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Readily biodegradable and low-toxic biocompatible ionic liquids for cellulose processing

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Abstract

Cellulose is an important feedstock for chemical industry, but its processing is a challenge. Considerable efforts have been invested in applying rules of green chemistry in developing environmentally more acceptable processes. Within this work we present an account on biocompatible, degradable ILs of low (eco)toxicity which could find potential applications in cellulose processing industry. We have tested ILs based on choline and betaine cations and levulinate anion. We have discovered that, among these structures, betaine-ester levulinate is the most degradable and the least toxic IL. All tested structures were to some, sometimes high, extent degradable and showed low to moderate toxicity towards *Daphnia magna*, *Vibrio fischeri* and rat leukaemia cells.

Keywords: ionic liquids, ecotoxicity, cytotoxicity, biodegradability, *Daphnia magna, Vibrio fischeri*

Introduction

Biomass gains increasing importance as a source of renewable raw materials that could, at least partially, replace fossil fuel derived chemicals. Cellulose is one of the most important renewable feedstocks and finds multiple industrial applications since it is available in vast quantities. Two main use directions include conversion to biofuels or to fine chemicals. Multiple inter- and intra-molecular hydrogen bonds make the supramolecular structure of cellulose very stable.[1] Cellulose is therefore, not soluble in water and many organic solvents.[2] The Viscose Process has been used for a long time to obtain cellulose fibre, but due to its high environmental impact is being replaced by a Lyocell process, which is a by-product free alternative. Nevertheless, the Lyocell process still requires high temperatures, relatively expensive solvent, and a cellulose activation step.[3] Recently ionic liquids (ILs) are gaining considerable attention as non-derivatising solvents for cellulose dissolution.[2]

Applications of choline/betaine based ILs

First studies on cellulose dissolution in ILs were conducted using imidazoliums and revealed that small hydrogen-bond accepting anions are preferred over larger non-coordinating ones.[2] Similarly, rather small, short chained ILs' cations are preferred over a long chained ones.[1,4] Even though, exact mechanism of dissolution is not known it is accepted that reducing the extent of intra-molecular hydrogen bonding is responsible for dissolution. The cellulose dissolved in IL can be precipitated by addition of an anti-solvent (water, methanol, ethanol etc.) and regenerated into a desired form.[4] As can be expected, the presence of water in ILs significantly reduces their cellulose solvation properties and is undesired. Choline based ILs were shown to be useful in extraction of antibiotics in aqueous biphasic systems[5] or dissolution of other biopolymers e.g. lignin and xylan.[6] There are still some obstacles that hamper wide scale application of ILs in processing/purification of biomolecules. Those include high cost of the ILs needed for that task, unfavourable physicochemical parameters (high viscosity, low melting point), and sometimes high toxicity or low biodegradability. A truly green IL-based process should employ possibly least environmentally harmful IL. Hereby, we present a study on biodegradability and (eco)toxicity of several ILs that might potentially find application in this area. Several ILs, based on choline or betaine derived cations and a biocompatible levulinate anion, were synthesised.[1] It was previously shown that ethyl cholinium-ether levulinate can dissolve up to 10% (w/w) of cellulose and significant amounts of lignin, xylan and starch.[7] This promising performance is making ILs interesting for biopolymer processing industry. In the current work, we examined the (eco)toxicity and biodegradability of several ILs based on choline and betaine. For the sake of systematically investigating the influence of the anion on biodegradability and toxicity, we have included a levulinate anion coupled with sodium. As a point of reference, we have also included a well-investigated 1-butyl-3-methyl-imidazole cation with levulinate anion. To investigate the possible environmental impacts of a 'real life' product we also tested a mixture of choline-based IL with cellulose.

Biodegradability of ILs

Research on developing structure-biodegradability rules for ILs has been going on for two decades now and a substantial amount of knowledge has been gathered.[8,9] Several rules of how to design more biodegradable ILs have been developed taking into account a special 'modular' character of ILs. A certain minimum length of six to eight carbons in the side chain is required for biodegradability of alkyl substituted cations.[8] Inserting structures that give microbes a point of leverage is beneficial. Therefore, incorporating ester groups, being a site of enzymatic hydrolysis, increases the biodegradability.[10] Using structures derived from biomaterials often results in higher biodegradability. In this sense lactate, benzoate, succinate anions or cholinium cation make, at least partially, degradable ILs.[8,9,11–13] The butanoate anion which differs from levulinate by lack of a ketone group has been previously shown to be degradable.[13] Due to, the 'modular nature' of ILs it is possible to produce chemicals composed of two ions out of which only one is

biodegradable. This was clearly shown for imidazolium coupled with the octylsulphate anion.[14] Usually, the non-degradable ions persist, though, an IL as a whole might even be rated 'readily biodegradable' in regulatory tests.[10] Therefore, the ultimate target should be to make both the anion and the cation biodegradable. The ILs investigated here take these rules into account.

