

2-HYDROXYETHYLAMMONIUM CARBOXYLATES – HIGHLY BIODEGRADABLE AND SLIGHTLY TOXIC IONIC LIQUIDS

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Highly hydrophilic ionic liquids easily form in reactions between 2-hydroxyethyl amines and carboxylic acids. They have low toxicity and high biodegradability, serve both as reaction solvents and catalysts in condensation reactions, and they may be reused several times without any purification.

Key words: *ionic liquids, biodegradation, toxicity, condensation reaction.*

INTRODUCTION

Ionic liquids (ILs) have gained incontestable popularity as promising solvents in organic chemistry and in other application areas during the last decades [1]. Their undisputable values are overshadowed only by their comparatively high toxicity and low biodegradability [1-9]. Albeit plenty of research has been done in order to improve the biodegradability and to reduce the toxicity of ILs during the last years, the best discovered ILs are a far cry from perfect materials for industrial utilization. Therefore, improved ILs are needed for large-scale applications, and especially for their exploitation as the solvents in the transformations of organic substances.

Recently some 2-hydroxyethylammonium carboxylates have been proposed as ILs, with application in electrochemistry and SO₂ removal from gases [10, 11]. These highly hydrophilic materials have also manifested a high solvating ability of organic, inorganic and polymeric compounds, including cellulose [10, 11].

Our preliminary experiments have shown that hydrophilic ILs are more favorable solvents than hydrophobic ones for a large group of syntheses including preparation of heterocyclic compounds [12]. Therefore, some 2-hydroxyethylammonium formates and acetates are elaborated, evaluated and compared in this communication, the main attention being paid to their biodegradability and toxicity.

EXPERIMENTAL

Materials

Formic acid (90%), 2-aminoethanol, 2-N-(2-hydroxyethyl) aminoethanol, 2-N,N-bis(2-hydroxyethyl)aminoethanol (*Aldrich*) were distilled before use. All the other chemicals were analytical grade (*Aldrich*) commercial products. Toxicity tests Rotokit F were obtained from *Microbiotests Inc.*, Deinze, Belgium.

Preparation of ionic liquids

2-Hydroxyethylammonium formate 2a. Formic acid (90 %, 5.11 g; 100 mmol) was added drop by drop to 2-hydroxyethylamine (**1**, 6.11 g; 100 mmol) at temperature of ~ 0 °C and under vigorous stirring. After this the mixture was stirred for extra 6 h at room temperature and dried for ~ 3 h at 50 °C in vacuum (1–2 torr). Colourless viscous liquid **2a** was obtained (10.60 g; 99 %) with the content of the basestock of ≥ 99 % (by HPLC/MS). ^1H NMR spectrum (400 MHz; DMSO- d_6 ; Me $_4$ Si), δ , ppm: 8.4 (1 H, s, $\underline{\text{H}}-\text{CO}_2$); 6.1 (4 H, m, $\text{NH}_3 + \text{OH}$); 3.5 (2 H, t, CH_2-O); 2.8 (2 H, t, CH_2-N). IR spectrum (nujol), ν , cm^{-1} : 3500–3100 (NH + OH); 1750–1600 (C=O + NH).

Other ILs (**4a**, **6a**, **8a**) were prepared in a similar way, their characteristics being presented in Table 1.

2-Hydroxyethylammonium acetate 2b. 2-Hydroxyethylamine (**1**, 6.11 g; 100 mmol) was placed in a flask equipped with a reflux condenser and a dropping funnel. Acetic acid (100%, 6.01 g, 100 mmol) was added drop by drop to the amine solution under vigorous stirring during ~ 1 h. Then the mixture was stirred for 8 h at room temperature. Ethyl acetate (50 ml) was added, and the mixture was stirred for 15 minutes; then the solvent was separated and the purification repeated for another time. The product was stirred and dried in vacuum (1–2 torr, 60–80 °C) for 4 hours to remove traces of any volatile liquid. IL **2b** (10.90 g; 90%) was obtained as a slightly yellow viscous liquid with the content of the basestock of $\geq 99\%$ (by HPLC/MS).

