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The role and indirect detection of amines in ionic liquids for biomass processing

Daria Osipova

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Author:	Daria Osipova
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Supervisor (Arcada):	Mirja Andersson
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Abstract:

Cellulose processing is a developing technique that is closely associated with various branches of the industry of materials processing. This paper discusses the technique with respect to application of ionic liquids for cellulose dissolution. Newly synthesized in Helsinki University, ionic liquids were designed for the purpose of cellulose dissolution and require detailed analysis because they are unknown compounds. Not only the characterization, but also the question of functionality is of interest to the present research. The question of influence of particular components on qualities of ionic liquids is not fully researched and in particular amines, which are present in cations of the studied ionic liquids, remains open today. The following work consists of a literature review chapter where the concepts of green chemistry and the relation of ionic liquids and biomass to it are discussed. Discussion of amines and their functionality with an emphasis on cellulose dissolution follows an overview of ionic liquids. The following chapter describes the method of capillary electrophoresis, which is used for the determination of aminesby means of separation and indirect detection. The resulting data is discussed further and summarized in a chapter entitled discussion and conclusions.

Keywords:	Amines, ionic liquids, capillary electrophoresis, green chemistry, cellulose, indirect detection
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ABBREVATIONS

BGE background electrolyte

CE capillary electrophoresis

EC effective concentration

EOF electro-osmotic flow

IL ionic liquids

LOD limit of detection

RSD relative standard deviation

SD standard deviation

UV ultra violet

1   INTRODUCTION

Materials science is the study of everything that surrounds our consciousness. Despite the fact that materials have been used from the dawn of humanity, how does one know which material to choose for a particular purpose? Knowledge about the characteristics of materials, which are used in everyday life, are passed from one generation to another. Nevertheless, more scientific questions are raised over time in accordance to technological developments. This means that study of materials will remain in demand and will hardly drain the potential for improving our understanding of the universe and enhancing the quality of our lives.

Wood is a primordial material, which is used by humanity for all sorts of purposes from construction to decoration. But not only raw wood is historically important, but also its processed derivatives constitute a crucial part of widely used materials. Processing of wood to produce pulp can be traced back to the second century, which is when China started producing paper. (Tsuen-Hsuin, 1985). To the present day one struggles to imagine our lives without paper; however, people strive to use it less today than before. That said there are other important applications for pulp, especially production of cellulose.

Keeping in mind significance of wood processing, topic for this research was chosen so to investigate one of the methods used for biomass processing and parameters that better outcome material. Understanding of biomass processing underlying processes is the key to melioration in directions affecting economic, environmental, humanitarian areas and, as a result, various institutions of our society.

Ionic liquids (ILs) were used for biomass processing for several decades. They show favourable qualities of good solvents and recyclable materials. Chemical composition of ILs obviously influence the abilities of ILs. The analysis of the ILs' composition and their comparison provide ground for synthesis of new, more sustainable, and more effective compounds. The department of Organic Chemistry in Helsinki University, Finland is working on the production of new ILs. As new materials are produced, they have to be characterized, because properties are hard to predict. The author completed practical

training in the Synthesis and Analysis department at the Helsinki University in order to analyse and characterize newly produced ILs.

1.1 Aims

ILs are known for their unique qualities of electrolytic salts. Moreover, various combinations of anions and cations are possible and allow a wide range of properties. The present work is focused on cations containing amine groups. The aims of the study are to analyse the role of amines within ILs for biomass and cellulose processing and discuss methods for indirect detection via capillary electrophoresis (CE).

To understand the role of amines in terms of a cellulose processing mechanism the cellulose dissolution should be studied first. The structure of biomass possesses molecular properties responsible for a choice of IL composition. The right composition of IL will react with structures of cells to a certain extent governed by intramolecular interactions. The correct choice of molecular composition will promote the dissolution, because IL would be able to break biomass structure.

Amines are predicted to have chemical and physical properties for enhancement of ILs potential. The highly electronegative Nitrogen included in the structure of amines is expected to be the initiator of the amines' qualities. Alkalinity of ILs will be studied to realize its influence on cellulose dissolution. Intermolecular interactions within amine containing cation such as resonance and hybridisation may influence IL's properties. Finally, features of amines govern conditions for their indirect detection. The CE technique involves the combination of electrolyte, buffer and other solutions chosen according to properties of analytes. As the name of the technique suggests, mostly electromagnetic properties are of interest. Furthermore, because an ultra-violet (UV) detector is used, the spectrum of detection for ions of interest ought to be taken into consideration.

ILs is the relatively new area for scientists and there are a lot yet to discover. Neither is the mechanism of cellulose dissolution completely understood. This would be the biggest limitation for this work as the author will have to investigate all available information and follow previously made hypotheses. Factors affecting interactions of ILs with biomass

would be studied alongside amine group properties and assumptions on their interactions would be made.

Moreover, the comparisons of different cations and anions constituting ILs would be excluded as no practical experiments were made to produce factual data for analysis. While researchers have described numbers of ILs variations, this project does not have the required resources to study the topic in the context of entire spectrum of ILs.

The biggest part of this work is based on research carried out in fields of green and analytical chemistry. Key research works concerning cellulose processing used for this thesis are firstly a publication of Swatloski et al. (2002) where they discuss the dissolution of cellulose with ionic liquids summarizing key aspects of the process, and secondly an article from the Royal Society of Chemistry by Li et al. (2018) where discussion of the same process is take on the level of contemporary technological advances to establish an anion/cation effect, physicochemical aspects and synergistic mechanism. The structure of biomass is well studied, and its description is widely available in literature. While the basic concepts are presented further in this chapter, it is worth noting that chemical and physical properties of structures of cellulose on the molecular level play the crucial role in one's understanding and the choice of IL for its dissolution. While studies in ILs are less extensive it is possible to understand the principle behind its function in biomass processing. Anions, cations and solvents constitute the environment for biomass dissolution, allowing interaction with cellulose polymers. The publication by Muhammad N. et al. (2015) describes the choice of the components for particular applications and analytical methods combined with a discussion of the results which provide prerequisites for theoretical part of this work.

Amines as a part of cations of IL is the essential focus of this paper. Literature concerning the role of amines in IL is limited. This led to the need for research on the general properties of amines in sources like Basic Principles of Organic Chemistry, organonitrogen compounds by Robert and Caserio (1977). Nitrogen, amine, and consequently cations containing amine groups contribute to bond breakage within a biomass. The extent of contribution is impossible to determine now; however, the conceptual framework can be found in available literature.

