products, however the lack of infrastructure makes that a challenging case.

Chapter 3: conclusion

This study demonstrates that ionic liquids can be applied as hydrophobic layer in the biphasic esterification of volatile fatty acids from a low titre aqueous solution, thus successfully addressing the separation barrier. Development of an economically sound process for the production of added-value bioproducts is needed to drive the transition of disposal of organic waste streams to valorisation and exploitation as sustainable resources. The proposed pipeline is especially interesting in a biorefinery context, where bio-ethanol and heat streams are available to perform the esterification and VFAs can be produced on-site from bio-industrial side streams and even from CO_2. In this way, esters can be made entirely from sustainable building blocks.

A proof of concept of the biphasic esterification confirmed the production of ethyl acetate, but also stressed the need for an analytical method to monitor the reaction. A practical and accurate analysis method was developed for the quantification of analytes in an ionic liquid matrix, through ^{1}H NMR and inverse gated ^{13}C NMR. A stepwise study of the biphasic esterification of acetic acid as model VFA identified the extraction as limiting step. The subsequent esterification takes place at a high rate and a high conversion, so that the extracted fraction of acetic acid is efficiently converted to ethyl acetate. Even for mild reaction conditions, the same conversion is reached, at a slower but reasonable rate. Exclusion of water from the IL layer is a crucial element to obtain a high conversion. Evaporation of the volatile ester from the non-volatile IL was shown to be a successful separation strategy, with an almost quantitative removal in a short time at mild conditions. The extraction capacity of the IL for butyric and valeric acid was shown to be higher compared to acetic and propionic acid. An equally high conversion into the corresponding ethyl ester was achieved, albeit at a threefold lower rate.

The successful proof of concept in this pioneering study gives an indication on the potential of the process, but on the other hand it demonstrates there is a vast valley to be crossed before possible implementation. There is much work to be done on the extraction capacity of the IL, by optimising the extraction conditions, by selecting a more suited IL and by providing higher VFA concentrations in the aqueous layer. Developments in the ionic liquid field, both in fundamental research and industry scale manufacture and applications, can provide a boost to the feasibility of ionic liquid driven processes. The pipeline to extract and upgrade biologically produced VFAs into esters via membrane separation and biphasic esterification may provide competitive edge to the resource recovery industry, if the separate steps can be further developed and integrated to a balanced and feasible production platform.

Chapter 4: materials and methods

1. Instrumentation

1.1. GC analysis

Gas chromatography analysis was performed on a Shimadzu GC-2014 with a DB-FFAP 123-3232 column (30m x 0.32 mm x 0.25 µm; Agilent, Belgium) and a flame ionization detector (FID). Samples were prepared with acetone as internal standard. The prepared sample (1 µL) was injected at 200 °C with a split ratio of 60 and a purge flow of 3 mL min^{-1}. The oven temperature increased by 6 °C min^{-1} from 110 °C to 165 °C where it was kept for 2 min. FID had a temperature of 220 °C. The carrier gas was nitrogen at a flow rate of 2.49 mL min^{-1}.

1.2. NMR spectroscopy

^{1}H NMR, ^{13}C NMR, ^{31}P NMR and ^{19}F NMR was performed at 400 MHz, 100 MHz, 162 MHz and 376.5 MHz respectively, on a Bruker Avance III Nanobay 400 MHz spectrometer. Unless stated otherwise, DMSO-d_6 was used as deuterated solvent, because it showed the best chemical shift separation of the C$\underline{H}_3$CO-peaks of ethyl acetate and acetic acid in ^{1}H NMR and an accurate integration of the peaks. Signals are reported relative to TMS.

1.3. Ion chromatography

The analysis was performed by a Metrohm 761 Compact IC with a Metrosep A sup 5-150 column and a Metrosep A Supp 4/5 guard column. The eluent was a 1.0 mM $NaHCO_3$, 3.2mM Na_2CO_3 and 5% v/v acetone solution at a flow rate of 0.7 mL min^{-1}.

1.4. HPLC-MS analysis

HPLC-MS analysis was performed on an Agilent 1200 series LC/MSD SL with a UV detector and a mass spectrometer with electrospray ionisation geometry (ESI 70 eV) and a quadrupole detector. The column was a Supelco Ascentis Express C18 column (L 3 cm x I.D. 4.6 mm) with 2.7 µm fused-core particles with 90 Å pore size.

2. Experimental procedures

2.1. NMR experiments

2.1.1. Relative quantification through ^{1}H NMR

The samples for ^{1}H NMR analysis were prepared by adding a small, accurately weighed amount (15-30 mg) of IL layer sample to a 2 mL vial, together with 400 µL of DMSO-d_6.The mixture was thoroughly stirred, after which it was transferred to an NMR tube. The ^{1}H NMR experiment was run with 8 scans with 1 second relaxation delay.

2.1.2. Relative quantification through IG ^{13}C NMR

The samples for inverse gated ^{13}C NMR (IG ^{13}C NMR) analysis were prepared by combining 300 mg of the IL layer with 300 mg $CDCl_3$ in a 2 mL vial. A droplet of TMS was added to mitigate calibration of the chemical shift in the concentrated samples. This was thoroughly mixed and transferred to an NMR tube. Experiments of 58 scans and 8 seconds relaxation time were run (i.e. an analysis time of 10 minutes), unless stated otherwise.

2.1.3. Absolute quantification through IG ^{13}C NMR

A predefined mixture of ethyl acetate, acetic acid and internal standard was prepared on a 1 g scale in a 2 mL vial, to increase the accuracy of the weighing. After homogenizing with a pipette, a accurately weighed amount (20-30 mg) of this mixture was added to 300 mg $P_{666,14}$ DCN in another vial. After addition of 300 mg $CDCl_3$ and stirring, the mixture was transferred to an NMR tube for IG ^{13}C NMR analysis. The amount of the target compound, in this case ethyl acetate and acetic acid, was calculated as follows:

$$n_c = \frac{A_c}{\varphi_c} * \frac{\varphi_{IS}}{A_{IS}} * n_{IS} \quad (1)$$

with: A_{IS}, A_c = signal peak area of internal standard and target compound resp.
φ_{IS}, φ_c = number of carbon nuclei corresponding to the signal, of the internal standard and the target compound resp.
n_{IS} = added amount of the internal standard
n_c = calculated amount of the target compound

The number of carbon nuclei for the target compound signal and the internal standard signal are both one (i.e. a signal of one CH_3). The formula can therefore be simplified to:

$$n_c = \frac{A_c}{A_{IS}} * n_{IS}. \quad (2)$$

In a subsequent experiment a response factor was calculated to give more accurate calculated amounts of the analytes in the sample. The response factor was calculated as:

$$Rf = \frac{\frac{A_{IS}}{\varphi_{IS}}}{\frac{A_c}{\varphi_c}} * \frac{n_c}{n_{IS}} \quad (3)$$

with: A_{IS}, A_c = signal peak area of internal standard and target compound resp.
φ_{IS}, φ_c = number of carbon nuclei corresponding to the signal, of the internal standard and the target compound resp.
n_{IS} = added amount of the internal standard
n_c = added amount of the target compound

In the case of quantification of ethyl acetate and acetic acid with 1,4-dioxane as internal standard, φ_{IS} is 4, since 4 carbon nuclei give rise to the 1,4-dioxane signal, while φ_c is 1 for both acetic acid and ethyl acetate. The same formula can be used to calculate n_c with a known Rf, this time as the calculated amount of the target compound instead of the added amount.

2.1.4. Absolute quantification through ^{1}H NMR:

The concentration of the compounds in the IL layer was determined through ^{1}H NMR analysis. WILMAD 1.8 mm coaxial NMR inserts were used to introduce a reference compound for quantification (Figure 4.1). In this way two potential sources of error are eliminated: reaction between the reference material and the sample is prohibited, and the coaxial insert can be used during multiple analyses, eliminating the error of dosing the reference compound to the sample.[117] Furthermore, a smaller sample volume is needed. For these reasons, use of coaxial inserts for quantitative ^{1}H NMR is a practical alternative to quantification by an internal standard. The drawbacks are that shimming is more difficult compared to a single-phase NMR sample, and the sensitivity is reduced due to the smaller volume of analyte.[118] The ^{1}H NMR experiments were run with 8 scans and 1 second relaxation delay.