^1H NMR spectrum (400 MHz; DMSO- d_6 ; Me $_4$ Si), δ , ppm: 5.9 (4 H, m, $\text{NH}_3 + \text{OH}$); 3.5 (2 H, t, CH_2-O); 2.7 (2 H, t, CH_2-N); 1.8 (3 H, s, CH_3-CO_2). IR spectrum (nujol), ν , cm^{-1} : 3450–3100 (NH + OH); 1740–1600 (C=O + NH).

Other ILs (**4b**, **6b**, **8b**) were prepared in a similar way, their characteristics being presented in Table 1.

Condensation reaction in ionic liquids

Synthesis of ethyl 2-cyano-3-phenylpropenoate 11. Benzaldehyde (0.53 g, 5 mmol), ethyl cyanoacetate (0.57 g, 5 mmol), and *bis*-(2-hydroxyethyl)ammonium formate (**4a**, 1.51 g, 10 mmol) were stirred at 90 °C for 1 h. The reaction mixture was cooled to room temperature and then extracted with diethyl ether (5 \times 2 ml). The joint ether solution was washed with water (2 \times 2 ml), dried with sodium sulfate for 16 h and evaporated. The residue was crystallized from ethanol. Colourless product **11** was obtained (0.98 g, 98%) with m.p. 50–51 °C (lit. [18] m.p. 51–53 °C). ^1H NMR spectrum (400 MHz; DMSO- d_6 ; Me $_4$ Si), δ , ppm: 8.13 (1 H, s, $\underline{\text{H}}-\text{C}=\text{}$); 7.79–7.33 (5 H, m, $\underline{\text{H}}-\text{C}_{\text{Ar}}$); 4.23 (2 H, q, CH_2-O); 1.42 (3 H, t, CH_2-CH_3). IR spectrum (nujol), ν , cm^{-1} : 3030 (=C–H), 2224 (C \equiv N), 1726 (C=O), 1607 (C=C).

The same IL (**4a**) without any purification was used for evaluation of its repeated exploitation and the results are collected in Table 5. Condensation reactions in other ILs **2**, **4**, **6**, **8** were performed in a similar way, and the results are presented in Table 4.

Measurements

^1H NMR spectra were registered on a *Varian 400 MR* spectrometer, using DMSO- d_6 as a solvent and TMS as an internal standard. FT-IR spectra were registered on an *Avatar 330 FT-IR Thermo Nicolet* spectrometer in nujol.

HPLC/MS was performed using a chromatograph *Waters Alliance 2690*. The stationary phase was *Atlantis[®] HILIC Silica*; (column 2.1 × 150 mm, particle size of the sorbent 3 μm). The mobile phase was water–methanol (3/7; v/v), its rate 0.2 ml/min at 30 °C. The detector was mass spectrometer *Micromass[®] Quattro Micro[™] API* with electrospray ionization. Water (moisture) content in ILs was measured by the Karl Fisher method using Karl Fischer titrator 836 *Titration Metrohm*. Reagent *Hydranal-Composite 5*, cylinder volume 20 ml and data processing program *Tiamo* (version 1.2.1.) were used in these analyses.

Toxicity tests for ILs solutions were performed as described in instructions for users according to [13]. Rotifers *Brachionus calyciflorus* that belong to the consumers trophic level of the aquatic food chain were used [14] as test organisms. Each sample was analyzed in triplicate using Toxicity Screening Test Rotoxkit F[™]. Mortality after 24 h was used as the endpoint for determination of acute toxicity. The dilution series to be tested in the definitive test spanned the range of the lowest concentration producing 100% mortality and the highest concentration producing 0% mortality in the range of test sensitivity. LC₅₀ was found by graphical interpolation procedure and was expressed in mg/l.

Oxygen demand was measured according to the standard method [16]. Solutions of ILs in water were oxidized in acidic (H₂SO₄) medium with K₂Cr₂O₇ at 145 °C in the presence of Ag₂SO₄ and HgSO₄ with the following spectrophotometric determination of Cr³⁺ concentration.

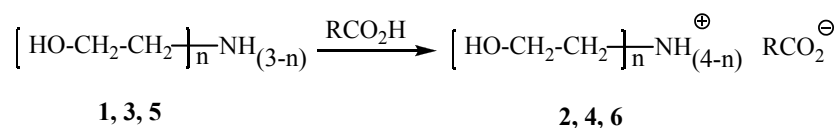
Biodegradation was measured according to the standard method [17]. Solution of IL was diluted with the necessary amount of water. Sludge as inoculum was added to the solution. The sample was kept during an incubation period of 5 days at 20 ± 1 °C in darkness in a completely filled tightly closed vessel in thermostat. The content of dissolved oxygen was measured at the start and at the end of the incubation period and expressed in mg O₂/l. Biodegradation of ILs was calculated as the ratio of biologically and chemically consumed oxygen. Glucose–glutamic acid mixture was used as a reference substance.

