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Synthesis of novel non-toxic naphthenic and benzoic acid ionic liquids. Structure-properties relationship and evaluation of their biodegradability potential.

Andromachi Tzani^a, Dimitrios Skarpalezos^a, Achilleas Papadopoulos^a, Dionysia Aravopoulou^b, Ioannis Kleidas^c, Efstathia Ioannou^c, Epaminondas Voutsas^d, Apostolos Kyritsis^b, Anastasia Detsi^{a*}

^aLaboratory of Organic Chemistry, Department of Chemical Sciences, School of Chemical Engineering, National Technical University of Athens, 15780 Athens, Greece

^bDepartment of Physics, School of Applied Mathematical and Physical Sciences, National Technical University of Athens, 15780 Athens, Greece

^cSection of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece

^dLaboratory of Thermodynamics and Transport Phenomena, Department of Process Analysis and Plant Design, School of Chemical Engineering, National Technical University of Athens, 15780 Athens, Greece.

ABSTRACT: Protic Ionic Liquids (PILs) are promising solvents with desirable physicochemical properties for a variety of applications. They are considered as greener alternatives to common organic solvents but in order to efficiently be characterized as green their environmental impact has to be assessed. In the present study, the design, synthesis and an initial potential hazard assessment of 11 naphthenic and benzoic acid based PILs is presented. PILs' "greenness" is enhanced considering that they are non-toxic according to the aquatic toxicity evaluation and most of them show satisfactorily to extremely high biodegradability level. Moreover, two of their most important physicochemical properties, viscosity and glass transition temperature (T_g), were determined for selected ILs. The effect of the aromatic and alicyclic ring on the anions, the size and the presence of a hydroxyl substituent at anions and cations has been also discussed for the studied environmental and physicochemical properties.

KEYWORDS Protic Ionic Liquids, Biodegradability, Toxicity, Viscosity, DSC

1. INTRODUCTION

Ionic Liquids (ILs) are organic salts with a wide range of liquid phase (in many cases near to the ambient temperature) which, during the last few decades, have received great attention as alternatives to commonly used volatile organic solvents (VOCs). They can be divided into two broad categories: protic ILs (PILs) and aprotic ILs (APILs) [1]. PILs, in comparison to APILs, are cheaper and easier to be prepared via a proton transfer reaction between a Brønsted acid and a Brønsted base without byproducts formation.

PILs have been less studied compared to AILs but they have attracted the scientific and, lately, the industrial community's interest, due to their remarkable properties such as high thermal and chemical stability, non-flammability, high solvation ability, high ionic conductivity etc [1,2]. The ability of PILs to adopt desirable properties by the targeted selection of ions, make them attractive candidates for use in numerous applications [1]. Among others, they have been used in: organic synthesis as solvents and catalysts [3], metallic nanoparticles synthesis [4,5], biocatalysis [6,7], separation of azeotropic mixtures [8,9], CO_2 capture [10], extractions [11,12] etc.

The considerable interest, the increasing production and use of ILs, raised environmental awareness issues, since their probable deposition in the environment is increased. In this context, the design of new environmentally friendlier ILs as alternatives to the commonly used hazardous VOCs consists a big challenge. Consequently, recent studies focusing on the ILs environmental impact have emerged evaluating factors such as chemical degradation, bioaccumulation, biodegradability and toxicity [13-19]. The use of biodegradable and non-toxic or of low toxicity compounds, are in accordance with the principles of green chemistry introduced in 1998 by Paul Anastas and John Warner [20]. Toxicity and biodegradability are crucial to be assessed together since toxicity may significantly affect the biodegradation

process. This is because the tested ILs could be toxic to the used microorganisms for the biodegradation process resulting in lower biodegradation percentages [21,22].

Taking advantage of the ILs structure-properties relationship, the design of biodegradable and non-toxic or of low toxicity ILs becomes a feasible objective. However, due to the numerous ILs that can potentially be designed through targeted ions selection, it is important to consider some factors that could affect their toxicity and biodegradation. The study of Boethling et al. [23], on the design of biodegradable chemicals, consists an important tool and suggests some guidelines that can be applied in the ILs design.

As a continuation of our study on the synthesis, characterization and structure-properties relationship of PILs [24], we hereby present the synthesis of 11 PILs, based on naphthenic and benzoic acids, the evaluation of their "greenness" and their structure-properties relationship. The ions selection was targeted in an effort to enhance their environmentally friendly character. Benzoic acids were selected due their aromatic character that, according to the Boethling guidelines, could possibly positively affect the biodegradability. Naphthenic acids were also selected since, they could be almost completely biodegraded and experimental studies on choline-based naphthenic acid ionic liquids (NAILs) have shown high biodegradability percentages [25].

Furthermore, the effect of modifications on the ILs' anions and cations structures on their environmental and some of their most important physicochemical properties has been investigated. More specifically, the alicyclic vs aromatic character of anions, the presence of hydroxyl groups on both ions and also the overall ILs' carbon load effect on their environmental (biodegradability and toxicity) and physicochemical (viscosity and T_g) properties were examined.

2. EXPERIMENTAL SECTION

2.1. Materials.

All the reagents were used without further purification (Table 1).

| Table 1 | | Chemicals | Used | S | vnthesized | Protic | Ionic | Lia | nids | and | their | Purities |
|----------|---|------------|-------|---|------------|--------|-------|-----|------|-----|-------|----------|
| I doic 1 | • | Chefficals | Useu, | D | ynniesizeu | TIONC | Iome | LIY | uius | anu | unen | 1 unites |

| Compound | Source | Purity | Analysis method |
|-------------------------------|-------------|---------------------|---------------------------------|
| | | (wt %) ^a | |
| Ethanolamine | Sigma | 99.99% | GC ^{b,c} |
| | Aldrich | | |
| Diethanolamine | Merck | >98% | GC ^{b,c} |
| 2-Methoxyethylamine | Alfa Aesar | 98% | GC ^{b,c} |
| Bis(2-methoxyethyl)amine | Sigma | 99% | GC ^{b,c} |
| 2 | Aldrich | | |
| Cyclohexanecarboxylic acid | Alfa Aesar | 98% | GC ^{b,c} |
| Cyclopentanecarboxylic acid | TCI | 98% | GC ^{b,c} |
| Benzoic acid | Alfa Aesar | 99% | GC ^{b,c} |
| 2-Hydroxy-benzoic acid | Alfa Aesar) | 99% | GC ^{b,c} |
| 2-Methoxy-ethylammonium | Synthesized | >98% | ¹ H NMR ^d |
| cyclopentane carboxylate (1) | | | |
| Bis(2-methoxy-ethyl) ammonium | Synthesized | >98% | ¹ H NMR |
| cyclopentane carboxylate (2) | | | |
| 2-Methoxy-ethylammonium | Synthesized | >98% | ¹ H NMR |
| cyclohexane carboxylate (3) | | | |
| Bis(2-methoxy-ethyl) ammonium | Synthesized | >98% | ¹ H NMR |

| cyclohexane carboxylate (4) | | | |
|----------------------------------|-------------|------|--------------------|
| Bis(2-hydroxy-ethyl) ammonium | Synthesized | >98% | ¹ H NMR |
| cyclohexane carboxylate (5) | | | |
| 2-Hydroxy-ethylammonium benzoate | Synthesized | >98% | ¹ H NMR |
| (6) | | | |
| Bis(2-hydroxy-ethyl) ammonium | Synthesized | >98% | ¹ H NMR |
| benzoate (7). | | ç | |
| 2-Methoxy-ethylammonium benzoate | Synthesized | >98% | ¹ H NMR |
| (8) | | 0 | |
| Bis(2-methoxy-ethyl)ammonium | Synthesized | >98% | ¹ H NMR |
| benzoate (9) | .0 | | |
| Bis(2-hydroxy-ethyl) ammonium 2- | Synthesized | >98% | ¹ H NMR |
| hydroxy-benzoate (10) | | | |
| Bis(2-methoxy-ethyl) ammonium 2- | Synthesized | >98% | ¹ H NMR |
| hydroxy-benzoate (11) | | | |

^a wt %: weight percent. ^b Given by the suppliers. ^c Gas Chromatography. ^d Proton Nuclear Magnetic Resonance.

