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UNIVERSITY OF TARTU
Press

Institute of Technology, Faculty of Science and Technology, University of Tartu, Estonia

Dissertation was accepted for the commencement of the degree of Doctor of Philosophy in Biomedical Engineering on 21st of April 2021 by the Council of the Institute of Technology, Faculty of Science and Technology, University of Tartu.

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Commencement: Auditorium 2075, Ravila 14A, Tartu, Estonia, at 14.15 on May 26th, 2021

Publication of this thesis is granted by the Institute of Technology, University of Tartu.

ISSN 2228-0855
ISBN 978-9949-03-603-5 (print)
ISBN 978-9949-03-604-2 (pdf)

University of Tartu Press
www.tyk.ee

ABSTRACT

The need for more sustainable progress in the industry creates a demand for solutions that fulfil the needs of society and bear no burden on nature. One promising way for this is to use soft polymer actuators. These materials are versatile and usable in many fields but often too toxic for applications that need to be used in contact with living things, like in the application of soft robotics.

Soft robotics is an emerging field enabling applications not attainable with more traditional rigid robotics. For this thesis, it was envisioned that choline ionic liquids could be used as electrolytes in soft polymer actuators, creating a harmless actuator without losing its effectiveness in functionality. Soft polypyrrole-gelatin and polypyrrole-PVdF trilayer ionic electromechanically active polymer actuators were prepared and characterized electrochemomechanically and toxicologically, also toxicologically characterizing the individual choline ILs.

The choline carboxylate ILs synthesized in this work were tested on human HeLa cell line and three different bacteria strains: Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and *Shewanella oneidensis*. Monocarboxylic choline ILs were more benign on the test organisms than di- and tricarboxylic choline ILs. The results of this work further confirm choline ILs as a group of harmless compounds. Also, the differences in toxicity of structurally similar compounds show that monocarboxylates should be preferred over di- and tricarboxylic anions when a low biological impact is essential.

The properties of the ILs can further be tailored by mixing them. Mixtures of ionic liquids tend to have lower melting points than pure components, as a eutectic mixture. This would widen the range of temperature these ionic liquids could be worked at. From the choline ILs, mixtures were made, and five binary phase diagrams tested for this thesis were eutectic. The maximum decrease in the melting point at the eutectic composition is in the mixture of choline acetate and choline 2-methylbutarate.

Finally, the electrochemomechanical characterization of the actuators with choline carboxylate ILs indicated that polypyrrole-gelatin actuators with choline ionic liquids are viable candidates for soft robotic applications. From the tested ionic liquids, choline acetate and choline isobutyrate showed the highest strain difference and highest efficiency in current to strain difference ratios with both studied membrane material. Polypyrrole-gelatin actuators with either choline acetate or choline isobutyrate outperformed the reference system containing 1-ethyl-3-methylimidazolium trifluoromethanesulfonate. Furthermore, the harmlessness of the actuators was also confirmed with disk diffusion tests. Thus, the harmless trilayered soft polymer actuator was achieved.

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications. The publications are sequenced to follow the structure of the thesis.

I. **Elhi, F.**; Priks, H.; Rinne, P.; Kaldalu, N.; Žusinaite, E.; Johanson, U.; Aabloo, A.; Tamm, T.; Põhako-Esko, K. (2020). Electromechanically active polymer actuators based on biofriendly choline ionic liquids. Smart Materials and Structures, *29*(5), 055021
II. **Elhi, F.**; Gantman, M.; Nurk, G.; Schulz, P. S.; Wasserscheid, P.; Aabloo, A.; Pohako-Esko, K. (2020). Influence of Carboxylate Anions on Phase Behavior of Choline Ionic Liquid Mixtures. Molecules, *25*(7), 1691.
III. **Elhi, F.**; Karu, K.; Rinne, P.; Nadel, K.-A.; Järvekülg, M.; Aabloo, A.; Tamm, T.; Ivaništšev, V.; Põhako-Esko, K. (2020). Understanding the Behavior of Fully Non-Toxic Polypyrrole-Gelatin and Polypyrrole-PVdF Soft Actuators with Choline Ionic Liquids. Actuators, *9*(2), 40.

Related publications:

Karu, K.; **Elhi, F.**; Põhako-Esko, K.; Ivaništšev, V. (2019). Predicting Melting Points of Biofriendly Choline-Based Ionic Liquids with Molecular Dynamics. Applied Sciences, *9*(24), 5367.

Author's contributions

In **Publications I–III**, the author was responsible for the majority of research in all phases. Co-authors contributed to the discussion, interpretation of the results, and writing of the manuscripts. Several procedures and measurements were conducted by co-authors and colleagues (Acknowledgements). For the laboratory experiments, the contributions of the author are as follows:

Publication I: The synthesis of the choline ILs, water content measurements, and FTIR characterization. Majority of the MIC and CC experiments. Measurement of the pH of solutions used in MIC and CC experiments. The fabrication of PPy-PVdF actuators. SEM and EDX imaging, electrochemomechanical characterization, and disk-diffusion tests of PPy-PVdF actuators.

Publication II: The synthesis of the choline ILs, water content measurements, and FTIR characterization. DSC sample preparation. DSC and TGA measurements.

Publication III: Fabrication of the PPy-gelatin actuators. SEM and EDX imaging, electrochemomechanical characterization, mechanical characterization, and disk-diffusion tests of PPy-gelatin actuators.

ABBREVIATIONS AND NOTATIONS

Listed in alphabetical order.

Abbreviation	Explanation
[BMIM][BF_4]	1-*n*-butyl-3-methylimidazolium tetrafluoroborate
[BMIM][PF_6]	1-*n*-butyl-3-methylimidazolium hexafluorophosphate
[BMIM][Tf_2N]	1-*n*-butyl-3-methylimidazolium bis(trifluoromethylsulphonyl)imide
[BzCh]Cl	benzyldimethyl(2-hydro-xyethyl)ammonium
[C_4mim]Cl	1-butyl-3-methylimidazoliumchloride
[C_4pyr]Cl	1-butylpyridinium chloride
[C_6mim]Cl	1-hexyl-3-methylimidazolium chloride
[Ch][2mb]	choline 2-methylbutyrate
[Ch][Ac]	choline acetate
[Ch][Bic]	choline bicarbonate
[Ch][But]	choline butonoate
[Ch][Cit]	choline citrate
[Ch][Diphosp]	choline dihydrogenophosphate
[Ch][Glu]	choline glutarate
[Ch][Ib]	choline isobutyrate
[Ch][Iv]	choline isovalerate
[Ch][Mal]	choline malonate
[EMIM][FSI]	1-ethyl-3-methylimidazolium bis(fluorosulfonyl)imide
[EMIM][OTf]	1-ethyl-3-methylimidazolium trifluoromethanesulfonate
[EMIM][TFSI]	1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide
^{1}H NMR	proton nuclear magnetic resonance
AS	as synthesized
CC	cytotoxic concentration
CC_{20}	20% cytotoxic concentration
CC_{50}	50% cytotoxic concentration
CC_{80}	80% cytotoxic concentration
CP	conductive polymer
CV	cyclic voltammetry
CV 5	actuated with triangle wave signal with 5 $mV \cdot s^{-1}$
CV 50	actuated with triangle wave signal with 50 $mV \cdot s^{-1}$
D	the peak to peak displacement divided by two in the strain difference formula
DES	deep eutectic solvent
DMEM	Dulbecco's Modified Eagle's Medium
DSC	differential scanning calorimetry
E. coli	*Escherichia coli*
EAP	electromechanically active polymer
EDX	energy-dispersive X-ray

E_m	heat fusion enthalpy of melting point
FTIR	Fourier-transform infrared spectroscopy
HeLa	human cervix carcinoma cells
IEAP	ionic electromechanically active polymer
IL	ionic liquid
L	the distance from the projection of the laser beam to the middle position of the actuator to the fixed end of the actuator in the strain difference formula
LB	lysogeny broth
MHB	Mueller Hinton Broth
MIC	minimum inhibitory concentration
MOPS	3-(N-morpholino)propanesulfonic acid
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NA	not available
ND	not detected in this study
pK_a	negative base-10 logarithm of the acid dissociation constant of a solution
PPy	polypyrrole
PVdF	polyvinylidene fluoride
Py	pyrrole
RH%	relative atmospheric humidity
S. aureus	*Staphylococcus aureus*
S. oneidensis	*Shewanella oneidensis* MR-1
SDBS	sodium dodecylbenzenesulfonate
SEM	scanning electron microscopy
sq	actuated with square wave voltage
$T_{d,\ 5\%,\ air}$	5% mass loss temperature in 80% N_2 and 20% O_2 atmosphere
$T_{d,\ 5\%,\ N2}$	5% mass loss temperature in 100% N_2 atmosphere
T_g	glass transition temperature
T_m	the melting temperature
$T_{m,\ A}$	melting point of pure component A in the eutectic mixture
$T_{m,\ B}$	the melting point of pure component B in the eutectic mixture
$T_{m,\ eu}$	melting point of eutectic mixture
W	the actuator average thickness (assuming constant thickness) in the strain difference formula
w%	water content
ΔC_p	the heat capacity at the glass-transition temperature
ε	the strain difference

1. INTRODUCTION

This thesis focuses on the development of the harmless trilayered electroactive actuator. The topmost priority for humanity has been and should be the conservation of Earth while keeping up the technological progress. This planet is our only home, and replacements are not available. Because of this situation, every solution to minimizing the impact of human activity on nature is welcome.

Growing awareness of the environmental impact of industry and the need for safe yet high-performance chemicals has generated broad interest in ionic liquids (ILs). ILs are liquid salts at ambient temperature (Clare et al., 2009). Their study has become an essential topic of research in materials science, chemical synthesis, cost efficiency of production, and reduced waste and toxic reagents, among many other globally essential issues.

Choline ILs have potential applications in the biomedical and environmental fields. These applications of ILs have been left on the side-lines because of the acute toxicity of many ILs used in other applications. The research here focuses on the rectification of this shortfall by applying choline ILs in a harmless ionic electromechanically active polymers (IEAP) actuator.

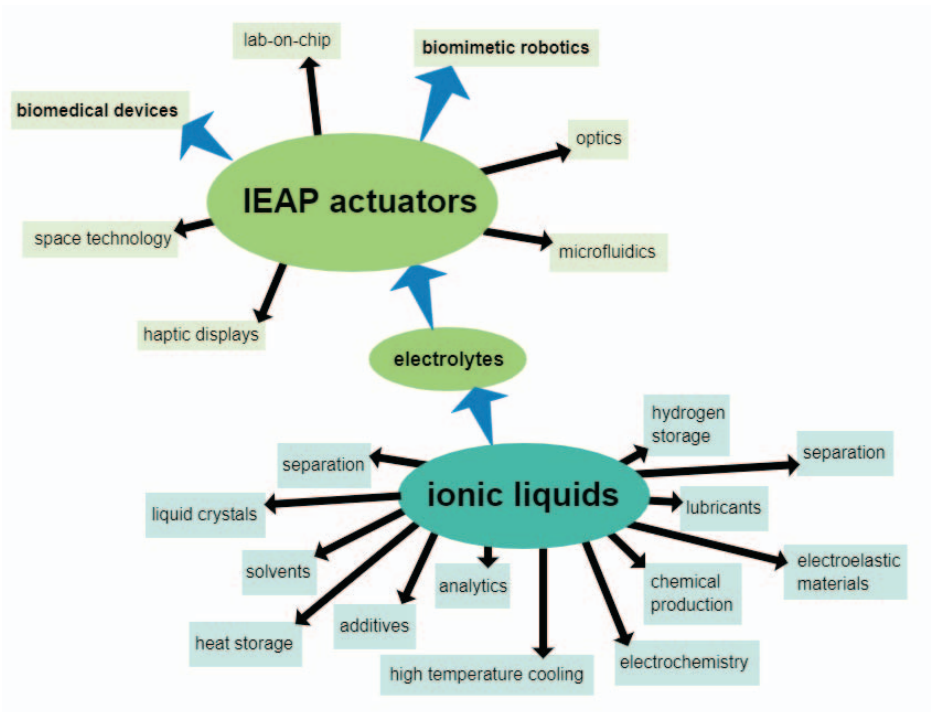


Figure 1: Selected applications of ILs and IEAP actuators.

1.1. Brief historic background

Smart materials react to environmental stimuli and can function according to their specific design. These materials have attracted the attention of the industry and have been heavily researched since the 1990s. Renewable technology uses resources that can be harvested from the environment without depleting their resources. The dream to utilize renewable resources has been at the forefront for several decades. So far, progress has been made by hybridizing renewable materials with inorganic compounds.

More widely used natural resources for smart materials are cellulose, chitin, and lignin. These materials have been combined with different carbon allotropes (carbon nanotubes, carbide-derived carbon, carbon aerogel), oxides (titanium dioxide, graphene oxide), conductive polymers (CPs), and ILs to improve their performance in smart material composites. Cellulose electroactive polymer paper has been the main biofriendly material combined with CPs to fabricate smart materials. From different configurations, trilayers have shown better performance than bilayers. (Kim et al., 2016)

Paul Walden (Walden, 1914) was the first to report a room temperature IL: ethyl ammonium nitrate (melting point: 12°C) in 1914. This area did not pick up until the 1970s and later decades. Classification by cations puts ILs into five major groups: ammonium, imidazolium, pyridinium, sulfonium, and phosphonium. Even with the same cation, ILs have shown drastically different properties when paired with different anions. (Gadilohar and Shankarling, 2017)

ILs have a long history and have been applied to IEAP actuators as electrolytes for several decades. Not much notice has been paid to their toxicity for this application. When toxicity was started to be tested for, all ILs were determined to be unsuitable for direct contact with living things. The ILs used in actuators in this work are choline ILs. Choline classifies under ammonium ILs and was first isolated from pig and ox bile in 1862 (Zeisel, 2012) by Adolph Strecker. It took three years until Oscar Liebreich was able to synthesize choline in the laboratory in 1865 (Zeisel, 2012).

1.2. The application of ionic liquids

ILs have many attractive properties, which include high stability at high temperatures (below 400°C), negligible vapour pressure, and the tailorability of features, like toxicity, melting point, solubility, acidity, among others (Bubalo et al., 2017; Egorova and Ananikov, 2018; Muhammad et al., 2012; Palumbo et al., 2017). All this has also led to the commercial use of ILs.

The limiting factor for IL use where a living thing or tissue is involved has been their toxicity when in direct contact with living things. Despite this, ILs with choline cation and carboxylate anions (1.3) are candidates for applications in environmental and medical fields because of their wide versatility in structure (Egorova and Ananikov, 2014) and tailorability (Bubalo et al., 2017; Egorova

and Ananikov, 2018; Muhammad et al., 2012) (Figure 1). According to Passino and Smith (Passino and Smith, 1987) a substance can be considered relatively harmless (or biofriendly) if it has no acute toxicity in concentrations higher than 1000 mg·L^{-1}. Practically harmless substances would have acute toxicity between 100 and 1000 mg·L^{-1}; slightly toxic between 10 and 100 mg·L^{-1}; moderately toxic between 1 and 10 mg·L^{-1}; highly toxic between 0.1 and 1 mg·L^{-1}; extremely toxic between 0.01 to 0.1 mg·L^{-1}; and supertoxic less than 0.01 mg·L^{-1}.

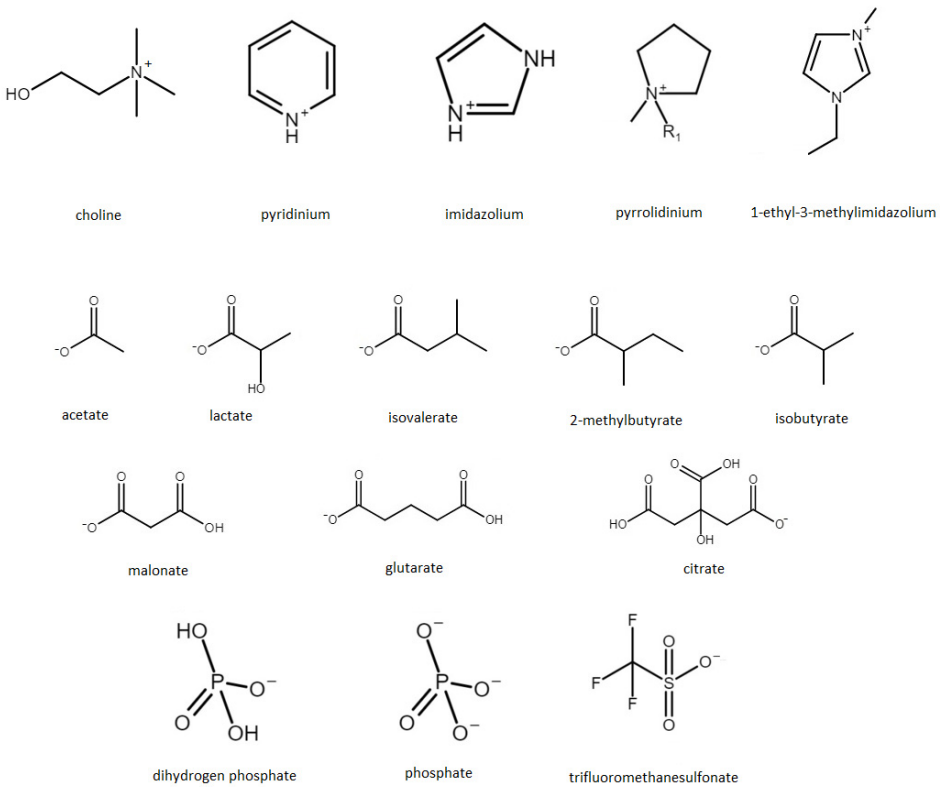


Figure 2: Selected IL cations and anions

Besides toxicity, ILs are tailorable for physical characteristics, like thermo-physical properties, solubility, acidity, among others (Muhammad et al., 2012; Palumbo et al., 2017). ILs have a lower melting point than other salts. To bring us several steps closer to their usage in low-temperature environments, ILs liquid at room temperature could be used at even lower temperatures (1.4). A low melting point is imperative for usage where humans or living things with normal or even elevated body temperature are involved.

The medical field has seen the growth in the application of soft electroactive polymer actuators. The physical performance of soft electroactive polymer

actuators does not cause harm to humans or other living creatures, but the main problem has been the toxicity of some of the components. Their electrodes and membranes can consist of materials biocompatible and entirely harmless for living things (1.5). Also, trilayered IEAPs have significantly benefitted from the use of ILs as the electrolyte, which is one of the main components responsible for their movement. Sadly, many ILs used in IEAPs are toxic and out of the question for medical and environmental applications (Egorova and Ananikov, 2014). Choline ILs are a viable alternative for more widely used ILs in IEAPs (1.3). By combining several IL for biocompatibility and peak physical characteristics, IEAP actuators can be used in many biomedical and environmental applications: biomimetic soft robotics (Sareh et al., 2013; Yeom and Oh, 2009), sensors (Pugal et al., 2010), space and other hazardous environment exploration (Punning et al., 2015), microfluidic systems (Sareh and Rossiter, 2013), among others.

1.3. The biological impact of choline ionic liquids

ILs have applications in numerous fields, including medicine and biotechnologies (Egorova and Ananikov, 2018; Hamzehzadeh et al., 2016; Housaindokht et al., 2013), where harmlessness is essential. Besides safe usage, it is crucial to consider the full life cycle of a compound together with possible routes to the environment and consequences. After the initial introduction, ILs were deemed to be benign towards the environment due to their negligible vapour pressure and overall stability. In the recent decade, the knowledge about ILs has become more nuanced, and it is generally acknowledged that in such a heterogeneous group of compounds like ILs, the toxicity also varies strongly depending on the specific cations and anions involved (Bubalo et al., 2017). It has been proven that ILs with choline (*N,N,N*-trimethylethanolammonium) cation are less toxic than the ILs with more widely used imidazolium or pyridinium cations (Couling et al., 2006) (Figure 2). Choline is biodegradable (Kortstee, 1970) and has many biological functions in the body (Zeisel and da Costa, 2009). Thus, it is considered an essential nutrient for humans (vitamin B4).

While choline is vital for building up cell membranes and neurotransmitters (Brock et al., 2007), there is a diverse body of data available concerning the biological effect of choline salts. Choline ILs have low toxicity towards marine life, e.g. *Daphnia magna* (Nockemann et al., 2007), *Vibrio fischeri* (Costa et al., 2015; Hernández-Fernández et al., 2015), and channel catfish ovary cell line (Radošević et al., 2016), brine shrimp, zebrafish, and green algae (Zhang et al., 2018). However, some choline ILs can also have more adverse effects on the life cycle of selected marine life compared to other ILs (Ventura et al., 2014; Younes et al., 2018) or common solvents (Santos et al., 2015), thus indicating that the toxicity of choline ILs is not as straightforward as first assumed.

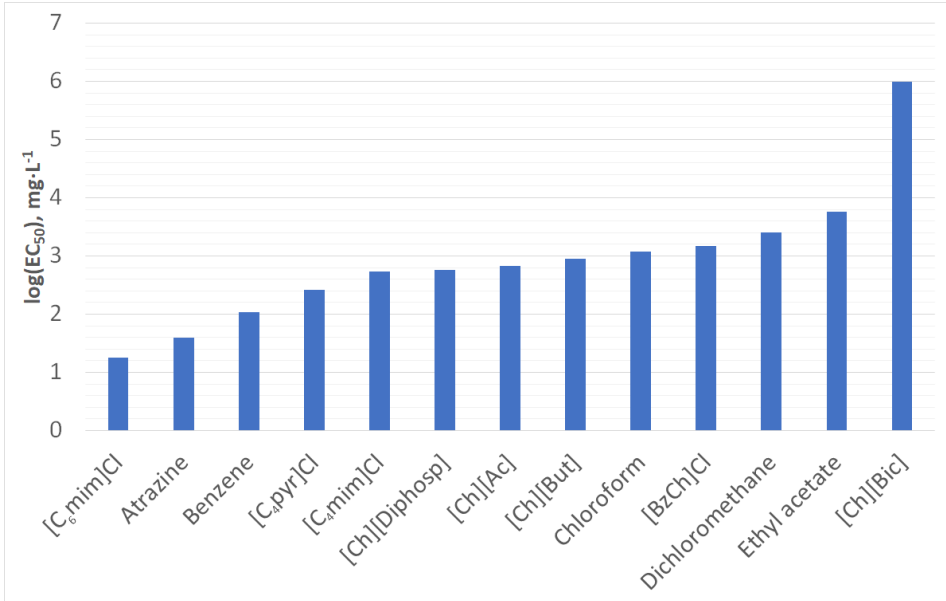


Figure 3: Vibrio Fischeri luminescence EC_{50} estimated at 30 min of exposure for selected ILs and other compounds. References: 1-butylpyridinium chloride ([C_4pyr]Cl) (Stolte et al., 2007); atrazine (Tchounwou et al., 2000); benzene, chloroform, dichlorometane, ethyl acetate (Kaiser and Palabrica, 1991); 1-hexyl-3-methylimidazolium chloride ([C_6mim]Cl), 1-butyl-3-methylimidazoliumchloride ([C_4mim]Cl), choline dihydrogenophosphate ([Ch][Diphosp]), choline acetate ([Ch][Ac]), choline butonoate ([Ch][But]), benzyldimethyl(2-hydro-xyethyl)ammonium ([BzCh]Cl), choline bicarbonate ([Ch][Bic]) (Ventura et al., 2014).

Choline ILs have low toxicity towards different microbial cultures like *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Listeria monocytogenes* (Hou et al., 2013), *Penicillium brevicompactum*, *P. glandicola*, *P. corylophilum*, and *P. diversum* (Petkovic et al., 2010, 2009).

Cytotoxicity of choline ILs is low in the case of mouse macrophage cells (Weaver et al., 2010) and different human tissue culture cells, including cervix carcinoma cells (HeLa) and keratinocytes (SK-Mel-28) (Klein et al., 2013; Rengstl et al., 2014), Caco-2 colon carcinoma cells and HepG2 hepatocellular carcinoma cells (Araújo et al., 2014), and breast cancer cell line MCF-7 (Muhammad et al., 2012).

Results from enzyme inhibition by ILs tested with cytochrome C oxidase (Costa et al., 2016) and acetylcholinesterase (Hou et al., 2013) indicated the low toxicity of choline ILs.

Choline salts find use in different applications, which require both the physicochemical properties of ILs and biofriendliness. So far, choline ILs have been used in hydrogels to combine the low biological impact of biopolymers and conductivity of ILs (Noshadi et al., 2017). Choline ILs have also been used to prepare ion gels with thixotropic nature (Sharma et al., 2014). Both types of materials

show promise in the actuator and medical fields. Also, choline ILs apply to protein binding (Ribeiro et al., 2016). Choline phosphate (Weaver et al., 2010), and choline dihydrogen phosphate (Hernández-Fernández et al., 2015) have found use in pharmaceuticals. More importantly, choline carboxylates have found use in a wider field of applications like separation processes (Mourão et al., 2014) and low toxicity microemulsions (Kunz et al., 2011).

The toxicity of carboxylates has been studied not only in the case of ILs but in a much broader context. Compared to sodium chloride, sodium salts of carboxylate anions, like acetate, can show lower minimum inhibitory concentrations when tested against *E. coli* or *S. aureus* cultures (Cebrián et al., 2014), but the difference is not drastic. On the other hand, sodium acetate and sodium citrate can be made antibacterial via the electro-activation of the aqueous solution (Liato et al., 2017; Liato and Aïder, 2017), and with the combination of highly antibacterial cations, like ionic silver (Greulich et al., 2012).

