

Antioxidant properties of ionic liquids based on vitamin C

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Introduction

An interest in ionic liquids unflagging for several decades is related to their application potential [1]. The term ionic liquids (ILs) refers to the compounds consisting of ions with melting point below 100°C [2]. The ILs are usually composed of an organic cation and anions, both organic and inorganic ones [3-7]. Moreover, the ILs are described as designable compounds because their physicochemical parameters can be controlled through the selection of both a cation and an anion [8]. They are used as environmentally friendly replacements of conventional, volatile solvents in organic synthesis [9] mainly due to their exceptional properties. Radicals are unstable molecules with an unpaired electron in the outer orbit. They usually include reactive forms of oxygen such as superoxide, hydroxyl, peroxy, alkoxyl radicals and various nitrogen oxides [10]. These compounds perform a double role in biological systems as they may have both a positive and a negative effect on the organism. Their activity inside the cells involves a defence against pathogens; they can also serve as signalling systems [11]. However, there is a large body of evidence indicating that radicals damage biomolecules by oxidation, which affects ageing and various diseases, mainly cardiovascular diseases, neurodegenerative disorders and cancer [12, 13]. Antioxidants are the substances that significantly reduce a negative effect of radicals on the functioning of the human body. They also include compounds that are metal chelators, inhibitors of oxidative enzymes and cofactors of antioxidant enzymes [14]. Natural antioxidants present in fruits and vegetables can be divided into three groups: vitamins, phenols and carotenoids [15]. Vitamin C (L-ascorbic acid) is a water soluble antioxidant in the form of six-carbon lactone produced from glucose in the livers of most mammals, excluding humans, monkeys and guinea pigs [16]. It participates in the production of steroid hormones, collagen synthesis and affects vascular permeability. In medicine, it is used for the regulation of blood coagulation, cell neogenesis and iron absorption [17]. Vitamin C is commonly used as a natural antioxidant, however its excess may lead to diarrhoea, stomach irritation and kidney stones [18].

Experimental part

Materials

4-methylmorpholin ($\geq 98\%$), 1-methylpiperidine (99%), 2-dimethylaminoethanol ($\geq 98\%$), 3-dimethylamino-1-propanol (99%), 1-bromobutane (99%), L-ascorbic acid (99%), sodium L-ascorbate (99%), 2,2-diphenyl-1-picrylhydrazyl (95%), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) ammonium salt ($\geq 98\%$), sodium persulfate ($\geq 98\%$) were supplied by Sigma-Aldrich and used without further refining. Other reagents and solvents were purchased from Avantor Performance Materials Poland S.A.

Analysis

The structure of the obtained compounds was confirmed using proton carbon nuclear magnetic resonance (^1H NMR) spectra taken with Varian Mercury 300 spectrometer. The melting point was measured using MP90 Melting Point System with operating temperature gradient of 5°C/min. The water content was measured using Karl Fischer titrator by Aquastar. The density of the obtained ionic liquids was measured using densimeter Density Meters by Rudolph Research Analytical. The refraction index was determined using the Automatic Refractometer J357 by Rudolph Research Analytical. The solubility test was performed in accordance with the Vogel's Textbook on Practical Organic Chemistry [19]. The thermal stability and phase transitions were measured by means of TGA and DSC using Mettler Toledo Stare TGA/DSC1.

Synthesis of quaternary bromides

0.1 mole of bromobutane and 50 cm³ of acetonitrile were added to the reaction flask. Then, 0.11 mole of an appropriate amine was added. The synthesis was carried out at 60°C for 24 hours; then, after the completion of the reaction, the solvent was evaporated using a vacuum evaporator. To precipitate the product from the final reaction mixture, acetone was added. The product in the form of white precipitate was separated by means of filtration and dried *in vacuo* at 60°C for 24 h.

Synthesis of ILs

A respective quaternary bromide (0.05 mole) was dissolved in 150 cm³ of methanol. 60 cm³ of ion-exchange resin was added in the form of methanol suspension. The reaction was carried out for 2 hours at ambient temperature. Then, the resin was separated from the reaction mixture by means of filtration and L-ascorbic acid (0.05 mole) was added to the filtrate. After 30 minutes of the reaction, the solvent was removed *in vacuo* and the final product was obtained. The compound was dried *in vacuo* for 48 hours at ambient temperature.

ABTS Method

ABTS radicals were obtained by mixing 7 mM ABTS solution with 2.45 mM sodium persulfate solution. After 16 hours, the obtained mixture was diluted with methanol to obtain the absorbance of 0.70 \pm 0.03 at 734 nm. Subsequently, 0.95 cm³ of freshly prepared ABTS solution was added to 0.05 cm³ of the aqueous solution of the tested compound. The reaction was conducted for 6 minutes at ambient temperature and the absorbance was measured at 734 nm. The antioxidative activity was calculated from the equation:

$$\% \text{ ABTS antioxidative activity} = \left(\frac{A_1 - A_2}{A_1} \right) \cdot 100\% \quad (1)$$

where: A_1 – absorbance of sample without the addition of the tested compound; A_2 – sample absorbance with the addition of the tested compound. The results are expressed as EC_{50} values.