2.2. Methods

¹H-NMR spectra were recorded on a Varian 300 MHz and 600 MHz spectrometers at the Institute of Chemical Biology of the National Hellenic Research Foundation, using DMSO-d₆ and CDCl₃ as solvents. The depletion of dissolved oxygen (DO) for the biodegradability assessment was measured with a Consort C1010 DO analyzer. Viscosities were measured on a Brookfield DV1. DSC measurements, were carried out on a TA instruments DSC Q200,

with temperature accuracy $+/-0.1^{\circ}$ C and sensitivity 0.2 μ W. The water content was determined in triplicate using a TitroLine Karl-Fischer Titrator.

2.3. General Procedure for the Synthesis of ILs

Method A. According to our previously applied procedure [24], the naphthenic acid based ILs (NAILs) **1-5** (Fig. 1) were prepared by direct neutralization of equimolar amounts of the corresponding amines and carboxylic acids.



Figure 1. Synthesized NAILs 1-5

Method B. The benzoic acid based ILs (BAILs) **6-11** (Fig. 2) were prepared according to Method A, slightly modified. The amine was placed in a two necked glass flask. The organic acid was added dropwise to the flask through a dropping funnel after dissolution with appropriate amount of ethanol. The reaction mixture was stirred for 24 h at laboratory temperature and then the solvent was removed by vacuum evaporation.



Figure 2. Synthesized BAILs 6-11

All the produced ILs (Table 1) were dried under high vacuum at 50° C, for a period never lower than 3 h in order to decrease the water content as much as possible. To confirm the synthesized products structures ¹H NMR were performed (Table 2).

2-Methoxy-ethylammonium cyclopentane carboxylate (1). Method A. 0.06 mol cyclopentanecarboxylic acid and 0.06 mol of 2-methoxyethylamine were used to obtain a yellow liquid.

Bis(2-methoxy-ethyl)ammonium cyclopentane carboxylate (2). Method A. 0.028 mol cyclopentanecarboxylic acid and 0.028 mol of bis(2-methoxyethyl)amine were used to obtain a dark orange liquid.

2-Methoxy-ethylammonium cyclohexane carboxylate (3). Method A. 0.017 mol cyclohexanecarboxylic acid and 0.017 mol of 2-methoxyethylamine were used to obtain a yellow liquid.

Bis(2-methoxy-ethyl)ammonium cyclohexane carboxylate (4). Method A. 0.014 mol cyclohexanecarboxylic acid and 0.014 mol of bis(2-methoxyethyl)amine were used to obtain a yellow liquid.

Bis(2-hydroxy-ethyl)ammonium cyclohexane carboxylate (5). Method A. 0.037 mol cyclohexanecarboxylic acid and 0.037 mol of diethanolamine were used to obtain a light orange liquid.

2-Hydroxy-ethylammonium benzoate (6). Method B. 0.008 mol benzoic acid diluted in 10 mL ethanol and 0.008 mol of ethanolamine were used to obtain a light yellow solid. (m.p. 139–141°C)

Bis(2-*hydroxy-ethyl*)*ammonium benzoate* (7). Method B. 0.104 mol benzoic acid diluted in 15 mL ethanol and 0.104 mol of diethanolamine were used to obtain a yellow liquid.

2-Methoxy-ethylammonium benzoate (8). Method B. 0.008 mol benzoic acid diluted in 4 mL ethanol and 0.008 mol of bis(2-methoxyethyl)amine were used to obtain an orange liquid.

Bis(2-methoxy-ethyl)ammonium benzoate (9). Method B. 0.014 mol of benzoic acid diluted in 7 mL ethanol and 0.014 mol of bis(2-methoxyethyl)amine were used to obtain a dark orange liquid.

Bis(2-hydroxy-ethyl)ammonium 2-hydroxy-benzoate (10). Method B. 0.053 mol of 2-hydroxy benzoic acid diluted in 17 mL ethanol and 0.053 mol of bis(2-hydroxyethyl)amine were used to obtain a very light yellow liquid.

Bis(2-methoxy-ethyl)ammonium 2-hydroxy-benzoate (11). Method B. 0.033 mol of 2-hydroxy benzoic acid diluted in 15 mL ethanol and 0.033 mol of bis(2-methoxyethyl)amine were used to obtain a brown liquid.

Table 2. ¹H NMR of the synthesized PILs

| PILs | ¹ H NMR |
|------|---|
| 1 | ¹ <i>H NMR</i> (300 <i>MHz</i> , <i>CDCl</i> ₃ , 26 o <i>C</i>) δ /ppm: 3.53 (t, <i>J</i> =5.1 Hz, 2H, -C <u>H</u> ₂ OCH ₃), 3.35 |
| | (s, 3H, –OCH ₃), 2.99 (t, <i>J</i> =5.1 Hz, 2H, –C <u>H</u> ₂ NH ₃)), 2.68 – 2.57 (m, 1H, –CHCOO ⁻), |
| | 1.87 – 1.55 (m, 8H, 4(–CH ₂ –)). |
| 2 | ¹ <i>H</i> NMR (300 MHz, CDCl ₃ , 26 ^o C) δ /ppm: 7.71 (br s, 2H, $-NH_2^+$ -), 3.56 (t, J=5.1 |
| | Hz, 4H, 2(-CH ₂ OCH ₃ -)), 3.37 (s, 6H, 2(-OCH ₃)), 2.95 (t, J=5.1 Hz, 4H, 2(- |
| | CH ₂ NH ₃)), 2.75 – 2.65 (quint, 1H, –CHCOO ⁻), 1.96 – 1.52 (m, 8H, 4(–CH ₂ –)). |
| 3 | ¹ H NMR (300 MH ₇ CDCl ₂ 26 °C) δ /npm: 6.75 (br.s. 3H –NH ₂ ⁺) 3.49 (t. I=5.1 Hz |

4H, 2(-C<u>H</u>₂OCH₃-)), 3.32 (s, 3H, (-OCH₃)), 2.95 (t, *J*=5.1 Hz, 4H, 2(-C<u>H</u>₂NH₃⁺)), 2.08 (tt, *J*=11.1, 4.2 Hz, 1H, -CHCOO⁻), 1.86 – 1.82 (m, 2H, -CH₂-), 1.71 – 1.57 (m, 3H, 2-CH₂-), 1.40 – 1.13 (m, 5H, -CH₂-).