Out of all the choline salts, choline chloride seems to be the most widely tested one and has been proven harmless towards different test organisms, like human cell lines (Radošević et al., 2015). Choline phosphate (Weaver et al., 2010) and choline dihydrogen phosphate (Hernández-Fernández et al., 2015) have medical uses. There is much to strengthen the argument for the use of choline ILs in biomedical fields. Studies show that choline carboxylates, in general, interact with liposome bilayer in a weak manner (Rengstl et al., 2014) and, more specifically, choline acetate inhibits enzymes in very high concentrations (Costa et al., 2016).

From the information provided above, it concludes that the research of the biological impact of choline salts, especially choline ILs has been rather unsystematic. Choline ILs with different anions have been tested on various living things and conditions, but these results are not easy to compare across studies (e.g. same choline IL tested for bacteria and cells). The evidence for the low toxicity of choline, the exact effect of specific anions paired with choline cations, has not been thoroughly analyzed. This gap in knowledge also includes the sensitivity of particular types of organisms and tissues to choline ILs, not to mention possible mechanistic analyses. It follows that more refined research of choline ILs by varying the functional groups of the ILs used, one aspect at a time, is needed. Bacteria and marine organisms have been the focus of toxicological IL studies so far (Heckenbach et al., 2016). If choline ILs are to be applied in medicine and biotechnologies, relevant cell cultures of mammalian origin need to be more thoroughly tested. Choline ILs show outstanding physico-chemical properties, and their potential applications can be unlocked by having a more systematic approach to their toxicological research.

1.4. The thermodynamic properties of ionic liquids and their mixtures

Whether an IL can be used as an electrolyte in an IEAP actuator is directly tied to the melting point. One can tailor the melting point by choosing the right functional groups in the anions and cations, making up the ILs in question. Another way to customize the melting point, among other physical qualities of the IL, is to mix the IL with other solvents, like other ILs, which can lead the mixture, at a specific ratio, to become eutectic. That means that the melting point of the mixture drops lower than the melting points of individual components.

Eutectic mixtures can form from different choline ILs accompanied by a range of carboxylic anions. One of the benefits of choline carboxylate IL mixtures is that their anion structures and, by extension, their properties can easily be modified.

Deep eutectic solvents (DESs) are defined as eutectic mixtures formed from Lewis or Brønsted acids and bases containing ionic species (Smith et al., 2014). They are often considered even more attractive than ILs, namely because of their tailorable properties. These changes in properties attractive for synthesis, like acidity and hydrophobicity often lead to increased biocompatibility for certain cation and anion combinations. These properties make DESs exploitable in various fields, like large-scale organic extractions (Abbott et al., 2009), electrochemistry (Nkuku and LeSuer, 2007), and organic reactions (Gore et al., 2011; Ilgen and König, 2009; Imperato et al., 2005).

As attractive as DESs using ILs and molecular solvents are, they often do not retain their non-volatility and other attention-drawing properties of ILs. Mixtures made from only ionic components can be used to overcome this hurdle.

Imidazolium (Alves et al., 2010; Kick et al., 2013) and pyrrolidinium (Stolarska et al., 2016) cations have been the most popular cations for eutectic mixtures from ILs. Even after achieving the lowered melting point, mixing several ILs for a DES increases the toxicity of the mixture (Hayyan et al., 2013). This increase in toxicity makes these mixtures limited to fields outside of biomedical and other areas where the materials used must be harmless. The specific harmlessness of choline ILs is discussed in 1.3. In the case of choline carboxylates being combined into eutectic mixtures, it would be a valuable addition to the materials used in biomedicine and other fields.

Based on the literature search, the most widely tested ammonium IL in eutectic mixtures is choline chloride (Kim and Park, 2015; Meng et al., 2016; Radošević et al., 2015; Singh et al., 2012). Studies also report choline chloride mixtures with acids, alcohols, and other types of salts. (Ruß and König, 2012) From these studies, the most well-known one concerns the extreme melting point decrease of choline chloride and urea mixture (Abbott et al., 2003) (Figure 4). Although DESs from choline ILs are useful as drug solubilization vehicles (Morrison et al., 2009), the choice used in literature for different applications has been minimal. One of the goals of this work is to fill this gap.

Because ILs have a negligible vapour pressure, the maximum temperature for their practical applications is their decomposition temperature and should be measured alongside other thermal properties. For example, glass transition temperature can be used to evaluate the suitability of a substance in electrolyte applications (Mousavisafavi et al., 2013) and to predict its viscosity at a specific temperature (Fletcher et al., 2010).

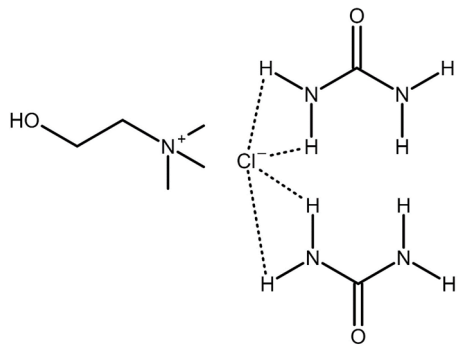


Figure 4: DES from choline chloride and urea

1.5. Ionic electromechanically active polymers and their applications

Electromechanically active polymers (EAPs) are a class of responsive materials, which can change their shape either by responding to an external stimulus or generating an electrical signal by being stimulated mechanically (Figure 5). This functionality puts them in a class of materials called “smart materials”. If an electrically driven flow of ions causes the actuation, these polymeric materials are called IEAPs, which exhibit either bending or linear movement can be synthesized in different compositions. Typically, a bending IEAP actuator consists of a trilayered structure: a membrane separating two electrodes, with an electrolyte permeating the whole material. Because of relatively simple structures, soundless operation (Madden et al., 2004), and low voltage operation (Maziz et al., 2016; Temmer et al., 2013; Yan et al., 2017), IEAP materials have gained positive attention in medicine, biology, biomimetics, and environmental monitoring. Other noteworthy properties of IEAPs include mechanical flexibility (Mirfakhrai et al., 2007), low mass density (Adewunmi et al., 2016), miniaturizability (Dubois et al., 2006), and self-sensing (Kruusamäe et al., 2015). Using these materials in any field that requires contact with living objects demands their harmlessness. Harmless materials for membranes and electrodes have already been used extensively, but harmless ILs as electrolytes suitable for soft actuators have yet to be achieved.

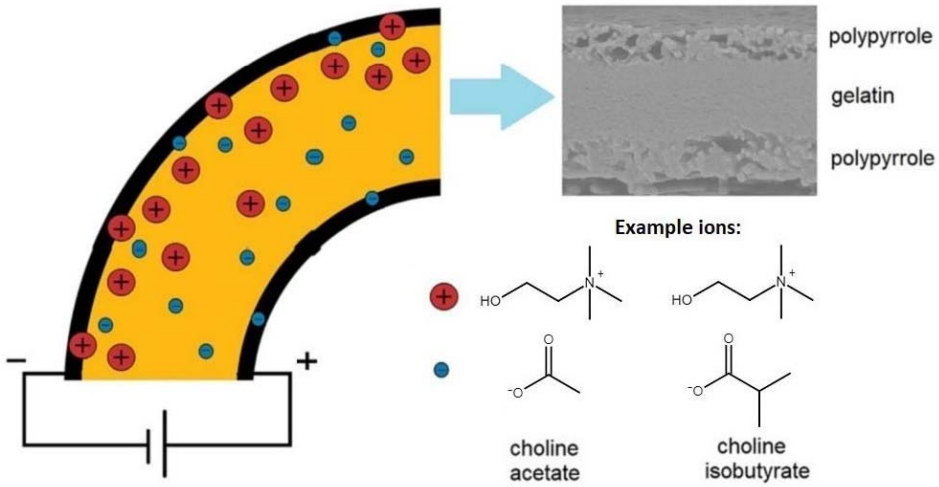


Figure 5: The working principle of an IEAP actuator illustrated using materials applied in the thesis

Electrodes of IEAPs can be made from CPs (Figure 6). IEAP materials are suitable as soft bending actuator electrodes for their high conductivity (10^2-10^3 $S \cdot cm^{-1}$), low operation voltage (<1 V), stability in the air, low cost, tailorable properties, and miniaturizability (Jager et al., 2000). The CP chosen for this research – polypyrrole (PPy) – is compatible with cell cultures (Svennersten et al., 2011), is implantable (George et al., 2005), can be used for controlled release of bioactive compounds (Svirskis et al., 2010), and can also be bio-degradable when functionalized (Shi et al., 2004). PPy is also suitable to work with nerve tissue (George et al., 2005; Wang et al., 2004), endothelial cells (Wong et al., 1994), mesenchymal stem cells (Castano et al., 2004), and human keratinocytes (Ateh et al., 2006). These results have lead to the application of PPy nanowires as biosensors to immobilize DNA (Tran et al., 2014). The right choice of dopant and conditions in PPy synthesis determine the properties of PPy, which are tunable for mechanical, electrochemical, biological properties, and stability. Most popular dopants for PPy films are different sulfonates, like sodium dodecylbenzenesulfonate (SDBS) and sodium p-toluenesulfonate (Figure 7). SDBS was chosen for this work for its biocompatibility, for example, with human primary osteoblasts (Fahlgren et al., 2015).

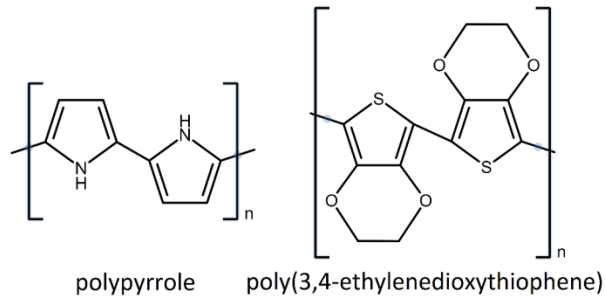


Figure 6: Selected CPs used in actuators

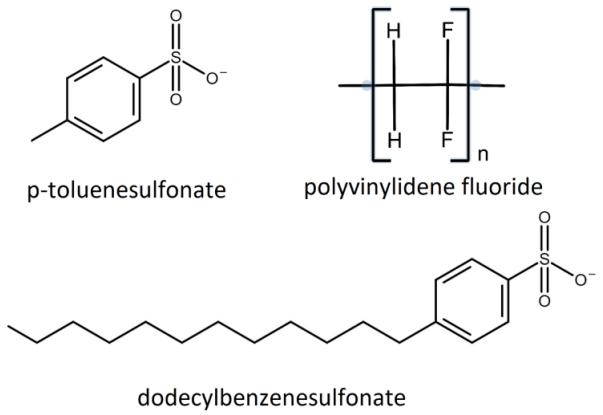


Figure 7: Selected materials used with PPy in actuators as membranes (polyvinylidene fluoride) or dopants (p-toluenesulfonate and dodecylbenzenesulfonate)

For the electrolyte to be able to move inside the IEAP actuator, the membrane must be ion permeable. Ideally, it is also inert and elastic. Usually, polymers, like PVdF (Figure 7), or fluoropolymers are possible choices. Biopolymers like gelatin (Chambers et al., 2014), chitosan (Cai and Kim, 2008), and cellulose (Kim et al., 2010) are also applicable to be membranes for IEAP constructions. From these natural polymers, gelatin was chosen, for making a non-toxic IEAP actuator, because of its biocompatibility and elasticity. It has found use in cardiac constructions (Kai et al., 2013), and similarly to electrode materials, solubility, mechanical, and other properties, can be tailored by cross-linking (Siimon et al., 2015).

However, one must remember that the biological influence of a material on its intended host system is a multifaceted issue that depends on a multitude of factors like degradation, aqueous solubility, biotic/abiotic transformation, sorption to solid phases, and toxicity. The first step towards harmless soft and smart EAP actuators in various systems is the elimination of all possible toxic com-

ponents and creating an active high-performance material made of known biofriendly components.

As established in previous paragraphs, materials with satisfactory properties electrodes and membranes already exist and can be enhanced even further. The main problem to solve is the choice or development of the electrolyte. ILs so far known to be suitable for IEAP electrolytes are toxic towards cells, bacteria, and higher organisms (Egorova and Ananikov, 2014).

1-alkyl-3-methylimidazolium tetrafluoroborates have severe toxicity issues (Frade and Afonso, 2010) but have remained widely used in IEAP actuators (Sun et al., 2018; Wang et al., 2016). Also, tetrafluoroborate salts are known to hydrolyze into hydrogen fluoride (Freire et al., 2010), which has corrosive properties. This constraint has limited IEAP actuator use in applications that need biocompatible or at least biofriendly solutions.

Because the properties of ILs are tunable with the choice of the ions, it naturally follows that so is their toxicity and other effects on living matter (Egorova and Ananikov, 2014). Current literature suggests that the smartest choice to replace harmful ILs in IEAPs for a biofriendly option, are choline based ILs (1.3).

(For more information about the materials used in biofriendly actuators, please refer to Figure 1 in Publication I.)

1.6. The goal and structure of thesis

To keep up with the technological progress, while conserving the Earth, industrial processes need to turn even more environmentally sustainable than they have become in the last half a century. Due to negligible vapour pressure ILs have gained attention as an alternative to volatile organic solvents in green technologies. However, their toxicity problem needs to be addressed before applying ILs into soft robotics.

The end goal of this thesis is to produce a harmless trilayered soft polymer actuator and characterize it thoroughly through measuring its electrochemomechanical properties, including the ionic conductivity of used electrolytes, cyclic voltammetry, strain difference, and mechanical properties. To be certain of its biological impact, the individual choline ILs must be tested on different test organisms, including bacteria and cell lines. To confirm the possibilities of enhancing the thermal properties of used electrolytes, the individual ILs need to be thermally characterized, including their melting points and the phase diagrams of their mixtures. The combination of natural polymers (e.g. gelatin) with electroactive polymers (e.g. PPy) and biologically benign ILs (e.g. choline ILs) leads to a soft polymer actuator which is all together biologically benign, without sacrificing its capability. The actuator is achieved with the right choice of starting materials and their careful combination based on the results obtained during this study.

To assess the ILs used for the making of the harmless IEAP actuator, the toxicological effects of seven choline ILs with carboxylic anions on three different microbial cultures (*Escherichia coli, Staphylococcus aureus, Shewanella oneidensis* MR-1) and human cervical cancer cells (HeLa) is studied. The hypothesis of this part of the study is that despite choline ILs being established as biologically benign substances, the changes in their anion structures still create differences in their toxicity. The anions vary by the length and branching of the alkyl chain and the number of carboxyl groups. All these characteristics are expected to affect the physico-chemical properties of the ILs, and also toxicity. Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* are popular choices for testing the influence of ILs on organisms. Gram-negative and Gram-positive bacteria react differently to ILs (Borkowski et al., 2017). Thus, both types of bacterial cultures should be tested. *Shewanella* strains are known for their interesting metabolic activities (Fredrickson et al., 2008). Based on recent literature, they have not been tested on choline ILs before. Because of these metabolic capabilities, *Shewanella oneidensis* MR-1 is used to see if this strain of bacteria can alter the chemical toxicity of choline ILs in differently oxygenated environments. HeLa cells are the choice as a human cell line very widely used for toxicology. The more detailed results for this investigation can be found in Publication I.

Once the harmlessness of the chosen choline ILs is established, the ILs are combined into mixtures aiming to find DESs that are liquid at room temperature. To properly design the IL mixtures used in any application, the thermal properties of the used materials need to be known beforehand. A useful fundamental tool for dealing with any kind of mixture system is a temperature-composition phase diagram. In this thesis, the phase behaviours of choline carboxylic IL mixtures are investigated, where the length of the carboxylic side chain of the anion and the number of carboxylic groups are varied. The hypothesis of this part of the study is that it is possible to achieve eutectic mixtures from choline ILs that would melt close to room temperature. To simplify the resulting systems and retain the low toxicity of choline ILs, the tested choline IL mixtures have only the anion varied. The eutectic points of selected systems and thermal stabilities of their components are reported in this thesis. The aim of this part of the thesis is to establish the eutectic points of the chosen choline IL mixtures and to investigate the thermal stability of the individual components. The more detailed results for this investigation can be found in Publication II.

Finally, IEAPs from harmless starting materials are fabricated. In this work, PPy-gelatin and PPy-PVdF actuators containing choline ILs as electrolytes are prepared and investigated to evaluate the potential of biofriendly actuators. Six different choline ILs are synthesized, characterized, and tested against 1-ethyl-3-methylimidazolium trifluoromethanesulfonate in both PPy-gelatin and PPy-PVdF actuators. The electrochemomechanical performance of both types of actuators is estimated. The final aim of this thesis is to make a biologically benign trilayered soft polymer actuator. The changes in the electrode material (e.g. expansion and contraction) in response to the electrical stimuli drive the

bending of an electroactive composite. However, the properties of the electrolyte play an essential role thereby. There are significant differences in actuator properties in the case of different ILs. However, all the actuators are cation-active, and all the ILs contained the same choline cation. A combination of high ionic conductivity of the IL, low viscosity, the difference in cation and anion sizes, and compatibility with the membrane and electrode structure and other aspects are known to contribute to high strain values in electroactive composites. In addition to their performance, the biological effect of complete actuators is also assessed. The more detailed results for this investigation can be found in Publications I and III.

2. MATERIALS AND METHODS

A more detailed description of the materials, their preparation, methods, and other necessary information can be found in the Materials and Methods sections of Publications I-III.

2.1. Characterization of individual choline ionic liquids

2.1.1. Synthesis and characterization of choline ionic liquids

The choline ILs used in this research were synthesized from choline bicarbonate and respective carboxylic acids (Muhammad et al., 2012). The obtained choline ILs and their relevant properties are presented in Table 1. The imidazolium-based ILs used for comparison in electrochemomechanical characterization (described in 2.3.4) and disk diffusion tests (described in 2.3.5) were 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([EMIM][OTf]) (water content 1.1%), (1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([EMIM][TFSI]), 1-ethyl-3-methylimidazolium bis(fluorosulfonyl)imide ([EMIM][FSI])).

Karl Fischer coulometric titration was used to measure the water content of the choline ILs. ^{1}H NMR spectra in D_2O and FTIR spectra were measured to identify their structures and confirm the purities of the synthesized choline ILs. (For results, please refer to 3.1.1, Table A 1, and Figure A 1.) (Publication I.)

2.1.1.1. Ionic conductivities of choline ionic liquids

One of the more critical factors to influence the performance of ILs as electrolytes in soft polymer actuators is the ionic conductivity of the ILs. Results from high-frequency real resistance impedance measurements using electrochemical impedance spectroscopy were used to calculate the ionic conductivities of choline ILs. Measurements were all done using a Parstat 2273 potentiostat-galvanostat and a Micrux microfluidic chip with interdigitated gold electrodes. The impedance of choline ILs were measured directly after synthesis. To assess the influence of water uptake on these hydroscopic IL, impedance measurements were repeated after 10 min, 1 h, and 3 weeks under ambient conditions (35.5 RH%, 25°C, normal atmospheric pressure). (For results, please refer to 3.3.1.) (Publications I and III.)

2.1.2. Microbial strains and HeLa cells

To test the effect of choline ILs on Gram-negative and Gram-positive bacteria, uropathogenic strain of *E. coli* (CFT073) and *S. aureus* DSM2569 (ATCC20213) were used as model organisms, respectively.

To see if the metabolic abilities of a *Shewanella* strain (discussed in 1.6) can influence the toxicological impact of choline ILs Gram-negative *S. oneidensis* MR-1 (ATCC700550) was used.

Human cervical cancer epithelial cells – HeLa – were used for cytotoxicity experiments of choline ILs to see the effect choline ILs have on a human cell line. (Publication I.)

2.1.3. Minimum inhibitory concentration assessments

To assess the toxicity of choline ILs towards bacteria, minimum inhibitory concentrations (MICs) were determined according to a protocol adapted from elsewhere (Andrews, 2001; Wiegand et al., 2008). Lysogeny broth was used for the inoculation environment for each microbial culture. These suspensions were put in suitable conditions for each bacterial strain.

3-(N-morpholino)propanesulfonic acid (MOPS) and tricin buffer pH 7.2 (MHB+MOPS) was added to Mueller Hinton Broth (MHB; cation adjusted) to adjust its pH. These MHB+MOPS solutions were used to prepare the stock solutions of choline ILs, which were sterilized through a 0.2 µm pore filter. These sterile solutions were further diluted to necessary concentrations (Table A 2) and used in MIC experiments.

MHB+MOPS was mixed with the resuscitated culture. 96-well microplates were used to seed the bacteria with IL solutions. Dark anoxic conditions were used to incubate *E. coli* and *S. aureus*. Dark anoxic and dark anaerobic conditions were used to incubate *S. oneidensis*. Wells with only bacteria in MHB+MOPS and wells with only MHB+MOPS were used as positive and negative controls, respectively.

The MICs of the ILs for choline ILs were determined from the optical density of the solutions in all the microtiter plates at 600 nm wavelength. Plates incubated in anoxic conditions were measured after 22 h, and for *S. oneidensis* in anoxic and anaerobic conditions after 48 h. (For results, please refer to 3.1.2.) (Supplementary Information of Publication I.)

2.1.4. HeLa CC_{50} assessments

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cell viability in this experiment. The experiment was done in fresh Dulbecco's Modified Eagle's Medium (DMEM).

DMEM was used to prepare the stock solutions of choline ILs, which were sterilized through a 0.2 µm pore filter. These sterile solutions were further diluted to necessary concentrations (Table A 2). The sterile IL dilutions were introduced to the attached cells.

Wells with only HeLa cells in DMEM and wells with only DMEM were used as positive and negative controls, respectively.

The cells were incubated in suitable conditions and optical density of the wells at 540 nm wavelength was measured to determine the CCs of the IL for HeLa cells from their concentration-response curves. (For results, please refer to 3.1.3.) (Supplementary Information of Publication I.)

2.1.5. pH of choline ionic liquid solutions

To assess the influence of ILs on the pH of the solutions, the pH of these solutions was measured with a pH/conductivity meter and confirmed using universal pH indicator paper. These solutions were made using three different solvents: pure water, MHB+MOPS, and DMEM, in the following IL concentrations: 1 M, 125 mM, and 15.65 mM. (For results, please refer to 3.1.4.) (Publication I.)

2.2. Thermal characterization of choline IL mixtures

The choline ILs used in their thermal characterization were firstly dried. This part of the study was conducted, firstly, to see whether it is possible to lower the melting points of choline ILs substantially by mixing them together. Secondly, to see what exactly in the structures of choline ILs influences this effect. For these experiments, differential scanning calorimetry measurements were conducted. Also, the upper temperature limit, before the ILs start degrading, was determined for the choline ILs. (Publication II.)

2.2.1. The preparation of choline IL mixtures for differential scanning calorimetry

All differential scanning calorimetry (DSC) samples were prepared in an argon environment and taken out to ambient conditions only for placement in the DSC device. In the search for choline IL eutectic mixtures, the ILs were combined as binary mixtures in an argon environment. These mixtures were prepared, taking count of their molar ratios from 0% to 100% of the first component, with 10% steps. These mixtures were weighed in the range of 2–9 mg to DSC crucibles and crimp sealed.

2.2.2. Differential scanning calorimetry measurement protocol

The differential scanning calorimetry (DSC) measurements were done in an inert nitrogen atmosphere under normal pressure 1 bar, with an empty sample pan with a small hole in the lid as the reference. Most samples were cooled from room temperature to -100°C at a fast screening rate of $10° \cdot min^{-1}$ and kept at an isotherm for 30 min. Afterward, the samples were heated at a slow temperature screening

rate of 5°·min^{-1} to 100°C. [Ch][Iv] and [Ch][Cit] were measured from -50°C to 150°C with the same screening rates for cooling and heating. Because samples with [Ch][Mal] and [Ch][Glu] often had trouble solidifying even with long isotherms, these samples were cooled to -100°C at a screening rate of 10°·min^{-1} and kept at an isotherm for 3 h, then heated to 100°C under nitrogen with a temperature screening rate of 5°·min^{-1}. The midpoint of the glass transition part of the DSC curve was taken as the glass transition temperature of the sample. The uncertainty of melting point and glass transition temperature results was estimated to be 1°C.

After measurements and fitting of eleven melting points with a 10% ratio difference, the eutectic ratio was determined for eutectic mixtures. An additional mixture was prepared for that ratio and measured.

These experiments were conducted using NETZSCH DSC 204 F1 Phoenix® differential scanning calorimeter equipped with nitrogen cooling accessories. (For results, please refer to 3.2.1., 3.2.2, and 3.2.3.)

2.2.3. Thermogravimetric measurements

Samples were placed in Al_2O_3 containers and heated initially from room temperature to 40°C. After that, the samples were heated with a heating rate of 10°·min^{-1} to 300°C. Two types of atmospheres were used: N_2 and synthetic air (80vol% N_2 and 20vol% O_2). NETZSCH STA449F3 instrument was used for these experiments. (For results, please refer to 3.2.4.)

2.3. Synthesis and characterization of PPy-gelatin and PPy-PVdF actuators

To achieve the final aim of this thesis – the harmless trilayered soft polymer actuator – PPy-gelatin and PPy-PVdF actuators were made via electrochemical synthesis of PPy on corresponding membranes and afterward soaking the electrode-membrane-electrode laminate into choline ILs. After being certain of the electrochemomechanical capabilities of the actuators, their harmlessness was tested via disk diffusion test.

2.3.1. Gelatin and PVdF membranes

The actuators used to test the performance of synthesized choline ILs were made using commercial PVdF (Millipore Immobilion-P; thickness of 110 μm, a pore size of 0.45 μm, porosity of 70%.) and electrospun gelatin membranes (thickness of 110 μm void space of 65%). (Publication III.)