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DPPH Method

Initially, 1.95 cm³ of freshly prepared 60 μM DPPH solution in methanol was added to 0.05 cm³ aqueous solution of the tested compound. The change in the absorbance at 515 nm was measured after a 30-minute incubation in the dark at ambient temperature. The antioxidative activity was calculated on the basis of the equation:

$$\% \text{ DPPH antioxidative activity} = \left(\frac{A_1 - A_2}{A_1} \right) \cdot 100\% \quad (2)$$

where: A₁ – absorbance of the sample without the addition of the tested compound; A₂ – absorbance of the sample with the addition of the tested compound. The results are expressed as EC₅₀ values.

Result discussion

The quaternary bromides (Fig. 1) that serve as a cation source in the IL synthesis were obtained in the Menshutkin reaction between tertiary amines and an quaternizing agent (Fig. 2). In all cases, bromobutane was used as an agent.

The obtained precursors were white, hygroscopic solids with melting temperatures (T_m) in the range from 131 to 241°C. The presence of the hydroxyl group and aliphatic substituent resulted in the significant reduction of T_m. The highest values were obtained for salts **1** and **2**.

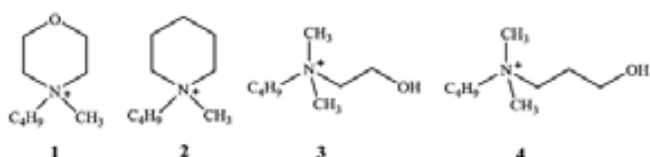


Fig. 1. Structure of cations of obtained ILs

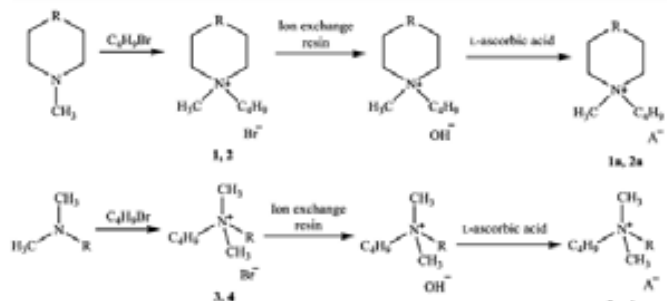


Fig. 2. Preparation of ILs with the ascorbate anion

The neutralisation reaction occurring between quaternary hydroxides derived from precursors and L-ascorbic acid was selected for the IL synthesis. The use of the anion resin allowed for the elimination of the need to further purify the compounds. The ILs were obtained with a high yield exceeding, in all cases, 98%. After each synthesis, the compounds were dried for 48 hours *in vacuo* at ambient temperature. All ILs with an ascorbate anion were orange liquids of very high viscosity. The elemental analysis was used to confirm the purity of the product, whereas the water content was determined by means of the Karl Fischer titration (below 1%).

The physicochemical properties were determined for the synthesised ILs at 20°C (Tab. 2). The lowest density was found for salts with morpholinium cation (**1a**) and piperidinium (**2a**) cation, for which the measurements gave the results of 1.22138 and 1.22993 g/cm³, respectively. The refraction index depends slightly on the type of cation in the IL structure. Unlike density, the highest value was observed for compounds **1a** and **2a**. Other ammonium ILs have similar results of approx. 1.53.

Table 1

Synthesized quaternary bromides and ILs

| Salt | R | Yield (%) | State at 25°C | T _m (°C) |
|-----------|----------------------------------|-----------|---------------|---------------------|
| 1 | O | 95 | solid | 216-218 |
| 2 | CH ₂ | 92 | solid | 240-241 |
| 3 | C ₂ H ₅ OH | 93 | solid | 123-125 |
| 4 | C ₃ H ₈ OH | 94 | solid | 131-133 |
| 1a | O | 99 | liquid | - |
| 2a | CH ₂ | 98 | liquid | - |
| 3a | C ₂ H ₅ OH | 98 | liquid | - |
| 4a | C ₃ H ₈ OH | 99 | liquid | - |

The obtained ILs have low glass transition temperatures (T_g) from -5.54 to 12.21°C. The presence of an oxygen atom in morpholinium cation causes a significant decrease in T_g in comparison to the compound **2a** with piperidinium cation. In case of the ILs **3a** and **4a**, the increase in the length of the alkyl substituent containing the hydroxyl group results in the increase in the value of T_g from 0.42 to 7.64°C. ILs with an ascorbate anion can be considered to be a thermally stable compound. The onset decomposition temperature (T_{onset}) in the worst case was 169°C for the salt **4a**. In case of other compounds, this value exceeded 190°C. In case of the T_{onset50} parameter, the presence of various cations has no significant effect on the stability.

