Journal of Molecular Liquids 247 (2017) 411–424

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

Physicochemical features and toxicity of some vitamin based ionic liquids

M. Vraneš [a,*], A. Tot [a], S. Papović [a], D. Četojević-Simin [b], S. Markov [c], A. Velićanski [c], M. Popsavin [a], S. Gadžurić [a]

[a] University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg D. Obradovića 3, 21000 Novi Sad, Serbia
[b] University of Novi Sad, Faculty of Medicine, Oncology Institute of Vojvodina, Put dr Goldmana 4, 21204 Sremska Kamenica, Serbia
[c] University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia

ARTICLE INFO

Article history:
Received 1 June 2017
Received in revised form 19 September 2017
Accepted 3 October 2017
Available online 05 October 2017

Keywords:
Ionic liquids
Vitamins
Toxicity
Antibacterial activity
Physicochemical characterization

ABSTRACT

A three novel vitamin based ionic liquids, cholinium nicotinate, cholinium biotinate and cholinium ascorbate were synthesized and characterized. For the first time the acidity constant for biotin was determined. Physicochemical properties such as density, electrical conductivity and viscosity were measured and nature of interactions was discussed from the obtained experimental results. Also, toxicity study of these ionic liquids has been performed using human non-tumor cell line (normal fetal lung fibroblasts, MRC-5) and rat liver hepatoma cell line (H-4-II-E). Antibacterial activity was determined by disc diffusion method on Gram negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* as well on Gram positive bacteria *Staphylococcus aureus* and *Listeria monocytogenes*.

1. Introduction

The chemistry of ionic liquids (ILs) has been developed rapidly during the last decade [1,2] founding applications in many classical areas of science. Also, they are important candidates to solve classical problems within several societal challenges, such as more soluble and more bioavailable pharmaceutical compounds, highly efficient carbon capture, clean and efficient energy, through the development of a new energy technologies. The potential of ionic liquids is further emphasised since their physical and chemical properties may be fine tuned by varying the cation and the anion [3]. In order to improve known ILs, or to create novel, so-called risk-conscious design and "thinking in terms of structure-activity relationships" should be applied [4]. According to this strategy, the best solution to reduce toxicity and enhance biodegradability is synthesis of ionic liquids from selected naturally-derived, already non-toxic materials [5,6]. Using this strategy it is possible to expand ILs application in food industry, for example as novel dietary supplements. Synthesis driven from non-toxic starting reagents does not necessarily give a product with lower toxicity [7], since the presence of hydrogen bond acceptors and donor ability significantly affect toxicity [8]. Also, so-called protic ionic liquids attracted attention of scientists recently, but still there is a lack of information about their toxicity in the literature [9].

There are numerous advantages of ionic liquids that can be of interest in food industry, such as good solubility in water, better bioavailability, designed lipophilicity that allows easier transport of the nutrients through the cell membrane and possibility of ILs synthesis with synergistic cation and anion performances. One of the most promising cations for this purpose is cholinium, also known as vitamin B_4, containing the quaternary ammonium ion with a polar hydroxyl group, which is reason for its low toxicity [7]. Also, choline is biologically widespread micronutrient completely degradable under aerobic conditions [10]. The use of choline in the human diet was officially recognized by the US Institute of Medicine's Food and Nutrition Board in 1998. Choline is essential for brain development of the fetus and improves visomotor performance of healthy humans. Lack of the choline causes serious liver and muscle damages [11–13]. Recently, a numerous cholinium based ILs has been synthesized, showing low toxicity and high biodegradability [14–16].

The anion also contribute to the overall toxicity of ILs, although its effect has often been overlooked, possibly due to the limited anion types reported. Vitamins, as important nutrients for humans, are the promising anion candidates for a new class of edible ionic liquids.

Importance of ascorbic acid (vitamin C) in nutrition is well-known: it increases iron absorption, improves collagen synthesis, acts as an antioxidant and biological blocking agent against nitrosamine formation [17,18].

Nicotinic acid, also known as niacin or vitamin B_3 is an essential vitamin required for processing fat in the body, lowering cholesterol levels and regulating blood sugar levels. Niacin was primarily used for the treatment of hypercholesterolemia and reduction of cardiovascular risk. A deficiency of niacin causes pellagra, a condition characterized by diarrhea, dermatitis, dementia, inflammation of the mouth, amnesia, delirium and if left untreated, death [19,20]. Even a slight deficiency of niacin can lead to irritability, poor concentration, anxiety, fatigue, restlessness, apathy and depression.