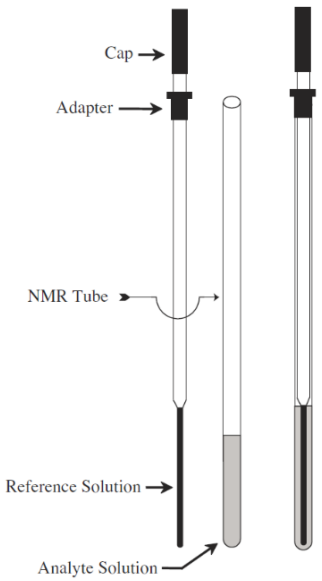


Figure 4.1: coaxial insert (left) inserted into an NMR tube (middle) to give an NMR sample with external standard (right)

1,4-dioxane was chosen as a standard. It has a signal that is well separated from the other signals from both the analyte and the IL, and it is a singlet. Furthermore this compound is readily available in a pure form. A stock solution was prepared of the reference compound in deuterated solvent: 24.6 mg of 1,4-dioxane was added to 2.5 mL DMSO-d_6 in a 5 mL vial, obtaining a solution of 0.112 mmol 1,4-dioxane per mL. After intense mixing, a small amount of this solution was transferred to the coaxial insert, to fill the capillary tube. The vial was closed, sealed with parafilm and stored in a desiccator to minimize water uptake by the DMSO-d_6. The same coaxial insert was used throughout an entire experiment, but it was emptied, cleaned and replenished with internal standard solution approx. every 14 days.

When NMR analysis is performed with an internal standard for quantification, the internal standard and the sample have to be a homogeneously mixed, single phase system, to make sure all the compounds in the NMR tube occupy the same volume. In this way, the number of nuclei in the scanned volume gives rise to a signal intensity that represents the concentration of the respective compounds, when acquired under quantitative conditions. Because of this, the ratio of the number of moles of the compound of interest to the reference compound (or: the ratio of the number of nuclei) equals the ratio of the signal peak areas:

$$\frac{n_c}{n_{IS}} = \frac{I_c}{I_{IS}} \qquad (4)$$

with: n = number of nuclei
A= signal peak area

Or $\frac{C_c}{A_c} = \frac{C_{IS}}{A_{IS}}$, since the compound of interest and the reference compound occupy the same volume, and thus the concentration of the compound of interest can be calculated by:

$$C_c = \frac{A_c}{A_{IS}} * C_{IS} \quad (5)$$

However, when an external standard is used, contained in a capillary insert, the reference compound and the sample do not occupy the same volume. Therefore, a correction factor has to be determined to account for this volume difference:

$$\frac{n_c}{n_{IS}} = \frac{A_c}{A_{IS}} \quad (6)$$

$$\text{or:} \quad \frac{n_c}{V_c} = \frac{A_c}{A_{IS}} * \frac{n_{IS}}{V_c} \quad (7)$$

$$\text{or:} \quad C_c = \frac{A_c}{A_{IS}} * \frac{n_{IS}}{V_{IS}} * \frac{V_{IS}}{V_c} \quad (8)$$

with: n_c, n_{ref} = number of moles of target compound and internal standard resp.
A_c, A_{IS} = signal peak areas of target compound and internal standard resp.
V_c =sample volume containing target compound
V_{ref} = capillary insert volume containing reference compound

From equation (8), the concentration of the compound of interest can be calculated from the signal intensities, the concentration of the stock solution in the coaxial insert ($\frac{n_{ref}}{V_{ref}}$), and the correction factor ($\frac{V_{ref}}{V_c}$). This correction factor has to be determined experimentally, as both V_{ref} and V_c, the effectively scanned volumes of the coaxial insert containing the reference compound and of the NMR tube containing the compound of interest, are a function of the length of the probehead receiver coils and the diameters of the coaxial inserts and the NMR tube. This means that this factor should be determined for every set of NMR spectrometer, coaxial insert and NMR tube.[117] The correction factor was experimentally determined by introducing the coaxial insert with the 1,4-dioxane external standard solution, into an NMR tube containing a known concentration of a compound, called the primary standard. In this way, the peak areas and the known concentrations of primary standard and the reference compound can be used to calculate the correction factor.

The samples were prepared by adding a small, quantified amount (15-30 mg) of IL layer sample to a 2 mL vial, together with 400 µL of DMSO-d_6.The mixture was thoroughly mixed, after which it was transferred to an NMR tube. The coaxial insert containing the external standard was added and the tube was sealed with a plastic cap.

2.2. Proof of concept: biphasic esterification with $P_{666,14}$ DCN

1 g $P_{666,14}$ DCN, 10 mL of a 0.33 M acetic acid solution and 323 mg H_2SO_4 (i.e. 1 equivalent compared to acetic acid) were added to a 15 mL pressure vial. This was heated to 75 °C in a temperature controlled oil bath. After addition of 759 mg ethanol (5 eq.) to the IL layer, the vial was sealed with a screw cap and the broth was magnetically stirred until homogenization. A control reaction was set up identically, but without ethanol addition. Sampling was done by stopping the stirrer to allow phase separation,

removing the cap and taking off 0.5 mL of the aqueous layer with a 1 mL syringe. The samples were subsequently filtered over a 0.2 µm filter and stored at 4 °C until the time of analysis. Immediately before the analysis, the samples were diluted and 100 µL of acetone was added as internal standard. GC analysis was performed as described in section 1.1.

2.3. Identification of product of biphasic esterification with $P_{666,14}$ DCN

100 mg of sodium dicyanamide (1.123 mmol) was added to a 10 mL flask, as well as 5166 mg ethanol (100 eq.), 202 mg water (10 eq.) and 110 mg sulphuric acid (1 eq.). This mixture was placed in a 75 °C temperature controlled oil bath and magnetically stirred. After reaction, $NaHCO_3$ was added until a pH of 7 was reached. The reaction was monitored with LC-MS, ^{1}H NMR and ^{13}C NMR analysis. NMR samples were analysed in DMSO-d_6.

5 mg of diethyl imidodicarbonate, 2.92 mg H_2SO_4 (1 eq.), 5.4 mg H_2O (10 eq.) and 137.1 mg ethanol (100 eq.) were added to a 2 mL vial. This was magnetically stirred and heated to 75 °C in a temperature controlled oil bath. The reaction was monitored with ^{1}H NMR and ^{13}C NMR analysis in DMSO-d_6.

2.4. Synthesis of the ILs

Five different ILs were tested, of which $P_{666,14}$ DCN, $P_{666,14}$ Tf_2N and $P_{666,14}$ Cl are commercially available and used as such. $P_{666,14}$ OAc and $P_{666,14}$ BF_4 were prepared through metathesis of $P_{666,14}$ Cl and acetic acid and sodium tetrafluoroborate respectively.

The procedure for the preparation of $P_{666,14}$ OAc is based on a procedure developed by Bradaric et al.[76] 5 g $P_{666,14}$ Cl (9.54 mmol) was combined with 859.1 mg acetic acid (14.3 mmol or 1.5 stoichiometric eq.) in the presence of an excess of NaOH (40% w/w solution in water: 763.6 mg NaOH and 1145.4 mg H_2O) into a 10 mL flask. This mixture was heated to 55 °C in a temperature controlled oil bath and magnetically stirred for 4 hours. The turbid aqueous bottom layer was removed; the pale yellow top layer was washed 3 times with 5 mL MilliQ-water. Residual water was removed by vacuum stripping at 40 mbar, at 135 °C for 1 hour. The product was analysed with IG ^{13}C NMR. Residual chloride content was determined through Mohr titration. A 0.1M $AgNO_3$ solution was used for titration, with 1 mL of a 0.25M K_2CrO_4 solution as indicator. 1g of the IL was combined with 2 mL Milli-Q water and the indicator solution. This was vigorously agitated at 50 °C. Titration was performed very slowly to enable exchange of the residual chloride with nitrate, so that the chloride can be detected. The titration continued until a red precipitate was formed.

The procedure for the preparation of $P_{666,14}$ BF_4 is described in Blundell et al.[119]

2.5. Extraction set-up

1 g of the ionic liquid was added to a 15 mL pressure vial, to which 10 mL of a 0.33 M acetic acid stock solution was pipetted. 49 mg H_2SO_4 was added to the aqueous phase, to obtain a concentration of 0.05 M. After sealing the vial with a screw cap, it was placed in a 75° C temperature controlled oil bath and magnetically stirred. Sampling was done by stopping the stirrer to allow phase separation, removing the cap and taking off 200 µL of the aqueous layer with a 1 mL syringe and a small amount of the IL layer with a second syringe, to be transferred and weighed in a 2 mL vial for quantitative ^{1}H NMR analysis.

