L-PROLINE BASED ALKYLMETHYLIMIDAZOLIUM IONIC LIQUIDS [$C_n$mim][Pro]: SYNTHESIS AND SOME PHYSICOCHEMICAL PROPERTIES OF THEIR AQUEOUS SOLUTIONS

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Abstract: A modification of synthesis of amino acid ionic liquids (AAILs) based on 1-alkyl-3-methylimidazolium cation and L-prolinate anion [$C_n$mim][Pro] (n = 4, 8, 12) was developed. The structures of the AAILs based on prolinate were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopies. Densities and refractive indices of aqueous solutions of these AAILs in the concentration range 0-97 wt % (weight percents) were measured at temperature 298.15 K. It has been found that in the field of high AAIL concentrations the dependence of the density value on the AAIL mole fraction for binary water + [$C_n$mim][Pro] solutions is nonlinear.

Keywords: ionic liquid, 1-alkyl-3-methylimidazolium cation, amino acid ionic liquid, L-proline, density, refractive index.

Introduction. During the last years amino acid ionic liquids (AAILs) have attracted much attention because of their high potential for applications in chemistry, biology, medicine etc. In recent years, the new data on the bioaccumulation, toxicity, and degradability of ionic liquids (ILs) were reported. The results show that some ILs may cause water or soil pollution as other commonly used chemicals [1, 2]; some ILs, like 1-alkyl-3-methyl-imidazolium halides (ILs with halide anions), may have negative effect on the soil micro-ecological system [3]. Toxicity test results have shown that [C_4mim]Cl inhibits the growth of soil microorganisms, including bacteria. AAILs are novel chiral ionic liquids derived from amino acids [4]. This is a special class of ILs because of their unique acid–base behavior, biological significance and applications in different fields, such as templates in synthetic chemistry, stabilizers for biological macromolecules etc. Their biodegradability and nontoxicity can also play an important role in pharmaceutics for drug formulations [5]. AAILs have been proposed for use in metal scavenging and heterogeneous catalysis, in the chiral liquid–liquid extraction etc. [4-6].

Materials. Commercially available 1-butyl-3-methylimidazolium bromide [C_4mim]Br (97 %), 1-octyl-3-methylimidazolium chloride [C_8mim]Cl (99 %) and 1-dodecyl-3-methylimidazolium chloride [C_{12}mim]Cl (98%) were purchased from Sigma-Aldrich Company and used without any additional purification. L- prolinate ($C_5H_9O_2$) was purchased from Sigma-Aldrich Company (≥ 98.5 %) and it was used also without any additional purification. Anion-exchange resin (type Amberlite IRA-400 chloride form) was purchased from Sigma-Aldrich Company and activated by the regular method before usage. Acetonitrile from Vekton (≥ 99.8 %) was used without further purification. Distilled water was used in the experiments.

Synthesis of [$C_n$mim][Pro]. Currently, one of the main methods for the synthesis of AAILs which are the derivatives of classic alkylmethylimidazolium ILs [$C_n$mim]X (X-anion) is the method proposed by K. Fukomoto and H. Ohno [7]. This method has found applications for 20 amino acids, including prolinate. The synthesis involves three stages. The first stage includes the ion exchange [$C_n$mim]Br → [$C_n$mim]OH using an anion exchange resin; in the second stage the resulting alkylmethylimidazolium hydroxide is mixed at 0ºC with the chosen amino acid taken in excess; at the end of the stage 2 the mixture contains AAIL with an excess of the amino acid; in the third step the AAIL solution is separated from the excess amino acid using MeOH/ACN (1:9) mixture, and the resultant product is dried under vacuum. This is the modification of the known AAIL synthesis method, which uses heating of the reaction mixture up to 96 ºC [8]. Essential disadvantages of the method are the low yield and the long duration of the synthesis time (12 hours + draining the reaction mixture for 48-72 hours at 80 °C). L-prolinate is dissolved in methanol and ethanol; however these solvents are not suitable for the separation of the excess amino acids from AAILs.

In our recent studies three AAILs based on 1-alkyl-3-methylimidazolium cation and L-glutamic acid anion [$C_n$mim][Glu] (n = 4, 6, 8) were synthesized, the Fukumoto method which we have modified partly being applied [9].

The successful modification of the method, carried out by us in the synthesis of AAIL prolinate [$C_n$mim][Pro] using the continuous gas extraction method [10], allowed to obtain during a day about 10 g of [$C_n$mim][Pro] (n = 4, 8, 12) with the purity ≥ 99.5 % (as it concerns organic constituents) and with the water content of 3-4 %.