2.3.1.1. Ionic conductivities of PVdF and gelatin membranes

Impedance spectroscopy described in 2.1.1.1 was used to determine the ionic conductivities of the membranes soaked with ILs, which is one of the more important factors to determine the functionality of the actuators. Choline ILs were kept under a nitrogen atmosphere, and both the electrospun gelatin and the commercial PVdF membranes were immersed in these choline ILs. The device used to measure impedance for membranes with choline ILs consisted of two round gold contacts (d = 8 mm), attached to a thickness gauge. Thus, the sample thickness could also be taken into account. The ionic conductivities were measured immediately after the membranes were removed from ILs and after 10 min in ambient conditions. (For results, please refer to 3.3.1.) (Publications I and III.)

2.3.2. Electrochemical synthesis of PPy electrodes

To be able to conduct the electrochemical synthesis of PPy on an ion-permeable non-conductive membrane, the membrane surface has to be made conductive. Both commercial PVdF and electrospun gelatin membranes were sputter-coated with a 20 nm gold layer.

PPy electrodes were deposited on both sides of the gold-coated membranes via galvanostatic electrochemical synthesis (Temmer et al., 2012), at -23°C±2°C, applying current density of 0.1 $mA \cdot cm^{-2}$ for 20 000 s in a one-compartment electrochemical cell, controlled with PARSTAT 2273 potentiostat/galvanostat. The gold-coated membrane and the symmetrical stainless steel (AISI 316L) mesh sheets were the anode and the cathodes, respectively. The synthesis was done in a mixture of MilliQ water and mono ethylene glycol (MEG) in a volume ratio of 1:1. The solution contained 0.2 M pyrrole (Py) and 0.2 M SDBS that later became the dopant in the PPy-electrode structure.

The laminate was cut into 3 mm x 14 mm strips that were soaked in choline ILs for a minimum of 72 h. After that, they underwent electromechanical characterization. (For results, please refer to 3.3.2.) (Publications I and III.)

2.3.3. Structure of obtained PPy actuators

After immersion to choline ILs, and the cross-sections of some samples were investigated with scanning electron microscopy (SEM; back-scattered electron detector, acceleration voltage of 15 kV). To investigate the distribution of $[DBS]^-$ ions, energy-dispersive X-ray (EDX) sulfur mapping was also conducted. (For results, please refer to 3.3.3.) (Publications I and III.)

2.3.4. Electro-chemo-mechanical characterization of PPy-gelatin and PPy-PVdF actuators

2 mm of the actuators was mounted sideways between gold contacts, and the rest (12 mm) was free to move. The displacement of the actuator was measured at 5 mm from the fixed end using a laser displacement meter. The actuators were driven using the following signals: square wave signal between ±1 V at 0.001 Hz, with neutral voltage period before each polarization step and triangular signals between ±1 V with scan rates: 5 $mV \cdot s^{-1}$ and 50 $mV \cdot s^{-1}$. Maximum strain differences were calculated relative to the middle position of a full bending cycle according to the formula (Sugino et al., 2009):

$$\varepsilon = \frac{2 \cdot D \cdot W}{L^2 + D^2}, \quad (1)$$

where ε is the strain difference, D is the peak to peak displacement divided by two, W the average actuator thickness (assuming constant thickness), and L is the distance from the projection of the laser beam to the middle position of the actuator to the fixed end of the actuator. The frequency of the applied square wave potential was 0.001 Hz and amplitude ±1 V.

Cyclic voltammetry (CV) of the actuators was measured using a PARSTAT 2273 potentiostat. Five cycles in the range of ±1 V, with a scan rate of 5 $mV \cdot s^{-1}$ and 50 $mV \cdot s^{-1}$, were recorded.

A mirrored logarithmic sweep sine signal was used for the frequency response characterization, ranging from 0.001 Hz to 400 Hz and having an amplitude of ±1 V.

For mechanical characterization, the synthesized PPy actuators were mounted side-ways between two gold pincer contacts. The resonance frequency of the mechanically excited oscillating actuator was measured for three different free lengths using a laser displacement meter. The equivalent elastic modulus of the composite was calculated from this data (Klesewetter et al., 1992).

All measurements described here were done under ambient conditions (35.5 RH%, 25°C, normal atmospheric pressure). (For results, please refer to 3.3.4.) (Publications I and III.)

2.3.5. Disk diffusion tests for PPy actuators

To test the harmlessness of the PPy-gelatin and PPy-PVdF actuators disk diffusion method adapted from an established method (Bauer et al., 1966; Hudzicki, 2009) was used. To examine the influence of actuators for both Gram-negative and Gram-positive bacteria *E. coli* and *S. aureus* (2.1.2) were used as test organisms. To ensure the repeatability and comparability of the results, 0.5 McFarland standard was used to adjust the density of the bacterial suspensions at 600 nm wavelength.

4x4 mm squares of PPy-gelatin and PPy-PVdF actuators with choline ILs were placed on the surface of the inoculated LB agar where *E. coli* and *S. aureus* were grown. Six of the seven choline ILs were liquid at room temperature at ambient conditions and thus suitable for use in actuators were compared with PPy-gelatin and PP-PVdF samples with [EMIM][OTf], [EMIM][TFSI], and [EMIM][FSI]. (For results, please refer to 3.3.5.) (Publications I and III.)

3. RESULTS AND DISCUSSION

In the present study, the fabrication of actuators from biocompatible and biofriendly starting materials was achieved. The aim of the study was also to investigate how the performance of the actuators is influenced by the different choline ILs as the electrolytes and by the membrane material.

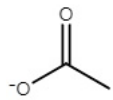
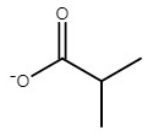
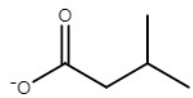
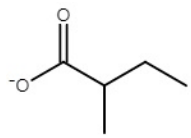
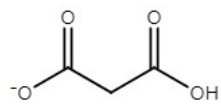
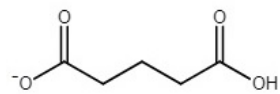
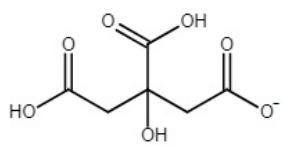
Firstly, the general properties of the synthesized ILs are described, with an emphasis on their toxicological properties (Publication I). Secondly, the results from thermophysical testing of mixtures from choline ILs are presented (Publication II). Finally, a comprehensive overview of the results pertaining to the use of choline ILs in PPy-gelatin and PPy-PVdF actuators is given (Publications I and III).

3.1. Toxicological properties of choline ILs

3.1.1. Synthesis and characterization of choline ILs

For the choline ILs used in this work, their yields, water contents after synthesis and drying, and the pK_as of the corresponding acids of their anions are in Table 1. Most of the syntheses resulted in >90% yields. For DSC and TGA measurements (measured as described in 2.2), choline ILs were dried further to minimal water content. The ^{1}H NMR and IR spectra (measured as described in 2.1.1) for all synthesized choline ILs are in Appendix 1 (Table A 1 and Figure A 1).

Table 1: The relevant properties of synthesized choline ILs

ionic liquid	anion structure	yield, %	water content, %	acid pK_a
choline acetate ([Ch][Ac])		97	5.4	pK_a = 4.76 (Braude et al., 1955)
choline isobutyrate ([Ch][Ib])		96	3.7	pK_a = 4.86 (Bjerrum et al., 1957)
choline isovalerate ([Ch][Iv])		93	3.6	pK_a = 4.77 (Lide, 2005)
choline 2-methylbutyrate ([Ch][2mb])		81	3.7	pK_a = 4.80 (Lide, 2005)
choline malonate ([Ch][Mal])		95	0.1	pK_{a1} = 2.83 pK_{a2} = 5.69 (Braude et al., 1955)
choline glutarate ([Ch][Glu])		89	0.5	pK_{a1} = 4.34 pK_{a2} = 5.42 (Braude et al., 1955)
choline citrate ([Ch][Cit])		86	0.7	pK_{a1} = 3.128 pK_{a2} = 4.761 pK_{a3} = 6.396 (Dean and Lange, 1999)

3.1.2. Growth inhibition of bacteria by choline ionic liquids

Microorganisms are outstanding for toxicological studies because of several attractive properties, including their impact on industrial and environmental research and the robustness of the toxicity assays. To assess the antibacterial activities of selected choline ILs, Gram-negative *E. coli* and *S. oneidensis* MR-1, and Gram-positive *S. aureus* (described more in detail in 2.1.2) were the choice model organisms for their short generation time and relevance in bio-technology and microbiology. Their MICs (measured as described in 2.1.3) are in Table 2 and Table 3. The structures of the carboxylic anions have a definite impact on the harmfulness of choline ILs. [Ch][Ac] was the most harmless out of all the tested choline ILs, which is also in line with MIC values previously

found in the literature (Hou et al., 2013) for other bacterial strains. This research confirms the low toxicity of choline ILs towards bacteria. Existing data for choline ILs with other types of anions based on other carboxylates (Petkovic et al., 2010) and amino acids (Hou et al., 2013) supports the argument that choline ILs have extraordinarily high MIC values compared to other popular substances (Figure 8). The MIC of [Ch][Ac] is one order of magnitude higher than [BMIM][OTf]. Even [Ch][Mal], which is one of the most toxic choline ILs tested in this work, has a higher MIC than other ILs and substances in this comparison. Triclosane and polihexanide are depicted to emphasize the drastic difference between choline ILs and substances antiseptics with differences in several orders of magnitude.

As stated in 1.2, a substance can be considered relatively harmless if it has no acute toxicity in concentrations higher than 1000 $mg \cdot L^{-1}$ (Passino and Smith, 1987). For all the choline ILs tested for this thesis, the highest of these concentrations, which needs to be exceeded for the tested choline IL to be considered relatively harmless, is 6.13 mM for [Ch][Ac]. This concentration is exceeded by all choline ILs used in this thesis for both MIC and CC experiments (Table 2, Table 3, Table 4, Table 5).

Table 2: The MIC values in mM for the tested choline ILs towards *E. coli*, *S. aureus*, and *S. oneidensis* MR-1, after 22 h anoxic, and 48 h of anoxic and anaerobic exposure. The values are presented with a comparative colour scale, and it should be noted that even the lowest MIC value in the table (22.5 mM) is still very high.

	Escherichia coli	*Staphylococcus aureus*	*Shewanella oneidensis* MR-1		
	anoxic 22h	anoxic 22h	anoxic 22h	anoxic 48h	anaerobic 48h
[Ch][Ac]	600	> 1000	250	350	350
[Ch][Ib]	500	> 1000	200	300	300
[Ch][Iv]	400	600	300	350	350
[Ch][2mb]	450	850	150	300	300
[Ch][Mal]	140	125	45	45	45
[Ch][Glu]	125	90	45	45	45
[Ch][Cit]	125	62	22.5	45	45

Table 3: The MIC values in mg·L for the tested choline ILs towards *E. coli*, *S. aureus*, and *S. oneidensis* MR-1, after 22 h anoxic, and 48 h of anoxic and anaerobic exposure. The values are presented with a comparative colour scale, and it should be noted that even the lowest MIC value in the table (6615 mg·L) is still very high.

	Escherichia coli	*Staphylococcus aureus*	*Shewanella oneidensis* MR-1		
	anoxic 22h	anoxic 22h	anoxic 22h	anoxic 48h	anaerobic 48h
[Ch][Ac]	97800	> 163000	40750	57050	57050
[Ch][Ib]	95500	> 191000	38200	57300	57300
[Ch][Iv]	82000	123000	61500	71750	71750
[Ch][2mb]	92250	174250	30750	61500	61500
[Ch][Mal]	28980	25875	9315	9315	9315
[Ch][Glu]	29375	21150	10575	10575	10575
[Ch][Cit]	36750	18228	6615	13230	13230

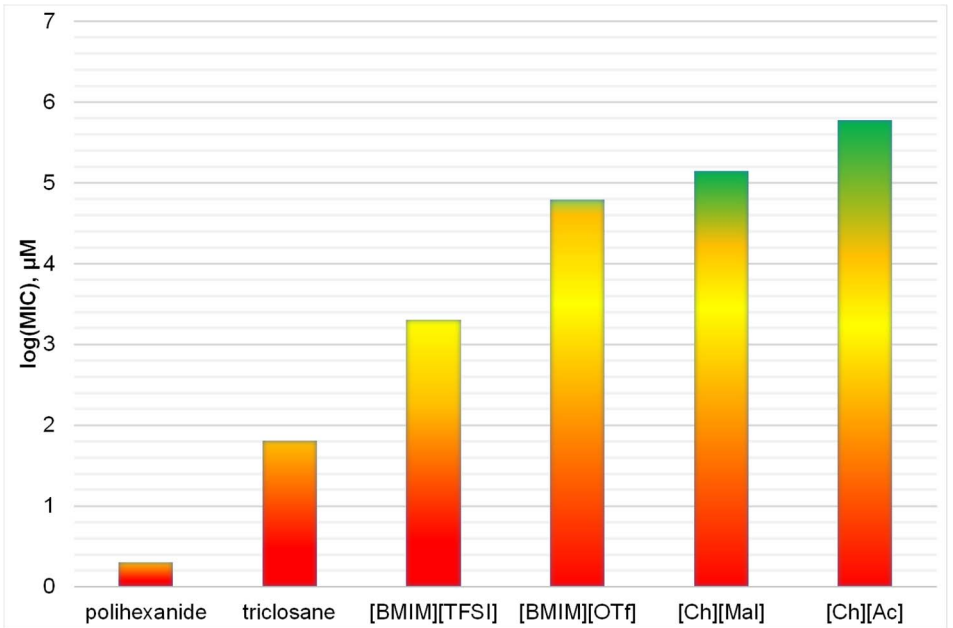


Figure 8: The logarithmic MICs (µM) of chosen substances towards *E. coli*: antiseptics polihexanide and triclosane for 20 h (Assadian et al., 2011), ionic liquids from literature 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([BMIM][TFSI]) 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([BMIM][OTf]) (upper limit) for 24 h (Łuczak et al., 2010), and two ionic liquids from this study for 22 h.

All the ILs used in the current study were hydrophilic and miscible with water in all proportions. It has been reported previously that hydrophobic ILs show higher toxicity in a suspension culture than hydrophilic ones (Lee et al., 2005). ILs in the present study were selected to systematically evaluate the toxicological effect of the following structural units in the carboxylate anions: length and branching of the alkyl chain, number of carboxyl groups.

An increase in the length and branching of the alkyl chains of carboxylic anions decreased the MIC values of many choline ILs. This is very likely tied to the increased hydrophobicity and interactions with biological membranes (Ventura et al., 2013), which has been observed in previous studies concerning the IL anions (Petkovic et al., 2010; Weaver et al., 2010) and cations (Borkowski et al., 2017; Zheng et al., 2016). It is not an IL-specific interplay (Sardessai and Bhosle, 2002) of how specific moieties affect the toxicity of substances in a general way. Any additional carbons added to the alkyl side chain increase the interaction between the bacteria and ILs. Despite this, looking at the results for [Ch][Iv] and [Ch][2mb] shows that the branching, i.e. the position of the methyl group, plays a minor role in IL toxicity also. Choline monocarboxylates demonstrated much higher MICs than [Ch][Mal], [Ch][Glu], and [Ch][Cit]. The probable cause for this effect is the decreased pH due to extra carboxylic groups of di- and tricarboxylic anions (3.1.4).

The reason behind the selection of the bacteria for the current study was to study the toxicological effect of ILs on both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. Gram-negative bacteria are covered by a lipopolysaccharide layer, which makes their cell walls less permeable (Samorì, 2011) and gives them extra protection against external biocidal agents. Gram-positive bacteria have only one thick porous cell wall. Hydrophobic alkyl chains can penetrate the cell wall of Gram-positive bacteria more quickly and lead to cell death. (Binder, 2008; Palermo et al., 2009) Consequently, Gram-positive bacteria are usually more susceptible to the effects of ILs with alkyl-side-chains when compared to Gram-negative bacteria (Li et al., 2012; O'Toole et al., 2012).

Despite this, gram-negative *E. coli* seem to be more susceptible to the antibacterial effect of choline monocarboxylates than Gram-positive *S. aureus* when it comes to experiments conducted in feeding solutions. Even with the general trend being that Gram-negative bacteria are more durable against environmental toxins and other effects, there are studies, which suggest that choline ILs have a different kind of an effect on Gram-negative and Gram-positive bacteria. It was found that Gram-negative *E. coli* and *S. enteritidis* were more negatively affected by choline amino acid ILs than the Gram-positive *S. aureus* and *L. monocytogenes* (Hou et al., 2013). Similar results were observed testing choline-cation containing DESs on *E. coli* (Wen et al., 2015).

The third bacteria strain used in the present study was *Shewanella oneidensis* MR-1, which is a model species of the genus, has been reported to contain at least 42 putative C-type cytochromes, of which 80% are located in the outer membrane (Heidelberg et al., 2002; Meyer et al., 2004), allowing it to couple organic matter oxidation to the reduction of wide range of electron accep-

tors (Fredrickson et al., 2008; Kracke et al., 2015). The aim of testing the choline ILs on this microbial culture was to investigate, are there any chemical changes occurring with the ILs as a result of bacterial metabolism, which might alter the toxicity of the ILs.

The anoxic and anaerobic 48 h MICs for *S. oneidensis* MR-1 showed the same results (Table 2, Table 3). From that, it follows that *S. onedensis* did not influence the toxicity of tested choline ILs. It is important to mention also that *S. oneidensis* MR-1 displays more sensitivity towards the tested choline ILs in an anoxic environment after 22 h of incubation.

The contrast between the results can be explained by various mechanisms that include the disruption of metabolic pathways and the interaction of choline ILs with the membranes of the test organisms. Overall, when studying the bio-friendliness or toxicity of ILs (or, in fact, any salts) with organic ions, specific sensitivities of certain types or species of organisms should not be excluded. While designing new ILs or choosing ones for a particular bio-interaction scenario, avoiding ions that are closely connected to the main metabolic pathways of cells, with potential inhibitory effect to those cycles, is recommended.

3.1.3. CC_{50} of choline ionic liquids exposure to HeLa cells

ILs needed in biomedical applications need to have remarkable biocompatibility towards cell lines. HeLa cells are widely tested cell line and easily comparable with other studies. The CC_{50} results (measured as described in 2.1.4) are in Table 4 and Table 5.

Table 4: The CC_{20}, CC_{50}, and CC_{80} values in mM for the tested choline ILs towards HeLa cells. The values are presented with a comparative colour scale, and it should be noted that even the lowest CC value in the table (8 mM) is still very high.

	CC_{20}	CC_{50}	CC_{80}
[Ch][Ac]	36	57	91
[Ch][Ib]	62	64	66
[Ch][Iv]	15	25	42
[Ch][2mb]	42	50	61
[Ch][Mal]	21	23	26
[Ch][Glu]	24	28	32
[Ch][Cit]	8	10	13

Table 5: The CC_{20}, CC_{50}, and CC_{80} values mg·L for the tested choline ILs towards HeLa cells. The values are presented with a comparative colour scale, and it should be noted that even the lowest CC value in the table (2352 mg·L) is still very high.

	CC_{20}	CC_{50}	CC_{80}
[Ch][Ac]	5868	9291	14833
[Ch][Ib]	11842	12224	12606
[Ch][Iv]	3075	5125	8610
[Ch][2mb]	8610	10250	12505
[Ch][Mal]	4347	4761	5382
[Ch][Glu]	5640	6580	7520
[Ch][Cit]	2352	2940	3822

Monocarboxylic choline ILs showed the lowest cytotoxicity, as previously shown lowest microbial toxicity. The ILs with the lowest CC_{50} values towards HeLa are [Ch][Ac], [Ch][Ib], and [Ch][2mb]. When comparing the structures and cytotoxicity of [Ch][Iv], it can be noted that this IL has the toxicity of more comparable dicarboxylic and tricarboxylic choline ILs. Here the importance of the branching of alkyl sidechains of carboxylic anions concerning their toxicity can be seen. [Ch][Mal], [Ch][Glu], and [Ch][Cit] had lower CC_{50} values than all monocarboxylic choline ILs. These CC_{50} values are comparable to the microbial toxicity of these di- and tricarboxylic choline ILs towards the tested bacteria described in 3.1.2. The general effect of di- and tricarboxylic anions on the pH of growth solutions is discussed more in 3.1.4. However, it can be suspected that the main mechanism of how these choline ILs lower the CC_{50} for HeLa, is through them lowering the pH of the environment. Despite this, the CC_{50} values for all choline ILs are still very high and can be considered benign towards HeLa (Figure 9). Other studies confirm this (Klein et al., 2013; Rengstl et al., 2014). [Ch][Ac] shows CC_{50} values almost six times higher than [BMIM][PF_6] in this comparison. Other imidazolium ILs in this comparison have CC_{50} values lower than the choline ILs by an order of magnitude or even more.

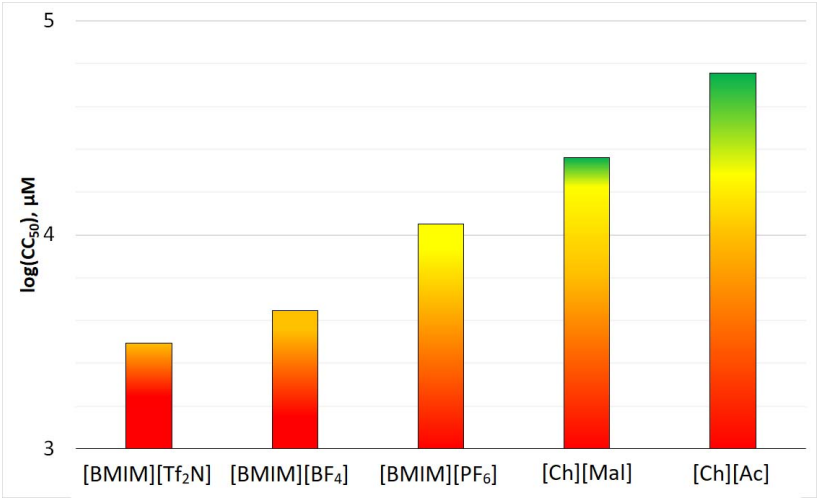


Figure 9: The logarithmic CC_{50}s (µM) of chosen substances towards HeLa: ionic liquids from literature 1-*n*-butyl-3-methylimidazolium bis(trifluoromethylsulphonyl)imide ([BMIM][Tf_2N]), 1-*n*-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-*n*-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) for 48 h (Cvjetko et al., 2012), and two ionic liquids from this study for 48 h.

3.1.4. The pH of choline ionic liquid solutions

There is a possibility that the influence of di- and tricarboxylic choline ILs on the pH of the growth media was the leading cause of their detrimental effect on bacteria and human cells. pH measurements were carried out in pure distilled water, DMEM, and MHB+MOPS at different concentrations (as described in 2.1.5). The pH of choline ILs with monocarboxylic anions remained around neutral. The pH of choline ILs with di- and tricarboxylic anions are in Table 6, and their corresponding pK_as are in Table 1.

Table 6: pH of the di- and tricarboxylic choline IL solutions in pure water, DMEM, or MHB+MOPS

medium	pure water			DMEM			MHB+MOPS		
IL/concentration, mM	1000	125	15.65	1000	125	15.65	1000	125	15.65
[Ch][Mal]	3.1	3.2	3.5	3.6	4.3	6.4	4.0	4.3	6.4
[Ch][Glu]	3.8	3.9	4.3	4.2	4.5	6.5	4.2	6.9	7.0
[Ch][Cit]	2.5	2.6	2.9	2.5	3.2	5.5	2.7	3.5	5.5

The increased number of carboxylic groups correlates with the sharp decrease of pH in both growth mediums from 7.4 to 5.5, even at the lowest tested [Ch][Cit] concentration (15.65 mM). Although most of the cell lines can exist in mildly acidic environments, diversion of pH from the optimal range can still affect the metabolic cycle and cellular growth. A change of even one unit can affect the growth of HeLa cells severely (Barton, 1971; Mackenzie, 1961; Xie et al., 2011). This kind of drop in pH is very apparent in all choline ILs solutions with dicarboxylic and tricarboxylic anions.

S. aureus can live at a pH range of 4-10 (Langsrud and Mat, 2009) with the optimal range of 6-7 (ICMSF, 1996; Stewart, 2003). *E. coli* can live at the pH up to 8.8 (Zilberstein et al., 1982) and can stay alive several hours under the pH 2-3 without multiplying (Goodson and Rowbury, 1989; Gorden and Small, 1993), but is accustomed to pH 5.5-7, being part of the large intestine microflora. *S. oneidensis* MR-1 is sensitive to shift in pH: even a slight change can lead to a significant drop in cell viability (Biffinger et al., 2008). The decrease of pH in [Ch][Cit], [Ch][Mal], and [Ch][Glu] solutions also showed a drastic drop in bacterial growth.

For monocarboxylates, the pH stayed neutral even at the higher concentrations. These ILs also showed higher MIC and CC values (Table 2, Table 3, Table 4, Table 5). Their differing MIC and CC values were due to other structure-related effects other than pH change and were most likely due to the effects caused by the branching and length of the alkyl side chains of their anions.

The MICs for all bacteria and the HeLa culture appear to be related to the pH, which is markedly lower for the ILs with di- and tricarboxylic anions. On the other hand, several other tendencies can be observed that cannot be traced back to the pH variations. Cell-level toxicity depends on a vast number of factors, including active and passive transport in and out of the cell, specific interactions with the membrane, the DNA, participation, or close relation to the metabolic cycles. Therefore, many specific structure-activity relations are explainable, which may differ between different species and strains.

3.2. The thermophysical properties of choline ionic liquids and their mixtures

3.2.1. The thermophysical properties of individual choline ILs

After establishing the chosen choline ILs as biologically benign, it was also essential to research the possibility of lowering the melting point of the electrolyte used in the actuators. This would present an opportunity to use otherwise solid choline ILs as a liquid mixture at lower temperatures. The hypothesis of this part of the thesis is that it is possible to achieve a eutectic mixture by mixing several choline ILs together. It is also essential to know how the structure of the carboxylic anions influences this effect.