Biotin (vitamin H or vitamin B_7) is necessary for cell growth, the production of fatty acids, metabolism of fats and amino acids and in

* Corresponding author.
E-mail address: milan.vranes@dh.uns.ac.rs (M. Vraneš).

https://doi.org/10.1016/j.molliq.2017.10.015
0167-7322/

maintaining a steady blood sugar level. Insufficient level of biotin can cause growth retardation, neurological disorders and dermatological abnormalities [21,22].

Therefore, in this paper a new vitamin based ionic liquids were synthesized and appropriate spectroscopy and thermal characterization was conducted. For the newly synthesized cholinium based ionic liquids: cholinium biotinate, [Chol][Biot], cholinium nicotinate, [Chol][Nicot] and cholinium ascorbate, [Chol][Asc], density, electrical conductivity and viscosity measurements were performed, together with their toxicity and antibacterial tests.

The antimicrobial activities were evaluated against a range of Gram positive and Gram negative bacteria, as well-established method for antibacterial activity determination of newly synthesized compounds [23–25].

In order to evaluate cytotoxicity of obtained ionic liquids, the starting compounds in the synthesis and applied standards (ascorbic acid, nicotinic acid and biotin), determination of cell growth effect in human fetal lung cell line MRC-5 derived from the healthy tissue and the rat hepatoma cell line H-4-II-E, were performed. Multi-endpoint bioassays that are based on whole-cell response in mammalian cell lines are powerful indicators of metabolic, biochemical, and genetic alterations that arise under the influence of evaluated compounds [26]. MRC-5 is a well characterized human diploid fibroblast cell line that is remarkably stable and retains the predominantly diploid normal karyotype of the original tissue cells [27]. The H-4-II-E cell line possess excellent growth characteristics and low basal, but highly inducible CYP1A activity. Mammalian studies have revealed the utility of the H-4-II-E cells to predict toxic effects [28]. The differences that are obtained under the influence of evaluated samples can point out a selective action and give bioassays in mammalian cell lines predictive power in terms of the risk to higher organisms [29].

2. Experimental section

2.1. General procedure for synthesis of vitamin based ILs

All applied chemicals were used without purification, the summary of the provenance and purity is given in Table A1 in Appendix. All cholinium based ILs (structures are presented in Fig. A1) were synthesized by an acid-base titration, measuring the pH change. The reaction was conducted by slow addition of aqueous solution of choline hydroxide (concentration $c = 1.7524\ \mathrm{mol\cdot dm^{-3}}$) to aqueous vitamin solution with stirring, until obtaining inflection point (pH values for [Chol][Biot] = 8.80; [Chol][Nicot] = 7.95; [Chol][Asc] = 7.66). From [Chol][Biot] and [Chol][Nicot] water was removed using a rotational evaporator under temperature of 343.15 K. After evaporation, obtained ILs were stored together with P_2O_5 and heated under the vacuum for the next two weeks. Knowing that [Chol][Asc] is unstable at higher temperatures [30], water was removed under room temperature in inert atmosphere using rotational evaporator and obtained [Chol][Asc] was stored in desiccator under P_2O_5. After drying, water content in the ILs were determined by the Karl Fisher titration (using Metrohm 831 Karl Fischer coulometer). The water content was found to be <200 ppm in all prepared ILs. The purities of these ILs were estimated from ^{1}H NMR spectra to be higher than 0.96 (mass fraction, Table A1). Two yellow compounds liquid at room temperatures with no tendency for crystallization during the work were obtained ([Chol][Biot] and [Chol][Asc]). [Chol][Nicot] was obtained as brown solid compound, with melting point below 100 °C (Thermal analysis section) and could be classified as ionic liquid, too.

2.2. Material and methods

For additional characterization, the IR and NMR spectra of newly synthesized cholinium based ILs were recorded. NMR spectra were recorded in D_2O at $T = 298.15$ K on a Bruker Advance III 400 MHz spectrometer. Tetramethylsilane was used as accepted internal standard for calibrating chemical shift for ^{1}H and ^{13}C. ^{1}H homodecoupling and 2D COSY method were used routinely for the assignation of obtained NMR spectra. ^{13}C NMR spectra were assigned by selective decoupling technique.