2.6. Esterification set-up

For the one-phase esterification in the ionic liquid, 1 g of the ionic liquid, 0.33 mmol acetic acid and 5 mg H_2SO_4 (i.e. 0.3 equivalent of protons compared to the acetic acid) were transferred to a 2 mL vial. After addition of a small stirring bar and sealing with a plastic cap with septum, the vial was placed in a temperature controlled oil bath. The esterification reaction was initiated upon addition of ethanol; 15.3 mg or a multiple of this, to obtain 1 equivalent ethanol or multiple equivalents respectively. Sampling was done with a 1 mL syringe, through the septum. In this way the need to open the vial was eliminated, which previously caused evaporation losses of the volatile product. The sample was transferred and weighed in a 2 mL vial for quantitative ^{1}H NMR analysis.

2.7. Evaporation set-up

1 g of ionic liquid was added to a 10 mL flask containing a stirring bar, to which 30 mg ethyl acetate was added. This was connected to a liquid nitrogen cooled cold trap, which was connected to a Vacuubrand vacuum pump with manometer. In this way a vacuum of 40 mbar was set. The flask was placed in a temperature controlled oil bath. The mixture was magnetically stirred. Time measurement started when the pressure started to drop. The vacuum was kept for a determined time, after which the pressure was allowed to rise by gradually decoupling the connecting tube from the flask. The flask was sealed with a glass stopper, after which the mass was determined , to calculate the evaporation efficiency.

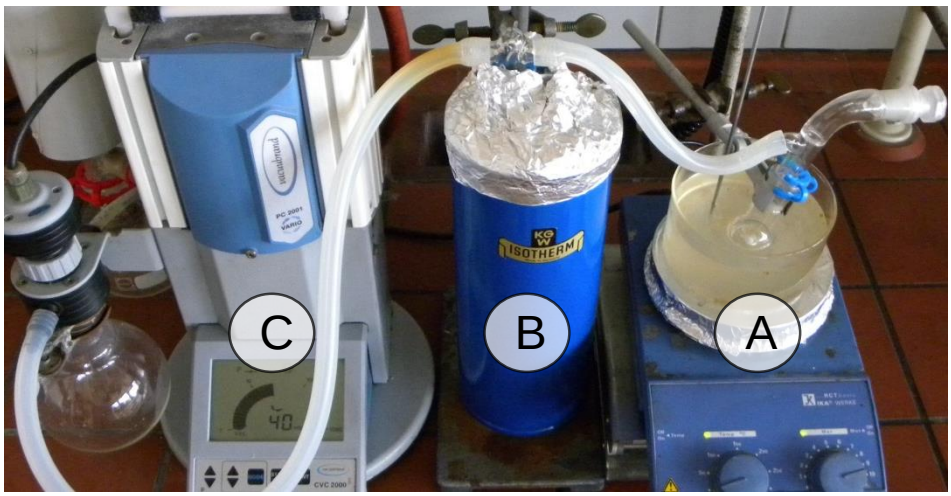


Figure 4.2: evaporation set-up, with flask in temperature controlled oil bath (A), liquid nitrogen cooled cold trap (B) and vacuum pump with manometer (C)

The condensate was analysed through ^{1}H NMR to determine its composition. Three control samples without ethyl acetate were run to verify there is no mass difference in this case. Moreover, the determination of evaporation efficiency based on mass difference was verified by quantitative ^{1}H NMR analysis before and after evaporation.

2.8. Anion exchange set-up

For the preliminary anion exchange experiment, a stock solution of 2 g/L of chloride, nitrate, phosphate and sulphate was made, by adding 516 mg H_2SO_4, 824 mg NaCl, 816 mg KNO_3 and 821 mg NaH_2PO_4 in a 250 mL volumetric flask, to which MilliQ-water was added. H_2SO_4 was used instead of a sulfate salt,

to adjust the pH to the pH range of the membrane electrolysis anolyte. For the anion exchange experiments in the comparison of the ILs, a stock solution of 1 g/L of the anions was used, to avoid saturation of the IL with one of the anions.

1 g of the ionic liquid was transferred to a 15 mL pressure vial, to which 10 mL of the anion stock solution was pipetted. After sealing the vial with a screw cap, it was placed in a 75° C temperature controlled oil bath and magnetically stirred. Sampling was done by stopping the stirring bar to allow phase separation, removing the cap and taking of approx. 0.8 mL of the aqueous layer with a 1 mL syringe. The sample was filtered over a 0.2 µm filter and stored at 4 °C. The samples were diluted immediately before ion-exchange chromatography analysis.

2.9. Determination of reaction parameters

For the experiments in which the influence of temperature and ethanol on the esterification was studied, the same experimental procedure as in 2.6. was performed, except for the temperature of the oil bath or the added amount of ethanol.

For the experiments in which the influence of water on the esterification was studied, the biphasic esterification was performed as the extraction described in 2.5, with addition of 455.4 mg ethanol (3 stoichiometric equivalents) to the IL layer after 2 hours of extraction, to initiate the esterification. Then the mixing was continued for 2 hours with frequent sampling of the IL layer to monitor the esterification. IL layer samples were analysed through quantitative ^{1}H NMR analysis. For the 'extraction + esterification' experiment, the extraction was conducted as described in 6., after which the aqueous layer was removed with a pipette. The acetic acid concentration was determined through quantitative ^{1}H NMR analysis. The IL layer was transferred to a 2 mL vial, to which 7,6 mg ethanol (approx. 1 eq.) and 5 mg H_2SO_4 (3 eq. of protons) were added. This was heated to 55 °C in a temperature controlled oil bath and magnetically stirred. Sampling was done with a 1 mL syringe, through the septum. The sample was transferred and weighed in a 2 mL vial for quantitative ^{1}H NMR analysis. In the 'extraction + centrifugation + esterification' experiment, the same procedure was followed, but the IL layer was centrifuged before it was transferred to the 2mL vial for esterification. The centrifugation was performed during 5 minutes by an Eppendorf 5430 R centrifuge, at 22 °C and 30 000 rcf. The IL layer was transferred and weighed in the 2 mL vial for esterification after removal of the supernatant.

3. Safety

All reactions in this thesis were performed in a fume hood. In general, mild conditions were applied with minimal safety risks. The compounds with the biggest safety and environmental risks are listed below. Experiments were always performed wearing a lab coat and safety goggles conform the general lab safety rules. Gloves were used when handling acidic or toxic compounds, as indicated below.

Trihexyl(tetradecyl)phosphonium ionic liquids: five ILs with different anions were used. ILs can interact with the cell membrane due to the hydrophobicity of the cation. It is therefore estimated they have a higher dermal toxicity compared to other ILs, but no toxicity tests have been carried out so far.[116] The MSDS file suggests to avoid contact with the skin. Given the possibility to interact with cell membranes and the very slow degradability, it is important that no IL can leach into the environment. The IL layers

were collected after the experiments in separate bottles per IL. Residues were cleaned with acetone and collected in halo- or non-halo waste vessels, depending on the IL anion.

Corrosive compounds such as sulfuric acid and the volatile fatty acids were handled with gloves.. The VFAs were handled in the fume hood to minimize odour nuisance in the lab and avoid inhalation.

Furthermore some chlorinated compounds were used: dichloromethane, chloroform and deuterated chloroform. These are very volatile and impose a risk of dermal and respirational toxicity. Their use is restricted in the lab conform EU rules. They were collected in a waste vessel for halogenated compounds, to make sure they did not end up in the wastewater or in non-halogenated organic waste fractions. Dichloromethane was used in very small quantities as internal standard for NMR samples. It was handled with gloves in the fume hood. Chloroform was used in one experiment as a last option diluent. Deuterated chloroform was used as deuterated solvent for NMR experiments, before it was replaced by DMSO-d_6. Both compounds were handled with the aforementioned precautions.

Deuterated benzene and deuterated toluene were used in one NMR experiment. These are flammable, mutagenic and carcinogenis compounds. They were handled with gloves in the fume hood, to avoid dermal contact and inhalation.