Seven different choline carboxylate ILs were investigated to study the influence of anions on the phase behaviour of choline ILs (Table 7). The ILs were selected similarly to the toxicological experiments, sharing a choline cation and differing in carboxylic cations, with little modifications (i.e., branching and length for the monocarboxylic choline ILs). The melting point of each IL and the solid-liquid phase diagrams of their mixtures were obtained from DSC data (measured as described in 2.2.1 and 2.2.2).

Table 7: Key thermodynamic properties of tested ILs

IL	T_g	ΔC_p	T_m	E_m	w%	$T_{d,\ 5\%,\ N2}$	$T_{d,\ 5\%,\ air}$
[Ch][Ac]	-65	1.4	81	115	2.7	178	176
[Ch][Ib]	-66	1.1	68	88	0.1	178	172
[Ch][Iv]	NA*	NA*	61	95	0.0	176	176
[Ch][2mb]	-61	0.9	90	70	0.3	175	174
[Ch][Mal]	-67	1.4	ND**	ND**	0.2	165	129
[Ch][Glu]	-58	1.8	ND**	ND**	0.0	212	204
[Ch][Cit]	NA*	NA*	105	116	0.0	169	167

T_g – glass transition temperature in °C
ΔC_p - the heat capacity at the glass-transition temperature in $J \cdot g^{-1} \cdot °C^{-1}$
T_m – the melting temperature in °C
E_m – heat fusion enthalpy of melting point in $J \cdot g^{-1}$
w% – water content after additional drying in a regenerating argon environment in %
$T_{d,\ 5\%,\ N2}$ – 5% mass loss temperature in 100% N_2 atmosphere in °C
$T_{d,\ 5\%,\ air}$ – 5% mass loss temperature in 80% N_2 and 20% O_2 atmosphere in °C
* NA – not available
** ND – not detected in this study

The melting points of tested choline ILs and various other vital properties, including glass transition and decomposition temperatures, are in Table 7. The data published in literature concerning choline carboxylate ILs have underestimated their melting temperatures. The melting points for [Ch][Ac] published in the literature are 51°C (Fukaya et al., 2007) and 72°C (Muhammad et al., 2012), which is in contrast with the melting temperature measured in this research 81°C. For [Ch][Ib], the difference is even more significant: 35°C (Petkovic et al., 2010) as opposed to 68°C in this study. The differences between the melting points can be explained with the difference in water content of the samples analyzed, which is not always measured or reported only as an upper limit for all ILs synthesized in the study. The importance of water content is apparent with the melting point of [Ch][Cit], which was 105°C in this research but 103°C with more water content in another publication (Mourão et al., 2014). Another highly possible explanation is the preference of onset of melting peaks to top values of melting peaks by some authors.

The decline of melting points of monocarboxylic choline ILs as the length of alkyl side-chains increases (except for [Ch][2mb]) is a trend that could be explained by the fact that larger and unsymmetrical ions tend to give ILs a lower melting point. This is because large unsymmetrical and bulky ions decrease the Coulombic attraction forces between ions and make it stereologically difficult to fit into a lattice, even at low temperatures.

The glass-transition values show a lesser discrepancy with values from the literature than melting point values. [Ch][Mal] has only a 2°C difference between the value of -67°C reported in this study and -65°C reported in the literature (Mourão et al., 2014). There is a more significant discrepancy between the glass transition values for [Ch][Glu] of -58°C reported here and -67°C in another study (Mourão et al., 2014). The difference in these glass transition temperatures can be attributed to the difference between the cooling rates that precede heating between studies.

In a recent study (Karu et al., 2019) several choline IL melting points, also measured for this thesis, were predicted: 57°C for [Ch][Ac]; 71°C for [Ch][Ib]; 62°C for [Ch][Iv] and [Ch][2mb]; 75°C for [Ch][Cit]. This was done to assess the correspondence between the experiment and simulation. The most accurate from these predictions are the melting points for [Ch][Ib] and [Ch][Iv]: 3° and 1° difference from the measured results, respectively. Overall, the root-mean-square error of the method is 18-24°, which is acceptable for the method to be used in the future also.

3.2.2. Eutectic choline IL mixtures

To research the possibility of enhancing the properties of electrolytes used in IEAPs, binary mixtures (two-component) of choline ILs were made. This creates the opportunity to lower the melting point of the overall mixture and makes it possible to start working with the same ILs at a temperature closer to ambient conditions. This thesis proposed that this is also possible for choline ILs and it can be confirmed by DSC measurements. Phase transformations cause peaks in DSC curves in the sample. The top values of these curves can be considered as the edge between the phases in question. Having several measured melting points for different compositions, a liquidus line can be comprised. Composing a liquidus line for the same ratios for mixtures where the first component is in excess, the liquidus line for the other half of the phase diagram can be comprised. The intersection of these two liquidus lines should be the eutectic temperature of the binary mixture. Thus, melting point vs. concentration phase diagrams were composed using the endothermic peak top values of the DSC curves.

The crystallization temperature can often come after supercooling, which is a probabilistic event. Because of that, the phase transition peak from the heating curve of DSC should be preferred for the interpretation of the melting point of the sample. This process is not dependent on the thermal history of the indi-

vidual sample and the conditions of the measurement. The eutectic points were later determined from isobaric phase diagrams (melting points vs. concentrations) and via the specific heat fusion of the eutectic peak.

The selection of ions the ILs are composed of determines their melting points. When pairing several ions with different bulkiness, one can achieve low melting points. When one extends this idea to eutectic mixtures, their melting point depressions become more natural to explain. Mixing a certain amount of the second IL to the first one, disrupts the possible crystal structure and lowers the melting point of the mixture.

Mixing two ILs with the same cation but different sized anions, like $[Ac]^-$ and $[2mb]^-$, it can be expected there is more significant melting point depression than mixing two choline ILs with similar kinds of anions, e.g. $[Iv]^-$ and $[2mb]^-$. This rationale can explain the more significant melting point depression in [Ch][Ac] and [Ch][2mb] mixture compared to other mixtures (Table 8).

Eutectic transition temperatures were detected in several compositions but were often difficult to determine because of overlap with the peak for solid-liquid transition peaks. Similar kinds of overlap have been seen in studies studying, for example, pyrrolidinium ILs (Annat et al., 2012).

There are five different eutectic mixtures with four different choline carboxylate ILs (Figure 10–14, Figure A 2). These types of mixtures use components that are biofriendly and readily available. The melting point depressions remained in the range of 13–45°C (Table 8). The most significant melting point depression from either component was 45°C concerning [Ch][2mb] in its mixture with [Ch][Ac].

Unfortunately, none of the eutectic mixtures were liquid at room temperature (Figure 10–14). The lowest achieved melting point in a eutectic mixture was 39°C in [Ch][Ac] and [Ch][Iv] mixture (Table 8). Eutectic mixtures with lower melting points could be achieved by mixing in more ILs to create an even less ordered arrangement in the mixture. Changing the structure of one of the carboxylates to a bulkier anion while keeping the other anion relatively small is another possibility.

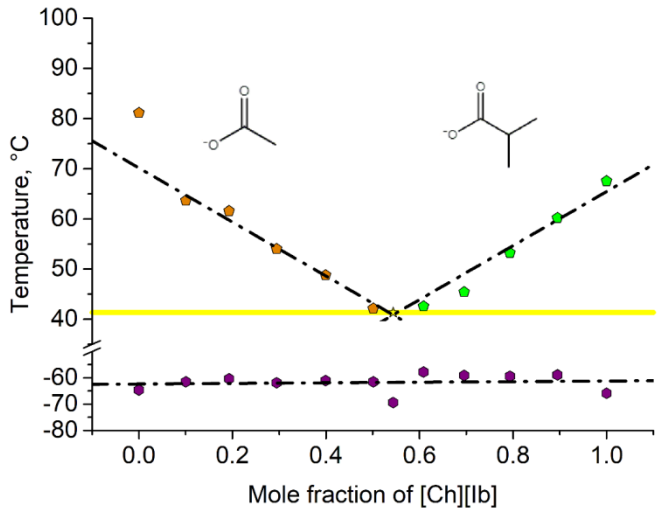


Figure 10: Temperature-composition phase diagram for the [Ch][Ac] and [Ch][Ib] mixture. Legend: ⬟ - melting point of the excess component [Ch][Ac] in the mixture; ⬟ - melting point of the excess component [Ch][Ib] in the mixture; ⬢ - glass transition temperature; ★ - eutectic point; — eutectic transition line.

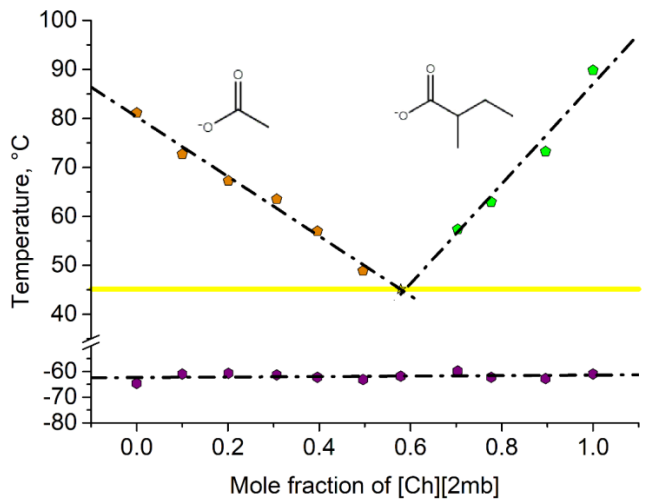


Figure 11: Temperature-composition phase diagram for the [Ch][Ac] and [Ch][2mb] mixture. Legend: ⬟ - melting point of the excess component [Ch][Ac] in the mixture; ⬟ - melting point of the excess component [Ch][2mb] in the mixture; ⬢ - glass transition temperature; ★ - eutectic point; — eutectic transition line.

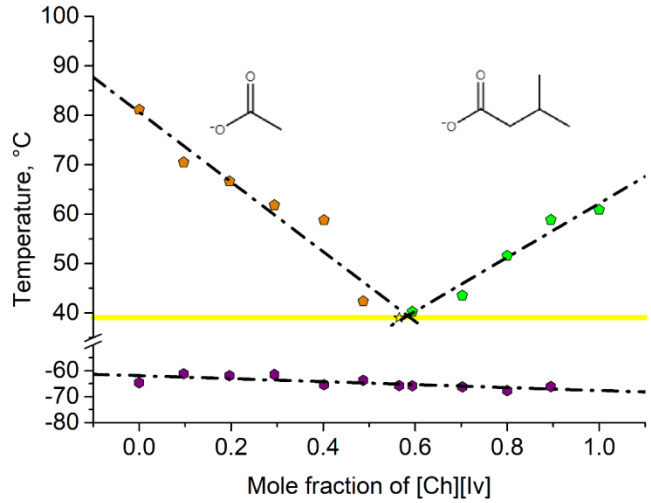


Figure 12: Temperature-composition phase diagram for the [Ch][Ac] and [Ch][Iv] mixture. Legend: ⬟ - melting point of the excess component [Ch][Ac] in the mixture; ⬟ - melting point of the excess component [Ch][Iv] in the mixture; ⬢ - glass transition temperature; ★ - eutectic point; — eutectic transition line.

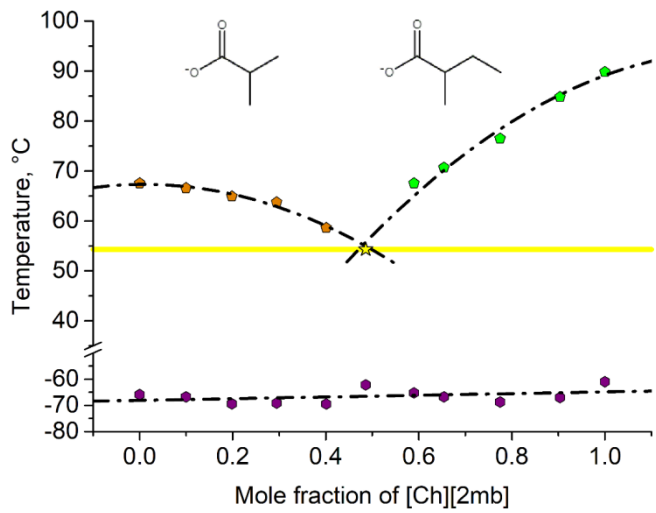


Figure 13: Temperature-composition phase diagram for the [Ch][Ib] and [Ch][2mb] mixture. Legend: ⬟ - melting point of the excess component [Ch][Ib] in the mixture; ⬟ - melting point of the excess component [Ch][2mb] in the mixture; ⬢ - glass transition temperature; ★ - eutectic point; — eutectic transition line.

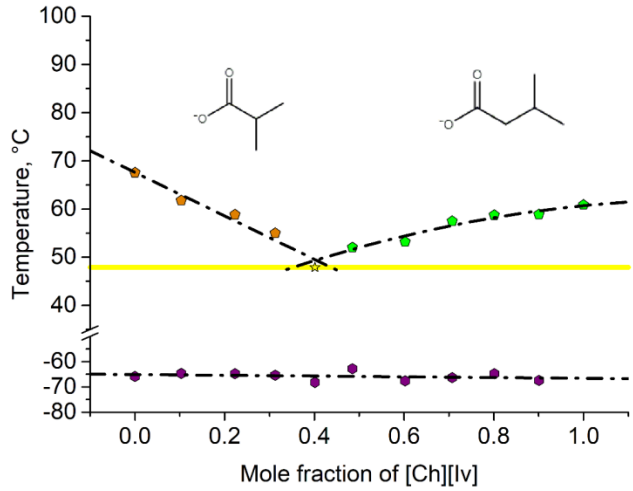


Figure 14: Temperature-composition phase diagram for the [Ch][Ib] and [Ch][Iv] mixture. Legend: ⬟ - melting point of the excess component [Ch][Ib] in the mixture; ⬟ - melting point of the excess component [Ch][Iv] in the mixture; ⬢ - glass transition temperature; ★ - eutectic point; — eutectic transition line.

All the eutectic points stayed between the 0.6 and 0.4 molar fraction range of both components. The summary of the properties of discovered eutectic mixtures (Table 8) allows a clear-cut comparison and analysis for the feature changes based on small variations to the chemical structures of choline carboxylate IL anions. Results show similarity to each other, which is not surprising. However, one aim of this thesis was also to see the differences in results based on small changes to the carboxylate structures of the anions.

Table 8: Melting points and melting point depressions of the eutectic solutions from choline ILs found in this study

Mixture			Eutectic composition		Melting point depression	
Comp. A	Comp. B	$T_{m,eu}$, °C	Comp. A	Comp. B	$T_{m,A}$, °C	$T_{m,B}$, °C
[Ch][Ac]	[Ch][Ib]	41	0.46	0.54	40	26
[Ch][Ac]	[Ch][2mb]	45	0.42	0.58	36	45
[Ch][Ac]	[Ch][Iv]	39	0.43	0.57	42	22
[Ch][Ib]	[Ch][2mb]	54	0.51	0.49	13	36
[Ch][Ib]	[Ch][Iv]	48	0.60	0.40	20	13

$T_{m,eu}$ – the melting point of eutectic mixture
$T_{m,A}$ – the melting point depression of pure component A
$T_{m,B}$ – the melting point depression of pure component B

3.2.3. Other thermal behaviour in choline IL mixtures

The binary mixtures show two different types of behaviour: besides eutectic, two diagrams exhibit no melting points after a certain concentration of [Ch][Mal] (Figure 15, Figure 16). All choline carboxylates ILs showed a glass transition temperature below -60°C. Other quaternary ammonium ILs have also exhibited glass transitions around the same temperature range (Sun et al., 1998). Because of their low glass transition temperature and the fact that no melting point was detected for the dicarboxylic choline ILs, it may conclude that [Ch][Mal] and [Ch][Glu] could find use as solvents in a wide temperature range. Because [Ch][Mal] and [Ch][Glu] were challenging to crystallize, a longer isotherm was used, as compared to other ILs in this work. Most mixtures of [Ch][Mal] researched in this study did not solidify into a solid-state even with the addition of a monocarboxylic choline ILs.

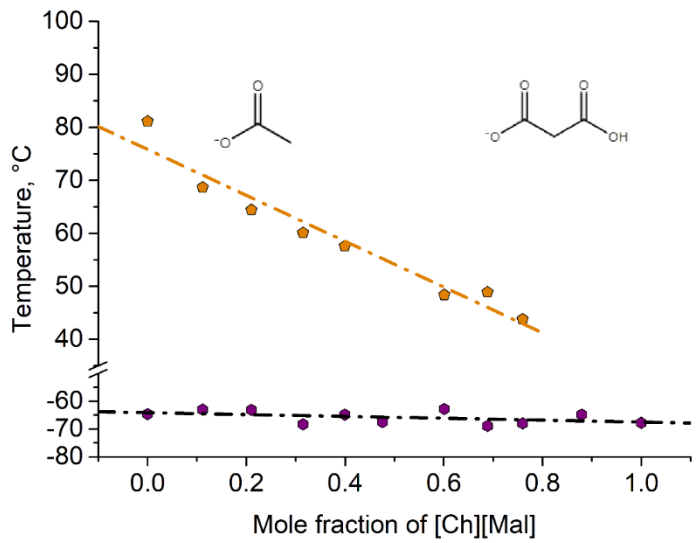


Figure 15: Temperature-composition phase diagram for the [Ch][Ac] and [Ch][Mal] mixture. Legend: ⬟ - melting points of mixture; ⬢ - glass transition temperatures of the mixture

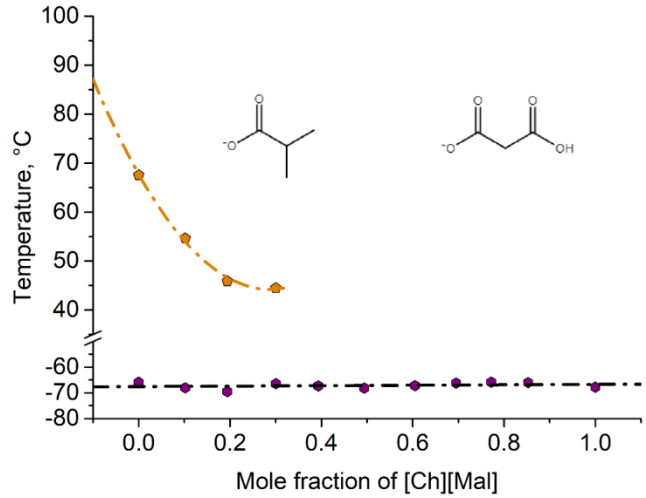


Figure 16: Temperature-composition phase diagram for the [Ch][Ib] and [Ch][Mal] mixture. Legend: ⬟ - melting points of mixture; ⬢ - glass transition temperatures of the mixture

Similar results have been achieved with so-called natural DES made from different compounds, including quaternary ammonium cations (e.g. choline cation) and various carboxylic acids (Dai et al., 2013). These natural deep eutectic solvents have excellent solubilizing properties. It follows that [Ch][Mal] and [Ch][Glu] are also possible candidates for solubility testing. Mixtures with no detectable melting point have been called low-transition mixtures before (Francisco et al., 2013, p.), and lack of crystallization is probably because of the high viscosity of the mixture (Morrison et al., 2009).

The absence of a melting point does not indicate that no melting point exists in these mixtures or pure dicarboxylic choline ILs (3.2.1) but can simply indicate that the melting point does not occur in the measurement conditions used in this study (2.2.2). The melting point can still be detected by having a longer isotherm to let the sample crystallize. A slow cooling rate of $1°C \cdot min^{-1}$ is also a possibility (Alves et al., 2010).

The mixture of [Ch][Mal] and [Ch][Ib] showed melting points only in the range of $0 < x_{[Ch][Mal]} < 0.3$ and the mixture of [Ch][Mal] and [Ch][Ac] in a much wider range of $0 < x_{[Ch][Mal]} < 0.8$, except for 1:1 mixture. The difference in the range where melting points can be detected may be due to the intrinsic differences between DESs formed between several ILs and DESs between IL and an H-bond donor. When choline chloride is mixed with malonic acid, a eutectic mixture forms at 50 molar % (Abbott et al., 2004). The formation of the eutectic mixture in that ratio could also be explained by the difference in ion size of Cl^- and carboxylic anions.

The particularly similar glass transition temperatures for all ILs used in this study and their mixtures can be explained with the results described before,

where it was found that the glass transition temperature is dependent on the number of carbons in the cation (Sun et al., 1998, p.).

3.2.4. Thermal decomposition of choline ILs

Most ILs have an extensive liquidus range, which gives them the potential to be used as solvents in a wide range of temperatures. The maximum temperature they can be used is limited by their decomposition temperature because of their almost negligible vapour pressure. The lack of boiling point is why it is crucial to know the decomposition temperature of ILs to know their limits in utilization to get the maximum value out of their usage. It is also essential to know this in different atmospheres to know how the environment plays a role in that.

So far, imidazolium ILs have been studied more widely than ammonium ILs (Maton et al., 2013), including choline ILs. Because the cation was the same for all ILs in TGA measurements, this research focuses on the influence of the anion on the decomposition temperatures of tested choline ILs.

The TGA results of 5% mass decomposition in nitrogen and artificial air (measured as described in 2.2.3) are in Table 7. The highest decomposition was determined for [Ch][Glu] and interestingly the lowest for [Ch][Mal] for both inert and oxygenated environments. From the TGA results, [Ch][Mal] shows a much lower decomposition temperature in synthetic air than the other tested choline ILs. The difference between the decomposition temperature of [Ch][Ac] and [Ch][Ib] in this study (178°C and 178°C) and literature (189°C (Fukaya et al., 2007) and 172°C (Petkovic et al., 2010)) are most probably due to the different measurement conditions used across studies.

Interestingly there was only a minor difference between decomposition for choline ILs temperatures measured in inert and oxygenated environments. The most probable explanation is that due to the short-term nature of the measurement, oxygen does not dissolve extensively into choline IL structure, like for many other ILs (Maton et al., 2013). This kind of ramped temperature method gives information about the short-term thermal stability, which suggests that choline ILs have the durability to survive at least short-term, even in oxygenated environments.

3.3. Biologically benign trilayered actuator

The final goal for this thesis was to develop a biologically harmless trilayered actuator. This was done after establishing the toxicity of chosen choline ILs and analyzing their thermal characteristics. To review the results for the developed PPy-gelatin and PPy-PVdF actuators with choline ILs, please refer to sections 3.3.4 and 3.3.5 of this thesis.

3.3.1. Ionic conductivity of choline ILs in PVdF and gelatin membranes

Because the actuator performance is partly dependent on the ionic movement in the membrane of the actuator, the ionic conductivity of the membrane was characterized, alongside the ionic conductivities of pure ILs (measured as described in 2.1.1.1; (Table 9). The water content in ambient conditions and the hygroscopic nature of gelatin increases the ionic conductivity of choline ILs. The water content also prevents crystallization of the ILs used in the actuators.

Table 9: Ionic conductivities for the synthesized choline ILs in pure form and in membranes. The conductivity was measured directly after synthesis, for the pure ILs. Additional measurements were done later, after kept in ambient conditions, after 10 min, after 1 h, and after 3 weeks in a fume hood. The conductivity of choline ILs in membranes, the ionic conductivity was measured directly after membrane immersion into a relevant IL in an inert atmosphere and after 10 min under ambient conditions.

ionic liquid	Ionic conductivity of ILs, $mS \cdot cm^{-1}$				Ionic conductivity of ILs in membranes, $mS \cdot cm^{-1}$			
	AS*	10 min	1 h	3 weeks	PVdF	PVdF, 10 min	gelatin	gelatin, 10 min
[Ch][Ac]	1.67	1.82	3.88	4.28	0.44	0.55	0.48	1.65
[Ch][Ib]	0.64	1.70	2.28	2.40	0.01	0.05	0.06	0.28
[Ch][Iv]	0.28	0.99	1.80	1.87	0.02	0.09	0.06	0.18
[Ch][2mb]	0.55	1.44	1.72	1.89	0.04	0.09	0.01	0.40
[Ch][Mal]	0.12	0.11	0.60	2.65	0.03	0.12	0.05	0.26
[Ch][Glu]	0.02	0.02	0.20	0.67	0.00	0.02	0.00	0.16

* AS – as synthesized

The ionic conductivity of choline ILs used to saturate the membranes was the primary determinant for the ionic conductivities of the membranes. [Ch][Ac] had the highest conductivity at all measurement conditions for pure ILs and in membranes. [Ch][Glu] had the lowest ionic conductivity in all those cases.

3.3.2. Electrochemical synthesis of PPy-gelatin and PPy-PVdF actuators

Virtually independent of the underlying membrane (described more in detail in 2.3.1), the chronopotentiograms (Figure 17) of all the synthesis rounds for gelatin and PVdF membranes were very similar (synthesized as described in 2.3.2). The shape of the potential curve is usual for this type of polymerization.