RESULTS AND DISCUSSION

2-Hydroxyethylammonium salts (**2**, **4**, **6**, **8**) are closely related to choline – 2-hydroxyethyltrimethylammonium hydroxide that participates in human biological processes. It was reasonable to expect that derivatives of this natural compound might be better acceptable to microorganisms than other ammonium salts used as ILs when the latter become a waste after their exploitation. On the other hand, 2-hydroxyethylammonium salts are highly hydrophilic substances due to the presence of O–H and N–H bonds in their structures. The elevated hydrophilicity is sometimes a crucial advantage to reach the high yields of the products in particular organic transformations. Therefore, some choline analogues are synthesized and described in this paper, and they look promising for utilization as solvents in syntheses.

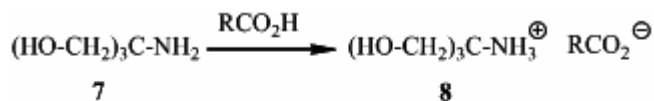
2-Hydroxyethylammonium salts were prepared according to a slightly modified recently described method [10] – by reaction of hydroxyethyl amines with formic or acetic acid (Scheme 1). 90% formic acid and 100% acetic acid were used in our experiments and the excess of water or absorbed moisture was separated from the reaction mixture after the salt formation by distillation in

high vacuum (2 torr at temperatures from 50 to 80 °C). ILs with various number of hydroxyl groups (**2**, **4**, **6**, **8**) were prepared this way.



where: **1,2**, n=1; **3,4**, n=2; **5,6**, n=3

a) R=H; b) R=CH₃



where: a) R=H; b) R=CH₃

Yields of ILs are high, and usually the obtained ILs are colourless viscous liquids. The removal of water at the end of the procedure has allowed us to obtain ILs with a low moisture content (mainly ≤ 2.7%), the latter being measured by the Karl Fisher method (Table 1). Fortunately, the small amount of water present does not affect our investigated condensation reactions.

Table 1. Characteristics of investigated ILs

Entry	IL	Yield, % ^a	Assay, % ^b	Water, % ^c	¹ H NMR, δ, ppm				
					H-CO ₂	CH ₃ -CO ₂	$\frac{\text{H-O}^-}{\text{H-N}^+}$	O-CH ₂ -	N-CH ₂
1	2a	99	≥ 99	2.2 ± 0.3	8.41 (s)		6.06 (m)	3.54 (t)	2.79 (t)
2	4a	99	≥ 99	2.5 ± 0.2	8.43 (s)		5.71 (m)	3.54 (m)	2.80 (m)
3	6a	97	≥ 97	2.7 ± 0.5	8.27 (s)		6.08 (m)	3.53 (m)	2.80 (m)
4	8a	99	≥ 99	2.5 ± 0.5	8.16 (s)		5.76 (m)	3.53 (s)	-
5	2b	90	≥ 97	1.3 ± 0.3		1.76 (s)	5.91 (m)	3.48 (t)	2.72 (t)
6	4b	99	≥ 99	1.1 ± 0.3		1.84 (s)	5.22 (m)	3.50 (t)	2.64 (t)
7	6b	91	≥ 99	0.4 ± 0.2		1.90 (s)	5.36 (m)	3.44 (t)	2.60 (t)
8	8b	97	≥ 98	0.6 ± 0.2		1.75 (s)	5.92 (m)	3.36 (s)	-

^a Yield of the isolated product;

^b analyzed by HPLC/MS;

^c determined by Karl Fisher method.

Structures of the obtained ILs were confirmed by their ¹H NMR and IR spectra. In the ¹H NMR spectra, the most characteristic signals of investigated ILs are those of the protons in the formate anion at 8.2–8.4 ppm or protons of the acetate anion at 1.8–1.9 ppm. Protons adjacent to the oxygen atom can be found by signals at 3.4–3.5 ppm, and protons adjacent to the ammonium nitrogen atom – at 2.6–2.8 ppm. IR spectra are less informative. The stretching vibrations at 3500–3100 cm⁻¹ and 1750 cm⁻¹ and NH bending vibrations at 1600 cm⁻¹ have witnessed only the presence of ammonium and carboxylate ions, respectively.