Chapter 5: references

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Chapter 6: addendum

1. Overview NMR experiments

1.1. Introduction

The required analysis methods for quantification of acetic acid and ethyl acetate as model compounds in the aqueous and IL layer are demonstrated in the next section. So far, quantification of VFAs and the corresponding esters in the aqueous phase is possible through GC analysis. The distribution of the acetic acid that is initially dosed to the aqueous layer, $n_{AA,0}$, partitions into different fractions during the biphasic esterification (Figure 6.1). This can be due to migration from the aqueous layer to the IL layer or evaporation, or by the conversion into ethyl acetate and subsequent distribution. A mass balance, expressed as amount of moles, will be drawn to demonstrate which fractions can be analysed and what analysis is needed for the others. It is assumed that acetic acid can only be converted to ethyl acetate and no other by-products are formed. Three different cases will be described. The first one is a simplified case in which only migration of acetic acid is considered. In the second case, biphasic esterification will be described and in the third case, the analysis after applying an evaporation step to remove ethyl acetate will be considered.

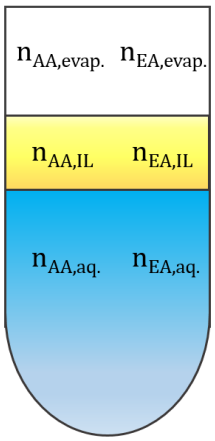

Figure 6.1: distribution into different fractions during biphasic esterification

1. Extraction
   During the extraction, the initial amount of acetic acid present in the aqueous layer distributes between the aqueous layer and the IL layer. The following material balance can be made:

$$n_{AA,0} = n_{AA,aq.} + n_{AA,IL} \quad (1)$$

   The initial amount of acetic acid, $n_{AA,0}$, is known from the concentration of the acetic acid solution and the volume of the aqueous layer. The amount of acetic acid in the aqueous layer, $n_{AA,aq.}$, can be determined through GC analysis. The amount of acetic acid in the IL layer can easily be calculated. In this case no analysis method for the IL layer is needed. It is however important that the amount of acetic acid lost by evaporation ($n_{AA,evap.}$ in Figure 6.1) is negligible, otherwise the IL layer concentration will be overestimated. This is a reasonable assumption if there is no acetic acid condensation in the headspace. Condensation is however possible since the vial is heated from the bottom, so the temperature at the top is lower than 75 °C.

2. Biphasic esterification
   The initial amount of acetic acid in the aqueous layer distributes into different fractions as depicted in Figure 6.1. If evaporation loss is negligible, the following material balance can be made:

$$n_{AA,0} = n_{AA,aq.} + n_{EA,aq.} + n_{AA,IL} + n_{EA,IL} \quad (2)$$

The initial amount of acetic acid is known from the concentration and the volume of the aqueous layer, and the aqueous concentrations can be determined through GC analysis. This means that relative quantification in the IL layer is sufficient: if the ratio of ethyl acetate to acetic acid can be determined, both terms can be calculated. It is again important that the evaporation loss is negligible, or the IL layer concentrations will be overestimated and the conversion of the esterification underestimated, since ethyl acetate is more volatile.

3. Analysis after evaporation
   The fraction of the acetic acid and ethyl acetate that evaporates goes through a tube into a liquid nitrogen cooled cold trap, where condensate is formed. The same material balance as in the biphasic esterification can be drawn for this case, with two additional terms:

$$n_{AA,0} = n_{AA,aq.} + n_{EA,aq.} + n_{AA,IL} + n_{EA,IL} + n_{AA,condens} + n_{EA,condens} \quad (3)$$

   The additional terms are the amount of acetic acid and ethyl acetate in the condensate. This is only quantitative provided all the vaporised acetic acid and ethyl acetate gets into the condensate. Moreover, the amounts in the condensate cannot be practically quantified due to the very small condensate volume and inevitable losses during sampling of the cold trap. This means that absolute quantification of the compounds in the IL layer is needed to make a decent mass balance.

The previous section demonstrated that relative quantification of ethyl acetate and acetic acid in the IL layer is sufficient for the biphasic esterification. However, when the evaporation is included, absolute quantification is required. It is also the safer method for biphasic esterification, to avoid errors due to evaporation losses. Relative quantification of compounds in a mixture can be achieved by ^{1}H NMR analysis. Absolute quantification is possible by addition of an internal standard. The development of a quantitative NMR analysis method for the IL layer is elaborated in the next sections.

1.2. Relative quantification through ^{1}H NMR

The analysis of the IL layer was initially performed by conventional ^{1}H NMR analysis (8 scans, 1 second relaxation delay) of a predefined mixture of ethyl acetate, acetic acid and $P_{666,14}$ DCN in $CDCl_3$. The $C\underline{H}_3CO$-peaks of ethyl acetate and acetic acid are however not entirely resolved. Different ratios of the IL layer mixture to $CDCl_3$ were tested to look for optimal peak separation. This was found to be 1% of the IL layer mixture in $CDCl_3$, but even then the peaks were not completely resolved (Figure 6.2), rendering quantification unreliable. It is expected that the $C\underline{H}_2$-peaks α to the carbonyl group of the longer chain VFAs and the corresponding esters will all be in a narrow chemical shift range of 1.95-2.25 ppm, of which a large part is already covered by a broad IL peak from 2.10-2.25 ppm (region (B) in Figure 6.3). Quantification based on the other aliphatic signals of higher VFAs and esters is also not possible, because of their similar chemical shift and even more

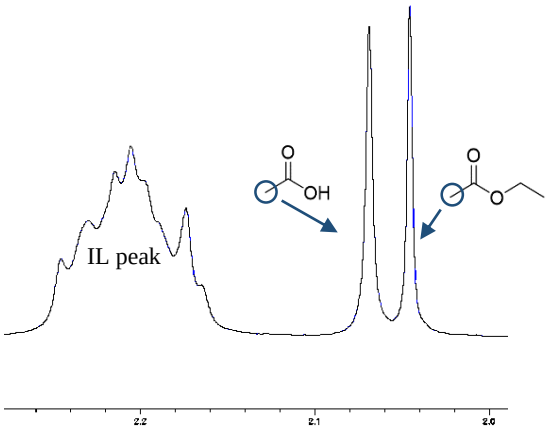

Figure 6.2: ^{1}H NMR spectrum of mixture of ethyl acetate, acetic acid and $P_{666,14}$ DCN in $CDCl_3$

complicated multiplicity. They are moreover all in the range 1.00-1.50 ppm, which is covered by a multitude of CH_2 and CH_3 signals of the IL cation (Figure 6.3, region (A)). Only the $C\underline{H}_2O$-peak of the ester is well separated from IL signal interferences. It is however in a narrow range of 4.00-4.15 ppm for different esters, making quantification of mixtures of different esters impossible (Figure 6.3, region (C)).

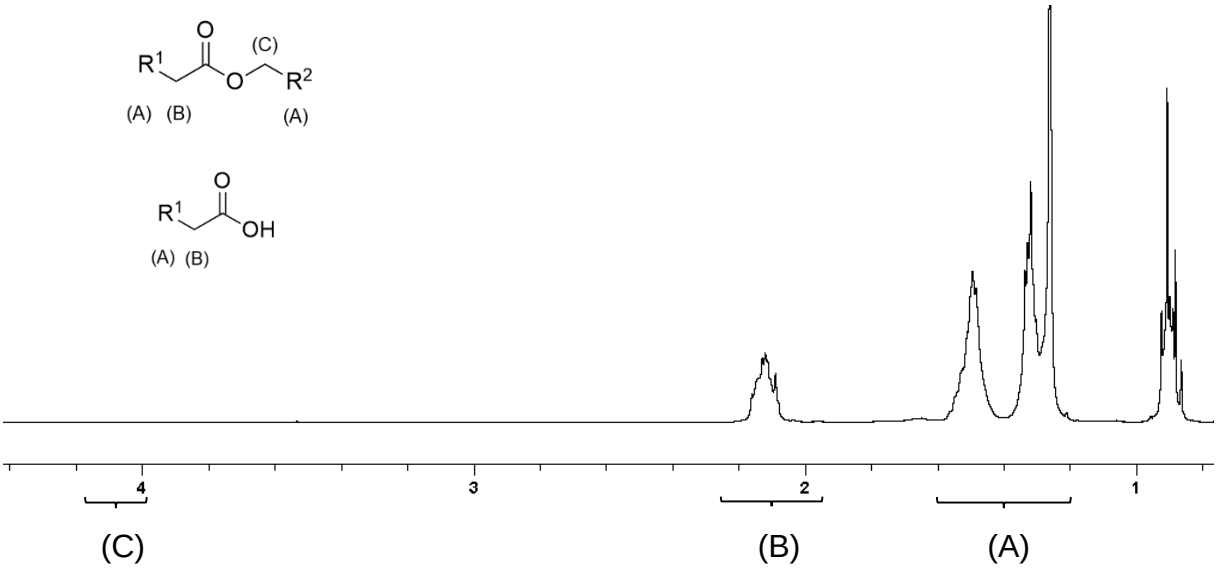

Figure 6.3: ^{1}H NMR spectrum of $P_{666,14}$ DCN in $CDCl_3$, with indicated regions for the signals of the VFA and the corresponding ester