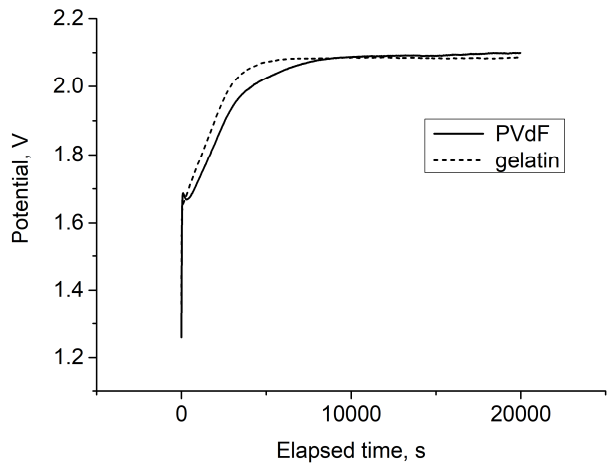


Figure 17: Chronopotentiograms of the electrochemical synthesis for gelatin (dashed line) and PVdF (solid line) actuators

3.3.3. Structure and morphology of PPy-gelatin and PPy-PVdF actuators

The average thickness of the actuators was 130 µm. The swelling from IL immersion was negligible, but after immersion, the mass of PPy-gelatin and PPy-PVdF actuators had doubled and increased by about 75%, respectively. The difference in structure, including porosity, of the fabricated actuators is apparent in SEM micrographs of the cross-sections (characterization method described more in detail in 2.3.3; Figure 18). PPy-PVdF actuators exhibit a typical uniform PPy layer (2–3 µm for this synthesis) on top of the PVdF membrane. The gelatin membrane has a fibrous structure inherent to electrospinning and a denser structure in the middle of the membrane. This denser structure forms possibly from partially dried fibers fusing during thermal treatment. The PPy layer follows the same kind of fibrous structure mentioned earlier.

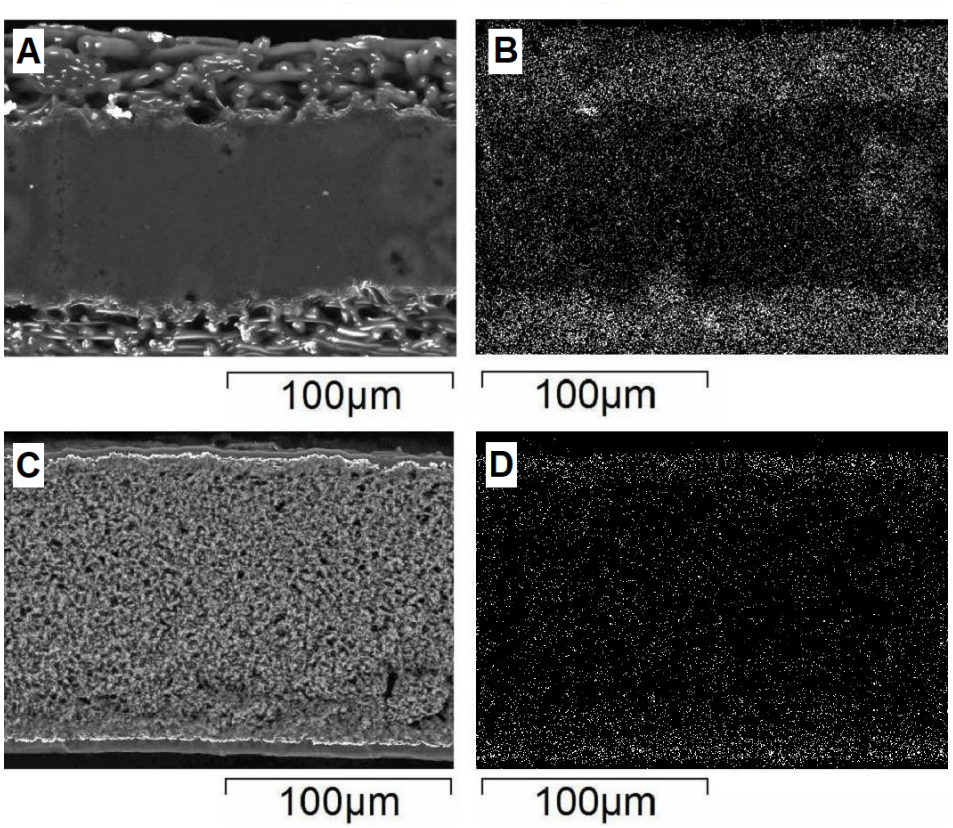


Figure 18: SEM images for the cross-sections (PPy-gelatin for A and PPy-PVdF for C) and the EDX sulfur maps that show the distribution of $[DBS]^-$ anions (PPy-gelatin for B and PPy-PVdF for D) of PPy-gelatin and PPy-PVdF actuators (adapted from Figure 1 of Publication III and Figure 4 of Publication I)

To investigate where PPy deposited during the chemical synthesis of PPy-gelatin actuators, the actuators were investigated with EDX sulfur mapping to determine the distribution of $[DBS]^-$ anions. These results are compared with the sulfur mapping of PP-PVdF actuators. The presence of PPy is marked by the higher concentration of $[DBS]^-$ anions. For PPy-gelatin actuators, $[DBS]^-$ anions can be found primarily in the fibrous layers that are predominant for 40 µm on both outer sides of the membrane.

3.3.4. Electro-chemo-mechanical properties of PPy-gelatin and PPy-PVdF actuators

After fabricating the trilayered actuator, it is important to choose the type of signal to drive it. Different signals that differ in their frequencies, shapes, and other characteristics, will vary in their effectiveness to move the actuator.

The different driving signals used to investigate the actuation properties of the PPy-gelatin and PPy-PVdF actuators are described in 2.3.4. Actuators were first driven with a square wave signal. From these results, actuators showed larger displacement values, i.e. with [Ch][Ac], were selected for further testing. Also, actuators with small displacement values were chosen as a comparison – actuators with [Ch][Ib] and [Ch][Mal]. In addition to actuators with choline ILs, actuators with a more popular imidazolium-based IL – [EMIM][OTf] – was used as a comparison to choline IL performance. (Publication I) These chosen actuators were driven with a triangular voltage signal (Figure 19) while recording the CV response. Change in the maximum displacement amplitude of the actuators in time was negligible, despite water from the atmosphere solubilizing into the choline IL present in the actuators. The displacement values were used to calculate the maximum strain difference (according to 1). The results for that are in Table 10. PPy-gelatin actuators with [Ch][Ac] or [Ch][Ib] demonstrated the highest strain difference values. Cationic activity was dominant for both PPy-gelatin and PPy-PVdF actuators.

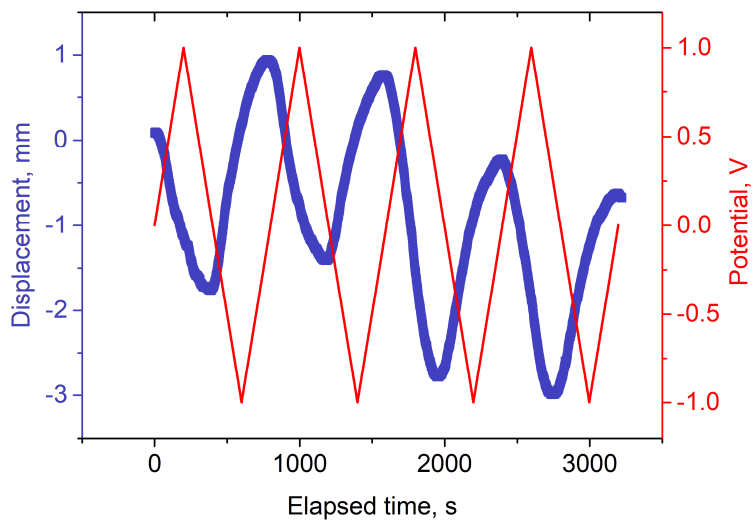


Figure 19: Displacement of PPy-gelatin actuators with [Ch][Ac] as the electrolyte. Driven with ±1 V CV-signal with a scan rate of 5 $mV \cdot s^{-1}$.

Table 10: Strain difference (%) of tested PPy-gelatin and PPy-PVdF actuators calculated from their maximum displacement according to 1. sq – actuated with square wave voltage between ±1 V at 0.001 Hz; CV 5 – actuated with triangle wave CV signal between ±1 V at 5 $mV \cdot s^{-1}$; CV 50 – actuated with triangle wave CV signal between ±1 V at 50 $mV \cdot s^{-1}$.

Ionic liquid	PPy-PVdF				PPy-gelatin			
	Strain difference, %			Elastic modulus, MPa	Strain difference, %			Elastic modulus, MPa
	sq	CV 5	CV 50		sq	CV 5	CV 50	
[Ch][Ac]	0.67±0.09	0.65±0.05	0.30±0.03	186	0.62±0.12	1.35±0.13	0.17±0.03	61
[Ch][Ib]	0.19±0.08	0.49±0.07	0.15±0.05	221	0.29±0.12	1.79±0.14	0.34±0.07	94
[Ch][Mal]	0.11±0.02	0.06±0.01	0.02±0.00	990	0.18±0.09	0.09±0.01	0.03±0.01	301
[EMIM][OTf]	0.16±0.02	0.05±0.01	0.04±0.01	914	0.07±0.01	0.05±0.01	0.03±0.00	222

Cyclic voltammetry (CV) was measured alongside maximum displacement. CV shapes and sizes are directly linked to the performance of the actuators and give an understanding of the electrochemical ability of the tested actuators. The chosen CVs (second cycle) for PPy-gelatin and PPy-PVdF actuators are in Figure 20. The CV surface amplitude and shape did not change noticeably from cycle to cycle, despite water from the atmosphere solubilizing into the choline IL present in the actuators. Interestingly, the response to changes in the scan-rate was quite different depending on the materials used. With PVdF membranes, neither [Ch][Mal] nor [EMIM][OTf] showed significant current density dependence on scan rate, while other ILs were incapable of keeping up with the faster scan-rate. With gelatin membranes, on the other hand, there was much more scan-rate dependence, but not equally so. Perhaps the most outstanding is the response of [Ch][Ac], where the shape of the voltammogram was changed but without losing much in overall exchanged charge.

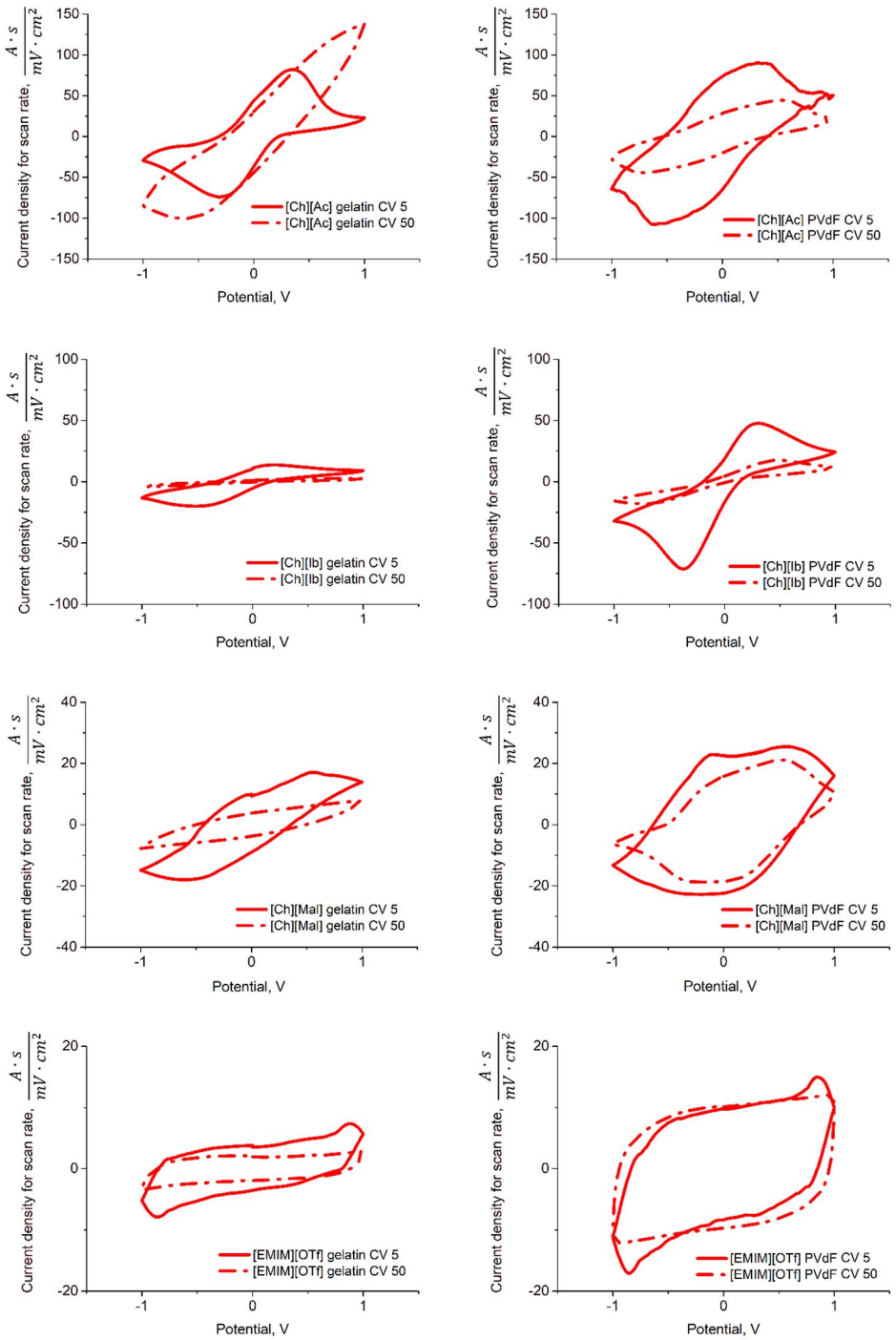


Figure 20: Cyclic voltammetry of PPy-gelatin and PPy-PVdF actuators

Different driving signals for different actuator materials exhibit varying charge-efficiencies (Table 11). The fact that increasing the scan-rate for PPy-gelatin actuators brings no decrease in their charge efficiency is an indicator of strong coupling between charge and the actuation (Publication III).

Table 11: The charge-efficiency of the tested actuators (%·Coulomb^{-1}·cm^{2}), defined as the strain difference (%) divided by charge density (Coulomb·cm^{-2}). sq – actuated with square wave voltage; CV 5 and CV 50 – actuated with triangle wave signal with 5 mV·s^{-1} and 50 mV·s^{-1}, respectively.

ionic liquid	PPy-PVdF sq	PPy-PVdF CV 5	PPy-PVdF CV 50	PPy-gelatin sq	PPy-gelatin CV 5	PPy-gelatin CV 50
[Ch][Ac]	10	11	12	16	74	77
[Ch][Ib]	5	11	12	13	81	85
[Ch][Mal]	3	3	2	2	5	4
[EMIM][OTf]	5	5	5	6	8	8

In the case of frequency response, the actuators exhibited similar displacements driven with a mirrored sinusoidal signal. The shape of the displacement curve shows that the materials retain their actuation properties even after being driven with high frequencies (400 Hz) (Figure 21, Figure 22). This result demonstrates the durability and wide application range of PPy-gelatin actuators using choline ILs.

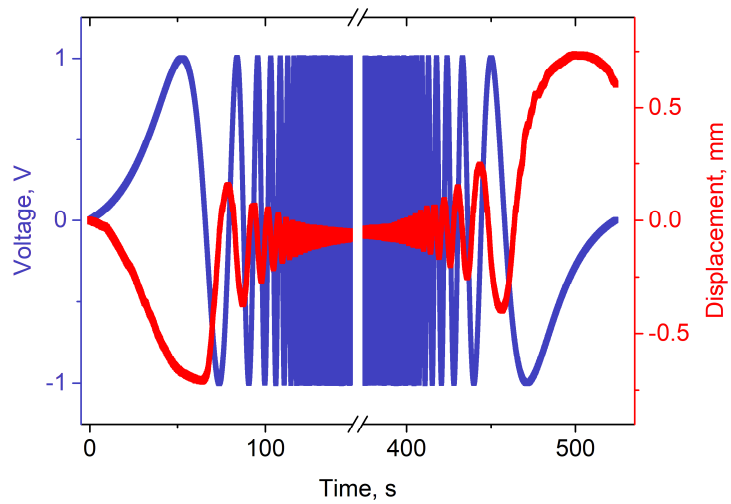


Figure 21: An example of a frequency response graph of PPy-gelatin actuator with [Ch][Ib]

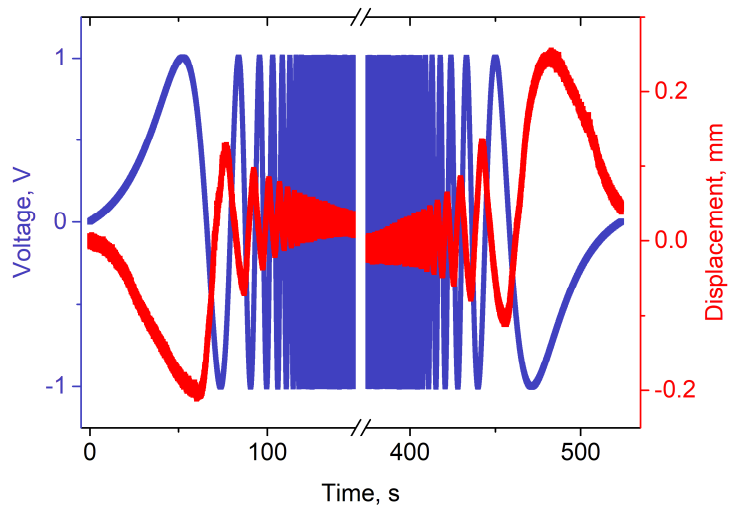


Figure 22: An example of a frequency response graph of PPy-gelatin actuator with [Ch][Mal]

Elastic moduli were determined from the resonance frequencies of the mechanically excited oscillating actuators (Table 10) to determine the effect of mechanical properties of the materials on the actuator performance. PPy-PVdF actuators were showed higher elastic moduli, thus being more rigid than the PPy-gelatin actuators. It is noteworthy to point out that the elastic moduli of the actuators are also influenced by the ILs used as well. Actuators previously immersed in ILs with smaller anions, i.e. [Ch][Ac] and [Ch][Ib], showed the most significant elasticity.

As the electrolytes play an essential role in the performance of the actuators, so does the membrane material. Their porosity and mechanical properties (i.e. elasticity) are their key indicators. The higher elasticity of PPy-gelatin actuators, compared to PPy-PVdF actuators, correlate well with their higher strain difference values (Table 10).

Despite all actuators being predominantly cation driven, there are still variations in their performance due to anions. The choline ILs most beneficial for actuator fabrication from the ILs tested in this work are [Ch][Ac] and [Ch][Ib]. The former for excellent charge retention and constant behavior regardless of the membrane material, explained by the highest ionic conductivity in both membrane materials. [Ch][Ib], on the other hand, showed the most massive displacement and very high charge efficiency. The optimal potential ranges differed depending on the IL used in the actuator material.

Triangle wave signals were more suitable for PPy-gelatin actuators, while the square wave signal gave optimal results for PPy-PVdF actuators. For triangle wave signal both voltage and current, and thus the movement of ions, increase more gradually in time than with square wave signal. For the square

wave signal, the electrode loses its conductivity because of abrupt reduction. This process leads to higher strain difference values for driving the actuators with a triangle wave signal.

The chosen electrolytes played an essential role concerning PPy-gelatin actuators, where ILs with smaller anions, i.e. [Ch][Ac] and [Ch][Ib] demonstrated higher strain differences with CV signals of 5 $mV \cdot s^{-1}$. Square wave signals gave larger strain difference values with larger ILs, i.e. [Ch][Mal] and [EMIM][OTf]. Results from Table 11 show that actuators with [Ch][Ac] and [Ch][Ib] have a higher charge efficiency (i.e. strain difference divided by charge density) than actuators with other ILs, which is very apparent when driving PPy-gelatin actuators with a CV signal of 5 $mV \cdot s^{-1}$. Comparing PPy-gelatin actuators with PPy-PVdF actuators, the option of gelatin membrane paired with [Ch][Ac] or [Ch][Ib] electrolyte gives the most efficient and high performing actuator from the choices tested in this work.

To determine to reason for the different behaviour of ILs in actuators on the atomic level, molecular dynamics simulations were run. Knowing the molar volumes of choline ILs is not enough to determine their performance in an actuator material. Neither does the conductivity of the tested choline ILs correlate strongly with the strain difference of the corresponding PPy-gelatin and PPy-PVdF actuators. Results calculated from molecular dynamics trajectory data show the actuation of the tested actuators is not from single mobile ions but the ions with their solvation shells. The model becomes more evident when properties of the membrane are considered, i.e. the hydrogen-bonds formed with the gelatin membrane. To predict the performance of the actuator more accurately, the whole actuator with all the peculiarities of each material component need to be taken into consideration. Despite this, the cation-cation correlation peak height can be used as a reliable predictor for strain difference. (Publication III)

The most combination of the tested materials that uses the most harmless choline ILs and also gives the highest strain difference in soft polymer actuators consists of PPy electrodes, gelatin membrane, and [Ch][Ac] or [Ch][Ib] as the electrolyte.

3.3.5. The harmlessness of PPy-gelatin and PPy-PVdF actuators

To test for the harmlessness of PPy-gelatin and PPy-PVdF actuators with choline ILs as a whole, disk diffusion tests were conducted (as described in 2.3.5), in addition to testing the the individual components in a solution. Also known as the Kirby-Bauer method, the disk diffusion method is a robust option for researching the effect of a material or a liquid on living things. *E. coli* and *S. aureus* were chosen to represent the Gram-negative and Gram-positive bacteria as the test organisms. [Ch][Cit] was excluded from these experiments because this IL is solid at ambient conditions and not suitable to be used as an electrolyte in actuators outside of solvent.

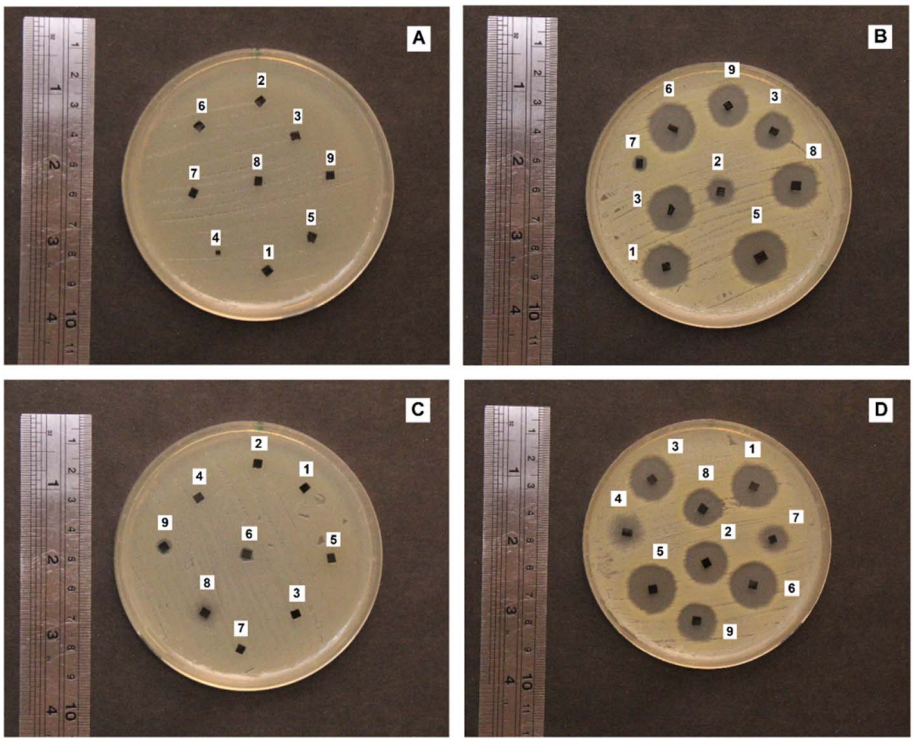


Figure 23: Disk diffusion assay for PPy-gelatin (*E. coli* for A and *S. aureus* for B) and PPy-PVdF (*E. coli* for C and *S. aureus* for D). Positions: 1 – [Ch][Ac], 2 – [Ch][Ib], 3 – [Ch][Iv], 4 – [Ch][2mb], 5 – [Ch][Mal], 6 – [Ch][Glu], 7 – [EMIM][OTf], 8 – [EMIM][TFSI], 9 – [EMIM][FSI].

There is a stark difference in disk diffusion test results for *E. coli* and *S. aureus* (Figure 23). For tests concerning *E. coli*, there are no inhibition rings for both PPy-gelatin and PPy-PVdF actuators previously immersed in choline ILs with monocarboxylate anions. In contrast, for choline ILs with dicarboxylic anions, there is a suggestion for minimal harmfulness, shown by their barely detectable inhibition rings around the samples. [EMIM][FSI] and [EMIM][TFSI], chosen for comparison, inhibited the bacterial growth more than choline ILs. The membrane material did not play an essential role in inhibiting *E. coli*.

S. aureus showed more sensitivity towards all ILs. In most cases, there is little difference between the results concerning choline and imidazolium ILs. The major differences occur in testing actuators with different membrane materials. For PPy-PVdF actuators, the only exceptions concerning the extent of inhibition are [EMIM][OTf] and [Ch][2mb] that demonstrated the smallest inhibition among imidazolium ILs and choline ILs, respectively. For PPy-gelatin actuators the least inhibiting ILs were two choline ILs: [Ch][2mb] and [Ch][Ac].

One possibility to explain the sensitivity of *S. aureus* is to look at the bacterial membrane. As a Gram-negative bacteria, *E. coli* has an extra protective layer in the structure of the membrane. This topic is also discussed in 3.1.2, where the opposite effect occurred for choline monocarboxylates in solution. In addition to this, the disk diffusion test results confirm the MIC and CC experiments described in 3.1.2 and 3.1.3, where choline monocarboxylates showed the least harmfulness.

To explain the smaller toxicity of choline ILs in disk diffusion experiments, there are several possibilities. One is to justify it with the hypothesis that the chosen imidazolium ILs diffuse more efficiently into the solid agar medium than to the chosen choline ILs. The other possibility is more straightforward and maybe even more probable: the ILs with larger inhibition rings are simply more toxic towards the tested bacteria. Imidazolium ILs harm the chosen bacterial strains more than choline ILs. When comparing choline ILs to each other, dicarboxylic choline ILs can also damage the bacteria more than their monocarboxylic counterparts because they increase the acidity of the solid agar medium.