1.2 Methods

The method section includes the theory behind CE and the methods for the separation of ions in ILs. This topic is relatively broadly studied by Holzgrabe and Wahl (2016). Also, the strategies for indirect amine detection suggest procedures that were used for the practical part of this work. The method described by Malinina et al. (2017) was successfully employed and a resulting data was sufficient to produce meaningful conclusions.

For the first part of this work the qualitative approach is used. Literature on topics of cellulose processing, ILs, amines and CE will be reviewed. This method will serve as the establishing point of the investigation. Basic concepts and theories would be underlined to create a framework for discussion about the role of amines and their characterization.

The role of amines in ILs would be analysed only on basis of literature review. Future conclusions are to be developed if they have to be verified. The verification ought to carry out by means of practical experiments and calculations. A separate method would be required to perform these verifications.

For amine indirect detection the CE method is used. This is a separation analytical method. The principle behind the CE is a separation of ions constituting an analyte under an electrical charge to be detected by a UV-detector. A resulting electropherogram supplies information on the charge of ions, the concentration of analyte etc.

The motivation for this work is partly based on the "green" nature of studied compounds and the methods. The importance of the circular economy today is increasingly important for sustaining the global environment. Green chemistry is a part of this concept and widely applied in leading industries worldwide. It allows the reuse pf the materials with the impact on costs and production time, meaning a reduction in energy consumption and a reduction in emissions.

ILs completely belong to the concept of green chemistry as they are non-toxic and recyclable. The structure of ILs allow their employment in processes that lead to emissions

free energy production. In addition, being a relatively new discovery, ILs have the potential for new applications leading to novel materials and technological discoveries.

ILs created for biomass dissolution may operate at elevated temperatures or in the presence of microwaves, but there are other modes. Researches have shown that ILs containing the amine group in cations are effective for biomass processing.

This thesis work consists of a theoretical investigation and a description of the practical method. The body of the work begins with a literature review chapter. The literature review introduces the structure of cellulose, its role in the industry and the characteristic features important for cellulose processing. The following subchapter discusses ILs. The focus of that section is on ILs which are dedicated for biomass processing and their corresponding properties. The recovery of ILs is also discussed there. As ILs are recyclable, the literature review includes a section where the topic of green chemistry is introduced. The final section of the literature review contains research on amines. The following chapter is method. In the chapter the technique of CE is described. It also includes the method for amine detection in ILs. Specifics of the equipment, necessary materials, strategies for signal improvement, and approaches for data analysis are presented. In chapter 4 the results of the CE method are presented and discussed. The discussion and the conclusions are the last sections of this thesis.

2 LITERATURE REVIEW

2.1 Green chemistry

Every year biomass attracts more attention from industry as a source of energy and materials with favorable characteristics. Similar to other renewable sources of energy, biomass is available all over the world and can be utilized in the constantly growing number of applications. By definition it is the organic matter of various origin that is converted

into energy or products. The generation of this energy is a unique process called photo-synthesis impossible without solar energy, carbon dioxide, and consequently water which in combination create organic carbohydrates compounds (Equation 1). (Yuanchun, 2013)

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2 \qquad (1)$$
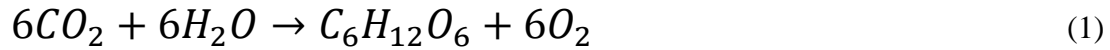
Biomass is considered as a sustainable material due to it renewable and recyclable features. Instead of becoming a waste it can continue its lifecycle as a source of energy after relatively inexpensive processing. Moreover, products resulting after biomass materials treatment are environmentally friendly and a cheap alternative to already existing materials used in industry and research. (Badgujar, et al. 2014)

There are different techniques that are used to convert biomass into energy. The choice of the suitable technique is dependent on the type of available biomass and a desired final product. Thermal conversion is one of the techniques that applies heat to transform biomass into other forms of energy. Another one, the thermochemical process, evidently uses heat combined with chemical reactions. The biochemical conversion with the help of microorganisms or enzymes generally transforms biological material into liquid fuels. Finally, biomass can be processed by means of the chemical conversion. (Wisconsin Grassland Bioenergy Network)

Processing of ILs decomposes a biomass on separate entities constituting the structure. Cellulose is separated during the dissolution process. A source of biomass may be grass, plants, wood powder and so on. Biomass studied in this project is called lignocellulosic due to its composition. Lignocellulose is a material found in a secondary layer of plant cell walls. After the production of cellulose fibrils wood pulp is extracted, which is the lignocellulosic material. It consists of three polymeric structures that are important for green chemistry: lignin, hemicellulose and cellulose. (Badgujar, et al. 2014)
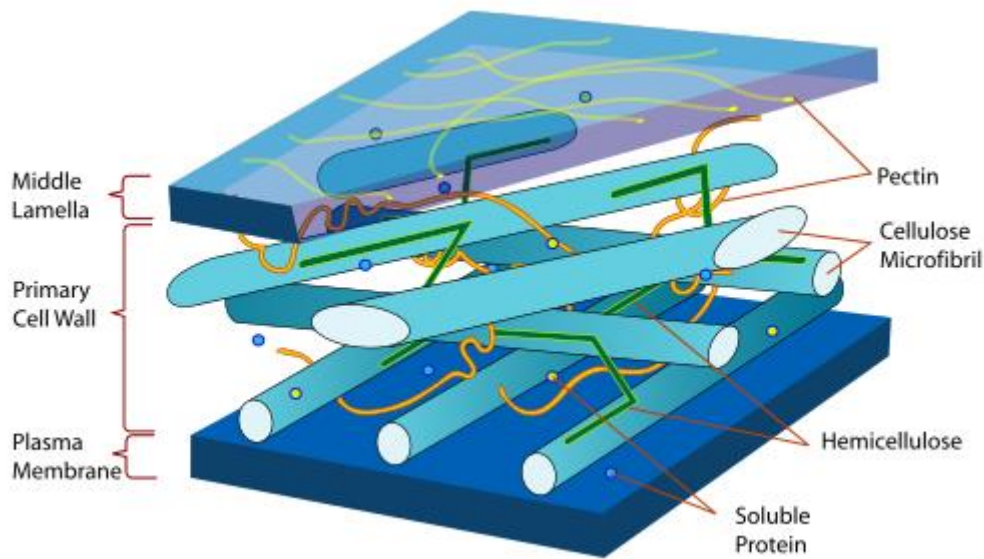
Figure 1. A section of the cell wall of a plant cell (LadyofHats, 2007)