1.3. Relative quantification through IG ^{13}C NMR

The resolution of peaks in ^{1}H NMR is difficult due to the limited chemical shift range in which they are located. Moreover, the presence of a multitude of broad IL peaks limits the number of peaks that can be used for quantification. Where it is already difficult to quantify a carboxylic acid and its corresponding ester due to peak separation, it is impossible for mixtures of different acids and their esters. The chances of finding well resolved peaks are higher when looking at the signals for the carbon atoms instead of the hydrogen atoms, since the chemical shift range for ^{13}C NMR is much larger compared to ^{1}H NMR (0-200 ppm and 0-12 ppm respectively). Conventional ^{13}C NMR is however not used for quantification, since the peak intensities are not proportional to the number of the respective ^{13}C atoms due to the Nuclear Overhauser Effect and differences in relaxation rates. This can be overcome in a so-called inverse gated decoupling experiment (IG ^{13}C NMR). In this experiment, broad band decoupling is performed during the radio frequency pulse and during the acquisition to eliminate C-H coupling, but it is disabled during relaxation delay, so that no Nuclear Overhauser Effect can build up. This results in a spectrum without C-H coupling and with a signal intensity representing the number of respective nuclei.[120] The spectrum of an equimolar mixture of ethyl acetate and acetic acid in $CDCl_3$ by conventional ^{13}C NMR (170 scans, 2 seconds relaxation delay) and inverse gated ^{13}C NMR (170 scans, 2 seconds relaxation delay) is depicted in Figure 6.4. The deviation of the conventional spectrum is 2.4%, while it is practically 0% in the case of the inverse gated experiment.

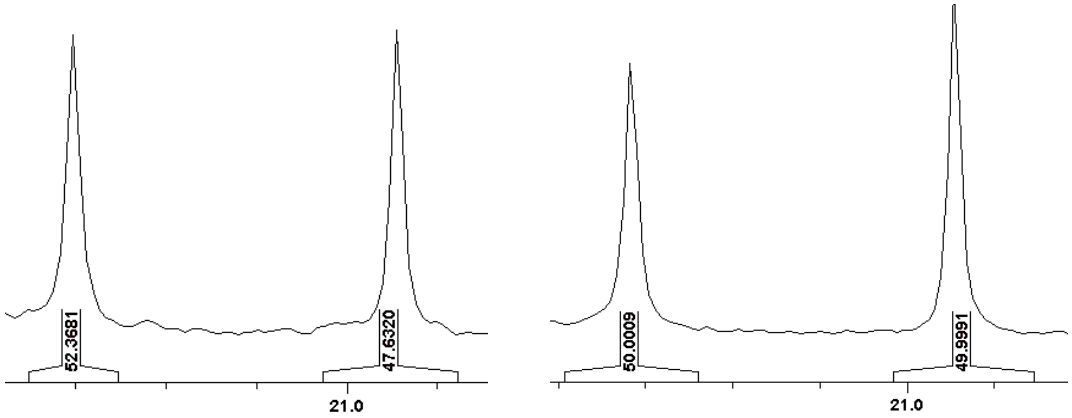

Figure 6.4: ^{13}C NMR spectrum of equimolar mixture of ethyl acetate and acetic acid in $CDCl_3$ with (left) and without (right) Nuclear Overhauser Effect

The accuracy of the method was verified for other ratios of acetic acid to ethyl acetate (Table 6.1). A more diluted sample of 100 mg IL layer mixture and 500 mg $CDCl_3$ was evaluated, but the deviations from the theoretical ratio were bigger. For a 95:5 ratio acetic acid to ethyl acetate, the ethyl acetate signal could not be distinguished from the noise. These results demonstrate the need for concentrated samples.

Table 6.1: theoretical vs. experimental ratios of ethyl acetate and acetic acid, determined through IG ^{13}C NMR

	$\%AA_{theo.}$	$\%EA_{theo.}$	$\%AA_{exp.}$	$\%EA_{exp.}$
300 mg analyte + 300 mg $CDCl_3$	50	50	50.00	50.00
300 mg analyte + 300 mg $CDCl_3$	60	40	60.20	39.80
300 mg analyte + 300 mg $CDCl_3$	95	5	95.31	4.69
300 mg analyte+ 300 mg $CDCl_3$	90	10	90.30	9.70
100 mg analyte + 500 mg $CDCl_3$	60	40	62.68	37.32

The same method was tested for analysis of the aqueous phase, to be able to use the same quantification method for both layers and to aim for a higher precision compared to the GC analysis. Another solvent is needed since $CDCl_3$ is not miscible with water. Both D_2O and CD_3CN were tested, each as 50% solution with the water layer containing acetic acid and ethyl acetate. In both cases, the acetic acid and ethyl acetate $\underline{C}H_3CO$-peaks were not resolved, even though they are in a pure D_2O and CD_3CN solution. The peaks are resolved when a 50% solution of the water layer in DMSO-d_6 is used.

The most suitable number of scans and relaxation delay was determined on basis of the deviation of the ratio ethyl acetate to acetic acid. Table 6.2 shows that a longer relaxation delay leads to smaller deviations. The length of the relaxation delay is limited by the length of a practical analysis, which was set at 10 minutes. A minimum number of scans is necessary to make sure the peaks are defined by data points (Figure 6.5).

Identical experiments were performed with an equimolar amount of acetonitrile as internal standard, to see the effect of the addition of an internal standard. Table 6.2 shows that the deviations are generally bigger.

Table 6.2: deviation of the experimentally determined to the theoretical ratio of ethyl acetate to acetic acid at different number of scans and relaxation delays for an IG ^{13}C NMR experiment

number of scans	relaxation delay	with CH_3CN	without CH_3CN
64	2	4.60%	ND
170	2	4.10%	ND
100	4	1.40%	3.00%
135	5	1.00%	1.90%
85	5	3.00%	ND
64	7	0.50%	2.20%
58	8	0.20%	1.00%

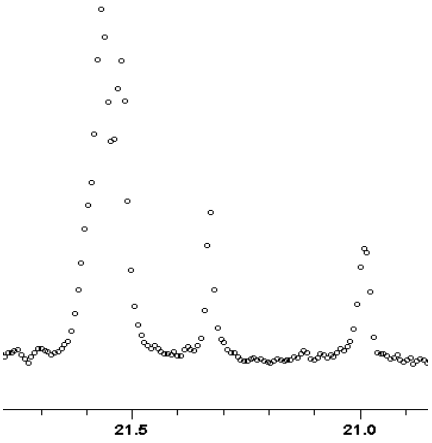

Figure 6.5: data points for a 58 scans IG ^{13}C NMR experiment

These experiments demonstrate that relative quantification of ethyl acetate and acetic acid in the IL layer can be performed through IG ^{13}C NMR analysis. An experiment of 58 scans with 8 seconds relaxation delay on a sample containing 300 mg IL layer and 300 mg $CDCl_3$ has shown to give the smallest errors of the peak areas. Absolute quantification of the analytes in the IL layer is however preferred, as discussed in section 1. This will be elaborated in the next section.