4. CONCLUSIONS

This thesis presented results on fabricating soft trilayered actuators from bio-friendly materials. The making of the actuators was possible through selecting the right starting materials and fine-tuning their combination further. The chosen choline ILs were from components that are harmless to tested organisms, readily available, and easy to synthesize, which makes them ideal candidates for solvents used in food, pharmaceutical, and other industries where biological and environmental friendliness is crucial.

Firstly, the hypothesis that even a small variation in the length and branching of carboxylic anion alkyl chains influences the biological impact of singular choline ILs is addressed. The toxicity of the used choline ILs was several magnitudes smaller compared to more commonly used ILs in IEAP actuators, both in solution and on solid medium. The lowest MIC value of 22.5 mM and CC_{50} value of 8 mM were both still very high. Despite high MIC and CC values, the influence of anions on the life cycle of the tested bacteria still varied. The longer alkyl side chains increased the toxicity of choline ILs, but the results did not differ drastically in that respect. The main factor of their toxicity was their influence on the pH of the solution. An increase in carboxyl groups increased to the toxicity of the choline ILs. The choline ILs with the shortest alkyl side-chains and one carboxyl group – [Ch][Ac] and [Ch][Ib] – both had MICs over 1 M and also the highest CC values.

Secondly, this thesis investigated the phase behaviour of the binary mixtures of choline ILs. The anion structures of choline ILs were deliberately selected from simple carboxylic acids. It was hypothesized that by mixing the choline ILs into a binary mixture at a certain ratio, the melting points of the mixtures would drop close to room temperature, and these mixtures could be used in PPy-gelatin and PPy-PVdF actuators. Five eutectic mixtures were identified with minimal structural changes to the anion alkyl chains. The melting point depressions in eutectic mixtures remained in the range of 13-45°C. The decomposition temperature of singular ILs depended on the number of carboxyl groups present and the length of the alkyl chain connecting them in the anion.

The final aim of this thesis was to prove that it is possible to make a harmless trilayered soft polymer actuator, that would also be effective in its use. It was hypothesized this could be done using harmless starting materials. PPy-gelatin actuators consistently outperformed their comparison – PPy-PVdF actuators. Furthermore, actuators with both PPy-gelatin and PPy-PVdF actuators achieved almost 3 times higher strain difference with choline acetate when compared to 1-ethyl-3-methylimidazolium trifluoromethanesulfonate. Based on the combinations from this study, the actuators with the highest strain difference and effectiveness were PPy-gelatin actuators with choline acetate or choline isobutyrate as the electrolyte. The strain difference values for PPy-gelatin actuators with choline acetate and choline isobutyrate were: 1.35% and 1.79% repectively, when driven with the triangular signal. The triangular voltage

signal was preferable to a square wave signal for the driving of the actuators because it facilitates the gradual movement of ions and reduces the electrode slower than the square wave signal. For example, when using choline acetate as the electrolyte, the strain difference was seven times greater with the triangular wave signal than the square wave signal.

For future work, the actuators developed for this thesis could be enhanced further by introducing mixtures of several choline ILs with a small addition of water in the mix as the electrolyte. Because these actuators are meant to work in at least partly in ambient conditions, water found in the atmosphere will eventually influence the overall behaviour of the actuators after it has solubilized into the electrolyte. Water will solubilize the choline IL mixture, making the electrolyte more conductive, as can be seen from the conductivity measurements in this thesis.

Moreover, binary eutectic mixtures identified in this thesis could also be used as electrolytes in PPy-gelatin actuators after a small amount of water has been introduced in the mix. Despite none of the mixtures identified in this thesis being liquid at room temperature, there is a significant chance that after water additive, they will be.

Also, the toxicity of choline IL eutectic mixtures towards bacteria and cell lines other than the ones discussed in this thesis should be tested in solution and solid agar media. Solutions could be used to test new choline carboxylate ILs, and solid media (disk diffusion test) would be suitable to test new types of actuators. Additionally, more biological objects, like other human tissues, should be used as test objects if soft IEAP actuators with choline ILs are to be used in medical applications. Human nerve tissues would be ideal for their sensitivity and importance. The actuators suitable for medical use could be incorporated into a robotic system that would work in a medical prototype (e.g. prosthesis limbs, probes into the vascular and lower intestinal systems).

5. SUMMARY IN ESTONIAN

Biopolümeeridest ja mittetoksilistest ioonvedelikest koosneva polümeerse ajami arendamine

Selles väitekirjas esitati tulemused pehmete kolmekihiliste täiturite valmistamise kohta loodussõbralikest materjalidest. Täiturite valmistamine oli võimalik eelkõige tänu õigete lähtematerjalide valimisele ja nende omaduste muutmisele. Valitud koliin ioonsed vedelikud sünteesiti komponentidest, mis on ohutud testitud organismidele, kergesti kättesaadavad ja hõlpsalt sünteesitavad, mis omakorda teeb neist ideaalsed kandidaadid solventidele, mida kasutatakse toidus, farmaatsias ja muudes tööstusharudes, kus bioloogiline ja keskkonnasõbralikkus on ülioluline.

Esiteks käsitleti hüpoteesi, et isegi karboksüülanioonide alküülahelate pikkuse ja hargnemise väike varieerumine mõjutab koliin ioonsete vedelike bioloogilist mõju. Kasutatud koliini ioonsete vedelike toksilisus oli mitu suurusjärku väiksem võrreldes sagedamini kasutatavate ioonsete vedelikega IEAP täiturites nii lahuses kui ka tahkel söötmel. Madalaim MIC väärtus 22,5 mM ja CC_{50} väärtus 8 mM olid mõlemad endiselt väga kõrged. Vaatamata kõrgetele MIC ja CC väärtustele, varieerus anioonide mõju testitud bakterite elutsüklile endiselt. Pikemad alküülkülgahelad suurendasid koliin ioonsete vedelike toksilisust, kuid tulemused ei erinenud selles aspektis drastiliselt. Testitud koliin ioonsete vedelike toksilisuse peamiseks põhjuseks oli arvatavasti nende mõju keskkonna pH-le. Karboksüülrühmade arvu suurenemine suurendas koliin ioonsete vedelike toksilisust. Kõige lühemate alküülkülgahelate ja ühe karboksüülrühmaga koliin ioonsetel vedelikel – [Ch][Ac] ja [Ch][Ib] – olid mõlemal MICid kõrgemad kui 1 M ja ka kõige kõrgemad CC väärtused.

Teiseks uuriti siinses väitekirjas koliini ioonete vedelike binaarsegude faasikäitumist. Koliini ioonsete vedelike anioonstruktuurid valiti sihilikult lihtsatest karboksüülhapetest. Oletati, et segades koliini ioonsed vedelikud teatud vahekorra juures binaarseguks, langevad segude sulamispunktid toatemperatuuri lähedale ning neid segusid võib kasutada PPy-želatiini ja PPy-PVdF täiturites. Tuvastati viis eutektilist segu, milleks oli vaja minimaalseid struktuurimuutusi anioonalküülahelates. Sulamispunkti langused eutektilistes segudes jäid vahemikku 13-45°C. Üksikute ioonsete vedelike lagunemistemperatuurid sõltusid karboksüülgruppide arvust ja neid ühendava alküülahela pikkusest anioonis.

Selle väitekirja lõppeesmärk oli tõestada, et on võimalik valmistada mittetoksilist kolmekihilist pehmet polümeertäiturit, mis oleks ka kasutusel efektiivne. Oletati, et seda saab teha mittetoksilistest algmaterjalidest. PPy-želatiini olid tõhusamad kui PPy-PVdF täiturid. Lisaks saavutasid nii PPy-želatiini kui ka PPy-PVdF täituritega täiturid koliinatsetaadiga võrreldes 1-etüül-3-metüülimidasooliumtrifluorometaansulfonaadiga peaaegu 3 korda suurema liigutusulatuse. Selle uuringu kombinatsioonide põhjal olid suurima liigutusulatuse ja efektiivsusega täituriteks PPy-želatiini täiturid, mille elektrolüütideks olid koliinatsetaat või koliinisobutüraat. Koliinatsetaadi ja koliinisobutüraadiga PPy-

želatiini täiturite liigutusulatused olid: 1,35% ja 1,79%, kolmnurkse pingesignaali korral. Kolmnurkpingesignaal oli aktuaatorite liigutamiseks tõhusam kui ruutpingesignaal, sest see hõlbustas ioonide järk-järgulist liikumist ja vähendas elektroodi aeglasemalt kui ruutpingesignaal. Näiteks kui kasutada elektrolüüdina koliinatsetaati, oli liigutusulatuse erinevus kolmnurkpingesignaaliga seitse korda suurem kui ruutpingesignaaliga.

6. ACKNOWLEDGEMENTS

I would like to thank the co-authors of the publications this thesis is based on and my family and friends who supported me in other ways. Special thanks to the co-authors for the following support:

Publication I: The NMR spectra of synthesized choline ILs were measured by Kaija Põhako-Esko. Ionic conductivity measurements of choline ILs in pure form and in membranes were conducted by Pille Rinne. The MIC measurements for *S. Oneidensis* were conducted with the help of Hans Priks. The HeLa cells for CC measurements were initially seeded by Eva Žusinaite. The disk diffusion assay was initially prepared by Niilo Kaldalu.

Publication II: The NMR spectra of synthesized choline ILs were measured by Kaija Põhako-Esko.

Publication III: Ionic conductivity measurements of choline ILs in membranes were conducted by Pille Rinne. The disk diffusion assay was initially prepared by Niilo Kaldalu. The simulation experiments were conducted by Karl Karu and Vladislav Ivaništšev. Gelatin membranes were made by Kadi-Anne Nadel.

This work was also supported by the Graduate School of Functional materials and technologies and the following funding: the research has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 793377 (BIOACT), European Regional Development Fund, Estonian Research Council grant No. 772 and Institute of Technology basic funding grant 20907, as well as Estonian Personal Research Projects PUT1107, PSG249, PRG772, and PRG1084.

Very special thanks to Hanna Marta Sirel for a sympathetic ear.

7. REFERENCES

Abbott, A.P., Boothby, D., Capper, G., Davies, D.L., Rasheed, R.K., 2004. Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. Journal of the American Chemical Society 126, 9142–9147. https://doi.org/10.1021/ja048266j

Abbott, A.P., Capper, G., Davies, D.L., Rasheed, R.K., Tambyrajah, V., 2003. Novel solvent properties of choline chloride/urea mixtures. Chemical Communications 70–71. https://doi.org/10.1039/b210714g

Abbott, A.P., Collins, J., Dalrymple, I., Harris, R.C., Mistry, R., Qiu, F., Scheirer, J., Wise, W.R., 2009. Processing of Electric Arc Furnace Dust using Deep Eutectic Solvents. Australian Journal of Chemistry 62, 341. https://doi.org/10.1071/CH08476

Adewunmi, A.A., Ismail, S., Sultan, A.S., 2016. Carbon Nanotubes (CNTs) Nanocomposite Hydrogels Developed for Various Applications: A Critical Review. Journal of Inorganic and Organometallic Polymers and Materials 26, 717–737. https://doi.org/10.1007/s10904-016-0379-6

Alves, M.B., Umpierre, A.P., Santos Jr., V.O., Soares, V.C.D., Dupont, J., Rubim, J.C., Suarez, P.A.Z., 2010. The use of Differential Scanning Calorimetry (DSC) to characterize phase diagrams of ionic mixtures of 1-n-butyl-3-methylimidazolium chloride and niobium chloride or zinc chloride. Thermochimica Acta 502, 20–23. https://doi.org/10.1016/j.tca.2010.01.023

Andrews, J.M., 2001. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy 48, 5–12.

Annat, G., Forsyth, M., MacFarlane, D.R., 2012. Ionic Liquid Mixtures—Variations in Physical Properties and Their Origins in Molecular Structure. The Journal of Physical Chemistry B 116, 8251–8258. https://doi.org/10.1021/jp3012602

Araújo, J.M.M., Florindo, C., Pereiro, A.B., Vieira, N.S.M., Matias, A.A., Duarte, C.M.M., Rebelo, L.P.N., Marrucho, I.M., 2014. Cholinium-based ionic liquids with pharmaceutically active anions. RSC Adv. 4, 28126–28132. https://doi.org/10.1039/C3RA47615D

Assadian, O., Wehse, K., Hübner, N.-O., Koburger, T., Bagel, S., Jethon, F., Kramer, A., 2011. Minimum inhibitory (MIC) and minimum microbicidal concentration (MMC) of polihexanide and triclosan against antibiotic sensitive and resistant Staphylococcus aureus and Escherichia coli strains. GMS Krankenhhyg Interdiszip. 6, 1–7.

Ateh, D.D., Vadgama, P., Navsaria, H., 2006. Culture of human keratinocytes on polypyrrole-based conducting polymers. Tissue Engineering 12, 645–655.

Barton, M.E., 1971. Effect of pH on the growth cycle of HeLa cells in batch suspension culture without oxygen control. Biotechnology and Bioengineering 13, 471–492. https://doi.org/10.1002/bit.260130403

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology 45, 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493

Biffinger, J.C., Pietron, J., Bretschger, O., Nadeau, L.J., Johnson, G.R., Williams, C.C., Nealson, K.H., Ringeisen, B.R., 2008. The influence of acidity on microbial fuel cells containing Shewanella oneidensis. Biosensors and Bioelectronics 24, 900–905. https://doi.org/10.1016/j.bios.2008.07.034

Binder, W.H., 2008. Polymer-Induced Transient Pores in Lipid Membranes. Angewandte Chemie International Edition 47, 3092–3095. https://doi.org/10.1002/anie.200800269

Bjerrum, J., Schwarzenbach, G., Sillen, L.G., 1957. Stability Constants of Metal-Ion Complexes, Part II, Inorganic Ligands. Chemical Society, London.

Borkowski, A., Kowalczyk, P., Czerwonka, G., Cieśla, J., Cłapa, T., Misiewicz, A., Szala, M., Drabik, M., 2017. Interaction of quaternary ammonium ionic liquids with bacterial membranes – Studies with Escherichia coli R1–R4-type lipopolysaccharides. Journal of Molecular Liquids 246, 282–289. https://doi.org/10.1016/j.molliq.2017.09.074

Braude, E.A., Nachod, F.C., eds., 1955. Determination of Organic Structures by Physical Methods. Academic Press, New York.

Brock, M., Nickel, A.-C., Madziar, B., Blusztajn, J.K., Berse, B., 2007. Differential regulation of the high affinity choline transporter and the cholinergic locus by cAMP signaling pathways. Brain Research 1145, 1–10. https://doi.org/10.1016/j.brainres.2007.01.119

Bubalo, M.C., Radošević, K., Redovniković, I.R., Slivac, I., Srček, V.G., 2017. Toxicity mechanisms of ionic liquids. Archives of Industrial Hygiene and Toxicology 68, 171–179. https://doi.org/10.1515/aiht-2017-68-2979

Cai, Z., Kim, J., 2008. Characterization and electromechanical performance of cellulose–chitosan blend electro-active paper. Smart Materials and Structures 17, 035028. https://doi.org/10.1088/0964-1726/17/3/035028

Castano, H., O'Rear, E.A., McFetridge, P.S., Sikavitsas, V.I., 2004. Polypyrrole Thin Films Formed by Admicellar Polymerization Support the Osteogenic Differentiation of Mesenchymal Stem Cells. Macromolecular Bioscience 4, 785–794. https://doi.org/10.1002/mabi.200300123

Cebrián, G., Arroyo, C., Mañas, P., Condón, S., 2014. Bacterial maximum non-inhibitory and minimum inhibitory concentrations of different water activity depressing solutes. International Journal of Food Microbiology 188, 67–74. https://doi.org/10.1016/j.ijfoodmicro.2014.07.011

Chambers, L.D., Winfield, J., Ieropoulos, I., Rossiter, J., 2014. Biodegradable and edible gelatine actuators for use as artificial muscles, in: Bar-Cohen, Y. (Ed.), p. 90560B. https://doi.org/10.1117/12.2045104

Clare, B., Sirwardana, A., MacFarlane, D.R., 2009. Synthesis, Purification and Characterization of Ionic Liquids, in: Kirchner, B. (Ed.), Ionic Liquids. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1–40. https://doi.org/10.1007/128_2008_31

Costa, S.P.F., Martins, B.S.F., Pinto, P.C.A.G., Saraiva, M.L.M.F.S., 2016. Automated cytochrome c oxidase bioassay developed for ionic liquids' toxicity assessment. Journal of Hazardous Materials 309, 165–172. https://doi.org/10.1016/j.jhazmat.2016.02.005

Costa, S.P.F., Pinto, P.C.A.G., Lapa, R.A.S., Saraiva, M.L.M.F.S., 2015. Toxicity assessment of ionic liquids with Vibrio fischeri: An alternative fully automated methodology. Journal of Hazardous Materials 284, 136–142. https://doi.org/10.1016/j.jhazmat.2014.10.049

Couling, D.J., Bernot, R.J., Docherty, K.M., Dixon, J.K., Maginn, E.J., 2006. Assessing the factors responsible for ionic liquid toxicity to aquatic organisms via quantitative structure–property relationship modeling. Green Chem. 8, 82–90. https://doi.org/10.1039/B511333D

Cvjetko, M., Radošević, K., Tomica, A., Slivac, I., Vorkapić-Furač, J., Srček, V., 2012. Cytotoxic Effects of Imidazolium Ionic Liquids on Fish and Human Cell Lines. Archives of Industrial Hygiene and Toxicology 63, 15–20. https://doi.org/10.2478/10004-1254-63-2012-2132

Dai, Y., van Spronsen, J., Witkamp, G.-J., Verpoorte, R., Choi, Y.H., 2013. Natural deep eutectic solvents as new potential media for green technology. Analytica Chimica Acta 766, 61–68. https://doi.org/10.1016/j.aca.2012.12.019

Dean, J.A., Lange, N.A. (Eds.), 1999. Lange's handbook of chemistry, 15. ed. ed, McGraw-Hill handbooks. McGraw-Hill, New York, NY.

Dubois, P., Rosset, S., Koster, S., Stauffer, J., Mikhaïlov, S., Dadras, M., Rooij, N.-F. de, Shea, H., 2006. Microactuators based on ion implanted dielectric electroactive polymer (EAP) membranes. Sensors and Actuators A: Physical 130–131, 147–154. https://doi.org/10.1016/j.sna.2005.11.069

Egorova, K.S., Ananikov, V.P., 2018. Fundamental importance of ionic interactions in the liquid phase: A review of recent studies of ionic liquids in biomedical and pharmaceutical applications. Journal of Molecular Liquids 272, 271–300. https://doi.org/10.1016/j.molliq.2018.09.025

Egorova, K.S., Ananikov, V.P., 2014. Toxicity of Ionic Liquids: Eco(cyto)activity as Complicated, but Unavoidable Parameter for Task-Specific Optimization. ChemSusChem 7, 336–360. https://doi.org/10.1002/cssc.201300459

Fahlgren, A., Bratengeier, C., Gelmi, A., Semeins, C.M., Klein-Nulend, J., Jager, E.W.H., Bakker, A.D., 2015. Biocompatibility of Polypyrrole with Human Primary Osteoblasts and the Effect of Dopants. PLOS ONE 10, e0134023. https://doi.org/10.1371/journal.pone.0134023

Fletcher, S.I., Sillars, F.B., Hudson, N.E., Hall, P.J., 2010. Physical Properties of Selected Ionic Liquids for Use as Electrolytes and Other Industrial Applications. J. Chem. Eng. Data 55, 778–782. https://doi.org/10.1021/je900405j

Frade, R.F., Afonso, C.A., 2010. Impact of ionic liquids in environment and humans: An overview. Human & Experimental Toxicology 29, 1038–1054. https://doi.org/10.1177/0960327110371259

Francisco, M., van den Bruinhorst, A., Kroon, M.C., 2013. Low-Transition-Temperature Mixtures (LTTMs): A New Generation of Designer Solvents. Angewandte Chemie International Edition 52, 3074–3085. https://doi.org/10.1002/anie.201207548

Fredrickson, J.K., Romine, M.F., Beliaev, A.S., Auchtung, J.M., Driscoll, M.E., Gardner, T.S., Nealson, K.H., Osterman, A.L., Pinchuk, G., Reed, J.L., Rodionov, D.A., Rodrigues, J.L.M., Saffarini, D.A., Serres, M.H., Spormann, A.M., Zhulin, I.B., Tiedje, J.M., 2008. Towards environmental systems biology of Shewanella. Nature Reviews Microbiology 6, 592–603. https://doi.org/10.1038/nrmicro1947

Freire, M.G., Neves, C.M.S.S., Marrucho, I.M., Coutinho, J.A.P., Fernandes, A.M., 2010. Hydrolysis of Tetrafluoroborate and Hexafluorophosphate Counter Ions in Imidazolium-Based Ionic Liquids †. The Journal of Physical Chemistry A 114, 3744–3749. https://doi.org/10.1021/jp903292n

Fukaya, Y., Iizuka, Y., Sekikawa, K., Ohno, H., 2007. Bio ionic liquids: room temperature ionic liquids composed wholly of biomaterials. Green Chem. 9, 1155–1157.

Gadilohar, B.L., Shankarling, G.S., 2017. Choline based ionic liquids and their applications in organic transformation. Journal of Molecular Liquids 227, 234–261. https://doi.org/10.1016/j.molliq.2016.11.136

George, P.M., Lyckman, A.W., LaVan, D.A., Hegde, A., Leung, Y., Avasare, R., Testa, C., Alexander, P.M., Langer, R., Sur, M., 2005. Fabrication and biocompatibility of polypyrrole implants suitable for neural prosthetics. Biomaterials 26, 3511–3519. https://doi.org/10.1016/j.biomaterials.2004.09.037

Goodson, M., Rowbury, R.J., 1989. Habituation to normally lethal acidity by prior growth of Escherichia coli at a sub-lethal acid pH value. Letters in Applied Microbiology 8, 77–79. https://doi.org/10.1111/j.1472-765X.1989.tb00227.x

Gorden, J., Small, P.L.C., 1993. Acid Resistance in Enteric Bacteria. Infect. Immun. 61, 364–367.

Gore, S., Baskaran, S., Koenig, B., 2011. Efficient synthesis of 3,4-dihydropyrimidin-2-ones in low melting tartaric acid–urea mixtures. Green Chemistry 13, 1009. https://doi.org/10.1039/c1gc00009h

Greulich, C., Braun, D., Peetsch, A., Diendorf, J., Siebers, B., Epple, M., Köller, M., 2012. The toxic effect of silver ions and silver nanoparticles towards bacteria and human cells occurs in the same concentration range. RSC Advances 2, 6981–6987. https://doi.org/10.1039/c2ra20684f

Hamzehzadeh, S., Majouy, A., Mokhtarani, B., 2016. Effect of the ionic liquid 1-butyl-3-methylimidazolium bromide as an additive on the formation of {polyethylene glycol+tri-potassium phosphate} aqueous biphasic systems: the role of polymer molecular weight. Journal of Molecular Liquids 213, 235–246. https://doi.org/10.1016/j.molliq.2015.10.039

Hayyan, M., Hashim, M.A., Hayyan, A., Al-Saadi, M.A., AlNashef, I.M., Mirghani, M.E.S., Saheed, O.K., 2013. Are deep eutectic solvents benign or toxic? Chemosphere 90, 2193–2195. https://doi.org/10.1016/j.chemosphere.2012.11.004

Heckenbach, M.E., Romero, F.N., Green, M.D., Halden, R.U., 2016. Meta-analysis of ionic liquid literature and toxicology. Chemosphere 150, 266–274. https://doi.org/10.1016/j.chemosphere.2016.02.029

Heidelberg, J.F., Paulsen, I.T., Nelson, K.E., Gaidos, E.J., Nelson, W.C., Read, T.D., Eisen, J.A., Seshadri, R., Ward, N., Methe, B., Clayton, R.A., Meyer, T., Tsapin, A., Scott, J., Beanan, M., Brinkac, L., Daugherty, S., DeBoy, R.T., Dodson, R.J., Durkin, A.S., Haft, D.H., Kolonay, J.F., Madupu, R., Peterson, J.D., Umayam, L.A., White, O., Wolf, A.M., Vamathevan, J., Weidman, J., Impraim, M., Lee, K., Berry, K., Lee, C., Mueller, J., Khouri, H., Gill, J., Utterback, T.R., McDonald, L.A., Feldblyum, T.V., Smith, H.O., Venter, J.C., Nealson, K.H., Fraser, C.M., 2002. Genome sequence of the dissimilatory metal ion–reducing bacterium Shewanella oneidensis. Nature Biotechnology 20, 1118–1123. https://doi.org/10.1038/nbt749

Hernández-Fernández, F.J., Bayo, J., Pérez de los Ríos, A., Vicente, M.A., Bernal, F.J., Quesada-Medina, J., 2015. Discovering less toxic ionic liquids by using the Microtox® toxicity test. Ecotoxicology and Environmental Safety 116, 29–33. https://doi.org/10.1016/j.ecoenv.2015.02.034

Hou, X.-D., Liu, Q.-P., Smith, T.J., Li, N., Zong, M.-H., 2013. Evaluation of Toxicity and Biodegradability of Cholinium Amino Acids Ionic Liquids. PLoS ONE 8, e59145. https://doi.org/10.1371/journal.pone.0059145

Housaindokht, M.R., Monhemi, H., Hosseini, H.E., Sadeghi Googheri, M.S., Najafabadi, R.I., Ashraf, N., Gholizadeh, M., 2013. It is explored that ionic liquids can be suitable solvents for nitrile hydratase catalyzed reactions: A gift of the molecular modeling for the industry. Journal of Molecular Liquids 187, 30–42. https://doi.org/10.1016/j.molliq.2013.05.012

Hudzicki, J., 2009. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol.