Ecotoxicity of ILs

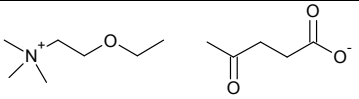
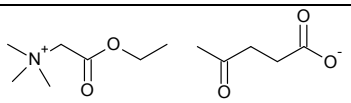
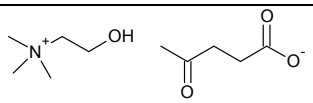
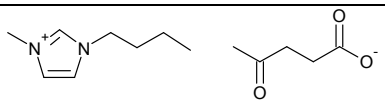
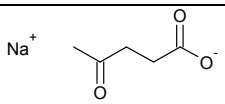
In terms of ecotoxicity of ILs there is a one general rule that stands out: hydrophobicity. In that terms the cation was shown to be a game-changer while the type of anion had rather minor influence.[8,15–19] Therefore, ILs substituted with longer alkyl chains lacking hydrophilic groups (e.g. hydroxyl, carboxyl) are usually more toxic.[15] Among anions the fluorinated ones were shown to influence toxicity either due to their high hydrophobicity or hydrolytic instability.[15] In general cholinium is considered an ecotoxicologically acceptable moiety due to its occurrence in biomolecules. Indeed the low toxicity of some cholinium based ILs has been proved.[13,15,20–22] Cholinium acesulfamate and saccharinate were shown to be non-cytotoxic in tests with colon carcinoma cell lines after short exposure.[21] The EC_{50} values towards *Daphnia magna* for same compounds were above 1 g L^{-1}.[20] The free levulonic acid was show to have low toxicity with EC_{50} towards *Daphnia magna* over 6 g L^{-1} and towards *Vibrio fischeri* over 5.5 g L^{-1}.[23] Biocompatible anions (including butanoate structurally similar to levulinate) coupled with cholinium cation were found to have lower antifungal properties than when coupled with sodium.[13] Cholinium levulinate was not cytotoxic in test with cardiac fibroblasts.[12] Therefore, there is a basis to expect that choline or betaine levulinates investigated here will be of low toxicity and could be considered as environmentally benign solvents for cellulose processing. In order to support the theory with experimental evidence, we have evaluated the biodegradability and (eco)toxicity of five biocompatible ILs. For evaluation of biodegradability we performed a ready biodegradability test (OECD 301F).[24] We also examined the cytotoxicity using a rat leukaemia cell line (IPC-81), the bacterial toxicity (luminescence inhibition test with *Vibrio fischeri*) and, the aquatic toxicity (immobilization assay with *Daphnia magna)* tests. Chosen test systems include both *in vitro* and *in vivo* tests with organisms on different trophic levels (daphnids and bacteria). A fast screening cytotoxicity test was previously proved to be useful in determining structure-activity relationships for ILs.[25] Both *Vibrio fischeri* and *Daphnia magna* tests are well established ecotoxicological tests targeting respectively marine and freshwater organisms. The test with *Daphnia magna* is additionally a recommended test in evaluation of acute aquatic toxicity of chemicals. All of the tests were previously widely used for assessment of ILs and a large pool of data is available.

Experimental

Ionic liquids

The structures, names and, abbreviations of ILs used throughout the paper are presented in Table 1. For the details regarding synthesis of ILs as well as physicochemical properties relevant for cellulose processing we refer the reader to the previous works.[5,7,26] ILs were isolated as pure products (purity > 99 % as determined by ^{1}H and ^{13}C NMR spectroscopy).[7]

Table 1. Names, structures, abbreviations of ILs tested

Symbol	Name	Structure
[Chol-ether][Lev]	2-ethoxy-*N*,*N*,*N*-trimethylethanaminium levulinate (oxopentanoate)	
[Bet-ester][Lev]	2-ethoxy-*N*,*N*,*N*-trimethyl-2-oxoethanaminium levulinate	
[Chol][Lev]	2-hydroxy-*N*,*N*,*N*-trimethylethanaminium levulinate	
[IM14][Lev]	1-butyl-3-methyl-1H-imidazolium levulinate	
[Na][Lev]	sodium levulinate	
[Chol-ether][Lev] + cellulose		[Chol-ether][Lev] + 10% (w/w) cellulose