Quantitative analyses of ILs are performed by HPLC/MS, and an instrument equipped with MS as a detector is successfully used. Only one signal is observed in all chromatograms, and mass spectrometry has served to confirm once more the right structures of the obtained ILs. The molecular ion M^+ has been the most intensive one in all the investigated MS.

Toxicity of the investigated ILs is also evaluated according to the standard method [13, 14]. As it was expected from the similarity of investigated ILs with choline, new ILs **2**, **4**, **6**, **8** have very low toxicity in comparison with imidazolium- or pyridinium-based ILs – more than 100 times lower (Table 2). Toxicity of the tested ILs is low also in respect to diethanolamine – a representative of 2-hydroxyethylamines [15].

Table 2. Toxicity of 2-hydroxyethylammonium salts

Entry	IL	Toxicity ^a	
		LC ₅₀ , mol/l	LC ₅₀ , mg/l
1	2a	0.0550 ± 0.0009	5885 ± 54
2	4a	0.0339 ± 0.0013	5119 ± 114
3	6a	0.0375 ± 0.0013	7313 ± 27
4	8a	0.0165 ± 0.0020	2755 ± 135
5	2b	0.0095 ± 0.0054	1149 ± 54
6	4b	0.0520 ± 0.0009	8580 ± 63
7	6b	0.0290 ± 0.0008	6061 ± 45
8	8b	0.0570 ± 0.0012	10317 ± 90

^a Determined as described in [13].

In order to evaluate how really “green” the described ILs are, their degradation has been investigated. Oxygen demand (chemical degradation) of the all synthesized ILs is measured according to standard method [16], albeit in our case these characteristics are necessary only for general evaluation of the possibility to decompose the investigated ILs. The expected results are in accordance with anticipations – all ILs have decomposed almost fully in the standard conditions. The obtained results are collected in Table 3. Our results confirm the idea that the chemical degradation of investigated ILs (chemical oxygen demand) proceeds in sufficiently drastic conditions to completely decompose all ILs, both hydrophilic 2-hydroxyethyl ammonium salts and more hydrophobic heterocyclic compounds – imidazolium and pyridinium salts.

Biodegradation of investigated ILs has been measured according to a well-known method – European Standard [17]. Biodegradation of some conventional ILs has been also performed in the same conditions for the comparison. Biodegradation of hydrophilic ILs (**2**, **4**, **6**, **8**) is ~ 10 times higher than that of traditional ILs (Table 3). In some cases, their degradation reaches 97% which makes these ILs next to the biodegradation level of the mixture of glucose and glutamic acid – an ordinary reference material. It is worth mentioning that monosubstituted 2-hydroxyethylammonium salts have the highest biodegradation level, the last decreasing with more branched ammonium salt structures.

Therefore, monosubstituted 2-hydroxyethylammonium salts may be considered as exceptionally harmless materials.

Table 3. Degradation of 2-hydroxyethylammonium salts

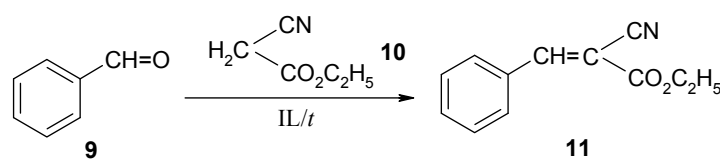
Entry	IL	Oxygen demand (chemical degradation degree), % ^a	Biodegradation degree,% ^b
1	2a	100	95
2	4a	100	55
3	6a	97	97
4	8a	100	40
5	2b	100	92
6	4b	100	57
7	6b	94	31
8	8b	100	26
9	[emim] CH ₃ SO ₃ ^c	93	7
10	[bmim] OTs ^c	100	7
11	[C ₁₂ mim] Br ^c	97	6
12	[C ₁₂ py] Br ^c	95	10

^a Determined according to [16], expressed as ratio of experimental and theoretical COD.

^b Determined according to [17], expressed as ratio of biological and chemical oxygen demand.