- ¹*H* NMR (300 MHz, CDCl₃, 26 °C) δ/ppm: 8.47 (br s, 2H, -NH₂⁺), 3.56 (t, J=5.1 Hz, 4H, 2(-CH₂OCH₃-)), 3.35 (s, 6H, 2(-OCH₃)), 2.95 (t, J=5.1 Hz, 4H, 2(-CH₂NH₂⁺)), 2.24 (tt, , J=11.1, 4.2 Hz, 1H, -CHCOO⁻), 1.93 (d, J=12.8 Hz, 2H, -CH₂-), 1.81 1.60 (m, 3H, -CH₂-), 1.49 1.24 (m, 5H, -CH₂-).
- ¹*H* NMR (600 MHz, CDCl₃, 26 °C) δ/ppm: 7.09 (br s, 4H, NH₂⁺ & 2(–OH)), 3.85 (t, J=4.5 Hz, 4H, 2(–CH₂OH)), 3.07 (t, J=4.8 Hz, 4H, 2(–C<u>H</u>₂NH₂⁺)), 2.12 (tt, J=11.1, 3.4 Hz, 1H, –CHCOO⁻), 1.88 (d, J=11.5 Hz, 2H, –CH₂–), 1.75-1.62 (m, 4H, 2(–CH₂–)), 1.45 1.16 (m, 5H, –CH₂–).
- ¹H NMR (300 MHz, DMSO-d₆, 26 °C) δ/ppm: 7.87 7.84 (m, 2H, aromatic H), 7.36
 7.26 (m, 3H, aromatic H), 3.56 (t, J=5.1 Hz, 2H, –CH₂OH)), 2.81 (t, J=5.4 Hz, 2H, –CH₂NH₃⁺).
- ¹*H* NMR (600 MHz, DMSO-d₆, 26 °C) δ/ppm: 7.93–7.88 (m, 2H, aromatic H), 7.43 (t, J=7.3 Hz, 1H, aromatic H), 7.37 (t, J=7.4 Hz, 2H, aromatic H), 3.66 (t, J=4.8 Hz, 4H, 2(-CH₂OH)), 2.96 (t, J=5.4 Hz, 4H, 2(-CH₂NH₃⁺)).
- ¹*H* NMR (300 MHz, CDCl₃, 26 °C) δ/ppm: 8.01 7.99 (m, 2H, aromatic H), 7.46 7.33 (m, 3H, aromatic H), 3.54 (t, J=4.8 Hz, 2H, -CH₂OH), 3.28 (s, 3H, (-OCH₃)), 3.08 (t, J=5.1 Hz, 2H, -CH₂NH₃⁺).
- ¹H NMR (300 MHz, CDCl₃, 26 °C) δ/ppm: 8.02 7.99 (m, 2H, aromatic H), 7.43 7.31 (m, 3H, aromatic H), 7.21 7.16 (br, 2H, -NH2⁺-), 3.61 (t, J=4.8 Hz, 4H, -2(-CH2OCH3)), 3.31 (s, 6H, 2(-OCH3)) 3.09 (t, J=4.8 Hz, 4H, 2(-CH2OCH3)).
- 10 ¹H NMR (600 MHz, CDCl₃, 26 °C) δ/ppm: 7.66 (dd, J= 7.5 Hz, 1H, aromatic H),
 7.15 (td, 1H, aromatic H), 6.64 6.59 (m, 2H, aromatic H), 3.67 (t, J=5.1 Hz, 4H,

 $2(-C\underline{H}_2OH))$, 3.02 (t, J=5.4 Hz, 4H, $2(-C\underline{H}_2NH_3^+))$.

¹*H* NMR (600 MHz, DMSO-d₆, 26 °C) δ/ppm: 7.66 (dd, J = 7.5 Hz, 1H, aromatic H),
7.14 (ddd, J=8.2, 7.2, 1.9 Hz, 1H, aromatic H), 6.64 – 6.58 (m, 2H, aromatic H),
3.59 (t, J=5.1 Hz, 4H, -2(CH₂OH-)),3.27 (s, 6H, 2(-OCH₃)), 3.12 (t, J=5.4 Hz, 4H,
2(-CH₂NH₃⁺)).

2.4. Biodegradability assessment

In order to determine the biodegradability potential of the synthesized ILs, the BOD₅ parameter was calculated. BOD₅ depicts the biochemical oxygen demand by suitable populations of microorganisms to metabolize the organic substance within 5 days. The BOD test starts with the carbonaceous stage, followed by both carbonaceous and nitrogenous stage. In order to ensure that only the carbonaceous oxygen demand is measured at the BOD₅ test, allyl thiourea (ATU) is used, and the results are reported as carbonaceous BOD₅ (CBOD₅). According to the method, in 300mL airtight bottles, a nutrient aerobic aqueous medium was added (containing specific quantities of necessary nutrients and micronutrients for the microorganisms development and activity), inoculated with microorganisms and an appropriate amount of known IL concentration. The tests were carried out in triplicate. The seed source of microorganisms was mixed liquor, collected from a secondary sedimentation tank of municipal wastewater from the Psyttaleia sewage treatment plant in Athens, Greece.

The BOD₅ is calculated as the difference between initial and final DO. The DO is measured both initially and after the 5-day incubation at 20 ± 1 °C, in darkness, using the Consort C1010 DO analyzer and corrected for uptake by the use of a blank inoculum run in parallel and then expressed as a percentage of the theoretical oxygen demand (ThOD) to give the biodegradation percentage.

In this study only the carbonaceous stage is taken into account since the nitrogenous stage according to bibliographic data begins after about 6 days, as nitrifying bacteria typically do not consume an appreciable amount of oxygen until 6-10 days have passed. In order to ensure that the nitrification can't occur within five days, ATU is added to the samples as an inhibitor, and so the results relate to carbonaceous BOD (CBOD) [26,27].

2.5. Toxicity assessment

Cysts of the brine shrimp A. salina were stored at -20 °C before use. Cysts were incubated for hatching in an Erlenmayer flask containing filtered seawater (3.2% salinity), at 25 °C under aerobic conditions. After 24 h, hatched larvae (instar 1) were transferred to a new Erlenmayer flask with fresh filtered seawater and incubated at 25 °C for a further 24 h under artificial light with air sparging. After 48 h from the start of the incubation, all larvae had moulted to the instar 2–3 stages, which were used for the toxicity evaluation. The phototropic nauplii were collected by disposable glass Pasteur pipettes from the illuminated side. Stock solutions and series of serial dilutions (at least three different concentrations) of the PILs were prepared in filtered seawater. For the toxicity evaluation, 2 mL of each dilution (or filtered seawater in the case of the control) were added to each well of a 24-well plate and ten A. salina nauplii were transferred in each well using disposable glass Pasteur pipettes. The plates were covered and maintained in an incubator at 25 °C. After 24 h of exposure, the number of surviving and dead nauplii in each well were counted under a stereoscope and recorded. The nauplii were considered dead if they did not show any forward movement for 10 sec after poking with a pipette. In case that dead nauplii were observed in the control wells, the data was corrected using Abbott's formula: corrected lethality (%) = [1 - (treatedpopulation/control population)] x 100.

The experiments were conducted in triplicate for each concentration. The LC_{50} values were calculated using the probit method [28] and expressed as a mean \pm standard deviation (p<0.05).