The excess amount of the prepared aqueous solution of [$C_n$mim]OH was added dropwise to the L-proline aqueous solution cooled to 0°C. The mixture was stirred under cooling and then heated up to 40 °C. The excess residual water was removed from the filtrate under reduced pressure by continuous gas extraction method. Thus, the reaction time was reduced to 12 hours for five cycles to obtain the same yield as in one cycle. Excess amino acids were separated from the AAILs using by acetonitrile. The products, [$C_n$mim][Pro] (n = 4, 8, 12), have being dried for 24 hours in the vacuum desiccator at the room temperature.

The results of ^{1}H NMR and ^{13}C NMR analysis of [C_8mim][Pro] are in a good agreement with the literature data [11]. The total peak integral in the ^{1}H NMR spectrum was found to correspond for all AAILs to a nominal purity higher than 99 % (see Table 1). The water content in the AAILs, determined by a volumetric Karl Fisher titration (V20 METTLER TOLEDO), was lower than 0.1 wt %.

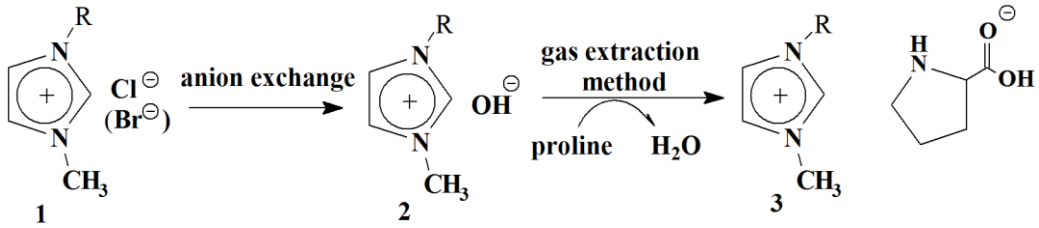

Figure 1 – Synthesis route of AAIL [$C_n$mim][Pro], where 1 is the [$C_n$mim]Br (or [$C_n$mim]Cl), 2 is the [$C_n$mim]OH, and 3 is the AAIL [$C_n$mim][Pro]. These are [$C_n$mim][Pro] molecules where C_n denotes R = C_4H_9, C_8H_{17}, $C_{12}H_{25}$; Pro denotes a prolinate anion

Table 1 – Specification of the synthesized compounds

Chemical Name	Molar Mass, g·mol^{-1}	Purification Method	Purity, Mole Fraction	Analysis Method
[C_4mim][Pro]	253.4	reprecipitation	0.995	^{1}H NMR, ^{13}C NMR
[C_8mim][Pro]	309.4	reprecipitation	0.996	^{1}H NMR, ^{13}C NMR
[C_{12}mim][Pro]	365.4	reprecipitation	0.996	^{1}H NMR, ^{13}C NMR

This procedure was tested on in the synthesis of [C_4mim][Glu]. The resulting AAIL was characterized by ^{1}H NMR and ^{13}C NMR spectra, the density and refractive index were measured. The data obtained are in a good agreement with the literature data [9].

Densities and Refractive Indices of Aqueous Solutions of [$C_n$mim][Pro]. The values of the density, ρ, and the refractive index, n_D, of aqueous solutions of [C_4mim][Pro], [C_8mim][Pro] and [C_{12}mim][Pro] with various contents of AAILs (0-97 wt %) at temperature 298.15 K are presented in Figures 2, 3. The correlation coefficients, R^2, of all linear regressions were larger than 0.99 and all values of the standard deviation, s, were within the experimental error.

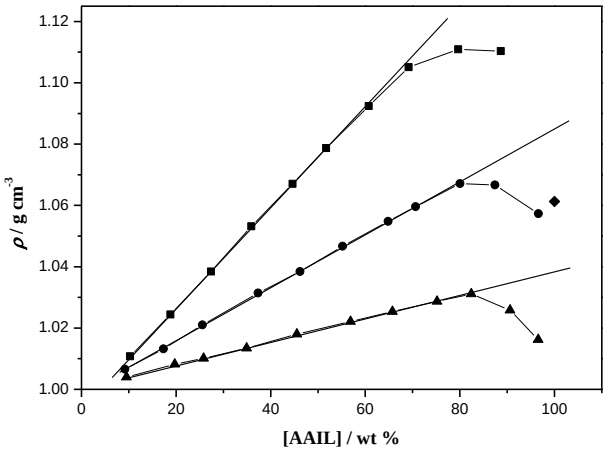

Figure 2 - Density values versus [$C_n$mim][Pro] concentration in aqueous solutions at 298.15 K: ■ – [C_4mim][Pro], ● - [C_8mim][Pro], ▲ - [C_{12}mim][Pro], ♦ - data for pure [C_8mim][Pro] from [11]