ICMSF, 1996. Staphylococcus aureus. Ch 17, in: Microorganisms in Food 5: Microbiological Specifications of Food Pathogens. Blackie Academic and Professional, London, UK, pp. 299–333.
Ilgen, F., König, B., 2009. Organic reactions in low melting mixtures based on carbohydrates and l-carnitine–a comparison. Green Chemistry 11, 848. https://doi.org/10.1039/b816551c
Imperato, G., Eibler, E., Niedermaier, J., König, B., 2005. Low-melting sugar–urea–salt mixtures as solvents for Diels–Alder reactions. Chem. Commun. 1170–1172. https://doi.org/10.1039/B414515A
Jager, E.W., Smela, E., Inganäs, O., 2000. Microfabricating conjugated polymer actuators. Science 290, 1540–1545.
Kai, D., Prabhakaran, M.P., Jin, G., Ramakrishna, S., 2013. Biocompatibility evaluation of electrically conductive nanofibrous scaffolds for cardiac tissue engineering. Journal of Materials Chemistry B 1, 2305. https://doi.org/10.1039/c3tb00151b
Kaiser, K.L.E., Palabrica, V.S., 1991. Photobacterium phosphoreum toxicity data index. Wat. Pollut. Res. J. 26, 361–431.
Karu, K., Elhi, F., Põhako-Esko, K., Ivaništšev, V., 2019. Predicting Melting Points of Biofriendly Choline-Based Ionic Liquids with Molecular Dynamics. Applied Sciences 9, 5367. https://doi.org/10.3390/app9245367
Kick, M., Keil, P., König, A., 2013. Solid–liquid phase diagram of the two Ionic Liquids EMIMCl and BMIMCl. Fluid Phase Equilibria 338, 172–178. https://doi.org/10.1016/j.fluid.2012.11.007
Kim, H.C., Mun, S., Ko, H.-U., Zhai, L., Kafy, A., Kim, J., 2016. Renewable smart materials. Smart Mater. Struct. 25, 073001. https://doi.org/10.1088/0964-1726/25/7/073001
Kim, J., Yun, S., Mahadeva, S.K., Yun, K., Yang, S.Y., Maniruzzaman, M., 2010. Paper Actuators Made with Cellulose and Hybrid Materials. Sensors 10, 1473–1485. https://doi.org/10.3390/s100301473
Kim, K.-S., Park, B.H., 2015. Differential Scanning Calorimetric Study on Binary Mixtures of Choline Chloride with Urea or 1,3-Dimethylurea. Journal of Chemical Engineering of Japan 48, 881–884. https://doi.org/10.1252/jcej.15we015
Klein, R., Müller, E., Kraus, B., Brunner, G., Estrine, B., Touraud, D., Heilmann, J., Kellermeier, M., Kunz, W., 2013. Biodegradability and cytotoxicity of choline soaps on human cell lines: effects of chain length and the cation. RSC Advances 3, 23347–23354. https://doi.org/10.1039/c3ra42812e
Klesewetter, L., Houdeau, D., Steckenbom, A., 1992. Determination of Young's moduli of micromechanical thin films using the resonance method. Sens Actuators A Phys. 32, 153–159.
Kortstee, G.J.J., 1970. The aerobic decomposition of choline by microorganisms: I. The ability of aerobic organisms, particularly coryneform bacteria, to utilize choline as the sole carbon and nitrogen source. Archiv für Mikrobiologie 71, 235–244. https://doi.org/10.1007/BF00410157
Kracke, F., Vassilev, I., Krömer, J.O., 2015. Microbial electron transport and energy conservation – the foundation for optimizing bioelectrochemical systems. Frontiers in Microbiology 6, 1–18. https://doi.org/10.3389/fmicb.2015.00575
Kruusamäe, K., Punning, A., Aabloo, A., Asaka, K., 2015. Self-Sensing Ionic Polymer Actuators: A Review. Actuators 4, 17–38. https://doi.org/10.3390/act4010017
Kunz, W., Maurer, E., Klein, R., Touraud, D., Rengstl, D., Harrar, A., Dengler, S., Zech, O., 2011. Low Toxic Ionic Liquids, Liquid Catanionics, and Ionic Liquid

Microemulsions. Journal of Dispersion Science and Technology 32, 1694–1699. https://doi.org/10.1080/01932691.2011.616109
Langsrud, S., Mat, N., 2009. 9 Biofilm formation by Gram-positive bacteria including Staphylococcus aureus, Mycobacterium avium and Enterococcus spp. in food processing environments, in: Biofilms in the Food and Beverage Industries. Woodhead Publishing Limited and CRC Press LLC, Cambridge, UK, pp. 250–269.
Lee, S.-M., Chang, W.-J., Choi, A.-R., Koo, Y.-M., 2005. Influence of ionic liquids on the growth ofEscherichia coli. Korean Journal of Chemical Engineering 22, 687–690. https://doi.org/10.1007/BF02705783
Li, P., Zhou, C., Rayatpisheh, S., Ye, K., Poon, Y.F., Hammond, P.T., Duan, H., Chan-Park, M.B., 2012. Cationic Peptidopolysaccharides Show Excellent Broad-Spectrum Antimicrobial Activities and High Selectivity. Advanced Materials 24, 4130–4137. https://doi.org/10.1002/adma.201104186
Liato, V., Aïder, M., 2017. Effect of electro-activated solutions of sodium acetate and sodium propionate on geosmin producing Streptomyces avermitilis strain. Chemosphere 188, 434–443. https://doi.org/10.1016/j.chemosphere.2017.09.011
Liato, V., Labrie, S., Aïder, M., 2017. Study of the antibacterial activity of electro-activated solutions of salts of weak organic acids on Salmonella enterica, Staphylococcus aureus and Listeria monocytogenes. Journal of Industrial Microbiology & Biotechnology 44, 23–33. https://doi.org/10.1007/s10295-016-1859-y
Lide, D.R. (ed.), 2005. CRC Handbook of Chemistry and Physics. CRC Press LLC.
Łuczak, J., Jungnickel, C., Łącka, I., Stolte, S., Hupka, J., 2010. Antimicrobial and surface activity of 1-alkyl-3-methylimidazolium derivatives. Green Chemistry 12, 593–601. https://doi.org/10.1039/b921805j
Mackenzie, C.G., 1961. The effect of pH on growth, protein synthesis, and lipid-rich particles of cultured mammalian cells. The Journal of Cell Biology 9, 141–156. https://doi.org/10.1083/jcb.9.1.141
Madden, J.D.W., Vandesteeg, N.A., Anquetil, P.A., Madden, P.G.A., Takshi, A., Pytel, R.Z., Lafontaine, S.R., Wieringa, P.A., Hunter, I.W., 2004. Artificial Muscle Technology: Physical Principles and Naval Prospects. IEEE Journal of Oceanic Engineering 29, 706–728. https://doi.org/10.1109/JOE.2004.833135
Maton, C., De Vos, N., Stevens, C.V., 2013. Ionic liquid thermal stabilities: decomposition mechanisms and analysis tools. Chemical Society Reviews 42, 5963. https://doi.org/10.1039/c3cs60071h
Maziz, A., Plesse, C., Soyer, C., Cattan, E., Vidal, F., 2016. Top-down Approach for the Direct Synthesis, Patterning, and Operation of Artificial Micromuscles on Flexible Substrates. ACS Applied Materials & Interfaces 8, 1559–1564. https://doi.org/-10.1021/acsami.5b09577
Meng, X., Ballerat-Busserolles, K., Husson, P., Andanson, J.-M., 2016. Impact of water on the melting temperature of urea + choline chloride deep eutectic solvent. New Journal of Chemistry 40, 4492–4499. https://doi.org/10.1039/C5NJ02677F
Meyer, T.E., Tsapin, A.I., Vandenberghe, I., De Smet, L., Frishman, D., Nealson, K.H., Cusanovich, M.A., Van Beeumen, J.J., 2004. Identification of 42 Possible Cytochrome C Genes in the *Shewanella oneidensis* Genome and Characterization of Six Soluble Cytochromes. OMICS: A Journal of Integrative Biology 8, 57–77. https://doi.org/10.1089/153623104773547499
Mirfakhrai, T., Madden, J.D., Baughman, R.H., 2007. Polymer artificial muscles. Materials today 10, 30–38.

Morrison, H.G., Sun, C.C., Neervannan, S., 2009. Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles. International Journal of Pharmaceutics 378, 136–139. https://doi.org/10.1016/j. ijpharm. 2009.05.039

Mourão, T., Tomé, L.C., Florindo, C., Rebelo, L.P.N., Marrucho, I.M., 2014. Understanding the Role of Cholinium Carboxylate Ionic Liquids in PEG-Based Aqueous Biphasic Systems. ACS Sustainable Chemistry & Engineering 2, 2426–2434. https://doi.org/10.1021/sc500444w

Mousavisafavi, S.M., Gharagheizi, F., Mirkhani, S.A., Akbari, J., 2013. A predictive quantitative structure–property relationship for glass transition temperature of 1,3-dialkyl imidazolium ionic liquids: Part 2. The nonlinear approach. J Therm Anal Calorim 111, 1639–1648. https://doi.org/10.1007/s10973-012-2208-7

Muhammad, N., Hossain, M.I., Man, Z., El-Harbawi, M., Bustam, M.A., Noaman, Y.A., Mohamed Alitheen, N.B., Ng, M.K., Hefter, G., Yin, C.-Y., 2012. Synthesis and Physical Properties of Choline Carboxylate Ionic Liquids. Journal of Chemical & Engineering Data 57, 2191–2196. https://doi.org/10.1021/je300086w

Nkuku, C.A., LeSuer, R.J., 2007. Electrochemistry in Deep Eutectic Solvents. The Journal of Physical Chemistry B 111, 13271–13277. https://doi.org/10.1021/jp075794j

Nockemann, P., Thijs, B., Driesen, K., Janssen, C.R., Van Hecke, K., Van Meervelt, L., Kossmann, S., Kirchner, B., Binnemans, K., 2007. Choline Saccharinate and Choline Acesulfamate: Ionic Liquids with Low Toxicities. The Journal of Physical Chemistry B 111, 5254–5263. https://doi.org/10.1021/jp068446a

Noshadi, I., Walker, B.W., Portillo-Lara, R., Shirzaei Sani, E., Gomes, N., Aziziyan, M.R., Annabi, N., 2017. Engineering Biodegradable and Biocompatible Bio-ionic Liquid Conjugated Hydrogels with Tunable Conductivity and Mechanical Properties. Scientific Reports 7. https://doi.org/10.1038/s41598-017-04280-w

O'Toole, G.A., Wathier, M., Zegans, M.E., Shanks, R.M.Q., Kowalski, R., Grinstaff, M.W., 2012. Diphosphonium Ionic Liquids as Broad-Spectrum Antimicrobial Agents: Cornea 31, 810–816. https://doi.org/10.1097/ICO.0b013e31823f0a86

Palermo, E.F., Sovadinova, I., Kuroda, K., 2009. Structural Determinants of Antimicrobial Activity and Biocompatibility in Membrane-Disrupting Methacrylamide Random Copolymers. Biomacromolecules 10, 3098–3107. https://doi.org/10.1021/bm900784x

Palumbo, O., Trequattrini, F., Navarra, M.A., Brubach, J.-B., Roy, P., Paolone, A., 2017. Tailoring the physical properties of the mixtures of ionic liquids: a microscopic point of view. Phys. Chem. Chem. Phys. 19, 8322–8329. https://doi.org/10.1039/C7CP00850C

Passino, D.R.M., Smith, S.B., 1987. Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish. Environmental Toxicology and Chemistry 6, 901–907. https://doi.org/10.1002/etc.5620061111

Petkovic, M., Ferguson, J., Bohn, A., Trindade, J., Martins, I., Carvalho, M.B., Leitão, M.C., Rodrigues, C., Garcia, H., Ferreira, R., Seddon, K.R., Rebelo, L.P.N., Silva Pereira, C., 2009. Exploring fungal activity in the presence of ionic liquids. Green Chemistry 11, 889–894. https://doi.org/10.1039/b823225c

Petkovic, M., Ferguson, J.L., Gunaratne, H.Q.N., Ferreira, R., Leitão, M.C., Seddon, K.R., Rebelo, L.P.N., Pereira, C.S., 2010. Novel biocompatible cholinium-based ionic liquids–toxicity and biodegradability. Green Chem. 12, 643–649.

Pugal, D., Jung, K., Aabloo, A., Kim, K.J., 2010. Ionic polymer-metal composite mechanoelectrical transduction: review and perspectives: Review of IPMC mechano-electrical transduction. Polym. Int. 59, 279–289. https://doi.org/10.1002/pi.2759

Punning, A., Kim, K.J., Palmre, V., Vidal, F., Plesse, C., Festin, N., Maziz, A., Asaka, K., Sugino, T., Alici, G., Spinks, G., Wallace, G., Must, I., Põldsalu, I., Vunder, V., Temmer, R., Kruusamäe, K., Torop, J., Kaasik, F., Rinne, P., Johanson, U., Peikolainen, A.-L., Tamm, T., Aabloo, A., 2015. Ionic electroactive polymer artificial muscles in space applications. Sci Rep 4, 6913. https://doi.org/10.1038/srep06913

Radošević, K., Cvjetko Bubalo, M., Gaurina Srček, V., Grgas, D., Landeka Dragičević, T., Radojčić Redovniković, I., 2015. Evaluation of toxicity and biodegradability of choline chloride based deep eutectic solvents. Ecotoxicology and Environmental Safety 112, 46–53. https://doi.org/10.1016/j.ecoenv.2014.09.034

Radošević, K., Železnjak, J., Cvjetko Bubalo, M., Radojčić Redovniković, I., Slivac, I., Gaurina Srček, V., 2016. Comparative in vitro study of cholinium-based ionic liquids and deep eutectic solvents toward fish cell line. Ecotoxicology and Environmental Safety 131, 30–36. https://doi.org/10.1016/j.ecoenv.2016.05.005

Rengstl, D., Kraus, B., Van Vorst, M., Elliott, G.D., Kunz, W., 2014. Effect of choline carboxylate ionic liquids on biological membranes. Colloids and Surfaces B: Biointerfaces 123, 575–581. https://doi.org/10.1016/j.colsurfb.2014.09.057

Ribeiro, R., Pinto, P.C.A.G., Azevedo, A.M.O., Bica, K., Ressmann, A.K., Reis, S., Saraiva, M.L.M.F.S., 2016. Automated evaluation of protein binding affinity of anti-inflammatory choline based ionic liquids. Talanta 150, 20–26. https://doi.org/10.1016/j.talanta.2015.12.009

Ruß, C., König, B., 2012. Low melting mixtures in organic synthesis – an alternative to ionic liquids? Green Chemistry 14, 2969. https://doi.org/10.1039/c2gc36005e

Samorì, C., 2011. Ionic Liquids and their Biological Effects Towards Microorganisms. Current Organic Chemistry 15, 1888–1904. https://doi.org/10.2174/138527211795703658

Santos, J.I., Gonçalves, A.M.M., Pereira, J.L., Figueiredo, B.F.H.T., e Silva, F.A., Coutinho, J.A.P., Ventura, S.P.M., Gonçalves, F., 2015. Environmental safety of cholinium-based ionic liquids: assessing structure–ecotoxicity relationships. Green Chemistry 17, 4657–4668. https://doi.org/10.1039/C5GC01129A

Sardessai, Y., Bhosle, S., 2002. Tolerance of bacteria to organic solvents. Research in Microbiology 153, 263–268. https://doi.org/10.1016/S0923-2508(02)01319-0

Sareh, S., Rossiter, J., 2013. Kirigami artificial muscles with complex biologically inspired morphologies. Smart Mater. Struct. 22, 014004. https://doi.org/10.1088/0964-1726/22/1/014004

Sareh, S., Rossiter, J., Conn, A., Drescher, K., Goldstein, R.E., 2013. Swimming like algae: biomimetic soft artificial cilia. J. R. Soc. Interface. 10, 20120666. https://doi.org/10.1098/rsif.2012.0666

Sharma, M., Mondal, D., Mukesh, C., Prasad, K., 2014. Preparation of tamarind gum based soft ion gels having thixotropic properties. Carbohydrate Polymers 102, 467–471. https://doi.org/10.1016/j.carbpol.2013.11.063

Shi, G., Rouabhia, M., Wang, Z., Dao, L.H., Zhang, Z., 2004. A novel electrically conductive and biodegradable composite made of polypyrrole nanoparticles and polylactide. Biomaterials 25, 2477–2488. https://doi.org/10.1016/j.biomaterials.2003.09.032

Siimon, K., Siimon, H., Järvekülg, M., 2015. Mechanical characterization of electrospun gelatin scaffolds cross-linked by glucose. Journal of Materials Science: Materials in Medicine 26. https://doi.org/10.1007/s10856-014-5375-1

Singh, B.S., Lobo, H.R., Shankarling, G.S., 2012. Choline chloride based eutectic solvents: Magical catalytic system for carbon–carbon bond formation in the rapid synthesis of β-hydroxy functionalized derivatives. Catalysis Communications 24, 70–74. https://doi.org/10.1016/j.catcom.2012.03.021

Smith, E.L., Abbott, A.P., Ryder, K.S., 2014. Deep Eutectic Solvents (DESs) and Their Applications. Chem. Rev. 114, 11060–11082. https://doi.org/10.1021/cr300162p

Stewart, C., 2003. Staphylococcus aureus and staphylococcal enterotoxins, in: Foodborne Microorganisms of Public Health Significance. Australian Institute of Food Science and Technology (NSW Branch), Sidny, Australia, pp. 359–380.

Stolarska, O., Rodríguez, H., Smiglak, M., 2016. Eutectic mixtures of pyrrolidinium-based ionic liquids. Fluid Phase Equilibria 408, 1–9. https://doi.org/10.1016/j.fluid.2015.08.007

Stolte, S., Matzke, M., Arning, J., Böschen, A., Pitner, W.-R., Welz-Biermann, U., Jastorff, B., Ranke, J., 2007. Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids. Green Chem. 9, 1170. https://doi.org/10.1039/b711119c

Sugino, T., Kiyohara, K., Takeuchi, I., Mukai, K., Asaka, K., 2009. Actuator properties of the complexes composed by carbon nanotube and ionic liquid: The effects of additives. Sensors and Actuators B: Chemical 141, 179–186. https://doi.org/10.1016/j.snb.2009.06.002

Sun, J., Forsyth, M., MacFarlane, D.R., 1998. Room-Temperature Molten Salts Based on the Quaternary Ammonium Ion. The Journal of Physical Chemistry B 102, 8858–8864. https://doi.org/10.1021/jp981159p

Sun, Z., Zhao, G., Song, W.L., Wang, J., Ui Haq, M., 2018. Investigation into Electromechanical Properties of Biocompatible Chitosan-Based Ionic Actuator. Experimental Mechanics 58, 99–109. https://doi.org/10.1007/s11340-017-0332-9

Svennersten, K., Berggren, M., Richter-Dahlfors, A., Jager, E.W.H., 2011. Mechanical stimulation of epithelial cells using polypyrrole microactuators. Lab of a Chip 11, 3287–3293.

Svirskis, D., Travas-Sejdic, J., Rodgers, A., Garg, S., 2010. Electrochemically controlled drug delivery based on intrinsically conducting polymers. Journal of Controlled Release 146, 6–15. https://doi.org/10.1016/j.jconrel.2010.03.023

Tchounwou, P.B., Wilson, B., Ishaque, A., Ransome, R., Huang, M.-J., Leszczynski, J., 2000. Toxicity Assessment of Atrazine and Related Triazine Compounds in the Microtox Assay, and Computational Modeling for Their Structure-Activity Relationship. IJMS 1, 63–74. https://doi.org/10.3390/ijms1040063

Temmer, R., Maziz, A., Plesse, C., Aabloo, A., Vidal, F., Tamm, T., 2013. In search of better electroactive polymer actuator materials: PPy versus PEDOT versus PEDOT–PPy composites. Smart Materials and Structures 22, 104006. https://doi.org/10.1088/0964-1726/22/10/104006

Temmer, R., Must, I., Kaasik, F., Aabloo, A., Tamm, T., 2012. Combined chemical and electrochemical synthesis methods for metal-free polypyrrole actuators. Sensors and Actuators B: Chemical 166–167, 411–418. https://doi.org/10.1016/j.snb.2012.01.075

Tran, T.L., Chu, T.X., Huynh, D.C., Pham, D.T., Luu, T.H.T., Mai, A.T., 2014. Effective immobilization of DNA for development of polypyrrole nanowires based bio-

sensor. Applied Surface Science 314, 260–265. https://doi.org/10.1016/j.apsusc.2014.06.068
Ventura, S.P.M., e Silva, F.A., Gonçalves, A.M.M., Pereira, J.L., Gonçalves, F., Coutinho, J.A.P., 2014. Ecotoxicity analysis of cholinium-based ionic liquids to Vibrio fischeri marine bacteria. Ecotoxicology and Environmental Safety 102, 48–54. https://doi.org/10.1016/j.ecoenv.2014.01.003
Ventura, S.P.M., Gonçalves, A.M.M., Sintra, T., Pereira, J.L., Gonçalves, F., Coutinho, J.A.P., 2013. Designing ionic liquids: the chemical structure role in the toxicity. Ecotoxicology 22, 1–12. https://doi.org/10.1007/s10646-012-0997-x
Walden, P., 1914. Ueber die Molekulargrösse und elektrische Leitfähigkeit einiger geschmolzenen Salze. Bulletin de l'Acad´emie Imp´eriale des Sciences de St.-P´etersbourg, VI 8, 405–422.
Wang, F., Jeon, J.-H., Park, S., Kee, C.-D., Kim, S.-J., Oh, I.-K., 2016. A soft biomolecule actuator based on a highly functionalized bacterial cellulose nano-fiber network with carboxylic acid groups. Soft Matter 12, 246–254. https://doi.org/10.1039/C5SM00707K
Wang, X., Gu, X., Yuan, C., Chen, S., Zhang, P., Zhang, T., Yao, J., Chen, F., Chen, G., 2004. Evaluation of biocompatibility of polypyrrole in vitro and in vivo. Journal of biomedical materials research Part A 68, 411–422.
Weaver, K.D., Kim, H.J., Sun, J., MacFarlane, D.R., Elliott, G.D., 2010. Cyto-toxicity and biocompatibility of a family of choline phosphate ionic liquids designed for pharmaceutical applications. Green Chemistry 12, 507–513. https://doi.org/10.1039/b918726j
Wen, Q., Chen, J.-X., Tang, Y.-L., Wang, J., Yang, Z., 2015. Assessing the toxicity and biodegradability of deep eutectic solvents. Chemosphere 132, 63–69. https://doi.org/10.1016/j.chemosphere.2015.02.061
Wiegand, I., Hilpert, K., Hancock, R.E.W., 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols 3, 163–175. https://doi.org/10.1038/nprot.2007.521
Wong, J.Y., Langer, R., Ingber, D.E., 1994. Electrically conducting polymers can noninvasively control the shape and growth of mammalian cells. Proceedings of the National Academy of Sciences 91, 3201–3204.
Xie, J., Wang, B.-S., Yu, D.-H., Lu, Q., Ma, J., Qi, H., Fang, C., Chen, H.-Z., 2011. Dichloroacetate shifts the metabolism from glycolysis to glucose oxidation and exhibits synergistic growth inhibition with cisplatin in HeLa cells. International Journal of Oncology 38, 409–417. https://doi.org/10.3892/ijo.2010.851
Yan, Y., Santaniello, T., Bettini, L.G., Minnai, C., Bellacicca, A., Porotti, R., Denti, I., Faraone, G., Merlini, M., Lenardi, C., Milani, P., 2017. Electroactive Ionic Soft Actuators with Monolithically Integrated Gold Nanocomposite Electrodes. Advanced Materials 29, 1606109. https://doi.org/10.1002/adma.201606109
Yeom, S.-W., Oh, I.-K., 2009. A biomimetic jellyfish robot based on ionic polymer metal composite actuators. Smart Mater. Struct. 18, 085002. https://doi.org/10.1088/0964-1726/18/8/085002
Younes, N., Salem, R., Al-Asmakh, M., Altamash, T., Pintus, G., Khraisheh, M., Nasrallah, G.K., 2018. Toxicity evaluation of selected ionic liquid compounds on embryonic development of Zebrafish. Ecotoxicology and Environmental Safety 161, 17–24. https://doi.org/10.1016/j.ecoenv.2018.05.064
Zeisel, S.H., 2012. A Brief History of Choline. Ann Nutr Metab 61, 254–258. https://doi.org/10.1159/000343120

Zeisel, S.H., da Costa, K.-A., 2009. Choline: an essential nutrient for public health. Nutrition Reviews 67, 615–623. https://doi.org/10.1111/j.1753-4887.2009.00246.x

Zhang, S., Ma, L., Wen, P., Ye, X., Dong, R., Sun, W., Fan, M., Yang, D., Zhou, F., Liu, W., 2018. The ecotoxicity and tribological properties of choline amino acid ionic liquid lubricants. Tribology International 121, 435–441. https://doi.org/10.1016/j.triboint.2018.01.063

Zheng, Z., Xu, Q., Guo, J., Qin, J., Mao, H., Wang, B., Yan, F., 2016. Structure–Antibacterial Activity Relationships of Imidazolium-Type Ionic Liquid Monomers, Poly(ionic liquids) and Poly(ionic liquid) Membranes: Effect of Alkyl Chain Length and Cations. ACS Applied Materials & Interfaces 8, 12684–12692. https://doi.org/10.1021/acsami.6b03391

Zilberstein, D., Agmon, V., Schuldiner, S., Padan, E., 1982. The Sodium/Proton Antiporter Is Part of the pH Homeostasis Mechanism in Escherichia coli. J. Biol. Chem. 257, 3687–3691.