Infrared spectra were recorded as neat samples from (4000–650) $\mathrm{cm^{-1}}$ on a Thermo-Nicolet Nexus 670 spectrometer fitted with a Universal ATR Sampling Accessory. The measurements were performed with a total of 60 scans, at $T = 298.15$ K, and a spectrum resolution of 2 $\mathrm{cm^{-1}}$ in a range of υ from (750 to 4000) $\mathrm{cm^{-1}}$. The software package Omnic version 6.2 was used in the data acquisition and spectral analysis.

The thermal stability of ionic liquids is checked applying thermogravimetric (TG) and differential scanning calorimetry (DSC) analysis. The thermal characterization of the sample was performed by thermogravimetric analysis using simultaneous TG/DSC thermal analyzer SDT Q600 (TA Instruments, USA). Sample of about 2.5 mg was placed in an open platinum pan. Measurements were carried out in nitrogen atmosphere (flow rate was 100 $\mathrm{cm^3\cdot min^{-1}}$) to 600 °C with a heating rate of 20 $\mathrm{°C\cdot min^{-1}}$. DSC measurements were performed by differential thermal analyzer DSC Q20 (TA Instruments, USA) with a heating rate of 20 $\mathrm{cm^3\cdot min^{-1}}$ in nitrogen atmosphere. Firstly, samples were heated up to 150 °C (in order to remove residual water), cooled to −60 °C and re-heated to 150 °C.

The density measurements were performed using vibrating tube Rudolph Research Analytical DDM 2911 densimeter equipped with Peltier-type thermostat within and automatically viscosity correction. Before each series of measurements calibration of the instrument was performed at the atmospheric pressure. Each experimental density value is the average of at least three measurements at temperatures ranged from (293.15 to 313.15) K. Repeated experimental measurements showed reproducibility within 0.01%, and an average value is presented in this work. Standard uncertainty of determining the density is $<7.5\cdot10^{-4}\ \mathrm{g\cdot cm^{-3}}$.

Viscosity of ionic liquids was measured using a Brookfield Viscosimeter DV II + Pro which is thermostated with an accuracy of ±0.01 K and filled with about 8 $\mathrm{cm^3}$ of pure liquid. The spindle type (SC4-18) was immersed and rate per minute (RPM) was set in order to obtain a suitable torque. A viscometer cell protected from moisture with the compartment made by the manufacturer was calibrated using the liquids of different viscosities purchased from the manufacturer. Viscosities of pure ILs were measured in the temperature range from (293.15 to 313.15) K with the rotation speed of 0.5 RPM. Presented experimental values are the mean of three measurements and the measurement uncertainty was found to be about 1%.

The electrical conductivity measurements were carried out in a Pyrex-cell with platinum electrodes on a conductivity meter Jenco 3107 using DC signal in the temperature range from (293.15 to 313.15) K. The cell constant of 1.0353 $\mathrm{cm^{-1}}$ was checked from time to time to control any possible evolution. The relative standard uncertainty for electrical conductivity was <1.5%. All obtained experimental values represent the mean of three measurements.

2.3. Cell lines and cytotoxicity tests

Sulforhodamine B (SRB) and antibiotic/antimycotic (amphotericin B) solution were purchased from Sigma Aldrich, fetal bovine serum (FBS) and Dulbecco's Modified Essential Medium (DMEM) was from PAA Laboratories GmbH, trypsin was from Serva and EDTA from Laphoma. All substances were diluted in 9 $\mathrm{mg\cdot cm^{-3}}$ NaCl and sterilized using 0.22 μm syringe filters. Ionic liquid and standards of ascorbic acid, biotin and nicotinic acid were investigated in the concentration range from (125 to 2000) $\mathrm{\mu g\cdot cm^{-3}}$.

Cell growth activity was evaluated *in vitro* in human fetal lung cell line MRC-5 (ECACC 05090501) and rat hepatoma cell H-4-II-E (ATCC CRL-1548). Cells were grown in Dulbecco's Modified Essential Medium supplemented with 10% heat inactivated FCS, 100 $\mathrm{\mu g\cdot cm^{-3}}$ of penicillin, 100 $\mathrm{\mu g\cdot cm^{-3}}$ of streptomycin and 0.25 $\mathrm{\mu g\cdot cm^{-3}}$ of amphotericin B. Cells were cultured in 25 $\mathrm{cm^3}$ flasks at 37 °C in the atmosphere of 5% CO_2 and high humidity, and sub-cultured twice a week. A single cell suspension was obtained using 0.1% trypsin with 0.04% EDTA.