As in the primary cell, cellulose is a basic structural component in the secondary cell with a composition between 40 and 55 percent of cell volume (Figure 1). Together with hemicellulose, constituting between 20 % and 30 %, cellulose is composed of carbohydrates polymers. Linear D-glucose chains with the degree of polymerization ranging from hundreds to thousands are connected by b(1->4) bonds from cellulose microfibrils (Figure 2, (a)). Hydrogen bonding makes the chains assemble into a crystalline rod-shaped structure with a diameter of 2-3nm (Figure 2, (b), (c)). Hemicellulose also feature throughout plant cell walls. This homopolymer consists of xylose, arabinose, galactose, mannose, glucose, acetyl, and other sugars characteristic for a specific plant. It is amorphous and has a lower degree of polymerization than cellulose. It forms the network with pectin around the cellulose scaffolds. Lignin creates a supportive structure for a plant cell wall filling between 15 and 20 percent of it with the cross-linked network formed of phenolic monomers and is linking to hemicellulose by covalent bonds. In addition to its mechanical properties, it can withstand rotting. Lignin is found in vascular plants and algae. (Li et al. 2018)

2.2 Cellulose

Cellulose can reach up to 10000-degree polymerization depending on a source of bio-mass. The structure of cellulose built out of rigid polymer strands, due to chair conformation of D-glucose, bond together by hydrogen bonding. The strands reach 20 nm in diameter. Bonding occurs between hydroxyl groups on glucose connecting them to form a polysaccharide matrix, which is relatively crystalline. The orientation of hydrogen bonds influence crystallization. Depending on the desired end product, cellulose can be treated differently to produce nanocrystals, nanofibrils and bacterial nanocellulose. (Angew Chem Int Ed Engl, 2005, 2011)
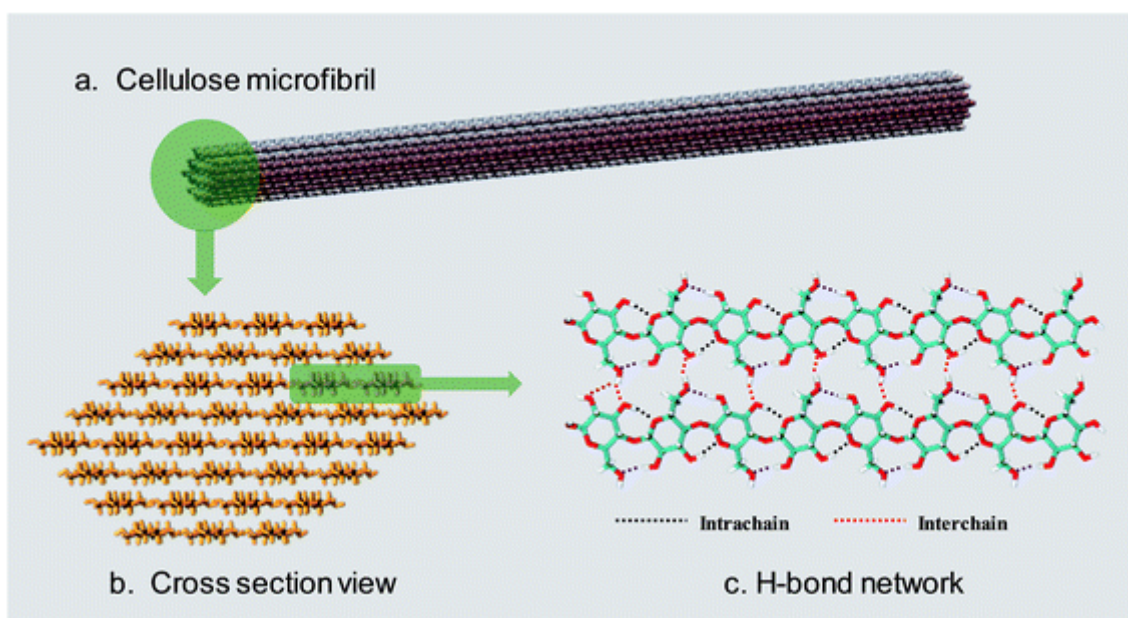
Figure 2. (a) The usual form of existence of cellulose–cellulose microfibril. (b) Cross-section view of a 36-chain cellulose elementary microfibril. (c) The H-bond network between cellulose chains. (Li, Y. et. al, 2018)

Biocompatibility and biodegradability, tensile strength, big surface area allowing interactions with surrounding conditions make nanocellulose derivatives attractive candidates for sustainable novel functional materials.

Properties of nanocellulose are suitable for biomedical applications include drug delivery, wound dressing, tissue and implant engineering. Manufacturing biocomposite scaffolds including nanocellulose structures creates the environment for cells to function and the permeability of such nutrients as water and oxygen. Cell activity will lead to a damaged area regeneration proliferated by nanostructure's properties. Nanocellulose can be functionalized and modified and enclosed in environments with various physical states that enlarges areas of applications. The production of nanosensors or drug releasing agents is possible owing to the synthesis possibilities and the reactivity of nanocellulose. (Liu, J., 2016)

2.3 Ionic liquids

In order to determine what is an ionic liquid (IL) it has to be specified what qualities make a substance to be qualifies as an IL. The same aim would serve the inquiry of what qualities materials lack so that they cannot be called IL.

ILs are commonly called liquid salts. These properties are apparent due to the fact that ions constituting the volume are smaller than the voids between them, that provides an increased mobility and thus low melting temperatures. In particular, the huge size of cations and small anions provide such a lattice structure (Figure 3). Not only the melting temperature but also the charge transfer abilities are resulting from such positioning. ILs are perfect electrolytes again, due to high ionic mobilities.
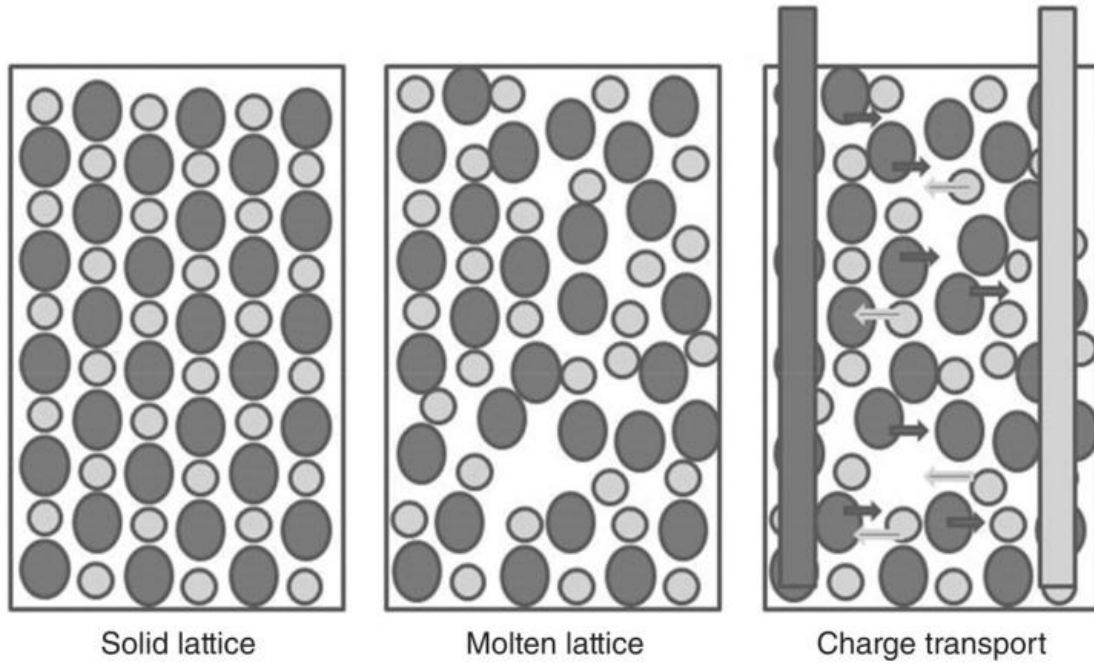
Figure 3. Schematic of the melting and charge transport processes in an ionic liquid. (Abbot, A. P., et. Al, 2015)