Biodegradation tests

Ultimate biodegradation was measured by manometric respirometry method according to OECD guideline 301F using automated, thermostatically controlled OxiTop© set (from WTW GmbH, Weilheim, Germany).[24] The activated sludge from the aeration tank of municipal wastewater treatment plant in Delmenhorst (Germany) was used as a source of inoculum. The flocs were allowed to settle and remaining supernatant was aerated for 4-7 days prior to use. The pre-concentrated medium was prepared and was added to the sludge to result in following final composition: 8.5 mg L^{-1} KH_2PO_4, 28.5 mg L^{-1} $K_2HPO_4 \cdot 3H_2O$, 33.4 mg L^{-1} $Na_2HPO_4 \cdot 2H_2O$, 0.5 mg L^{-1} NH_4Cl, 36.4 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 22.5 mg L^{-1} $MgSO_4 \cdot 7H_2O$ and 0.25 mg L^{-1} $FeCl_3$ (pH 7.4). The inoculum used in the test had a dry mass of approximately 0.5 g L^{-1} and bacteria cell density of approximately 10^4 cells L^{-1} (determined using a Paddle Tester; Hach Europe, Düsseldorf). The concentration of ILs was chosen to correspond of theoretical oxygen demand (ThOD) of 200 mg O_2 L^{-1} meaning: 100 mg L^{-1}

for [Chol-ether][Lev], 113 mg L^{-1} for [Bet-ester][Lev] and [IM14][Lev], 110 mg L^{-1} for [Chol][Lev], and 164 mg L^{-1} for [Na][Lev]. The vessels were closed with gas-tight stoppers and kept protected from light at 20 ± 0.5 °C. Sodium hydroxide was used to absorb the evolved carbon dioxide inside the sample vessels. The oxygen consumption was determined manometrically. Biodegradation of the test substance was calculated as the biological oxygen uptake (BOD) for the test substance (corrected by the oxygen demand of the blank samples) with respect to the ThOD of the substance and the amount of substance present in the sample. Test lasted at least 28 days and was performed in two replicates for each compound accompanied by two blanks (inoculated media without test substance) and two positive controls (inoculated media with benzoic acid). The whole test was repeated twice to account for variability of inoculum.

Cytotoxicity tests with IPC-81

The colorimetric WST-1 assay with IPC-81 promyelocytic leukaemia rat cell line was used to investigate the influence of ILs on cell viability. The IPC-81 cell line originates from promyelocytic leukaemia in Brown Norway rats. The cell line was kindly donated by dr. M. Lanotte who first established the culture. The test is based on spectrophotometric assessment of intensity of red colour caused by enzymatic reduction of WST-1 reagent (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) which is inversely proportional to cytotoxicity. The WST-1 test was carried out in RPMI medium (with L-glutamine, without $NaHCO_3$, supplemented with 1% penicillin–streptomycin and 1% glutamine, pH 7) with 10% horse serum at 37 °C in atmosphere containing 5% CO_2. The exact procedure is described in detail elsewhere.[16] The test was repeated on three different days using three independently prepared solutions. Each dose response curve was therefore recorded for at least nine parallel dilution series on three different 96-well plates. Solutions were prepared directly in medium and diluted in 1:1 series with medium.

***Vibrio fischeri* luminescence inhibition test**

This test with the marine luminescent bacterium *Vibrio fischeri* was performed according to DIN EN ISO 11348-2.65. The freeze-dried bacteria were purchased from Dr Lange GmbH (Düsseldorf, Germany). Each substance was tested three times, using independently prepared solutions, with two replicates at each concentration level and accompanied by at least four positive controls (2% w/w NaCl solution in phosphate buffer). All solutions were prepared directly in phosphate-buffer (0.02 M, pH 7.0, including 2% NaCl). The tests were performed at 15 °C using thermostats (LUMIStherm, Dr Lange GmbH, Düsseldorf, Germany). The freeze-dried bacteria were rehydrated according to the test protocol, and then 500 µL aliquots of the bacteria solution were pre-incubated for 15 min at 15 °C. After measuring the initial luminescence, 500 µL of the samples were added. The bioluminescence was measured again after an incubation time of 30 min using a luminometer (LUMIStox 300, Dr Lange GmbH, Düsseldorf, Germany). The relative toxicity of the samples was expressed as a percentage inhibition compared to the controls. In the range finding test two concentrations were chosen: approximately 500 mg L^{-1} and 100 mg L^{-1}. For all tested compounds except

[IM14][Lev] this did not cause any significant effect. For those compound the test was repeated once more in the same two concentrations. For [IM14][Lev] series of concentrations between 1000 and 125 mg L^{-1} was tested in definite test, this test was also performed twice.