^c Abbreviations: [emim] – 1-ethyl-3-methylimidazolium; [bmim] – 1-butyl-3-methylimidazolium; [C₁₂mim] – 1-dodecyl-3-methylimidazolium; [C₁₂py] – 1-dodecylpyridinium; OTs – *para*-toluenesulfonyl.

Practical suitability of the investigated ILs has been checked in the condensation reaction of benzaldehyde (**9**) with ethyl cyanoacetate (**10**). Results obtained in ILs media have been compared with those obtained in ethanol solution (Table 4). Our investigated ILs are ammonium salts, and therefore, they themselves may serve as catalysts for the condensation reaction. No other catalysts are necessary. Yields of the condensation product (**11**) are high, and the addition of an extra catalyst, glycine, resulted only in a meaningless increase of the reaction yields (Table 4).



Investigated 2-hydroxyethylammonium-based ILs have been planned for utilization in such organic syntheses that need highly hydrophilic media. Syntheses of ethyl 2-cyano-3-phenylpropenoate (**11**) in 2-hydroxyethylammonium-based ILs have fully confirmed these expectations. ILs have served both as solvents and catalysts in these condensation reactions that demand the presence of catalytic amounts of both acids and bases. The best performance was reached in ILs **4a**, **6b** and **8b** (Table 4). No clear relation between the structure of investigated ILs and the result of condensation reaction can be seen.

Table 4. Yields of the condensation reaction product **11** in ILs and ethanol ^a

Entry	Solvent	Yield, % ^b	Yield, % ^c
1	2a	88	87
2	4a	98	92
3	6a	94	88
4	8a	77	87
5	2b	74	50
6	4b	71	73
7	6b	99	90
8	8b	99	94
9	EtOH	34	89 ^d

^a Yield of the isolated condensation reaction product **11** after 1 h at 90 °C with the molar ratio benzaldehyde : ethyl cyanoacetate : ionic liquid = 1:1:2; the found content of **11** in the isolated product always was $\geq 99.0\%$ (measured by GC-MS method).

^b Yield without any catalyst.

^c Yield of the product **11** in the presence of a catalyst – glycine in the molar ratio benzaldehyde : ethyl cyanoacetate : ionic liquid : glycine = 1:1:2: 0.2.

^d Reaction in ethanol in the presence of glycine according to a described [18] procedure.

Isolation of the product in our investigated reaction is possible in two ways: 1) by dissolving the used IL in water; 2) by extracting the product with an organic solvent – diethyl ether in our case. The last method allows the condensation reaction to be repeated for several times (in the same IL). Repeated reactions proceed without any substantial decrease of their yields (Table 5).

Table 5. Yields of the condensation reaction product in the reused ionic liquid

Entry	Run	Yield, % ^a
1	1st	98
2	2nd	99
3	3rd	98
4	4th	99
5	5th	98

^a Yield of the isolated condensation reaction product – ethyl 2-cyano-3-phenylpropenoate (**11**) after 1 h at 90 °C with the molar ratio benzaldehyde : ethyl cyanoacetate : ionic liquid (**4a**) = 1:1:2.

The reuse of ILs is highly efficient – no significant loss of the yield is observed up to the 5th run in the same IL (Table 5). Therefore, 2-hydroxyethylammonium-based ILs can be considered very perspective solvents for such organic transformations that usually require highly hydrophilic media and the presence of acids and bases, both in laboratories and in industry.

CONCLUSIONS

2-Hydroxyethylammonium carboxylates are only slightly toxic and highly biodegradable ionic liquids. Condensation reactions in these ILs provide high yields of the products without any catalyst and permit repeated exploitation of the same ionic liquid for at least five times.

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**2-HIDROKSIETILAMONIJA KARBOKSILĀTI –
VIEGLI BIODEGRADĒJAMI UN NEDAUDZ TOKSISKI
JONU ŠĶIDRUMI**

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K O P S A V I L K U M S

Ievērojami hidrofilu jonu šķidrums veidojas reakcijās starp 2-hidroksi-
etilamīniem un karbonskābēm. Tiem piemīt zems toksiskums un augsta bio-
degradējamība. Šie materiāli kalpo vienlaikus par šķīdinātājiem un kataliza-
toriem dažās kondensācijas reakcijās, un tos var lietot vairākkārt bez jebkādas
attīrīšanas.

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