2.6. Differential scanning calorimetry (DSC) spectroscopy

The thermal behavior some of the synthesized ILs was investigated using a TA Q200 series DSC instrument with Tzero functionality. Helium was used as a purge gas (25 mL/min). Empty furnace and sapphire runs were previously performed to calibrate the capacitance and resistance values of the Tzero Heat Flow Circuit. Then, Indium was used for temperature and enthalpy calibration. Ionic liquid samples of similar size, 7-12 mg, were sealed in Low mass - Tzero Aluminum Hermetic pans while cooling and heating rates were fixed at 10 °C/min. The usual protocol of the measurements consisted of heating from 20 °C up to 150 °C, then cooling down to -150 °C and heating to 150 °C (second heating). The temperature accuracy +/-0.1°C and sensitivity 0.2 μ W.

2.7. Temperature-Dependent Viscosity

The tested synthesized NAILs and BAILs were dried under high vacuum at 50°C, for a period never lower than 3 h in order to decrease the water content. The water content was determined with Karl Fischer titration and is reported in Table 3. Then, their viscosity was measured in triplicate in a broad temperature range (20 - 90 °C) using Brookfield digital viscometer (LV-DVI-E) using a sample adapter (chamber SC4-13R) and spindle SC4-18. The IL's were waited using a KERN balance with an accuracy of ±0.0001 g for all measurements. The samples temperature was maintained to within ±0.01 °C using a Julabo F12 thermostat bath. Each measurement was carried out at a constant time of about seven minutes to investigate if there is any time dependency of the viscosity. Viscosities were measured with an accuracy of ± 2%.

3. RESULTS AND DISCUSSION

In this work, eleven naphthenic and benzoic acid-based protic ILs were synthesized and structurally characterized, via a simple proton transfer reaction between an organic carboxylic acid and a substituted amine. The % water content (% wt) was determined in triplicate using a TitroLine Karl-Fischer Titrator and calculated below 1% w/w. Table 3 shows the average % wt of the tested ILs. The % wt of IL 7 (which is expected to be one of the most hygroscopic ILs in this study) was also determined after the viscosity measurements in order to quantify the % wt change. The measurements confirmed a slightly % wt increase after the viscosity measurement (from 0.8483% to 0.9489%).

Table 3. % water content and Standard Deviation (SD) of the synthesized PILs

| PIL | % wt | SD |
|-----------------|---------------------|-----------|
| <mark>2</mark> | <mark>0.4547</mark> | 0.0021 |
| <mark>4</mark> | 0.1737 | 0.0031 |
| <mark>5</mark> | 0.9247 | 0.0038 |
| <mark>7</mark> | 0.8483 | 0.0047 |
| <mark>9</mark> | 0.6487 | 0.0078 |
| <mark>10</mark> | <mark>0.7397</mark> | 0.0025 |
| <mark>11</mark> | 0.6577 | 0.0060 |

3.1. Biodegradability assessment

The ILs biodegradability assessment is essential in order to possibly label them as green. Biodegradation is the natural process of removal of organic substances from the environment. According to the Organization for Economic Cooperation and Development (OECD)

standards, organic compounds are classified as "readily biodegradable", when the percentage of biodegradation level is equal or exceed the $\geq 60\%$ in 28 days [26].

All BOD₅ tests were run in triplicate and the average amount of ILs biodegradability potential expressed as the ratio CBOD₅/UCBOD (Table 4) where:

CBOD₅: Oxygen demand by microorganisms for degradation of the organic compound within five days (only carbonaceous stage).

UCBOD: Oxygen demand by microorganisms for the ultimate degradation of the organic compound referring only to the conversion of organic carbon to carbon dioxide, water, and new microbial cellular constituents (ultimate carbonaceous demand). The UCBOD coincides with the ThOD for complete conversion of the organic carbon to carbon dioxide.

| PIL | CBOD ₅ / UCBOD (%) |
|-----|-------------------------------|
| 1 | 44.5 <mark>± 0.6</mark> |
| 2 | 24.5 ± 0.1 |
| 3 | 52.3 ± 1.5 |
| 4 | 52.8 ± 3.2 |
| 5 | 79.0 <mark>± 13.7</mark> |
| 6 | 91.5 ± 8.8 |
| 7 | 90.3 ± 4.0 |
| 8 | 73.7 ± 0.9 |
| 9 | 27.1 ± 3.9 |
| 10 | 33.1 ± 2.8 |
| 11 | 30.6 <mark>± 0.58</mark> |

Table 4. (%) Biodegradability evaluation of ILs 1-11

From this study, it is concluded that the synthesized ILs are susceptible to biodegradation, especially those whose biodegradation of organic carbon exceeds 50% and, in some cases, reaches up to 90% even within the first five days. Through a more extensive examination of the anions' and cations' effect on the biodegradability of the examined ILs, some important information can be gathered.

3.1.1. Cation effect on the ILs biodegradability

According to the CBOD₅/UCBOD results of the benzoic acid-based ILs (BAILs, Table 5), it is clearly observed that the effect of the cationic part is considerable on the organic carbon removal. More specifically, by comparing PIL **6** with **8**, and **7** with **9** it can be easily concluded that the hydroxyl groups favour the biodegradation process. This observation is in accordance with other authors' findings where it is remarked that the insertion of hydroxyl groups into long chained ILs might be advantageous for biodegradability [29]. Moreover, according to the study of Boetheling et al. [23], it is proposed that groups which consist possible sites of enzymatic hydrolysis, like hydroxyl groups, may lead to increased aerobic biodegradability of the examined substance.

| BAILs | Structure | CBOD ₅ /UCBOD (%) |
|-------|---|------------------------------|
| 6 | O O H ₃ N OH | 91.5 <mark>± 8.8</mark> |
| 7 | | 90.3 <mark>± 4.0</mark> |
| 8 | O O H ₃ N CH ₃ | 73.7 <mark>± 0.9</mark> |

Table 5. Effect of BAILs cation on their (%) biodegradability

9
$$H_3C^{O} \to H_3C^{O} \to H_3C^{O} \to H_3$$
 27.1 ± 3.9

Almost the same trend seems to be followed by the biodegradability rates of the cyclohexanecarboxylic acid-based ILs **3-5** (Table 6). In particular, by maintaining the cyclohexanoate anion, it is observed that the IL **5** shows an increased biodegradability up to 26% compared to **4**.

Moreover, from these results it can also be assumed that the biodegradability is not affected by the amine's class since the biodegradability percentage from the primary amine-derived IL **3** is almost identical to secondary amine-derived IL **4**. This observation agrees with the results of ILs **6** and **7**.

 Table 6. Effect of cyclohexanecarboxylic acid based-NAILs cation on their (%)

 biodegradability

| NAILs | Structure | CBOD ₅ /UCBOD (%) |
|-------|---|------------------------------|
| 3 | | 52.3 <mark>± 1.5</mark> |
| 4 | $\bigcup_{H_3} C^{O} \xrightarrow{\oplus}_{H_2} C^{O} CH_3$ | 52.8 <mark>± 3.2</mark> |
| 5 | | 79.0 <u>± 13.7</u> |

Comparing ILs 1 and 2 (Table 7), a different trend is observed. It seems that the primary amine-derived IL 1 exhibits overall almost double biodegradation rate compared to the secondary amine-derived IL 2.