Ecotoxicity test with *Daphnia magna*

The 48 h acute immobilization test with the freshwater crustacean *D. magna* was assessed using the commercially available Daphtoxkit F (MicroBioTest Incorporation, Gent, Belgium) referred to in OECD guideline 202.[27] The tests with neonates less than 90 h old, obtained by the hatching of dormant ephippia, were performed at 20 °C in the dark. Five pre-fed animals were incubated with the toxicants in a volume of 10 mL of mineral medium. For each test, five different concentrations of the ILs in four parallels and four controls were investigated. Positive controls using potassium dichromate were also included. All the experiments were performed twice. The numbers of immobilized organisms were checked after 24 and 48 h.

Live subject statement

All experiments were performed in compliance with the relevant laws (German Animal Welfare Act and European Union Directive 2010/63/EU) and institutional guidelines of the University of Bremen. Working with cladoceran and bacterial species or animal cell lines *in vitro* does not require permission from the Ethics Commission.

Results

Biodegradability

Results of biodegradability tests are presented in Figure 1. The extent of degradation of positive control (benzoic acid) reached 75-85% thereby fulfilling the validity criterion of the ready biodegradability test (at least 60% degradation for positive control). The level of degradation, even for easily degradable compounds, usually is somewhat lower than 100% since low molecular weight transformation products can be directly built into the biomass instead of being completely transformed to CO_2, causing no observable oxygen consumption.

All tested ILs were to some extent biodegradable. The [IM14][Lev] and [Chol-ether][Lev] showed the lowest degree of degradation not exceeding 30%. The extent of [Chol-ether][Lev] biodegradation in our test system suggests that only the anion was degraded as approximately 33% of biologically oxidisable carbon is in the anion. This would suggest that the cation resist degradation. The [Chol-ether] cation contains C–O–C linkage which is known to resist degradation.[28] Similar results were indeed observed before for imidazolium IL substituted with ethoxyethyl- sidechain.[22,29] The IM14 cation was shown to be non-biodegradable and the result observed here confirms that most probably only the anion was degraded. As in the case of [Chol-ether][Lev] 33% of available carbon is in the anion which is close to maximum biodegradation level of 25% which was observed here.[29]

The [Chol][Lev], [Na][Lev] and [Bet-ester][Lev] were considerably degradable (> 60%) and showed degradability levels similar to positive control. These three ILs can be classified as readily biodegradable according to OECD 301 criterion (they reached a pass level of 60% degradation within 10 days window). Results of two independent tests for [Bet-ester][Lev] differ somewhat more than for other compounds but remain within acceptable limits.