1.4. Absolute quantification through IG ^{13}C NMR

In a next experiment, samples of ethyl acetate and acetic acid in $P_{666,14}$ DCN were analysed in $CDCl_3$ with CH_3CN as internal standard, to see how suitable the method is for absolute quantification. Table 6.3 shows in the left three columns the theoretical ratios of the internal standard (acetonitrile), ethyl acetate and acetic acid. The peak areas from the IG ^{13}C NMR spectrum (58 scans, 8 seconds relaxation delay) are given in the middle three columns. The peak areas of ethyl acetate and acetic acid closely resemble the theoretical ratios. The deviation of the internal standard peak area is however substantial: it ranges from 42.4 to 66.0, while it should be 50 for all samples. In the right column, the calculated amount of ethyl acetate and acetic acid relative to the initial ratios is given, based on the peak areas in the middle columns. This is calculated according to equation (2) in section 2.1.3, chapter 4:

$$n_c = \frac{A_c}{A_{IS}} * n_{IS} \qquad (2)$$

The calculated amounts of the analytes display a bigger deviation from the theoretical amounts than the peak areas, with an increased deviation for the more uneven ratios (10-90 and 5-95 ethyl acetate to acetic acid). In these cases, the amount of acetic acid is strongly underestimated. These results demonstrate that the bottleneck here is the stability of the internal standard peak area over different target compound ratios. Other internal standards were therefore screened in a next experiment.

Table 6.3: peak areas and calculated ratios of ethyl acetate and acetic acid in $P_{666,14}$ DCN, based on IG ^{13}C NMR spectral information with acetonitrile as internal standard.

n_{IS}	n_{EA}	n_{AA}	A_{IS}	A_{EA}	A_{AA}	$n_{EA,exp.}$	$n_{AA,exp.}$
50	50	50	45.7	48.9	51.1	53.5	55.9
50	50	50	42.4	50.8	49.2	59.9	57.9
50	50	50	55.7	50.5	49.5	45.4	44.5
50	50	50	53.8	51.3	48.7	47.7	45.3
50	40	60	49.9	41.6	58.4	41.7	58.6
50	40	60	57.7	42.3	57.7	36.7	50.0
50	10	90	61.3	9.0	91.0	7.3	74.2
50	10	90	57.1	12.9	87.1	11.3	76.2
50	5	95	66.0	6.7	93.3	5.0	70.7

Five different internal standards were selected from a list of easily available compounds with tabulated chemical shifts, according to signals that are in a range that is free from interfering peaks (Figure 6.6).[121]

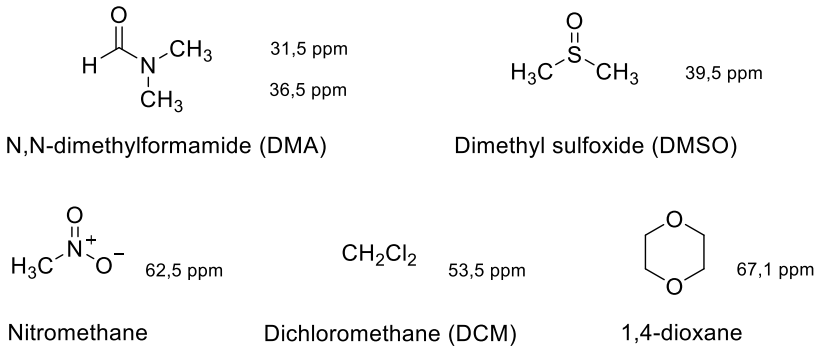

Figure 6.6: selected internal standards with ^{13}C NMR chemical shift in $CDCl_3$

These standards were added to equimolar mixtures of acetic acid and ethyl acetate in $P_{666,14}$ DCN and water for IL layer and aqueous layer samples respectively. The IL layer samples consisted of 50% IL layer and 50% $CDCl_3$, while the aqueous samples consisted of 50% aqueous layer and 50% DMSO-d_6. Since DMSO is already used as a solvent in the aqueous samples, it was decided not to investigate this compound as internal standard. The deviations of the ethyl acetate to acetic acid ratio as determined by the peak areas to the theoretical ratios is given in Table 6.4, as well as the internal standard peak areas for the four internal standards. Nitromethane (CH_3NO_2) and 1,4-dioxane (dioxane) were selected as the most suitable internal standards, because the integration errors and the deviation in internal standard peak area are the smallest.

Table A.6: Integration errors in IG ^{13}C NMR of equimolar mixture of ethyl acetate and acetic acid in $P_{666,14}$ DCN and water for the IL layer and aqueous layer respectively, with DMF, DCM, CH_3NO_2 and dioxane as internal standards

	IL layer		Aqueous layer	
IS	Integration error	IS area	Integration error	IS area
DMF	5.8%	51.9	5.1%	62.2
	3.6%	49.7	2.0%	59.8
	2.6%	62.7	0.4%	52.3
DCM	6.4%	66.9	1.2%	53.5
	2.8%	60.3	1.7%	48.9
	3.3%	59.1	1.8%	52.6
CH_3NO_2	0.1%	43.3	0.1%	45.0
	1.8%	48.0	0.8%	52.3
	0.1%	46.9	1.2%	52.5
Dioxane	1.5%	247.0	0.1%	206.0
	3.9%	261.3	1.0%	212.4
	2.4%	260.0	0.5%	213.1

Subsequently other ratios of ethyl acetate and acetic acid were tested with 1,4-dioxane and nitromethane as internal standards, to see if the internal standard peak area is more stable throughout the different ratios than was the case with acetonitrile. The results in Table 6.6 demonstrate that the peak areas of ethyl acetate and acetic acid represent the theoretical ratios (A_{EA} and A_{AA} vs n_{EA} and n_{AA}), but the calculated amounts deviate from the theoretical ratios ($n_{EA,exp.}$ and $n_{AA,\ exp.}$ vs n_{EA} and n_{AA}), as was previously the case with acetonitrile as internal standard. This effect is bigger for the more uneven ratios (10-90 and 5-95), and for the IL layer samples compared to the aqueous layer samples. This is again due to an unstable peak area of the internal standard signal, which is more pronounced in the IL layer samples than in the aqueous layer samples. The same trends are observed for 1,4-dioxane, as depicted in Table 6.6.

Table 6.5: theoretical ratios, IG ^{13}C NMR peak areas and calculated amounts of ethyl acetate and acetic acid for nitromethane as internal standard, in the IL layer and the aqueous layer

Theoretical ratios			IL layer					Aqueous layer				
n_{IS}	n_{EA}	n_{AA}	A_{IS}	A_{EA}	A_{AA}	$n_{EA,exp.}$	$n_{AA,exp.}$	A_{IS}	A_{EA}	A_{AA}	$n_{EA,exp.}$	$n_{AA,exp.}$
50	50	50	43.3	49.9	50.1	57.6	57.8	45.0	49.9	50.1	55.4	55.7
50	50	50	48.0	48.2	51.8	50.2	53.9	52.0	49.2	50.8	47.3	48.8
50	40	60	53.6	40.2	59.8	37.5	55.8	46.8	43.0	57.0	45.9	60.9
50	40	60	53.8	37.8	62.2	35.1	57.8	42.5	41.6	58.4	48.9	68.7
50	10	90	53.1	10.1	89.9	9.5	84.6	45.5	10.8	89.2	11.9	98.0
50	10	90	59.3	10.1	89.9	8.5	75.9	49.2	9.6	90.4	9.8	91.9
50	5	95	61.2	5.1	94.9	4.2	77.5	48.8	4.7	95.3	4.8	97.6
50	5	95	53.4	7.2	92.8	6.7	86.9	47.6	5.1	94.9	5.4	99.7

Table 6.6: theoretical ratios, IG ^{13}C NMR peak areas and calculated amounts of ethyl acetate and acetic acid for 1,4-dioxane as internal standard, in the IL layer and the aqueous layer

Theoretical ratios			IL layer					Aqueous layer				
n_{IS}	n_{EA}	n_{AA}	A_{IS}	A_{EA}	A_{AA}	$n_{EA,exp.}$	$n_{AA,exp.}$	A_{IS}	A_{EA}	A_{AA}	$n_{EA,exp.}$	$n_{AA,exp.}$
50	50	50	247.0	48.5	51.5	39.3	41.7	206.0	49.9	50.1	48.4	48.6
50	50	50	261.3	46.1	53.9	35.3	41.3	212.0	49.0	51.0	46.2	48.1
50	40	60	243.1	43.8	56.2	36.0	46.2	213.2	40.5	59.5	38.0	55.8
50	40	60	247.2	41.3	58.7	33.4	47.5	202.0	37.4	62.6	37.0	62.0
50	10	90	286.2	8.2	91.9	5.7	64.2	204.0	11.1	88.9	10.9	87.2
50	10	90	277.3	10.4	89.6	7.5	64.6	202.0	9.0	91.0	8.9	90.1
50	5	95	284.6	2.6	97.4	1.8	68.4	207.0	8.5	91.5	8.2	88.4
50	5	95	266.4	4.3	95.7	3.2	71.8	218.5	2.0	98.0	1.8	89.7