 Table 7. Effect of the cyclopentanecarboxylic acid based NAILs cation on their (%)

 biodegradability



Regarding the 2-hydroxy-benzoic acid based ILs **10** and **11**, similar biodegradation rates are exhibited (Table 8).

 Table 8
 Effect of the 2-hydroxy-benzoic acid based BAILs cation on their (%)

 biodegradability

| BAILs | Structure | CBOD ₅ /UCBOD (%) |
|-------|--|------------------------------|
| 10 | | 33.1 ± 2.8 |
| 11 | $\bigcup_{OH}^{O} H_3C^{O} \bigcup_{H_2}^{O} O_{CH_3}$ | 30.6 <mark>± 0.58</mark> |

3.1.2. Anion effect on the ILs biodegradability

The influence of the anion moiety of the ILs on their biodegradability is identified by comparing the results presented in Table 9. More specifically, the effect of the aromatic ring and the alicyclic ring on the biodegradation rate is examined, by maintaining the selected

amine constant each time. According to literature data, ILs derived from surrogate naphthenic acids possess biodegradability levels up to 60% during 28 day incubation [25].

| | | CBOD ₅ / | | | CBOD ₅ / |
|-----|--|---------------------|-----|--|-------------------------|
| ILs | Structure | UCBOD (%) | ILs | Structure | UCBOD (%) |
| 1 | | 44.5 <u>± 0.6</u> | 7 | | 90.3 <mark>± 4.0</mark> |
| 2 | $ \begin{array}{c} O \\ O \\ O \\ H_3 C \end{array} \begin{array}{c} O \\ O \\ H_3 C \end{array} \begin{array}{c} O \\ O \\ H_2 \end{array} \begin{array}{c} O \\ O \\ H_2 \end{array} \begin{array}{c} O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O \\ O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O \\ O \\ O \\ O \\ C \\ H_3 \end{array} \begin{array}{c} O \\ O $ | 24.5 ± 0.1 | 8 | | 73.7 <mark>± 0.9</mark> |
| 3 | O O H ₃ N O CH ₃ | 52.3 ± 1.5 | 9 | о С Ф Ч ₃ С-О Ч ₂ Ф Ч ₂ О С Ч ₃ | 27.1 <mark>± 3.9</mark> |
| 4 | $\bigcup^{O} \overset{\Theta}{\underset{H_3}{\cup}} C^{\mathcal{O}} \overset{\Theta}{\underset{H_2}{\vee}} O_{\mathcal{C}} H_3$ | 52.8 <u>± 3.2</u> | 10 | | 33.1 <mark>± 2.8</mark> |
| 5 | | 79.0 <u>± 13.7</u> | 11 | $\bigcup_{OH}^{O} H_3C^{O} \bigcup_{H_2}^{O} CH_3$ | 30.6 <u>± 0.58</u> |

Table 9. Effect of NAILs and BAILs anion on their (%) biodegradability

It is observed that between ILs 8 and 3, as well as between ILs 7 and 5, the ILs with an aromatic ring on the anion exhibit higher biodegradation rates (up to 20%) compared to cyclohexane carboxylate ILs. It is possible that the aromatic character positively affects the biodegradation process. This observation comes in agreement with other bibliographic data [30] according to which the aromatic ring positively affects aerobic biodegradation. However, this trend is not followed by ILs 9 and 4.

Moreover, the number of carbon atoms of the alicyclic ring seems to affect the ILs' biodegradability. ILs **3** and **4** (derived from cyclohexanoic acid) produce higher

biodegradation rates (between 10-28%) compared to ILs **1** and **2** (derived from cyclopentanoic acid).

Regarding the presence of the hydroxyl group as anion substituent of the BAILs, it seems that biodegradability is strongly hindered, since IL 7 (derived from benzoic acid and diethanolamine) presents a biodegradability of 90.3% while the biodegradability of IL 10 (derived from 2-hydroxy-benzoic acid and diethanolamine) is only up to 33.1%. On the contrary, the presence of the hydroxyl groups on the cations has a positive effect on biodegradation compared to ILs which have a methoxy group at the corresponding position in the cation.

Overall, from the experimental data obtained, it is observed that both the anionic and cationic part greatly contribute to the final biodegradability level of the whole series of the studied PILs, which seems to be in agreement with other bibliographic references [23].

3.2. Toxicity Evaluation

The majority of ILs are water soluble so their aquatic toxicity is considered important to be evaluated in case of a possible realize in the aquatic ecosystem. In this work, the brine shrimp lethality assay was used to assess the toxicity of the synthesized PILs. The lethality exerted on the nauplii of the brine shrimp *A. salina* has been widely used in laboratory bioassays as a predictor of chemical toxicity on organisms in aquatic environments through the estimation of the medium lethal concentration (LC₅₀). The crustacean *A. salina* L. (Artemiidae), is an invertebrate organism residing in saline aquatic ecosystems and has been widely used in laboratory bioassays for toxicological applications [31]. This test has been already successfully used in the estimation of ILs toxicity [24,32].

The toxicity results of the synthesized PILs on the brine shrimp after 24 h of incubation are presented in Table 10 and it is worth noting that all the tested PILs, were non-toxic to the nauplii of *A. salina*, while, most of them exhibited LC_{50} values above 1000 mM.

| Table 10. ILs Toxic | ty on the nau | plii of the brine | shrimp A.salina |
|---------------------|---------------|-------------------|-----------------|
|---------------------|---------------|-------------------|-----------------|

| IL | LC ₅₀ (mM) | |
|----|---------------------------|--|
| 1 | 331.9 ± 15.7 | |
| 2 | >1000 | |
| 4 | 283.8 <mark>± 11.3</mark> | |
| 5 | >1000 | |
| 6 | >1000 | |
| 7 | >1000 | |
| 9 | >1000 | |
| 10 | 403.6 <u>± 14.6</u> | |
| 11 | >1000 | |
| | | |

3.3. Differential Scanning Calorimetry (DSC)

Many applications demand ILs with low viscosity values and high conductivity at room temperature. Both these properties are correlated and strongly depended on glass transition temperatures (T_g), so the T_g value determination is considered crucial. The thermal behavior and T_g of the studied ILs have been investigated by DSC.

The (second) heating DSC plots of selected NAILs and BAILs are illustrated in Fig.3-4 and Fig. 5-6 respectively and the estimated T_g along with heat capacity increment ΔC_p (J/g °C), are presented in Table 11. The temperature accuracy +/-0.1°C and sensitivity 0.2 μ W. Due to the fact that aging effects contribute to all heating thermograms (overshooting in the

heating scans), the T_g was determined by using the fictive temperature method in Universal Analysis 2000 software (TA Instruments) for all the ILs.