8. APPENDIX 1

Table A 1: ^{1}H NMR data for choline ILs synthesized in this research

[Ch][Ac]	^{1}H NMR (D_2O, 400 MHz): δ/ppm = 3.99 (m, 2H, NC**H**$_2$), 3.45 (m, 2H, C**H**$_2$OH), 3.13 (s, 9H, N(C**H**$_3$)$_3$), 1.84 (s, 3H, C**H**$_3$)
[Ch][Ib]	^{1}H NMR (D_2O, 400 MHz): δ/ppm = 4.04 (m, 2H, NC**H**$_2$), 3.50 (m, 2H, C**H**$_2$OH), 3.18 (s, 9H, N(C**H**$_3$)$_3$), 2.36 (sep, *J* = 6.9 Hz, 1H, C**H**(CH$_3$)$_2$), 1.04 (d, *J* = 6.8 Hz, 6H, CH(C**H**$_3$)$_2$)
[Ch][Iv]	^{1}H NMR (D_2O, 400 MHz): δ/ppm = 4.02 (m, 2H, NC**H**$_2$), 3.48 (m, 2H, C**H**$_2$OH), 3.16 (s, 9H, N(C**H**$_3$)$_3$), 2.03 (d, *J* = 6.9 Hz, 2H,C**H**$_2$COOH), 1.92 (m, 1H, C**H**(CH$_3$)$_2$), 0.88 (d, *J* = 6.5 Hz, 6H, CH(C**H**$_3$)$_2$)
[Ch][2mb]	^{1}H NMR (D_2O, 400 MHz): δ/ppm = 4.04 (m, 2H, NC**H**$_2$), 3.49 (dd, *J* = 4.3, 5.5 Hz, 2H, C**H**$_2$OH), 3.17 (s, 9H, N(C**H**$_3$)$_3$), 2.16 (sex, *J* = 7.2 Hz, 1H, C**H**COOH), 1.41 (m, 2H, C**H**$_2$CH$_3$), 1.01 (d, *J* = 7.1 Hz, 3H, CHC**H**$_3$), 0.83 (t, *J* = 7.5 Hz, 3H, CH$_2$C**H**$_3$)
[Ch][Mal]	^{1}H NMR (D_2O, 400 MHz): δ/ppm = 4.02 (m, 2H, NC**H**$_2$), 3.48 (m, 2H, C**H**$_2$OH), 3.16 (d, *J* = 3.9 Hz, 9H N(C**H**$_3$)$_3$)
[Ch][Glu]	^{1}H NMR (D_2O, 400 MHz): δ/ppm = 4.02 (m, 2H, NC**H**$_2$), 3.49 (t, *J* = 4.8 Hz, 2H, C**H**$_2$OH), 3.18 (s, 9H, N(C**H**$_3$)$_3$), 2.27 (t, *J* = 7.5 Hz, 4H, C**H**$_2$COOH), 1.81 (quin, *J* = 7.5 Hz, 2H,CH$_2$ C**H**$_2$CH$_2$)
[Ch][Cit]	^{1}H NMR (D_2O, 400 MHz): δ/ppm = 3.93 (m, 2H, NC**H**$_2$), 3.39 (m, 2H, C**H**$_2$OH), 3.01 (s, 9H, N(C**H**$_3$)$_3$), 2.77 (d, *J* = 15.6 Hz, 2H, C**H**$_2$), 2.65 (d, *J* = 15.2 Hz, 2H, C**H**$_2$)

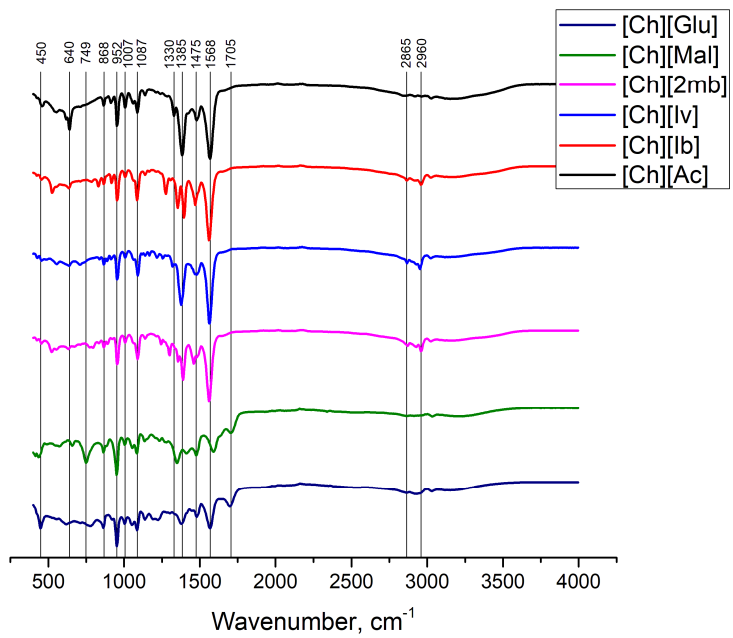


Figure A 1: FTIR spectra of ILs used in PPy-gelatin and PPy-PVdF actuators

Table A 2: Exposure concentrations of ionic liquids on test organisms

IL	Exposure concentrations (mM)																		
	Escherichia coli																		
[Ch][Ac]	15.625	31.25	62.5	125	250	500	550	600	700	750	800	900							
[Ch][Ib]	15.625	31.25	62.5	125	250	500	550	600	650	700	750	800	900						
[Ch][Iv]	15.625	31.25	62.5	125	250	300	350	400	450	500	550	600	750						
[Ch][2mb]	15.625	31.25	62.5	125	250	300	350	400	450	500	550	600	750						
[Ch][Mal]	15.625	25	30	31.25	50	62.5	70	75	100	125	140	250	500	750					
[Ch][Glu]	15.625	31.25	60	100	125	150	200	250	300	500	750								
[Ch][Cit]	15.625	25	31.25	62.5	75	100	125	150	175	200	250	500	750						
	Staphylococcus aureus																		
[Ch][Ac]	15.625	31.25	62.5	125	250	500	600	700	750	800	850	900	1000						
[Ch][Ib]	15.625	31.25	62.5	125	250	500	600	700	750	800	850	900	1000						
[Ch][Iv]	15.625	31.25	62.5	125	250	500	550	600	650	700	750	800	900						
[Ch][2mb]	15.625	31.25	62.5	125	250	500	600	700	750	800	850	900	1000						
[Ch][Mal]	15.625	31.25	50	62.5	70	90	110	120	125	130	140	250	500	750					
[Ch][Glu]	15.625	20	30	31.25	40	50	55	60	62.5	70	90	125	250	500	750				
[Ch][Cit]	10	15.625	17.5	20	25	30	31.25	40	62	62.5	125	250	500	750					
	Shewanella oneidensis MR-1																		
[Ch][Ac]	3.75	7.5	15	31.25	56.25	62.5	112.5	125	200	225	250	300	325	350	400	450	500	1000	
[Ch][Ib]	1.875	3.75	7.5	15	31.25	37.5	62.5	75	100	125	150	200	250	300	325	500	1000		
[Ch][Iv]	3.75	7.5	15	30	31.25	56.25	62.5	112.5	125	200	225	250	300	325	350	400	450	500	1000
[Ch][2mb]	1.875	3.75	7.5	15	31.25	37.5	62.5	75	125	150	250	300	325	500	1000				
[Ch][Mal]	0.5625	1.125	2.25	4.5	5.625	15	22.5	31.25	45	62.5	125	250	325	500	1000				
[Ch][Glu]	0.5625	1.125	2.25	4.5	5.625	11.25	15	22.5	31.25	45	62.5	125	250	325	500	1000			
[Ch][Cit]	0.5625	1.125	2.25	4.5	5.625	11.25	15	22.5	31.25	45	62.5	125	250	325	500	1000			

IL	Exposure concentrations (mM)													
	HeLa cells													
[Ch][Ac]	0.001	0.01	0.1	1	5	10	15	30	45	60	75	90	105	125
[Ch][Ib]	0.001	0.01	0.1	1	5	10	15	30	45	60	75	90	105	125
[Ch][Iv]	0.001	0.01	0.1	1	5	10	15	25	35	45	55	65	75	
[Ch][2mb]	0.001	0.01	0.1	1	5	10	15	30	45	60	75	90	105	125
[Ch][Mal]	0.001	0.01	0.1	1	5	10	15	25	35	45	55	65	75	
[Ch][Glu]	0.001	0.01	0.1	1	5	10	15	25	35	45	55	65	75	
[Ch][Cit]	0.001	0.01	0.1	1	5	10	15	20	25	30	35	40		

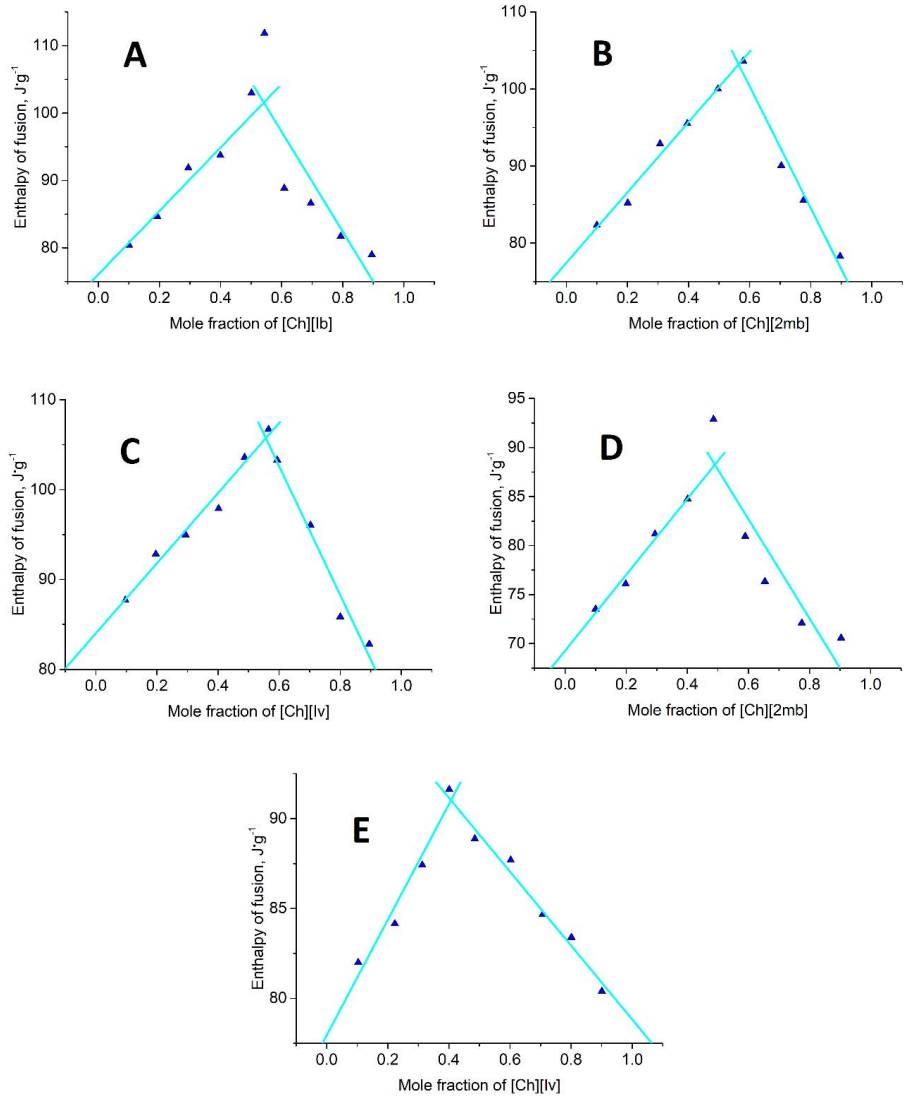


Figure A 2: A) Tammann diagram of the [Ch][Ac] + [Ch][Ib] mixture; B) Tammann diagram of the [Ch][Ac] + [Ch][2mb] mixture; C) Tammann diagram of the [Ch][Ac] + [Ch][Iv] mixture; D) Tammann diagram of the [Ch][Ib] + [Ch][2mb] mixture; E) Tammann diagram of the [Ch][Ib] + [Ch][Iv] mixture

PUBLICATIONS

CURRICULUM VITAE

Name: Fred Elhi
Date of Birth: October 20, 1990.
Citizenship: Estonian
Address: Kadaka pst 75, Tallinn, Estonia
Phone: +372 53320474
E-mail: elhi.fred@gmail.com
Position: PhD student

Education:
- University of Tartu, 2016, Master's degree
- University of Tartu, 2013, Bachelor's degree

Language skills:
- Estonian – native language (high fluency)
- English – excellent in speech and writing (fluent)
- Finnish – basic knowledge (low fluency)
- Danish – basic knowledge (low fluency)
- Swedish – basic knowledge (low fluency)

Professional career:

2018–2020	TBD-Biodiscovery OÜ, Industrial PhD
2018–2019	TBD-Biodiscovery OÜ, Quality Control
2019–2020	TBD-Biodiscovery OÜ, Technical Evaluator
2019	Biotatec OÜ, Bioleaching; separation of natural gas from minerals and precious metals from electronics with the help of bacterial cultures
2017	Hairvel OÜ, Development of novel hair colouring agent
2014	University of Tartu, Institute of Technology Making cellulose-carbon composites with ionic liquid
2014	Tallinn University of Technology, The Department of Chemistry, Engineer
2013	University of Tartu, Institute of Technology Developing method for making of carbon electrodes using spin coating

Research and development work
Main fields of research:
- Electroactive polymers
- Ionic liquids
- Choline
- IEAP actuators
- Biocompatible materials

– Nanocarbon
– Aerogels
– Microprinting
– Eutectic mixtures

A list of publications:

1.1 publications listed in Thompson Reuters Web of Science database:

Elhi, F.; Peikolainen, A.-L.; Kiefer, R.; Tamm, T. (2020). Cellulose-Multiwall Carbon Nanotube Fiber Actuator Behavior in Aqueous and Organic Electrolyte. Materials, *13*(14), 3213.

Elhi, F.; Karu, K.; Rinne, P.; Nadel, K.-A.; Järvekülg, M.; Aabloo, A.; Tamm, T.; Ivaništšev, V.; Põhako-Esko, K. (2020). Understanding the Behavior of Fully Non-Toxic Polypyrrole-Gelatin and Polypyrrole-PVdF Soft Actuators with Choline Ionic Liquids. Actuators, *9*(2), 40.

Khuyen, N. Q.; Kiefer, R.; **Elhi, F.**; Anbarjafari, G.; Martinez, J. G.; Tamm, T. (2020). A biomimetic approach to increase soft actuator performance by friction reduction. Polymers, *12*(5), 1120.

Elhi, F.; Priks, H.; Rinne, P.; Kaldalu, N.; Žusinaite, E.; Johanson, U.; Aabloo, A.; Tamm, T.; Põhako-Esko, K. (2020). Electromechanically active polymer actuators based on biofriendly choline ionic liquids. Smart Materials and Structures, *29*(5), 055021

Elhi, F.; Gantman, M.; Nurk, G.; Schulz, P. S.; Wasserscheid, P.; Aabloo, A.; Pohako-Esko, K. (2020). Influence of Carboxylate Anions on Phase Behavior of Choline Ionic Liquid Mixtures. Molecules, *25*(7), 1691.

Karu, K.; **Elhi, F.**; Põhako-Esko, K.; Ivaništšev, V. (2019). Predicting Melting Points of Biofriendly Choline-Based Ionic Liquids with Molecular Dynamics. Applied Sciences, *9*(24), 5367.

Elhi, F.; Aid, T.; Koel, M. (2016) Ionic liquids as solvents for making composite materials from cellulose. Proceedings of the Estonian Academy of Sciences, *65*(3), 255–266.

5.2 Conference theses:

Karu, K.; **Elhi, F.**; Põhako-Esko, K.; Ivaništšev, V. (2019). Determining the melting point of biocompatible ionic liquids with molecular dynamics simulations. *FMTDK teeside kogumik: FMTDK konverents 2019.* Tartu: FMTDK.

Elhi, F.; Küppar, K.-A.; Tamm, T.; Järvekülg, M.; Põhako-Esko, K.; Aabloo, A. (2017). Biocompatible Ionic Electroactive Polymer Actuators. *2017 MRS Fall Meeting & Exhibit Abstracts.*

Elhi, F.; Põhako-Esko, K.; Küppar, K.-A.; Tamm, T.; Järvekülg, M.; Aabloo, A. (2017). Biocompatible Ionic Electrochemically Active Polymer Actuators. *FMTDK Teaduskonverentsi teeside kogumik.FMTDK Teaduskonverents*

Elhi, F.; Kisand, V.; Peikolainen, A.-L.; Koel, M.; Aabloo, A. (2016) Carbon aerogel-cellulose films and fibres with electrical conductivity and anti-

bacterial properties against cyanobacteria, *Proceedings 3rd International Seminar On Aerogels: Synthesis-Properties-Applications*, 180.

Kesküla, A.; **Elhi, F.**; Peikolainen, A.-L.; Mäeorg, U.; Aabloo, A. (2013) Polymerized ionic liquid membranes for electrochemical actuator and sensor applications. *International Conference on Materials and Applications for Sensors and Transducers Abstracts Book: 3rd International Conference on Materials and Applications for Sensors and Transducers, ICT-MAST 2013.* Ed: Hristoforou, E.; Vlachos, D.S., 1–45.

Research grants and scholarships:

- Dora Plus PhD mobility stipend (T1.2) 2018 Archimedes

Other administrative and professional activities.

- 2018 Sept – 2019 Jan Visiting PhD student at Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute of Chemical Reaction Engineering

Teaching work

1. Information regarding the teaching work carried out at universities.

- Partly supervising 2017/2018 (spring), 2018/2019 (spring), 2019/2020 (spring), and 2020/2021 (spring) course "Working with Modern Lab Equipment" (LTTI.00.017, 6 EAP). Supervising practical laboratory work on the topic of infrared spectroscopy.

2. Supervision.

- Volobujeva, J. (2019) Deep Eutectic Solvents as Electrolytes in Ionic Electromechanically Active Polymer Actuators. *Bachelor's thesis in Science and Technology.* University of Tartu, Tartu, Estonia.

ELULOOKIRJELDUS

Nimi: Fred Elhi
Sünniaeg: 20. oktoober 1990
Kodakondsus: Eesti
Aadress: Kadaka pst 75, Tallinn, Eesti
Telefon: +372 53320474
E-mail: elhi.fred@gmail.com
Töökoht: PhD tudeng

Haridus:
- Tartu Ülikool, 2016. a., magister
- Tartu Ülikool, 2013. a, bakalaureus

Keelteoskus:
- eesti keel – emakeel (kõrgtase)
- inglise keel – hea kõnes ja kirjas (kesktase)
- soome keel – baasteadmised (algtase)
- taani keel – baasteadmised (algtase)
- rootsi keel – baasteadmised (algtase)

Teenistuskäik:

2018–2020	TBD-Biodiscovery OÜ, Tööstusdoktorant
2018 –2019	TBD-Biodiscovery OÜ, Kvaliteediosakond
2019 –2020	TBD-Biodiscovery OÜ, Tehniline hindaja
2019	Biotatec OÜ Töö kirjeldus: Bioleostamine; bakterikultuuride abil kivimitest maagaasi ning vanaelektroonikast väärismetallide eraldamine
2017	Hairvel OÜ Töö kirjeldus: uudse juuksevärvi väljatöötamine
2014	Tartu Ülikooli Tehnoloogiainstituut Töö kirjeldus: tselluloos-süsinikomposiitide valmistamine ioonse vedeliku abil
2014	Tallinna Tehnikaülikooli Keemiainstituut Töö kirjeldus: insener
2013	Tartu Ülikooli Tehnoloogiainstituut Töö kirjeldus: Vurrkatturiga süsinikelektroodide valmistamise metoodika väljatöötamine

Peamised uurimisvaldkonnad
- Elektroaktiivsed polümeerid
- Ioonsed vedelikud
- Koliin
- IEAP aktuaatorid

– Biosobivad materjalid
– Nanosüsinikud
– Aerogeelid
– Mikroprintimine
– Eutektilised segud

Publikatsioonide loetelu

Thompson Reuters Web of Science andmebaasis kajastatud 1.1 teadusartiklid:

Elhi, F.; Peikolainen, A.-L.; Kiefer, R.; Tamm, T. (2020). Cellulose-Multiwall Carbon Nanotube Fiber Actuator Behavior in Aqueous and Organic Electrolyte. Materials, *13*(14), 3213.

Elhi, F.; Karu, K.; Rinne, P.; Nadel, K.-A.; Järvekülg, M.; Aabloo, A.; Tamm, T.; Ivaništšev, V.; Põhako-Esko, K. (2020). Understanding the Behavior of Fully Non-Toxic Polypyrrole-Gelatin and Polypyrrole-PVdF Soft Actuators with Choline Ionic Liquids. Actuators, *9*(2), 40.

Khuyen, N. Q.; Kiefer, R.; **Elhi, F.**; Anbarjafari, G.; Martinez, J. G.; Tamm, T. (2020). A biomimetic approach to increase soft actuator performance by friction reduction. Polymers, *12*(5), 1120.

Elhi, F.; Priks, H.; Rinne, P.; Kaldalu, N.; Žusinaite, E.; Johanson, U.; Aabloo, A.; Tamm, T.; Põhako-Esko, K. (2020). Electromechanically active polymer actuators based on biofriendly choline ionic liquids. Smart Materials and Structures, *29*(5), 055021

Elhi, F.; Gantman, M.; Nurk, G.; Schulz, P. S.; Wasserscheid, P.; Aabloo, A.; Pohako-Esko, K. (2020). Influence of Carboxylate Anions on Phase Behavior of Choline Ionic Liquid Mixtures. Molecules, *25*(7), 1691.

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Elhi, F.; Aid, T.; Koel, M. (2016) Ionic liquids as solvents for making composite materials from cellulose. Proceedings of the Estonian Academy of Sciences, *65*(3), 255–266.

5.2 Konverentsiteesid:

Karu, K.; **Elhi, F.**; Põhako-Esko, K.; Ivaništšev, V. (2019). Determining the melting point of biocompatible ionic liquids with molecular dynamics simulations. *FMTDK teeside kogumik: FMTDK konverents 2019.* Tartu: FMTDK.

Elhi, F.; Küppar, K.-A.; Tamm, T.; Järvekülg, M.; Põhako-Esko, K.; Aabloo, A. (2017). Biocompatible Ionic Electroactive Polymer Actuators. *2017 MRS Fall Meeting & Exhibit Abstracts.*

Elhi, F.; Põhako-Esko, K.; Küppar, K.-A.; Tamm, T.; Järvekülg, M.; Aabloo, A. (2017). Biocompatible Ionic Electrochemically Active Polymer Actuators. *FMTDK Teaduskonverentsi teeside kogumik.FMTDK Teaduskonverents*

Elhi, F.; Kisand, V.; Peikolainen, A.-L.; Koel, M.; Aabloo, A. (2016) Carbon aerogel-cellulose films and fibres with electrical conductivity and antibacte-

rial properties against cyanobacteria, *Proceedings 3rd International Seminar On Aerogels: Synthesis-Properties-Applications*, 180.

Kesküla, A.; **Elhi, F.**; Peikolainen, A.-L.; Mäeorg, U.; Aabloo, A. (2013) Polymerized ionic liquid membranes for electrochemical actuator and sensor applications. *International Conference on Materials and Applications for Sensors and Transducers Abstracts Book: 3rd International Conference on Materials and Applications for Sensors and Transducers, ICT-MAST 2013.* Ed: Hristoforou, E.; Vlachos, D.S., 1–45.

Saadud uurimistoetused ja stipendiumid:

- Dora Plus Doktorantide õpirände stipendium (T1.2) 2018 Archimedes

Muu teaduslik organisatsiooniline ja erialane tegevus

- 2018 sept – 2019 jaan Vahetusdoktorant Friedrich-Alexander-Universität Erlangen-Nürnbergis, Institute of Chemical Reaction Engineering

Õppetöö

1. Andmed kõrgkoolis tehtud auditoorse õppetöö kohta

- Õppetöö juhendamine 2017/2018 (kevad), 2018/2019 (kevad), 2019/2020 (kevad) ja 2020/2021 (kevad) aastatel kursuse "Kaasaegsed laboritehnikad" (LTTI.00.017, 6 EAP) raames. Juhendati praktikumi infrapunaspektroskoopia teemal.

2. Juhendamine

Volobujeva, J. (2019) Deep Eutectic Solvents as Electrolytes in Ionic Electromechanically Active Polymer Actuators. *Bakalaureusetöö tehnika ja tehnoloogia erialal.* Tartu Ülikool, Tartu.

DISSERTATIONES TECHNOLOGIAE UNIVERSITATIS TARTUENSIS

1. **Imre Mäger**. Characterization of cell-penetrating peptides: Assessment of cellular internalization kinetics, mechanisms and bioactivity. Tartu 2011, 132 p.
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55. **Eva-Maria Tombak**. Molecular studies of the initial amplification of the oncogenic human papillomavirus and closely related nonhuman primate papillomavirus genomes. Tartu, 2019, 150 p.
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57. **Jelena Beljantseva**. Small fine-tuners of the bacterial stringent response – a glimpse into the working principles of Small Alarmone Synthetases. Tartu, 2020, 104 p.
58. **Egon Urgard**. Potential therapeutic approaches for modulation of inflammatory response pathways. Tartu, 2020, 120 p.
59. **Sofia Raquel Alves Oliveira**. HPLC analysis of bacterial alarmone nucleotide (p)ppGpp and its toxic analogue ppApp. Tartu, 2020, 122 p.
60. **Mihkel Örd**. Ordering the phosphorylation of cyclin-dependent kinase Cdk1 substrates in the cell cycle. Tartu, 2021, 228 p.