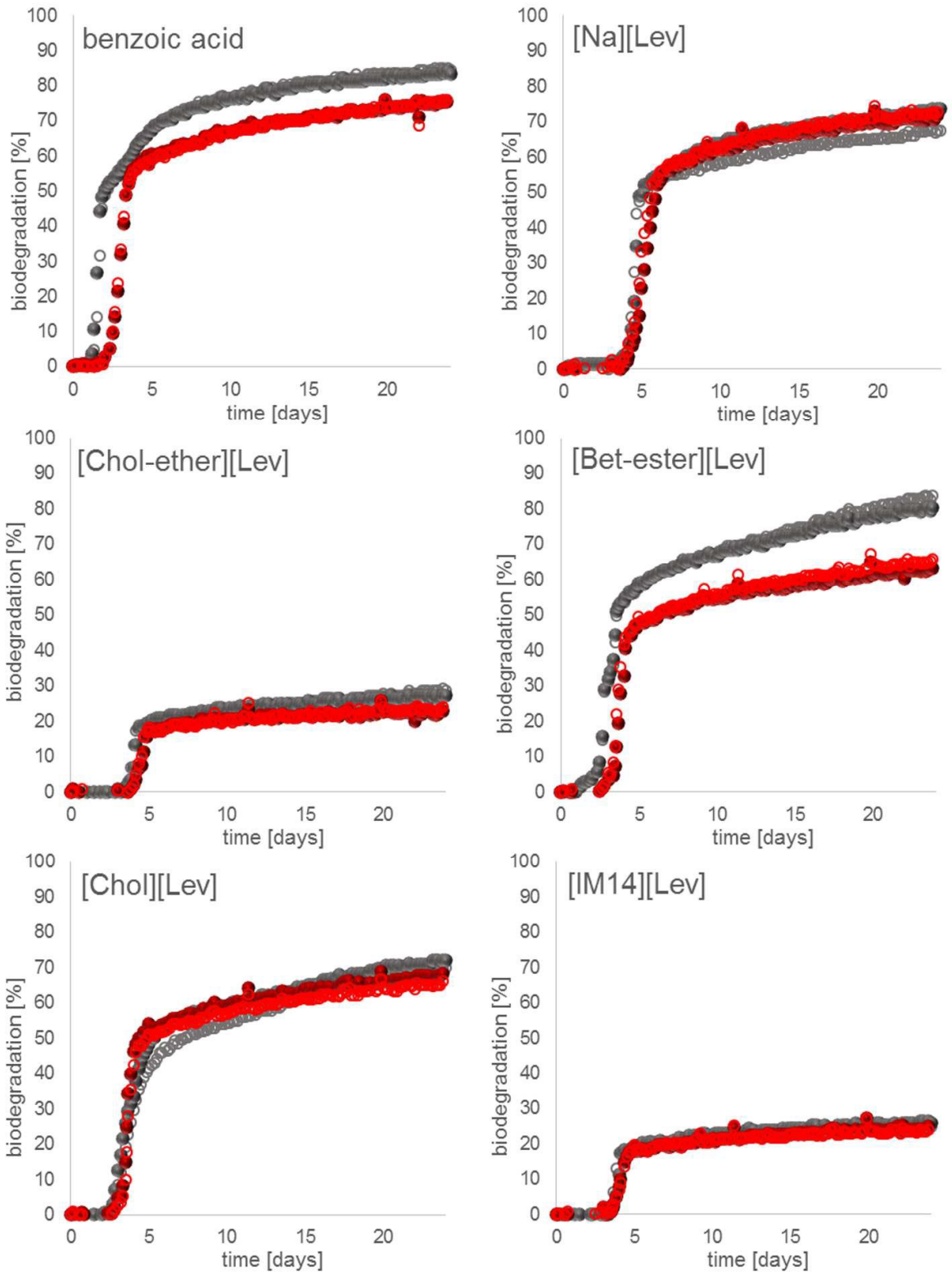


Figure 1. Biodegradability of ILs in manometric respirometry test. Open and closed symbols in the same colour are replicates within one test, curves in two colours come from two independent tests.

The extent of degradation of [Bet-ester][Lev] and [Chol][Lev] exceeds the levels that would correspond to degradation of either cation or anion alone suggesting that both of them must have been degraded to significant extent. This proves that [Chol][Lev] and [Bet-ester][Lev] form easily biodegradable ILs and are expected to breakdown rapidly and completely in the environment. The presence of ester bond in [Bet-ester] is a site for the enzymatic attack by esterases and is known to increase degradability.[8] The terminal hydroxyl and carboxyl groups in cholinium cation and levulinate anion are also easily degradable. Because degradation of alkyl substituted ILs usually proceeds by introduction of hydroxyl and carboxyl group they might be thought of as already 'pre-degraded' structures.[29,30]

(Eco)toxicity

The summary of results of (eco)toxicity tests is given in Table 2. In most of cytotoxicity and bacterial toxicity tests we did not observe any significant effects in highest tested concentration which were very high (in excess of 1 g L^{-1} and 0.5 g L^{-1} respectively). In such cases, the EC_{50} value is reported as higher than the highest tested concentration. In these two tests only for [IM14][Lev] did we observe a concentration dependent effect but a full dose-response curve could not be fitted. Nevertheless, at the highest tested concentration we observed approximately 50% inhibition of cell viability or luminescence in tests with IPC-81 cell line and *Vibrio fischeri* respectively. Therefore, we reported the EC_{50} as approximately 4000 µmol L^{-1} in both tests (see Table 2). All of the EC_{50} values in these two tests are close to or higher than 1000 µmol L^{-1} which proves that all ILs tested are of low toxicity.