The T_g is indicative of the ILs cohesiveness. So, low cohesive energy means low T_g values. The cohesion energy is reduced by the Pauli's repulsion energy due to the orbits overlapping, while is increased through Coulomb, van der Waals and hydrogen bonds attractive interactions [33]. On the other hand, the glass transition temperature is also determined by the agility of the structural units and the steric hindrances that may exist in their movement. The combination of intermolecular interactions and mobility of structural units greatly determines the T_g . Thus, the ILs with the lowest T_g values are characterized by greater mobility of their molecules and, possibly, weaker intermolecular interactions. According to previous studies on alkylammonium PILs, the T_g increases when the alkyl chain length increases [33] but also increases with the presence of a hydroxyl group [24,33]. Low T_g values could be an indication that the ILs are likely to have desirable physicochemical properties, such as low viscosity and high ionic conductivity at room temperature [33,34].

| IL | Structure | $T_{g}(^{\circ}C)$ | $\Delta C_p (J/g \ ^\circ C)$ |
|----|--|--------------------|-------------------------------|
| 1 | | -80.5 | 0.38 |
| 2 | $ \underbrace{ \begin{array}{c} \bullet \\ \bullet $ | -89.4 | 0.13* |
| 4 | $\bigcup_{n=1}^{O} \bigcup_{n=1}^{O} \bigcup_{n$ | -78.9 | 0.46 |
| 5 | | -61.8 | 0.54 |
| 6 | O O H ₃ N OH | -105.7 | 0.10* |

| 7 | O O HO HO HO HO HO O HO | -49.9 | 0.54 |
|----|---|-------|------|
| 9 | | -74.4 | 0.50 |
| 10 | | -46.1 | 0.53 |
| 11 | | -59.7 | 0.44 |

*Semi-crystalline structure

The NAILs T_g values derived from cyclopentane carboxylic acid range between -80.5 to -89.4 °C (Fig. 3). As for the IL **2**, the curve during the second heating run, exhibits an endothermic peak ($\Delta H = 134.6 \text{ J/g}$) at 92.2 °C that possibly corresponds to melting effects. Consequently, the reduced value of ΔC_p (=0.13 J/g °C) may be attributed to the semi-crystalline morphology of the sample.



Figure 3. DSC behavior of NAILs 1-2

For the ILs 4 and 5 which possess a cyclohexane carboxylate anion, the T_g ranges between – 61.8 to –78.9 °C. In Fig. 4 is perceived the effect of methoxy group on the NAILs cations

compared to the hydroxyl group presence. It seems that the hydroxyl groups in the cationic part favor the formation of hydrogen bonds within the IL and thus the IL exhibits higher T_g values.



Figure 4. Comparison of DSC behavior of NAILs 4-5

This is an observation that is also confirmed by the results of our previous study [24] where cyclopentane carboxylate ILs derived from ethanolamine and diethanolamine exhibited higher T_g values (-68.5 and -66.8 °C respectively) compared to NAILs **1** (T_g = -80.5 °C) and **2** (T_g = -89.4 °C).

The same trend is followed by the BAILs (Fig. 5). Specifically, diethanolamine derived BAILs that possess two hydroxyl groups on the cations scaffolds, like ILs 7 (T_g = -49.9 °C) and 10 (T_g = -46.1 °C) respectively, exhibit higher T_g values compared to those that have one hydroxyl group (IL 6, T_g = -105.7 °C) or no hydroxyl group (IL 11, T_g = -59.7 °C). The presence of a hydroxyl group on the anionic part also affects the T_g values. The effect is slight in case of diethanolamine based BAILs (7 and 10) but seems to be more intense in case of bis(2-methoxy-ethyl)ammonium ILs 9 and 11 where no hydroxyl groups are present on the

cation. Furthermore, considering all 4 ILs we observe that T_g increases with increasing total number of OH-groups ($T_{gIL9} < T_{gIL11} < T_{gIL7} < T_{gIL10}$).



Figure 5. Comparison of DSC behavior of BAILs 6-7 and 9-11

From the examined ILs, IL **6** is solid at room temperature. From the DSC analysis, the first cooling scan gives two exothermic peaks at 65.7 °C and 29.9 °C with Δ H values equal to 84.57 J/g and 4.53 J/g respectively (Fig. 6, left) indicating the ILs crystallization. Therefore, in the following heating stage (second heating for the specimen), the IL is in a semicrystalline state something that is indicated from the very small glass transition step (Δ C_p). The second heating scan, shows that IL **6** has a melting point at 133.4 °C giving an endothermic peak with Δ H = 134.6 J/g, while at 3.12 °C appears an exothermic peak due to cold crystallization (Δ H = 22.55 J/g) (Fig. 6, right). Thus, DSC measurements reveal the strong tendency of IL **6** to crystallize.



Figure 6. DSC Diagram of 1st cooling cycle (left) and of 2nd heating cycle (right) of BAIL 6

3.4. Viscosity Measurements

Viscosity is an important factor of understanding ILs' behavior as well as one of the most useful properties to determine the possible applications of ILs. Viscosity is the result of numerous interactions such as Coulombic, Van der Waals and hydrogen bonding and is highly dependent on many parameters such as the shape and the size of the ILs ions [35-37].

It is common ILs to show a non-Arrhenius behavior, hence the variation of the viscosity as a function of temperature is well described by the Vogel–Tamman–Fulcher (VTF) equation 1 [35].

$$n(T) = n_o \exp\left[\frac{B}{T - T_o}\right]$$
 Eq. 1

In this work, the viscosity of some of the synthesized ILs is studied with respect to chemical structure variations on the anions and cations. More specifically, the effect of hydroxyl groups' existence on either ions of ILs on their viscosities is investigated. Viscosity was measured at temperatures from 20 to 90 °C, and the results span a wide viscosity range (cP), with viscosities decreasing as temperature increases (Table 12). For the studied ILs, viscosity increases rather rapidly at low temperatures and decreases asymptotically to smaller values towards higher temperatures.

The viscosity of ILs 4 and 5 and also ILs 7 and 10 was determined with an accuracy of $\pm 2\%$ and reported at Table 12.

| IL | 4 | 5 | 7 | 10 |
|-------|---------------------|-----------------------|-----------------------|-----------------------|
| T(°C) | n(cP) | | | |
| 20 | 87 <mark>.</mark> 8 | - | - | - |
| 25 | 63 <mark>.</mark> 6 | 6310 <mark>.</mark> 0 | - | 9860 <mark>.</mark> 0 |
| 30 | 46 <mark>.</mark> 2 | 3900 <mark>.</mark> 0 | 7360 <mark>.</mark> 0 | 3436 <mark>.</mark> 7 |
| 40 | 26 <mark>.</mark> 7 | 1668 <mark>.</mark> 7 | 2644 <mark>.</mark> 3 | 1406 <mark>.</mark> 7 |
| 50 | 16 <mark>.</mark> 7 | 779 <mark>.</mark> 7 | 1109 <mark>.</mark> 0 | 646 <mark>.</mark> 0 |
| 60 | - | 398 <mark>.</mark> 2 | 523 <mark>.</mark> 8 | 327 <mark>.</mark> 3 |
| 70 | - | 217 <mark>.</mark> 5 | 273 <mark>.</mark> 2 | 183 <mark>.</mark> 5 |
| 80 | - | 126 <mark>.</mark> 5 | 154 <mark>.</mark> 1 | 109 <mark>.</mark> 6 |
| 90 | - | 78 <mark>.</mark> 2 | 93 <mark>.</mark> 8 | - |

Table 12. Viscosity values of ILs 4, 5, 7 and 10 at atmospheric pressure (accuracy $\pm 2\%$).

Concerning the structure-logarithm of viscosity dependence, the data of this study (Fig. 7) indicates a significant potential effect on the viscosities of the ILs by the presence of hydroxy group on both anions and cations. The effect of the hydroxyl groups in the cationic part is apparently stronger than the effect evident by a hydroxyl group in the anionic part.



Figure 7. Logarithm of viscosity dependence of ILs 4, 5, 7 & 10 in different temperatures.