To understand where the properties of ILs originate the concept of diffusion has to be discussed on an example featured on Equation 2, which was derived by Watanabe (Tokuda, H., et al., 2004) from Nernst-Einstein and Stokes-Einstein relations.

$$\Lambda_{NMR} = \frac{N_A e^2 (D_+ + D_-)}{kT}$$

(2)

Where:

Λ_{NMR} theoretical molar conductivity

D diffusion coefficient of ions

T absolute temperature

The voids in the molecular lattice allow the ions to diffuse under the applied current. Moreover, there is a chance that the ions will interact, but that does not affect the conductivity. Mostly these calculations are applied for establishing ion interactions in water solution, the most common solute for ILs, although aprotic solutions are used for cellulose

dissolution. The whole model can be represented as ions that move from hole to hole. So, as the mechanics of ionic movements can be expressed with the equation as the voids that are occupied during the process, the structure of IL can be expressed and modelled. To conclude, voids in the IL's lattice structure and not ion interaction are responsible for electrolytic properties in ILs. Such the mechanism of charge transfer is a unique characteristic that distinguishes them from other materials. (Abbot, A. P., 2015)

There is a vast amount of possible composition of ILs and each of them will have different properties. These properties are tied to the molecular structure of IL and it is important to understand what they are based on to make the right choice for desired application.

In the pure form, ILs' properties are based on charge-charge interactions between anions and cations, when the ions are comparatively small in size. When the deviation in sizes occurs new forms of intermolecular interactions appear either directional or as a reduction in anion-cation interaction. These changes cause shift in anion behaviour towards surrounding cations as well as relating to the whole system. In case of interactions of IL with a solute, the size of anion governs the nature of reactions. Small anions form hydrogen bonds with the solute while cations play a miniscule role. However, with increasing size of anions, cations take their part in the solvation. (Ranieri,G., et. al, 2008)

While after change of anion, several properties of IL can change, adjusting cations may shift only one of the target properties, such as the charge distribution, the size, or the shape, which in turn influence all possible characteristics of IL: molar volume, glass transition temperature, melting point, viscosity, surface tension, density, and electrical conductivity. In addition, the cations affect the reaction of the dissolution, for example, in terms of the rate.

2.3.1 Cellulose dissolution

To follow trends of green chemistry ILs found their application in cellulose dissolution. The era of cellulose processing with ILs starts with the publications of Graenacher in

1934 and Swatloski et al. after a long lull in 2002. The mechanism of cellulose dissolution is still not clear to researchers, but certain processes can be underlined (Figure 4).
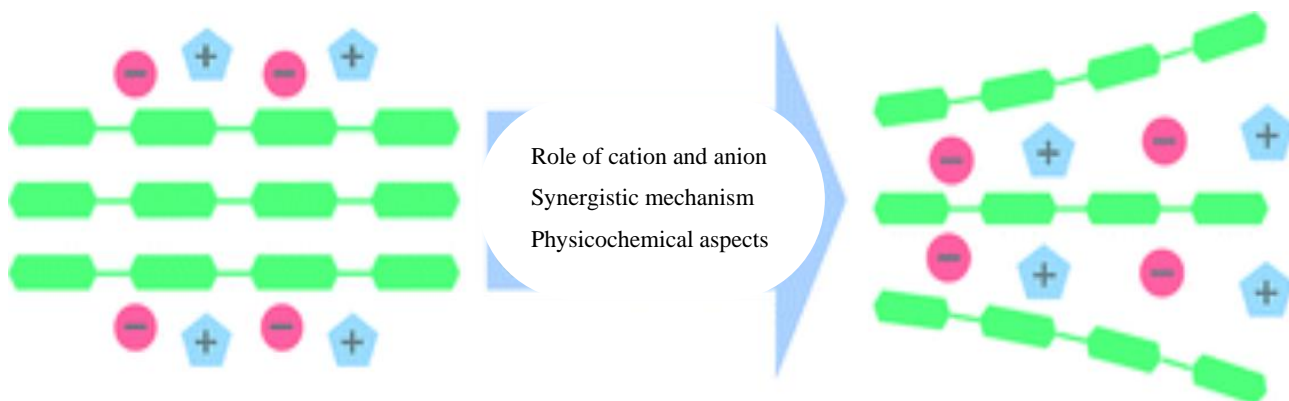
Figure 4. Aspects governing cellulose dissolution by ILs. (Li, Y. et. al, 2018)

Swatlowski et al. (2002) writes that for IL to be a biomass solvent, constituting anion should be a strong hydrogen bond acceptor to break the intramolecular bonds between the hydroxyl groups on the cellulose fibres.

The solvents for IL are also important to consider as they can mobilize ions and accelerate the process of dissolution. It may be caused by activation of anions in hydrogen bonds breaking. On the other hand, the presence of water decreases the solvent abilities because water molecules may interrupt the dissolution by bonding with the cellulose fibres. (Swatlowski et al., 2002)

2.4 Why amines?

Here the structures of studied amines with IL anion are introduced.

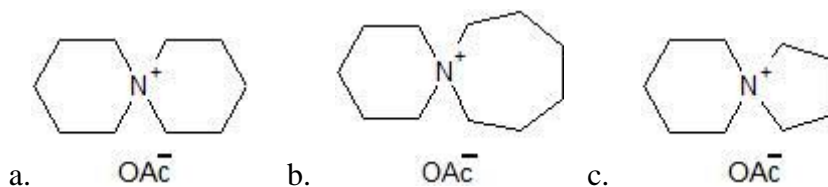
Figure 5. ILs (a) GP113, (b) GP131, (c) GP132.