Table 2. Results of cytotoxicity test with IPC-81 cell line, luminescence inhibition test with Vibrio fischeri *and acute immobilization test with* Daphnia magna *are shown. The EC_{50} values are given in µmol L^{-1} and mg L^{-1}, the confidence intervals are given in brackets. CR represents the "cation effect ratio" (see explanation within the text).*

IL	EC_{50}						
	IPC-81		*Vibrio fischeri*		*Daphnia magna*		
	µmol L^{-1}	mg/L	µmol L^{-1}	mg/L	µmol L^{-1}	mg/L	CR
[Chol-ether][Lev]	> 4040	> 1000	> 2500	>620	20 (15-26)	4.9 (3.6-6.3)	183
[Bet-ester][Lev]	>3820	>1000	> 2050	>535	880 (712-1033)	230 (186-270)	4
[Chol][Lev]	>4560	>1000	> 2620	>575	155 (112-182)	33.9 (24.6 - 39.8)	23
[IM14][Lev]	approx. 4000	approx. 1000	approx. 4000	approx. 1000	70 (55-99)	17.8 (14.1-25.1)	51
[Na][Lev]	>7240	> 1000	> 3950	> 545	> 3620	>500	1
[Chol-ether][Lev] + cellulose		> 1000		> 540		22.9 (14.8-38.9)	

For all the ILs except [Na][Lev] we were able to calculate the EC_{50} values in the test with freshwater crustacean (*Daphnia magna*) showing that this test system is the most sensitive one. In case of [Na][Lev] we did not observe immobilization of daphnids up to the concentration of 500 mg L^{-1} proving that levulinate

anion shows low toxicity in all the three test systems employed in this work. The aquatic toxicity of other ILs varies from low for [Bet-ester][Lev] through slightly higher for [Chol][Lev] and [IM14][Lev] to end with [Chol-ether][Lev] on the other side of the spectrum. According to Globally Harmonized System for labelling of chemicals (GHS) based on acute *Daphnia magna* test [Chol-ether][Lev] would be assigned to Acute category II, both [Chol][Lev] and [IM14][Lev] to Acute category III and [Bet-ester][Lev] shows toxicity low enough that it would not to be classified within GHS environmental acute toxicity.[31] A mixture of [Chol-ether][Lev] with cellulose has lower toxic effect than pure [Chol-ether][Lev] most probably due to lower amount of the IL and cellulose acting as a ballast. This also means that none of tested ILs exceeded the threshold in *Daphnia magna* test set by REACH PBT assessment (EC_{50}< 1 mg L^{-1}) to identify toxic substances and would the therefore not be identified as toxic based on this test.[32]

Because, all the ILs are built with the same anion, the differences in their toxicities can be assumed to be caused by the cation. Based on that, a following order of cation toxicity was established [Chol-ether]>[IM14]>[Chol]>[Bet-ester]>[Na]. To give this order a more quantitative meaning, we calculated a cation effect ratio (CR) in analogy to anion effect ratio proposed before.[16] The CR is defined as a ratio between the EC_{50} values for reference IL containing intrinsically non-toxic cation (sodium) and any other cation since they all share the levulinate anion. In this way the CR shows the extent to which cations from the pool tested within this work influence the toxicity of IL (see Table 2). Based on CR we can conclude that [Bet-ester] contributes to the toxicity to minor extent (CR = 4), both [Chol] and [IM14] are comparably and approximately ten times more potent (CR of 23 and 51 respectively). Lastly, [Chol-ether] has the highest impact on toxicity as evident by the CR of 183.

If the cytotoxic impact was to be based solely on hydrophobicity, implying only baseline toxicity, the following order of toxicities would be expected: [IM14]>[Chol-ether]>[Bet-ester]>[Chol] following the general order of polarities of organic chemicals: alcohols>esters>ethers>alkanes (e.g. normalised solvent polarities for four-carbon molecules with these functional groups are: butanol = 0.59, ethylacetate = 0.23, dimethylether = 0.12).[33] The higher toxicity of [Chol-ether] as compared to [Bet-ester] might therefore arise from different hydrophobicity. The [Chol] would be expected to be significantly less toxic because it contains terminal OH group which renders it significantly more polar than ester (due to being able to act as a hydrogen bond donor and acceptor) and has a shorter alkyl chain (only two carbons as opposed to four in [Bet-ester] and [Chol-ether]). Indeed, Stolte *et al.* observed three-fold lower cytotoxicity for N,N-dimethylethanaminium substituted with hydroxyethyl chain than for methoxyethyl chain.[17] Nevertheless, in the current study, [Chol] is actually more toxic than [Bet-ester] suggesting that some specific mode of toxic action is involved. The type of interaction involved driving [Chol] cytotoxicity is so specific that replacing one of the methyl groups at quaternary nitrogen with an ethyl group as reported previously completely eliminates that specificity and restores 'normal' hydrophobicity governed order of toxicities.[17] Choline is an essential biomolecule fulfilling various physiological functions. Among others, it is a precursor of

phosphatidylcholine and sphingomyelin (building blocks of biological membranes), signalling and messenger biomolecules, or neurotransmitter acetylcholine.[34] Nevertheless since choline is a charged hydrophilic molecule it cannot easily cross the membranes and several transporter systems exists that facilitate the transport of choline.[35] It is conceivable that higher than expected choline toxicity results from its increased uptake.