According to the data above, IL 5, which possesses two hydroxyl groups on the cation, has almost twice the viscosity value of IL 4 that lacks a hydroxyl group. In the case of IL 10, derived from diethanolamine and 2-hydroxy-benzoic acid, an even greater increase of viscosity is observed compared to IL 7 (derived from diethanolamine and benzoic acid). These observations can be considered reasonable since the increase of the hydrogen bonds, due to the presence of the hydroxyl groups, leads to the increased cohesion energy.

Having determined the T_g of the examined ILs and utilizing the VFT equation, viscosity values can be compared with each other on the basis of the T_g -scaled Arrhenius plots. In most cases, the logarithm of viscosity on T_g/T is used to assess the fragility of the materials based on whether viscosity follows the Arrhenius equation at temperatures higher than the T_g ($0 < T_g/T < 1$). A greater deviation from the linear trend indicates increased fragility, which means that as the temperature increases, the viscosity decreases at a faster rate than the Arrhenius ratio. Therefore, strong liquids are those in which a linear increase in the logarithm of viscosity as a function of T_g/T is observed, i.e. the viscosity follows the Arrhenius equation. The fragility of liquids above the T_g is of great importance as the increased fragility corresponds to a greater viscosity reduction as temperature increases [33,34].

In the inset of Fig. 8 we present the viscosity data of the selected ILs in Arrhenius plots where the absolute temperature is scaled to the T_g of each IL.



Figure 8. Logarithm of viscosity vs. T_g/T (in Kelvin) for the PILs studied. In the inset we show the fragility plot for ILs 4, 5, 7 & 10.

Considering the viscosity values of the ILs in the T_g – scaled plots in Fig. 8 we observe that the hydroxyl groups in the cations of the cyclohexane carboxylate ILs have strong influence on the viscosity since IL 5 exhibits the highest values among the studied ILs. On the other hand, for BAILs 7 and 10, which both possess hydroxyl groups in the cations, the presence of OH group in the anion has actually no effect on the viscosity. This finding may imply that cations' mobility may have stronger influence on the viscosity and the fragility of the ILs. Moreover, BAIL 7 (derived from diethanolamine and benzoic acid) can be characterized as more fragile than IL 5 (derived from diethanolamine and cyclohexanecarboxylic acid). This observation is consistent with bibliographic data where it appears that the aromatic character of the compounds enhances their fragility. For example, toluene is very fragile, while paraffin analogues such as methylcyclohexane have an intermediate fragility [34,38].

4. CONCLUSIONS

In this work novel NAILs and BAILs have been successfully synthesized by an atom efficient and waste free neutralization reaction between selected acids and corresponding substituted amines. Their biodegradability and toxicity have been evaluated since these are the key properties to determine their green character. Biodegradability varies between 25 to 92% according to the BOD test after five days of incubation. The wide biodegradability range could be attributed to the structure variations of both ions. Specifically, it appears that ILs with lower carbon load in the cations chain, exhibit higher biodegradation rates, whereas those with methoxy-substituted cations exhibit lower biodegradability compared to those having hydroxyl substituents. On the other hand, with regard to the anion, the aromatic rings' substitution by phenolic groups seems to adversely affect the biodegradability of the ILs. It seems that the aromatic ring of the anion, positively affects the biodegradation compared to the alicyclic ring. Moreover, ILs toxicity was assessed using the brine shrimp lethality assay and all the tested PILs were characterized as non-toxic to the nauplii of A. salina. Some of the most important ILs' physicochemical properties were also measured, the $T_{\rm g}$ and viscosity, and their structure-properties relationship has been investigated. Hydroxyl substituent on the ILs ions seems to be the primary parameter that strongly affects the examined properties since it enhances hydrogen bond interactions. T_g values are affected from the shape, the size and the hydroxyl substitution of the ions. We found that Tg increases monotonously with increasing total number of hydroxyl groups in both, cations and anions. As regards the carbon load effect, the cyclohexanoate ILs give higher Tg values compared to the cyclopentane carboxylate. Furthermore, benzoate ILs give lower values compared to the cyclopentane and cyclohexane carboxylate ILs. Concerning viscosity, it seems that it is mainly governed by the presence of hydroxyl groups on the ions that lead to higher viscosities due to the hydrogen bonding. As far as ILs fragility is concerned, the logarithm of viscosity vs. Tg/T diagrams

proposes that the studied PILs exhibit fragile behaviour. The most fragile is BAIL **4** possibly due to the absence of hydroxyl groups comparing to ILs **5**, **7** and **10** that carry in their scaffolds two to three hydroxyl substituents. Our data imply that cations' mobility has the major influence on the viscosity and its temperature dependence. This structure-property relationship study, is supposed to contribute and promote the future synthesis of novel PILs with desirable properties and environmentally friendly character for specific applications.

AUTHOR INFORMATION

Corresponding Author

*Laboratory of Organic Chemistry, Department of Chemical Sciences, School of Chemical Engineering, National Technical University of Athens, 15780 Athens, Greece
E-mail: adetsi@chemeng.ntua.gr, Tel +30 2107724126, Fax: +30 2107723072.

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REFERENCES

[1] T.L. Greaves, C.J. Drummond, Protic Ionic Liquids: Evolving Structure–Property Relationships and Expanding Applications, Chem. Rev.115 (2015) 11379-11448.

[2] J.D. Holbrey, Rogers, R. D., Mantz, R. A., Trulove, P. C., Cocalia, V. A., Visser, A. E., Anderson, J. L., Anthony, J. L., Brennecke, J. F., Maginn, E. J., Welton, T. and Mantz, R. A..
Physicochemical Properties. In Ionic Liquids in Synthesis, in P. Wasserscheid and T. Welton (Eds.), 2008

[3] A. Tzani, A. Douka, A. Papadopoulos, E.A. Pavlatou, E. Voutsas, A. Detsi, Synthesis of Biscoumarins Using Recyclable and Biodegradable Task-Specific Ionic Liquids, ACS Sustain. Chem. Eng. 1 (2013) 1180-1185.

[4] A. Tzani, S. Koutsoukos, D. Koukouzelis, A. Detsi, Synthesis and characterization of silver nanoparticles using biodegradable protic ionic liquids, J. Mol. Liq. 24 (2017), 212-218.
[5] A.-V. Mudring, T. Alammar, T. Bäcker, K. Richter, Nanoparticle Synthesis in Ionic Liquids, in: Ionic Liquids: From Knowledge to Application, Am. Chem. Soc. 1030 (2009) 177-188.

[6] A.A. Papadopoulou, A. Tzani, A.C. Polydera, P. Katapodis, E. Voutsas, A. Detsi, H. Stamatis, Green biotransformations catalysed by enzyme-inorganic hybrid nanoflowers in environmentally friendly ionic solvents. Environ. Sci. Pollut. Res. Int. 25(2018) 26707-26714.

[7] A.A. Papadopoulou, A. Tzani, D. Alivertis, M.H. Katsoura, A.C. Polydera, A. Detsi, H. Stamatis, Hydroxyl ammonium ionic liquids as media for biocatalytic oxidations, Green Chem. 18 (2016) 1147-1158.

[8] N. Gjineci, E. Boli, A. Tzani, A. Detsi, E. Voutsas, Separation of the ethanol/water azeotropic mixture using ionic liquids and deep eutectic solvents, Fluid. Phase Equilib. 424 (2016) 1-7.