The previous results show that the biggest deviations are observed in the IL layer samples. This could be due to longer relaxation times in the IL, because of a higher ionic strength. We subsequently performed an experiment with chromium(III) acetylacetonate. This is a relaxation agent, of which the addition results in a reduced spin-lattice relaxation time. This means the acquisition time is shorter, or more relaxation and therefore a more quantitative result can be obtained in the same analysis time.[122] A chromium(III) acetylacetonate concentration of 0.025 M was used, by addition of 5.2mg to the samples. An equimolar mixture of acetic acid and ethyl acetate in $P_{666,14}$ DCN was used (300 mg), with $CDCl_3$ as solvent (300mg). The results in Table 6.7 show that the deviation of the peak area (integration error) to the theoretical ratios is bigger than in previous experiments. The peak area of the internal standard signal still shows a big fluctuation, from 180 to 212. The only improvement is that the peak areas of the carbonyl signals of ethyl acetate and acetic acid are closer to the theoretical ratios (50-50), as can be seen from the last two columns.

Table 6.7: integration error, internal standard peak area and peak areas of the carbonyl signals of ethyl acetate and acetic acid from IG ^{13}C NMR experiments of the IL layer with chromium(III) acetylacetonate as relaxation agent

# scans	t_{relax}	integration error	IS area	CO area EA	CO area AA
58	8	5%	202	48.6	47.6
58	8	3%	180	44.1	45.6
58	8	4%	212	48.7	50.2
58	8	6%	196	55.9	60.3
58	8	3%	185	48.6	49.4
200	8	4%	191	48.5	49.1
100	4	3%	188	48.7	44.9
100	4	3%	184	46.1	44.9
100	4	2%	200	54.6	53.8
170	2	5%	198	50.1	48.7
170	2	6%	190	39.4	38.2

In a next experiment we aimed to determine a response factor and see if the calculated amounts of ethyl acetate and acetic acid show a lower deviation compared to the theoretical ratios when calculated with the response factor. 1,4-dioxane was selected as the internal standard, because the deviation of the peak area was smaller than for nitromethane, as can be seen in Table 6.6 compared to Table 6.5. A double concentration of 1,4-dioxane was used, in order to have a more stable signal peak area. The response factor was calculated according to equation (3) in section 2.1.3 in chapter 4:

$$Rf = \frac{\frac{A_{IS}}{\varphi_{IS}}}{\frac{A_C}{\varphi_C}} * \frac{n_c}{n_{IS}} \quad (3)$$

In this case, φ_{IS} is 4, since 4 carbon nuclei give rise to the signal, while φ_c is 1 for both acetic acid and ethyl acetate. The same formula can be used to calculate n_c with a known Rf, this time as the calculated amount of the target compound instead of the added amount.

An average Rf of 1.23 ± 0.11% for ethyl acetate and an average Rf of 1.35 ± 0.18% for acetic acid was determined in this way. The amounts of ethyl acetate and acetic acid as calculated with the response factor show closer resemblance to the theoretical ratios than previous experiments in which no response factor was used (Table 6.8). This shows that the use of a response factor gives more accurate results.

Table 6.8: experimentally determined response factors for ethyl acetate and acetic acid, and the calculated amounts as compared to the initial ratios. Values are based on 4 experiments

n_{IS}	n_{EA}	n_{AA}	Rf_{EA}	Rf_{AA}	$n_{EA,exp,Rf\ gem}$	$n_{AA,exp,Rf\ gem}$
50	5	95	1.2 ± 0.3	1.4 ± 0.0	5.5 ± 1.2	89.1 ± 1.5
50	10	90	1.2 ± 0.2	1.5 ± 0.0	9.9 ± 0.1	82.2 ± 0.7
50	40	60	1.3 ± 0.2	1.4 ± 0.0	38.9 ± 0.6	56.1 ± 1.2
50	50	50	1.4 ± 0.0	1.5 ± 0.1	45.2 ± 1.0	45.3 ± 1.9
50	60	40	1.2 ± 0.0	1.3 ± 0.0	61.3 ± 1.4	40.9 ± 1.4
50	90	10	1.2 ± 0.0	1.3 ± 0.1	93.6 ± 0.7	10.8 ± 1.1
50	95	5	1.2 ± 0.0	1.0 ± 0.2	98.5 ± 2.4	6.8 ± 1.2

We speculated that the reason for the higher deviation in ionic liquid samples compared to aqueous samples might be due to the high ionic strength of the ionic liquids samples. An increased ionic strength results in increased T_1 relaxation times, making the analysis less suitable for quantification.[118] An experiment was set up to measure the effect of the ionic strength by determining the 90° H-pulse length, which is related to the ionic strength. This could however only be determined for samples containing maximum 25 mg IL in 500 mg $CDCl_3$, in which case the pulse length was very close to the normal value: 7.31 µs compared to 7.50 µs. It remains speculative what the ionic strength effect is in samples containing 300 mg IL layer, as normally applied in IG ^{13}C NMR.

The previous experiments demonstrate that quantification through IG ^{13}C NMR is challenging. Relative quantification of the target compounds can be performed, since the peak areas represent the molar ratios. Absolute quantification by the addition of an internal standard leads however to bigger

deviations, especially for more uneven ratios of ethyl acetate to acetic acid. It is possible to determine a response factor for a more accurate calculation of the number of moles of the target compound in a sample. The optimal conditions for a practical IG ^{13}C NMR experiment (i.e. run time of 10 minutes) have been determined as 58 scans and 8 seconds relaxation delay. The IL layer samples are prepared by diluting a 300 mg IL layer mixture with 300 mg $CDCl_3$. For the aqueous samples, 250 µL of the water layer is mixed with 250 µL DMSO-d_6. The samples should be vigorously stirred in both cases.

1.5. Absolute quantification through ^1H NMR

We then decided to elaborate on quantitative ^1H NMR analysis for ethyl acetate and acetic acid detection in IL layer samples, because preliminary experiments showed that the $C\underline{H}_3CO$-peaks of these compounds can be separated in DMSO-d_6. A much faster analysis is possible with ^1H NMR compared to IG ^{13}C NMR (1 minute vs 10 minutes respectively), and smaller IL layer samples are needed (20 mg vs 300 mg respectively). In this way, reaction rate experiments with frequent sampling are possible, without strong interference of sampling by volume reduction. This quantitative ^1H NMR analysis method could be used during the first phase of experiments, in which the extraction and esterification of acetic acid as model VFA is studied. It is clear from previous sections that it cannot be applied for mixtures of VFAs, as the peaks are in a too narrow chemical shift range which is already partially covered by IL peaks (see section 1.1).

An equimolar mixture of ethyl acetate and acetic acid in $P_{666,14}$ DCN was tested in six different deuterated solvents, to determine in which ones the $C\underline{H}_3CO$-peaks are completely resolved and the integration closely reflects the 50:50 ratio in which ethyl acetate and acetic acid were added. ^1H NMR spectrum outtakes of this experiment are depicted in Figure 6.7. There is no complete peak resolution in the case of $CDCl_3$ (A), CD_3CN (C) and acetone-d_6 (D). In the case of DMSO-d_6 (B) , benzene-d_6 (E) and toluene-d_8 (F), the peaks are resolved. The peak areas are the most accurate in the case of DMSO-d_6. It was therefore decided to use DMSO-d_6 in further quantitative ^1H NMR experiments. The same technique was applied in aqueous samples, but there peak separation was not possible, and the sensitivity was lower due to the aqueous conditions.

The previous experiment demonstrated that relative quantification of ethyl acetate and acetic acid in ^1H NMR with DMSO-d_6 is possible. Absolute quantification by addition of an internal standard is however more attractive, to rule out the errors corresponding to possible losses by evaporation and sampling. The previously selected internal standard 1,4-dioxane was added in the same concentration range as the analytes acetic acid and ethyl acetate. However, due to the small concentrations of these compounds in the IL layer and the small IL layer volume used in ^1H NMR, the added amount of internal standard is very small and impractical to quantitatively add to every new sample. Addition of the standard with a 0.1-10 µL micropipette was attempted, but even then the internal standard peak area was not stable enough for quantification.