Molecules have structural features that affect the basicity of compounds and rule the reactions outcome in terms of the basicity of product. Lying in the base of all cations of studied ILs is a Nitrogen compound found in many organic compounds. Amines are one of the biggest classes of nitrogen compounds and are met for example in plants (morphine, caffeine etc.) or in animals (adrenaline, vitamin B etc.). Amines have a pair of lone electrons that makes them basic and nucleophilic.

Robert (1977) points out that the saturated ring of azacyclohexane increases the basic strength that can be said about GP113,131,132 (Figure 5). At the same time the inductive effect provided by saturated groups make cations even more basic. When the alkyl group is attached to the amine it acts as an electron donor because one of the carbons in the group has a pair of valence electrons that are not involved in covalent bonding. Due to electron-donating nature of alkyl, the electrons are drawn towards the amine and the positive charge is braced on the nitrogen, making it the stronger base compared to amines without the alkyl group. These interactions lower the electric potential energy that causes the increase in basicity.

Non-conjugated amines are stronger bases than conjugated ones. This is caused by the inability of vacant electrons to resonate leading to electron density stability. For this reason, the aliphatic compounds are more stable and thus more basic than the aromatic ones.

2.4.1 Amines in ionic liquids

For the successful cellulose dissolution IL has to have a low viscosity that will depend on a size and a shape of the anions and the cations and the nature of their interactions. Also, the higher basicity of IL better undergoes dissolution. These characteristics are positively influenced by the presence of amine groups in ILs.

If the cations with nitrogen present are to be discussed, the N-alkyl chain variations mostly affect the properties of IL. Firstly, changing the length of the N-alkyl chain will vary glass transition temperature, density, and viscosity. The increasing length of the chain density will decrease due to the change in the molecule packing caused by the chain flexibility. The viscosity will increase with the increased chain length as the molecule will not be able to move freely, the internal friction and the dispersion forces rise as well. This process will decrease the mass transfer during the cellulose dissolution process. This will also affect the electrical conductivity in a negative sense for the same reason. The similar trend is noted when the cation is modified with rings; however, it is variant. The variations can be explained by the electronic distribution in the molecule and, as a consequence, the interaction with its surroundings. Increased electronegativity can also influence the molecule packing as ions are stronger attracted towards each other. As a result, the viscosity and the density are increased on the account of the electron-withdrawing nature. (Hawker, R. R., et al., 2018)

3   EXPERIMENTAL METHOD

The characterisation of newly synthesised ILs and any new materials in general is a first step towards its application. The materials with established qualities are placed into the existing framework of materials science. Knowing the rules of material behaviour and the variables for its prediction assists the development of technological advances.

The CE technique is a separation method for the detection of ions. Amine detection is of interest and discussed in this section. In general, a concentration of analyte can be determined by indirect UV detection. Indirect detection requires comparison of an analyte of interest with a standard with known properties for a selected method. Measuring a response of a series of solutions with changing concentrations of the standard sample and the analyte it is possible to determine the unknown component.

The method for present work was adopted from Malinina et al. (2017). The publication describes the procedure for amine indirect UV detection by CE. The technique was used in Helsinki University Polymer Chemistry laboratory. In this chapter the CE method will be discussed, the materials and the preparatory procedures for this method, the results, and the analytical processing of results.

3.1 Capillary electrophoresis

Capillary electrophoresis (CE) is a separation technique used for the detection of ions that are set in motion by means of a high voltage application. Figure 6 depicts the common setup for a CE instrument. An electrolyte passes through a capillary and is detected by an ultra UV detector, aligned to a transparent sector of the capillary. Apart from an analytes, the CE requires a background electrolyte (BGE) or a buffering salt solution that is used to condition the capillary walls and to provide a constant environment for a sample ion migration. The capillary is placed between the electrodes connected to the anode and the cathode with the help of which current from a high-voltage output travels through the capillary creating electrophoretic and electroosmotic flows.
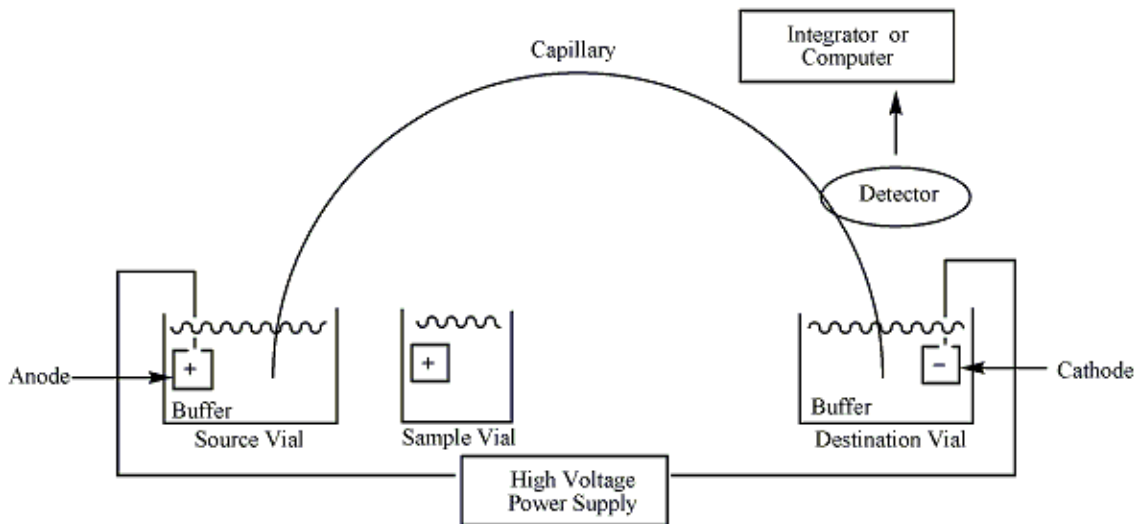
Figure 6. The system of the capillary electrophoresis instrument setup. (Apdlum, 2004)

When voltage is applied to a filed with the electrolyte capillary, the ions within it separate according to their number and a type of charge and size. Smaller ions are faster than the bigger ones and a higher charge ion are passing also faster. This principle can be expressed by Equation 3:

$$\mu_{ep} = (z/f \times e) \times \eta \times r \tag{3}$$

Where:

μ_{ep} electrophoretic mobility

z number of charges

f friction coefficient

e elementary charge

η solution viscosity

r radius of the ion

Thus, knowing the electrophoretic mobility, it can be theoretically predicted when a certain ion should appear on an electropherogram resulting from the CE measurement using Equation 4:

$$v = \mu_{ep} \times E \qquad\qquad (4)$$

Where:

V velocity of the ion

E applied voltage (V/cm)
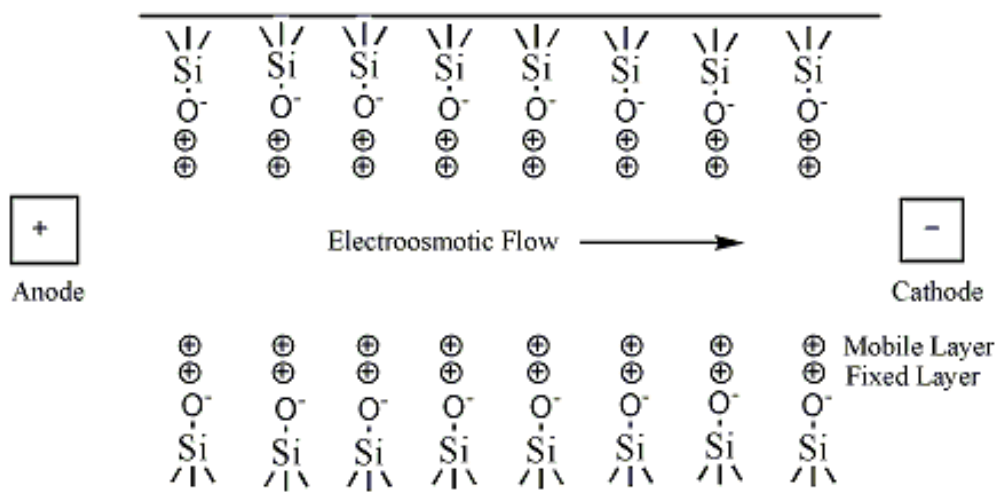
Figure 7. Interior of fused-silica gel capillary in the presence of buffer solution. (Apdlum, 2004)

Electro-osmotic flow phenomenon is also called an electric pump, which created by an interaction of migrating to a cathode solution and a negatively charged walls of the capillary (Figure 7). The cations accumulate on the walls of the capillary with a rate dependent on electrolyte concentration and pH value. The EOF provides not only cations but also an anions passage through a detector. EOF can be measured by using Equation 5:

$$\mu_{eo} = (\varepsilon \times \varsigma / \eta) \qquad\qquad (5)$$

Where:

μ_{eo} EOF mobility

ε dielectric constant of electrolyte

η relative permittivity of the solution

ς electrokinetic potential

Increasing a concentration of a buffer decreases the velocity of EOF, that is always directed toward the electrode, which has the same charge as the capillary wall. (Whatley 2001)

3.2 Safety

Most of the materials for CE are handled in a chemistry laboratory. Before starting any laboratory work participants must become familiar with the general safety regulations and the guidelines. Moreover, there are specific safety rules to an equipment and a materials, that has to be outlined in a laboratory work plan created before a practical work begins.

General safety awareness starts with the knowledge about a closest evacuation paths and a safety exits, a location of laboratory safety equipment, for example, a fire extinguisher, a medical kit, a shower, etc. An emergency phone number should also be indicated within the laboratory. Any laboratory experiments cannot proceed without attendance and the work is always performed in the presence of at least two people. Eating and drinking are not permitted in a chemical laboratory environment. Laboratory workers should always use personal protection equipment within a laboratory premises. Chemicals are never to be smelled directly nor injested. When glassware or other equipment is used it is always has to be checked for integrity. Any accident in a laboratory should be reported. Lab regulations should never be ignored and in case of any violation a laboratory supervisor is to be informed.

Before any work is done with chemicals, material safety data sheet has to be introduced. It will provide a general information about the chemical, a hazards, a specifics of

handling, an applicable safety procedures, and a disposal instructions. The method for this work does not cover the use of highly hazardous materials. Although, it should be noted that chemicals can never come into contact with the skin.

CE technique has hazards that also have to be taken into consideration. Firstly, the availability of safety manual from a manufacturer is a prerequisite and its content familiarized with before the first use of the equipment. Each time before switching on the equipment all the parts must be checked for any presence of mechanical defects. After approximately 72 hours of use the electrodes and other parts that contact with a chemicals have to be cleaned. Sometimes cleaning is necessary even earlier if the chemicals leave excessive amounts of traces inside the equipment. When cleaning the CE machine, it has to be disconnected from a power source. A procedure for disassembling the equipment is provided by the manufacturer.

3.3 Materials

The experiment was executed using the Hewlett Packard G1600AX - 3D CE that included the UV/Vis diode-array detector (190-600 nm with accuracy ±1 nm) with a deuterium lamp light source. Polypropylene 700 µl vials with releasing snap caps were used as vessels for the instrument. A fused silica capillary with an inner diameter of 50 µl was used in the instrument.

The following chemicals were used in the experiment: acridine, acetic acid, Tween 20, DABCO, ILs (GP_113, GP_131, GP_132, and GP_049 (Figure 8)). Water used for the experiment was purified by filtration and deionisation by the Arium® mini ultrapure water system.
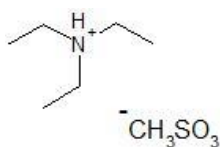
Figure 8. IL GP049.

Indirect detection requires the presence of a probe in a BGE. The probe ion, emitting light, is displaced by an ion of analyte with the similar charge. While the system stays electrically neutral, an intensity of the signal registered by the detector decreases (Marina, M. L., 2005). The acridine (Figure 9) is used as a probe both because its mobility is similar to mobilities of amines and because it has strong fluorescent qualities. The probe molecule size should correspond to a size of the analytes' molecules. Also, depending on the size, the probe should be charged sufficiently high to move through the capillary; however, an excessive charge may disrupt the flow. If the mobility of a probe differs from the mobility of an analyte peaks on an electropherogram would be distorted due to inconsistent migration. To maintain ionization of acridine, acetic acid (Figure 8) is used to decrease pH value. (Altria, K. D., 1996)
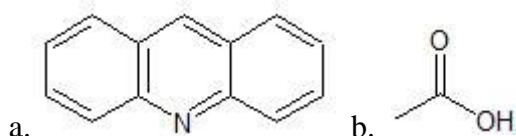
Figure 9. Acridine (a) and acetic acid (b).