[9] C. Tsanas, A. Tzani, A. Papadopoulos, A. Detsi, E. Voutsas, Ionic liquids as entrainers for the separation of the ethanol/water system, Fluid. Phase Equilib. 379 (2014) 148-156.

[10] J.-G. Lu, H. Ge, Y. Chen, R.-T. Ren, Y. Xu, Y.-X. Zhao, X. Zhao, H. Qian, CO2 capture using a functional protic ionic liquid by membrane absorption, J. Energy Inst. 90 (2017) 933-940.

[11] E.C. Achinivu, Protic Ionic Liquids for Lignin Extraction—A Lignin Characterization Study, Int. J. Mol. Sci, 19 (2018) 428. [12] Z. Li, J. Xu, D. Li, C. Li, Extraction process of sulfur compounds from fuels with protic ionic liquids, RSC Adv. 5 (2015) 15892-15897.

[13] S.P.F. Costa, A.M.O. Azevedo, P.C.A.G Pinto, M.L.M.F.S Saraiva, Environmental Impact of Ionic Liquids: Recent Advances in (Eco)toxicology and (Bio)degradability, Chem. Sus. Chem. 10 (2017) 2321-2347.

[14] A. Jordan, N. Gathergood, Biodegradation of ionic liquids - a critical review, Chem.Soc. Rev. 44 (2015) 8200-8237.

[15] R. Biczak, B. Pawłowska, P. Bałczewski, P. Rychter, The role of the anion in the toxicity of imidazolium ionic liquids, J. Hazard Mater. 274 (2014) 181-190.

[16] B. Peric, E. Martí, J. Sierra, R. Cruañas Terradas, M. Garau, Green chemistry: Ecotoxicity and biodegradability of ionic liquids, Recent Advances in Pharmaceutical Sciences II, 2012 *in*: D. Muñoz-Torrero, D. Haro and J. Vallès (Eds), Chapter 6, 2012, p. 89-113.

[17] J. Neumann, O. Grundmann, J. Thoming, M. Schulte, S. Stolte, Anaerobic biodegradability of ionic liquid cations under denitrifying conditions, Green Chem. 12 (2010) 620-627.

[18] S.P.F. Costa, P.C.A.G. Pinto, R. Lapa, M.L.M..F.S. Saraiva, Toxicity assessment of ionic liquids with Vibrio fischeri: An alternative fully automated methodology, J. Hazard Mater. 284 (2015) 136-142.

[19] N. Gathergood, P.J. Scammells, M.T. Garcia, Biodegradable ionic liquids Part III. The first readily biodegradable ionic liquids. Green Chem. 8 (2006) 156-160.

[20] P.T. Anastas, J.C. Warner, Green chemistry: theory and practice, Oxford University Press; New York, 1998

[21] M. Markiewicz, M. Piszora, N. Caicedo, C. Jungnickel, S. Stolte, Toxicity of ionic liquid cations and anions towards activated sewage sludge organisms from different sources --

consequences for biodegradation testing and wastewater treatment plant operation, Water Res. 47 (2013) 2921-2928.

[22] A.S. Wells, V.T. Coombe, On the freshwater ecotoxicity and biodegradation properties of some common ionic liquids, Org Process Res Dev. 10 (2006) 794-798.

[23] R.S. Boethling, E. Sommer, D. DiFiore, Designing small molecules for biodegradability, Chem. Rev. 107 (2007) 2207-2227.

[24] A. Tzani, M. Elmaloglou, C. Kyriazis, D. Aravopoulou, I. Kleidas, A. Papadopoulos, E. Ioannou, A. Kyritsis, E. Voutsas, A. Detsi, Synthesis and structure-properties relationship studies of biodegradable hydroxylammonium-based protic ionic liquids, J. Mol. Liq. 224 (2016) 366-376.

[25] Y. Yu, X. Lu, Q. Zhou, K. Dong, H. Yao, S. Zhang, Biodegradable Naphthenic Acid Ionic Liquids: Synthesis, Characterization, and Quantitative Structure–Biodegradation Relationship, Chem. Eur. J., 14 (2008) 11174-11182.

[26] OECD Revised Guidelines for Tests for Ready Biodegradability: Ready Biodegradability: Closed Bottle Test. Method 301 D,. OECD Chemical Group: Paris, France, 1993.

[27] G.C. Delzer, and McKenzie, S.W., 2003, Five-day biochemical oxygen demand: U.S.
Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, section
7.0, November, accessed May 2018 from http://pubs.water.usgs.gov/twri9A/.

[28] D. J. Finney, The adjustment for a natural response rate in probit analysis. Ann Appl Biol. 36 (1949) 187-195.

[29] J. Neumann, S. Steudte, C.-W. Cho, J. Thoming, S. Stolte, Biodegradability of 27 pyrrolidinium, morpholinium, piperidinium, imidazolium and pyridinium ionic liquid cations under aerobic conditions, Green Chem. 16 (2014) 2174-2184.

[30] D. Coleman, N. Gathergood, Biodegradation studies of ionic liquids, Chem Soc Rev., 39(2010) 600-637.

[31] B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, J.L. McLaughlin,Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents, Planta Med. 45(1982) 31-34.

[32] W. Gouveia, T.F. Jorge, S. Martins, M. Meireles, M. Carolino, C. Cruz, T.V. Almeida,M.E.M. Araújo, Toxicity of ionic liquids prepared from biomaterials, Chemosphere, 104 (2014) 51-56.

[33] T.L. Greaves, A. Weerawardena, C. Fong, I. Krodkiewska, C.J. Drummond, Protic Ionic Liquids: Solvents with Tunable Phase Behavior and Physicochemical Properties, J. Phys. Chem. B. 110 (2006) 22479-22487.

[34] W. Xu, E.I. Cooper, C.A. Angell, Ionic Liquids: Ion Mobilities, Glass Temperatures, and Fragilities, J. Phys. Chem. B. 107 (2003) 6170-6178.

[35] P.K. Chhotaray, R.L. Gardas, Thermophysical properties of ammonium and hydroxylammonium protic ionic liquids, J. Chem. Thermodyn. 72 (2014) 117-124.

[36] A. Bhattacharjee, J.A.P. Coutinho, M.G. Freire, P.J. Carvalho, Thermophysical properties of two ammonium-based protic ionic liquids, J. Solution Chem. 44 (2015) 703-717.

[37] G.Yu, D. Zhao, L. Wen, S. Yang, X. Chen., Viscosity of ionic liquids: Database, observation, and quantitative structure property relationship analysis. AIChE J. 58 (2012) 2885-2899.

[38] L.-M. Wang, V. Velikov, C.A. Angell, Direct determination of kinetic fragility indices of glassforming liquids by differential scanning calorimetry: Kinetic versus thermodynamic fragilities, J. Chem. Phys. 117 (2002) 10184-10192.

Highlights

- Eleven Protic Ionic Liquids (PILs), were synthesized and structurally characterized
- Most of the tested ILs show satisfactory to extremely high biodegradability level
- The tested PILs are non-toxic to nauplii of Artemia salina (up to LC₅₀>1000mM)
- Structure variations of PILs ions affect their biodegradability and toxicity
- Structure-properties relationship for T_g and viscosity is discussed

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Conflict of Interest

The authors declare that they have no conflict of interest to this work.

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