Conclusions

Within this work we have tested the (eco)toxicity and biodegradability of five ILs that were considered as solvents of cellulose. We have shown that levulinate anion exhibits both low toxicity and high biodegradability. Depending on the cation it is coupled with, it forms highly to moderately biodegradable IL. [Bet-ester][Lev] was shown to be the most favourable from the environmental perspective being both readily degradable and showing low toxicity in tests systems used here. It is followed by [Chol][Lev], showing equally good degradability yet somewhat higher toxicity. Coupling levulinate with either [IM14] or [Chol-ether] gives partially degradable IL, where the degradability is most probably due to anion only. In the same time, the latter is the most toxic in the tested set and therefore the least desired. From environmental hazard point of view our preliminary assessment suggest that in particular [Bet-ester][Lev] has a great potential to be used as green material in applications such as biopolymers extraction or processing due to its excellent biodegradability/toxicity profile. Nevertheless, other aspects pertaining to both technological performance and process safety (i.e. thermal stability, viscosity, possibility of recycling etc.) need to be investigated in more details if these ILs are to be used on broader scale.

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References

1. F. Boissou, A. Mühlbauer, K. De Oliveira Vigier, L. Leclercq, W. Kunz, S. Marinkovic, B. Estrine, V. Nardello-Rataj, and F. Jérôme, *Green Chem.*, 2014, **16**, 2463.

2. R. P. Swatloski, S. K. Spear, J. D. Holbrey, and R. D. Rogers, *J. Am. Chem. Soc.*, 2002, **124**, 4974–4975.

3. D. Klemm, B. Heublein, H.-P. Fink, and A. Bohn, *Angew. Chem. Int. Ed. Engl.*, 2005, **44**, 3358–93.

4. P. Mäki-Arvela, I. Anugwom, P. Virtanen, R. Sjöholm, and J. P. Mikkola, *Ind. Crops Prod.*, 2010, **32**, 175–201.

5. S. Shahriari, L. C. Tomé, J. M. M. Araújo, L. P. N. Rebelo, J. A. P. Coutinho, I. M. Marrucho, and M. G. Freire, *RSC Adv.*, 2013, **3**, 1835–1843.

6. Q.-P. Liu, X.-D. Hou, N. Li, and M.-H. Zong, *Green Chem.*, 2012, **14**, 304–307.

7. A. Mühlbauer, PhD theseis, University of Lille, 2014.

8. S. Stolte, S. Steudte, A. Igartua, and P. Stepnowski, *Curr. Org. Chem.*, 2011, **15**, 1946–1973.

9. D. Coleman and N. Gathergood, *Chem. Soc. Rev.*, 2010, **39**, 600–637.

10. N. Gathergood, P. J. Scammells, and M. T. Garcia, *Green Chem.*, 2006, **8**, 156–160.

11. Y. Fukaya, Y. Iizuka, K. Sekikawa, and H. Ohno, *Green Chem.*, 2007, **9**, 1155–1157.

12. D. R. Vijayaraghavan, B. C. MacFarlane, R. Kumar, R. Thompson, S. Surianarayanan, P. K. Sehgal, and M. Aishwarya, *Chem. Commun.*, 2010, **46**, 294–296.

13. M. Petkovic, J. L. Ferguson, H. Q. N. Gunaratne, R. Ferreira, M. C. Leitão, K. R. Seddon, L. P. N. Rebelo, and C. S. Pereira, *Green Chem.*, 2010, **12**, 643–649.

14. M. T. Garcia, N. Gathergood, and P. J. Scammells, *Green Chem.*, 2005, **7**, 9–14.

15. M. Petkovic, K. R. Seddon, L. P. N. Rebelo, and C. S. Pereira, *Chem. Soc. Rev.*, 2011, **40**, 1383–1403.

16. S. Stolte, J. Arning, U. Bottin-Weber, M. Matzke, F. Stock, K. Thiele, M. Uerdingen, U. Welz-Biermann, B. Jastorff, and J. Ranke, *Green Chem.*, 2006, **8**, 621–629.