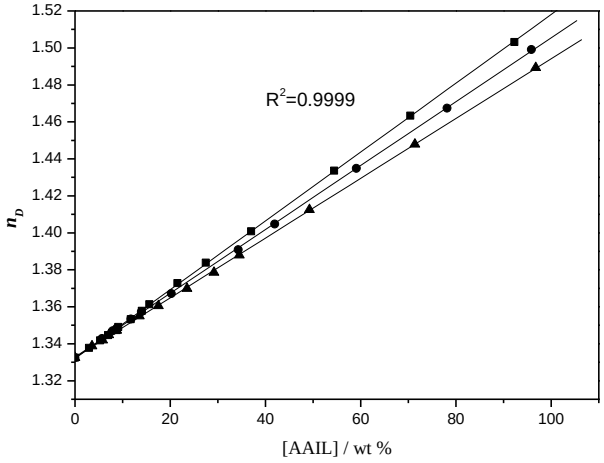

Figure 3 – Refractive index values versus [$C_n$mim][Pro] concentration in aqueous solutions at 298.15 K: ■ – [C_4mim][Pro], ● - [C_8mim][Pro], ▲ - [C_{12}mim][Pro]

Figures 2 and 3 show that the densities and the refractive indices of the aqueous solutions with the same concentration of AAIL decrease in the following sequence: [C_4mim][Pro] > [C_8mim][Pro] > [C_{12}mim][Pro]. In the field of high concentrations of [$C_n$mim][Pro] a nonlinear change in the density values was observed. The density value of the aqueous solution containing 88.68 % [C_4mim][Pro] is 1.1103 g·cm^{-3}. For the aqueous solutions containing 96.59 % [C_8mim][Pro] and 96.57 % [C_{12}mim][Pro] at 298.15 K the density values are 1.1057 and 1.0162 g·cm^{-3}, respectively. The density value for 96.59 % [C_8mim][Pro] at 298.15 K obtained in the present work is compared with the value ρ = 1.0613 g·cm^{-3} for pure IL presented in literature [11] (see Figure 2).

The values of refractive index, n_D, for pure [$C_n$mim][Pro] as results of linear extrapolation are presented in Table 2.

Table 2 – The values of refractive index, n_D, of [$C_n$mim][Pro] (n = 4, 8, 12) at 298.15 K[a]

AAIL	n_D
[C_4mim][Pro]	1.512
[C_8mim][Pro]	1.505
[C_{12}mim][Pro]	1.494

[a] Standard uncertainties: u(n_D) = 0.001, u (T) = 0.05 K

Conclusion. The successful modification of the method has been proposed by us for the synthesis of AAIL prolinate [$C_n$mim][Pro] using the continuous gas extraction.The data obtained have shown that the density and refractive index for [C_{12}mim][Pro] solutions are lower than those for solutions of [C_4mim][Pro] and [C_8mim][Pro]. It was found that in the field of high concentrations of the binary system water + [$C_n$mim][Pro] the change in the density values versus AAIL concentration is nonlinear.

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ОСОБЕННОСТИ ЭНЕРГЕТИЧЕСКОГО МЕТАБОЛОМА ЭРИТРОЦИТОВ У БОЛЬНЫХ РАКОМ РАСПРОСТРАНЕННЫХ СТАДИЙ

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Аннотация. В эритроцитах 74 больных гастроинтестинальным раком, раком легких распространенных стадий исследованы особенности ферментативной активности глюкозо-6-фосфатдегидрогеназы, глицеральдегид-3-фосфатдегидрогеназы, лактатдегидрогеназы, глутатионпероксидазы. Установлено повышение активности ферментов гликолиза и снижение активности ключевого фермента прямого пути окисления глюкозы. При этом отмечено нарастание уровней 2,3-дифосфоглицерата. Это может быть связано с изменением энергообмена эритроцитов и развитием дисфункции. Установлена обратная связь между нарастанием активности глицеральдегид-3-фосфатдегидрогеназы и снижением активности глутатионпероксидазы (показатель корреляции Спирмена ρ = -0,69, т.е. отрицательная связь). Последнему способствовало снижение активности глюкозо-6-фосфатдегидрогеназы, как следствие дефицит НАДФН+H^+. Следовательно, дисметаболические процессы в эритроцитах могут быть связанными с нарушениями процессов тканевой оксигенации у больных раком распространенных стадий.

Ключевые слова: глюкозо-6-фосфатдегидрогеназа, глицеральдегид-3-фосфатдегидрогеназа, лактатдегидрогеназа, глутатионпероксидаза, рак, эритроциты