During mobility, the analyte can adsorb into the capillary walls due to the charge difference. This will result in a peak tailing (an asymmetric peak shape) or an increase in the peak width. Use of surfactants helps to prevent this issue. Tween 20 (Figure 10) was used as the surfactant. By adsorbing into silanol groups on the surface of the capillary it prevents a deceleration of analyte ions.
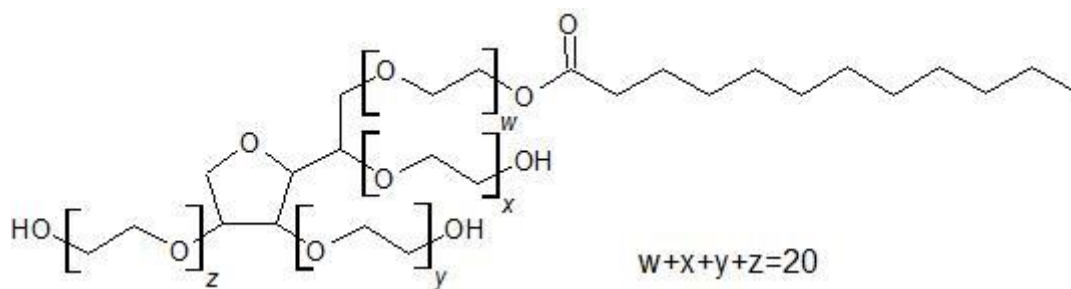
Figure 10. Tween 20.

DABCO (Figure 11) was used as an internal standard for analysis of the linearity of the experiment. The internal standard, which has a known concentration, is added to every sample for later reference on the resulting signal.
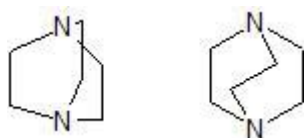
Figure 11. DABCO.

3.4 Preparation

BGE is required to sustain the electrophoretic current and the medium where analyte ions migrate keeping the ionic strength constant. In preparation of BGE 2 mM acridine and an amount of 0,05 wt% Tween 20 were filtered with a 0,45 µm syringe filter and mixed with 20 mM acetic acid.

Stock solutions of ILs were prepared by dissolving them in water.

Each day the capillary was conditioned with H_2O (5 min), 1 M HCl (5 min), H_2O (5 min), 0,5 M NaOH (5 min), H_2O (5 min), and BGE (5 min). This was required to rinse a

capillary surface from unwanted residues after previous injections. Contamination of the capillary may cause a change in the EOF. It was also used if a capillary was out of use for some time and the surface needs to be rehydrated. It is important to note that the capillary should be submerged into water at all times when not in use. After the rinse the capillary is filled with the electrolyte to adjust the pH environment.

Before each injection run the capillary was rinsed with 0,1 M NaOH (3 min), H_2O (1 min), and BGE (3 min). The purpose of this rinse is same as the purpose of the daily conditioning. This is a standard procedure during CE separations and the time of flushing can be increased if the time of migration rises with each run.

At the end of every operation day the capillary was flushed with H_2O (5min), 1 M HCl (5min), H_2O (5 min), 0,5 M NaOH (5 min), and H_2O (5 min). This rinse was also made to remove residues from previous injections from the capillary walls.

All injections were done at 20 °C and with 25 kV voltage. The detection wavelength was set to 254 nm. All separations were done in accordance with the capillary zone electrophoresis method.

4 RESULTS

4.1 Migration

For the measurements a concentration of 4 analytes were 0,01 mg/ml, and then in a range from 0,1 mg/ml to 1 mg/ml with a step of 0,1 mg/ml. The internal standard concentration was 0,4 mg/ml for all the measurements. Each sample with the internal standard was separated six times to obtain six electropherograms to establish repeatability and achieve accurate results.

To acquire data from obtained electropherograms can be processed in manufacturer's software OpenLab CDS ChemStation. The migration times and the area of internal

standard and the analyte peaks are important for present research. Figures 12 and 13 show the electropherogram of all components of interest in one matrix.

The electropherogram on Figure 12 shows a good separation of the analytes' peaks. This result is satisfactory. While Figure 13 depicts the electropherogram with unresolved peaks of GP049 and GP132. This indicates that some parameters in the method have to be changed. An increasing length of the capillary will increase the time of migration but also separate the analytes better. Decreasing the applied voltage will also cause the flow to slow, thus causing the peaks to separate.

Figure 12. Electropherogram of mixture internal standard solution and amines: peak 1 – DABCO, 0,4 mg/ml; 2 – GP132, 0,05 mg/ml; 3 – GP131, 0,05 mg/ml; 4 – GP113, 0,05 mg/ml. Electrolyte: 2 mM acridine, 20 mM acetic acid, 0,05 % Tween 20, pH3,8. Indirect UV detection.
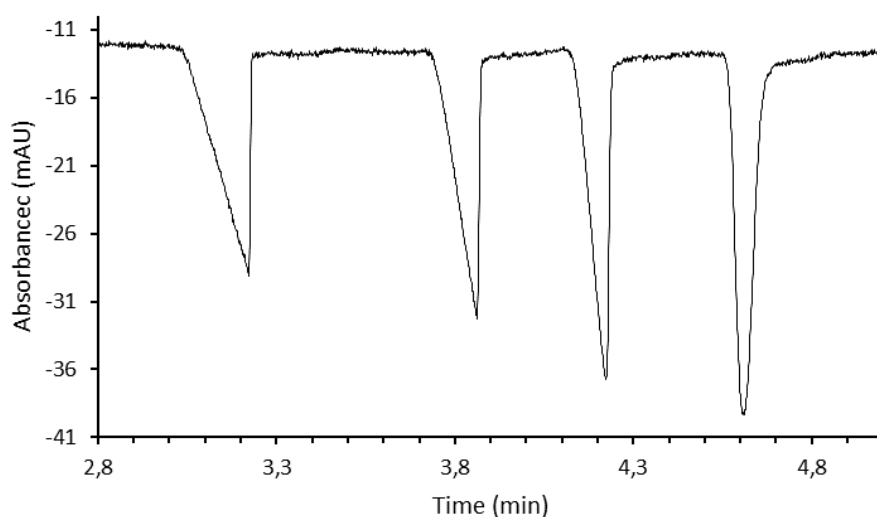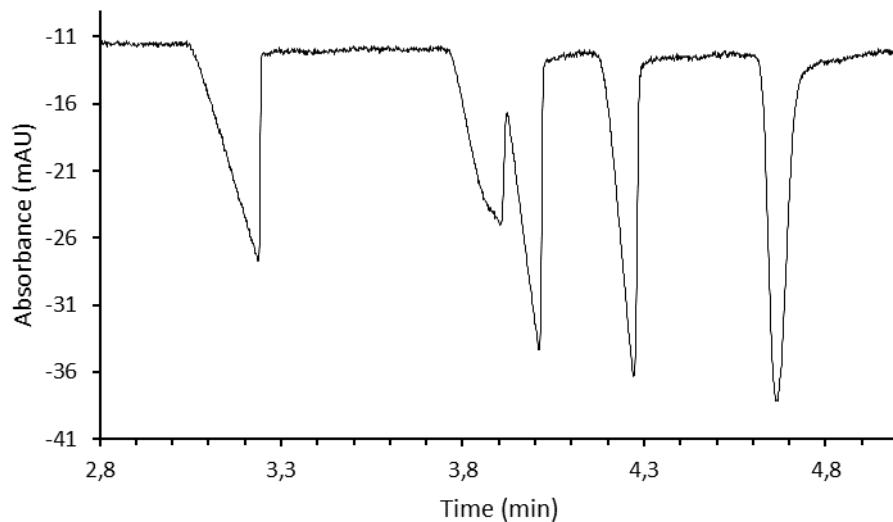
Figure 13. Electropherogram of mixture internal standard solution and amines: peak 1 – DABCO, 0,4 mg/ml; 2 - GP049, 0,05 mg/ml; 3 – GP132, 0,05 mg/ml; 4 – GP131, 0,05 mg/ml; 5 – GP113, 0,05 mg/ml. Electrolyte: 2 mM acridine, 20 mM acetic acid, 0,05 % Tween 20, pH3,8. Indirect UV detection.