17. S. Stolte, J. Arning, U. Bottin-Weber, A. Müller, W.-R. Pitner, U. Welz-Biermann, B. Jastorff, and J. Ranke, *Green Chem.*, 2007, **9**, 760–767.

18. C. Pretti, C. Chiappe, I. Baldetti, S. Brunini, G. Monni, and L. Intorre, *Ecotoxicol. Environ. Saf.*, 2009, **72**, 1170–6.

19. T. P. T. Pham, C.-W. Cho, and Y.-S. Yun, *Water Res.*, 2010, **44**, 352–372.

20. P. Nockemann, B. Thijs, K. Driesen, C. R. Janssen, K. Van Hecke, L. Van Meervelt, S. Kossmann, B. Kirchner, and K. Binnemans, *J. Phys. Chem. B*, 2007, **111**, 5254–5263.

21. R. F. M. Frade, A. Matias, L. C. Branco, C. A. M. Afonso, and C. M. M. Duarte, *Green Chem.*, 2007, **9**, 873–877.

22. S. Stolte, S. Steudte, O. Areitioaurtena, F. Pagano, J. Thöming, P. Stepnowski, A. Igartua, and J. Thoeming, *Chemosphere*, 2012, **89**, 1135–1141.

23. L. Lomba, S. Muñiz, M. R. Pino, E. Navarro, and B. Giner, *Ecotoxicology*, 2014, **23**, 1484–93.

24. OECD, *Guideline for testing of chemicals 301 - Ready Biodegradability*, 1992.

25. J. Ranke, S. Stolte, R. Stormann, J. Arning, and B. Jastorff, *Chem. Rev.*, 2007, **107**, 2183–2206.

26. S. Stevanovic, A. Podgorsek, L. Moura, C. C. Santini, A. A. H. Padua, and M. F. Costa Gomes, *Int. J. Greenh. Gas Control*, 2013, **17**, 78–88.

27. OECD, *Guidelines for the Testing of Chemicals 202: Daphnia sp. Acute Immobilisation Test*, 2004.

28. G. F. White, N. J. Russell, and E. C. Tidswell, *Microbiol. Rev.*, 1996, **60**, 216–232.

29. S. Stolte, J. Arning, A.-K. Blomeyer-Nienstedt, U. Bottin-Weber, M. Matzke, B. Jastorff, and J. Thöming, *Green Chem.*, 2008, **10**, 214–224.

30. M. Markiewicz, S. Stolte, Z. Lustig, J. Luczak, M. Skup, J. Hupka, and C. Jungnickel, *J. Hazard. Mater.*, 2011, **195**, 378–382.

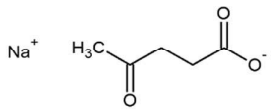
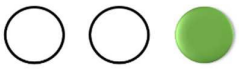
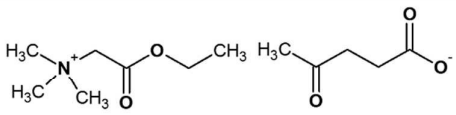
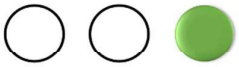
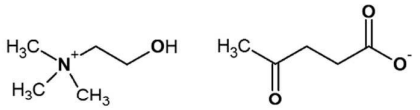
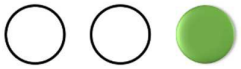
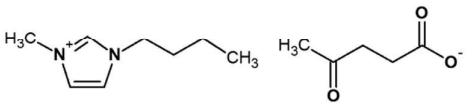
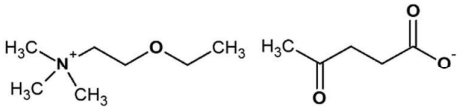
31. *Globally Harmonized System of Classification and Labelling of Chemicals (GHS): Fourth Revised Edition*, 2011.

32. ECHA, in *Guidance on information requirements and chemical safety assessment*, European Chemical Agency, 2012.

33. C. Reichardt, *Chem. Rev.*, 1994, **94**, 2319–2358.

34. J. K. Blusztajn, *Science*, 1998, **281**, 794–795.

35. P. R. Lockman and D. D. Allen, *Drug Dev. Ind. Pharm.*, 2002, **28**, 749–771.

Ionic liquid	biodegradability	(eco)toxicity
		
		
		
		
		

Ecotoxicity and biodegradability of several ILs intended for cellulose processing was examined. Betaine-ester levulinate based IL exhibits low environmental hazard potential (full degradability, low toxicity). Other partially degradable and moderately toxic biomolecule-based ILs are also reported.