It was therefore decided to introduce the standard in a coaxial insert. In this way, a solution of dioxane in DMSO-d_6 can be prepared on a bigger scale, making more accurate weighing possible. The coaxial insert can be reused for multiple samples, eliminating deviations in amount of standard added as source of error. The use of a coaxial insert requires experimental determination of a correction factor, as discussed in section 2.1.4, chapter 4.

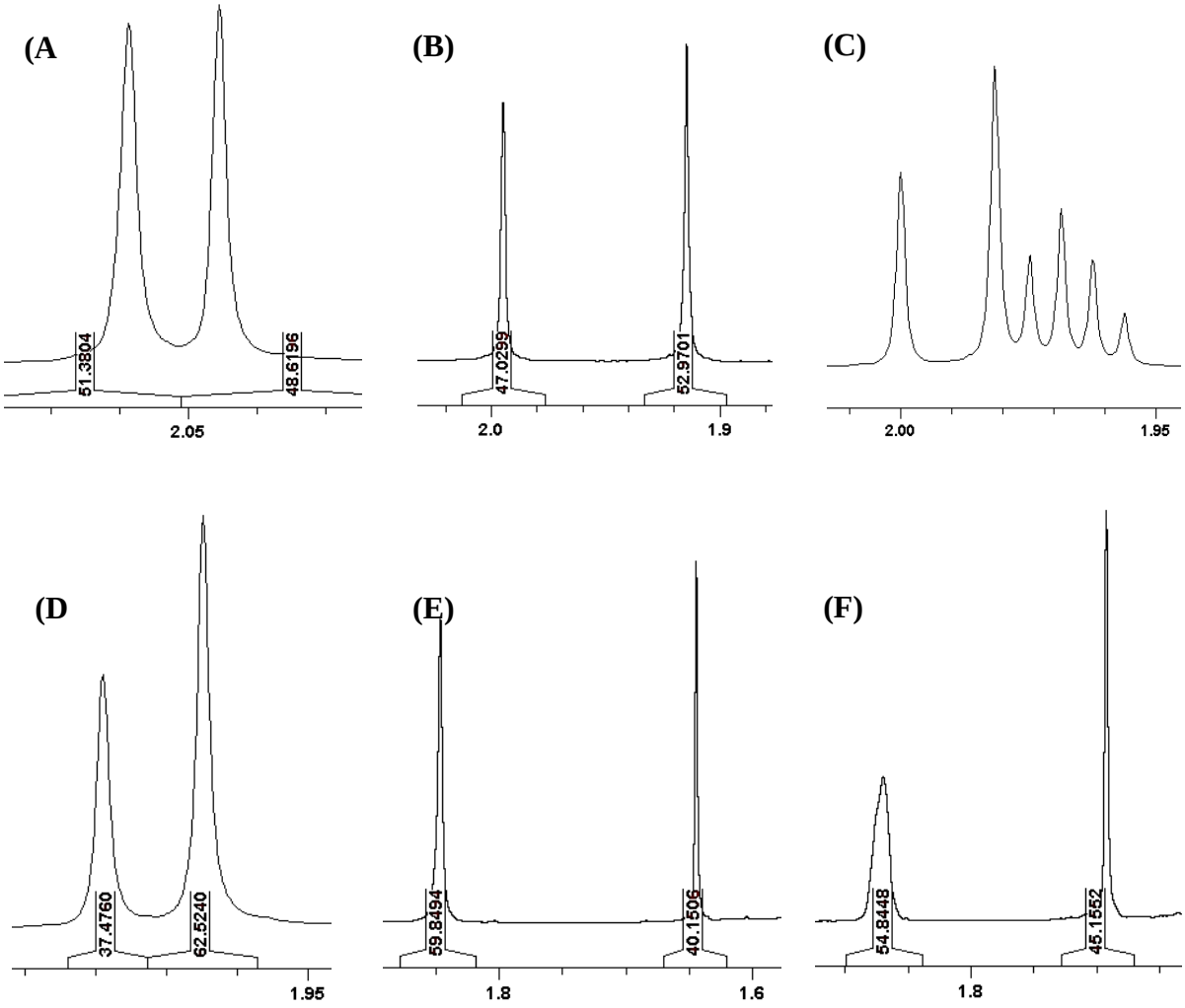

Figure 6.7: $C\underline{H}_3CO$-peaks and corresponding areas of a 50-50 mixture of ethyl acetate and acetic acid in $P_{666,14}$ Tf_2N through ^1H NMR, in $CDCl_3$ **(A)**, DMSO-d_6 **(B)**, CD_3CN **(C)**, acetone-d_6 **(D)**, benzene-d_6 **(E)** and toluene-d_8 **(F)**

The correction factor is determined through equation 8:

$$C_c = \frac{A_c}{A_{IS}} * \frac{n_{IS}}{V_{IS}} * \frac{V_{IS}}{V_c} \tag{8}$$

In which V_{IS}/V_c is the correction factor, that corrects for the difference between the volume containing the standard (inside the coaxial insert) and the volume containing the analyte (in the NMR tube, around the coaxial insert). This factor has to be determined by using a sample with a known analyte concentration C_c and a known standard concentration in the coaxial insert (n_{IS}/V_{IS}), over a range of different analyte concentrations. From the spectral information (A_c, A_{IS}), the correction factor can be calculated.

This was practically performed by adding an accurately weighed amount of acetic acid in $P_{666,14}$ Tf_2N, of which a certain mass (5-40 mg) was added to 500 mg DMSO-d_6. In this case, equation 8 can be converted to:

$$\frac{C_c}{\frac{A_c}{A_{IS}}c_{IS}} = \frac{V_{IS}}{V_c} \quad (9)$$

or: $$\frac{C_{AA,theo.}}{c_{AA,exp.}} = \frac{V_{IS}}{V_c} \quad (10)$$

The results are depicted in Table 6.9. Based on these results, a correction factor of 0.255 ± 0.107 is obtained, of which the average value is further used for the calculation of analyte concentrations.

Table 6.9: determination of correction factor for quantitative ^{1}H NMR with coaxial insert

mass IL layer (mg)	c_{IS} (mol/mL)	c_{AA} (mol/mL)	$c_{AA,\ theo.}$ (mol/mL)	Correction factor
40	1.07E-04	2.36E-04	5.93E-05	0.252
30	1.07E-04	1.73E-04	4.45E-05	0.258
20	1.07E-04	1.16E-04	2.97E-05	0.255
10	1.07E-04	5.25E-05	1.48E-05	0.283
5	1.07E-04	3.12E-05	7.41E-06	0.238
5	1.07E-04	3.27E-05	7.41E-06	0.227

This correction factor was determined identically for IG ^{13}C NMR, for which a value of 0.254 ± 0.030 was obtained (Table 6.10). This demonstrates that the coaxial insert can also be used in IG ^{13}C NMR, which makes it more practical due to smaller IL layer samples that are needed. It might moreover result in an improved accuracy.

Table 6.10: determination of correction factor for quantitative IG ^{13}C NMR with coaxial insert

mass IL layer (mg)	c_{IS} (mol/mL)	c_{AA} (mol/mL)	$c_{AA,\ theo.}$ (mol/mL)	Correction factor
40	1.07E-04	1.59E-03	3.33E-04	0.210
30	1.07E-04	9.20E-04	2.50E-04	0.272
20	1.07E-04	6.06E-04	1.67E-04	0.275
10	1.07E-04	3.18E-04	8.33E-05	0.262

During the experiments in which the quantification with a coaxial insert was applied, a gradual decrease of the correction factor over time was observed. We speculate this might be due to a slow loss of the 1,4-dioxane to the headspace of the coaxial insert. Therefore, the correction factor was tested in triplicates at the start of each experiment and the average value of the three measurements was used for calculations. The coaxial insert was cleaned and replenished with a fresh internal standard solution every 2 weeks.

These experiments demonstrate that absolute quantification of acetic acid and ethyl acetate in the IL layer can be accurately performed through ^{1}H NMR by addition of a standard in a coaxial insert. In this way, a fast analysis of the IL layer is possible. The use of a coaxial insert is also possible in IG ^{13}C NMR experiments for analysis of higher VFAs or mixtures of VFAs and esters. It is more practical and can be beneficial for the accuracy of the method.