4.2 Analytical

The calibration curve is used to measure a concentration relative to an unknown concentration of a substance in a sample solution. However, in the present experiment the calibration curve was used to establish the relative standard deviation (RSD). To create the calibration curve, an average correlation area between the internal standard peak areas and the analyte peaks areas were plotted against the corresponding concentrations of analytes. Then, a line was fitted, and its slope indicates a regression line or a correlation coefficient R^2 showing how well the data fits the curve (Table 1). RSD is calculated with the standard deviation (SD) of the correlation area and the average correlation area and shows the extent of the standard deviation comparing to the mean of the data set.

The limit of detection (LOD) indicates the lowest concentration of an analyte that can be detected by a device. It is usually found to predict an accuracy of a measurement. To determine the LOD span of the signal's noise span have to be measured and multiplied

by the factor of three (Figure 14). From that number the concentration is calculated, and several runs are made to testify the predicted height of the ions concentration peak. If needed, the concentration can be decreased or increased so that the height is corresponding to three times noise height. The LOD is also fitted in the calibration curve.
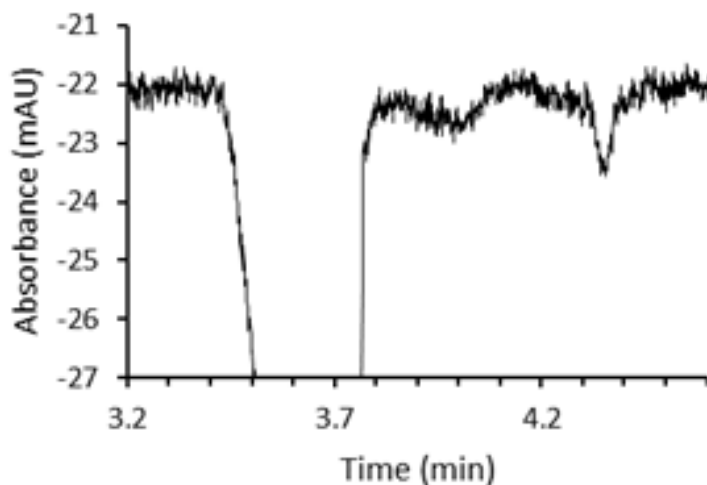
Figure 14. Example of electropherogram for LOD detection for GP132.

Table 1 represents the summary data collected after each injection. The correlation coefficient is close to an ideal but indicates that the method should be adjusted for improvement of the results.

Table 1. Analytical data obtained after practical work.

Ana-lyte	Concentration, mg/ml	Average migration time, min	RSD, %	R^2	LOD, mg/ml
GP113	0,01 - 1	4,75	1,93	0,9967	0,003
GP131	0,01 - 1	4,73	1,76	0,9974	0,004
GP132	0,01 - 1	4,26	2,19	0,9961	0,002
GP049	0,01 - 1	4,08	1,23	0,9973	0,003

The new ILs cations are determined. Now these parameters can be used in future research for the discussed analytes detection. This data will also assist an interpretation of results obtained from other instruments.

5 DISCUSSION AND CONCLUSIONS

The synthesis of ILs opens new perspectives on biomass dissolution and other applications. To ensure potential applications of ILs in cellulose processing their properties must be defined and the mechanism of interactions with cellulose structure studied. This thesis work attempted to uncover general properties of ILs created for biomass dissolution. With the focus on the cationic component of ILs, role of amine group present in the compounds of interest was important to discuss. By combining theoretical inquiry with analytical techniques, the foundation for future research of applications and methods for detection is created.

In the light of the changing global environment a change in approach for its sustainability is needed. Renewable and recyclable materials are at the center of attention today. Providing research of the possible solutions for industry improvements may lead to positive changes towards green solutions. Renewable sources of energy are attractive because they always replenish, and one of them is biomass. Plants and cellulose in particular have a wide variety of applications. ILs used for cellulose production are non-toxic and recyclable with about 1 % loss. The constant development of new ILs is explained by the huge possibilities for their synthesis and their ability to combine different anions and cations constituting IL. The determination is required to start an experimental search for applications. Indirect detection was studied in this work as an effective method for the separation of ions. This technique, capillary electrophoresis, detects the presence of compounds of interest.

The structure of cellulose is of interest as a functional material that can be used in biomedical applications, the food industry, the textile industry, the construction industry, and so on. Rigid D-glucose polymer strands connected by hydrogen bonds forms amorphous

and crystalline regions. By breaking H-bonds with IL, cellulose nanofibers and nanocrystals are produced.

As was discussed in the literature review section, the ion mobility of ILs depend on the ion diffusion and the temperature. The ion mobility together with the lattice that ions form affect the speed of reactions. The nature of cations and anions constituting IL and participating in its dissolution also affect the process. A combination of right conditions will proliferate cellulose dissolution. Amines in ILs' cations have been noted to successfully break hydrogen bonds between cellulose polymers. (Li, J., et. al, 2018)

The practical part of this report was held in the Polymer laboratory at the University of Helsinki with developed organic chemistry laboratory IL compounds. The structure of the IL compound was presented in the materials part of the method section. The amine groups found in cations of every IL have shown to proliferate the process of the cellulose fibers dissolution. The N-alkyl group makes cation basic and allows it to establish bond between cations and hydroxyl groups on cellulose polymer due to its nucleophilicity. The aliphatic, non-conjugated cations are more basic and thus more reactive with cellulose.

To allow new IL into a production, it has to be determined and studies done to establish physical and chemical properties as toxicity, charge etc. The method section of this work presented CE technique, which is an analytical technique used for ion separation. It was combined with an indirect detection to create a relation between a standard and the ILs themselves based on their electrophoretic mobilities.

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