Table 2

Physicochemical properties of obtained ILs

| Ionic liquid | T _g ^a (°C) | T _{onset} ^b (°C) | T _{onset50} ^c (°C) | n _d ^d | Density ^d (g/cm ³) |
|--------------|----------------------------------|--------------------------------------|--|-----------------------------|---|
| 1a | -5.54 | 205 | 231 | 1.53886 | 1.22138 |
| 2a | 12.21 | 212 | 238 | 1.54196 | 1.22993 |
| 3a | 0.42 | 194 | 225 | 1.52600 | 1.23483 |
| 4a | 7.64 | 169 | 228 | 1.52585 | 1.26519 |

^a glass transition temperature, ^b decomposition temperature, ^c decomposition temperature of 50% sample, ^d at 20°C

Table 3

Solubility of compounds at 25°C^a

| Solvent | Compound | | | | | |
|---------------|-----------|-----------|-----------|-----------|-----------------|-----------------|
| | 1a | 2a | 3a | 4a | AA ^a | SA ^b |
| water | + | + | + | + | + | + |
| methanol | + | + | + | + | - | - |
| DMSO | + | + | + | + | + | - |
| acetonitrile | - | - | - | - | - | - |
| acetone | - | - | - | - | - | - |
| 2-propanol | ± | + | ± | - | - | - |
| ethyl acetate | - | - | - | - | - | - |
| chloroform | - | - | - | - | - | - |
| toluene | - | - | - | - | - | - |
| hexane | - | - | - | - | - | - |

^a "+", complete solubility; "±", limited solubility; "-", insoluble., ^b L-ascorbic acid, ^c sodium L-ascorbate

The solubility of ionic liquids is presented in Table 3. The tested salts showed a very good solubility in polar solvents such as water, methanol and DMSO. The presence of the piperidinium cation in the compound **2a** resulted in the significant increase in the isopropanol

solubility. All ILs were insoluble in low-polarity solvents such as hexane and toluene, as well as in ethyl acetate, chloroform, acetone and acetonitrile. L-ascorbic acid (AA) and sodium L-ascorbate (SA) show a similar solubility, whereas the greatest difference was observed for the low solubility in methanol and isopropanol.

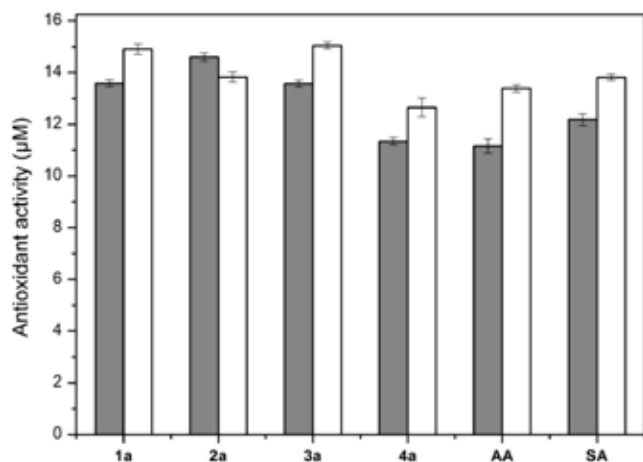


Fig. 3. Antioxidant activity (EC₅₀) measured using ABTS (gray columns) and DPPH (white columns) methods. L-ascorbic acid (AA) and sodium L-ascorbate (SA) were used as reference

In case of DPPH method, the tested compounds showed a slightly lower antioxidative activity in comparison to the ABTS method (Fig. 3). The compound **2a** was an exception. The synthesised ILs containing an ascorbate anion was characterised by the ability to inhibit DPPH radicals in the EC₅₀ range from 12.65 ± 0.37 to 15.04 ± 0.13 µM. In case of the ABTS tests, the range was 11.34 ± 0.14 to 14.60 ± 0.15 µM. L-ascorbic acid and its sodium salt were used as reference compounds. The tests showed that vitamin C in the form of salt has a slightly lower effectiveness of the elimination of free radicals. Similar relations were observed for ILs. The lowest activity in the presence of acid was observed for salts **1a** and **3a**. However, thanks to the use of an ammonium cation with hydroxypropyl substituent, it was possible to obtain the compound of a higher efficacy in case of the DPPH method or a similar one for the ATBS method.

Summary

To conclude, it is possible to obtain antioxidative ILs containing cations with a short alkyl substituent and anion from a naturally occurring vitamin C. As a result of the neutralisation reaction, compounds of high purity and antioxidative activity were obtained. The selection of anion was related to its wide availability and low price in comparison to other acids showing an antioxidative activity. The obtained compounds were viscous liquids at ambient temperature. They showed a high thermal stability. Moreover, the tests showed that the selection of cation may affect the efficacy of the ability to capture radicals. The application of cheap anions for the synthesis of ILs is another step in the research, which will make it possible to expand their application potential.

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