SOME PHYSICOCHEMICAL PROPERTIES OF AMINO ACID IONIC LIQUIDS BASED ON ALKYLMETHYLIMIDAZOLIUM

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Abstract. A modification of synthesis of amino acid ionic liquids (AAILs) based on 1-alkyl-3-methylimidazolium cation and L-Serinate anion [$C_n$mim][Ser] (n = 8, 10, 12) was developed. The structures of the AAILs based on serinate were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopies. The electrical conductivity in aqueous mixtures of ionic liquids from the family 1-alkyl-3-methylimidazolium serinate [$C_n$mim][Ser] (n = 8, 10, 12) at 298.15 K were measured. The results of the electrical conductivity study clearly show that the general features of micellar aggregation of relatively long-chain imidazolium IL are analogous to those in the case of traditional surfactants. Critical micelle concentrations (cmc) of AAILs, degree of counterion binding (β), standard Gibbs energy of micellization (ΔG°_m) for them were estimated from the experimental data. The dependences of the cmc, β and ΔG°_m, on the length of the alkyl chain of the cations were discussed. These compounds were initially screened for biology activity by a simple zone of inhibition study against *Staphylococcus aureus* ATCC 5638 (gram-positive bacteria), *Escherichia coli* ATCC 25922 (gram-negative bacteria) and *Candida albicans* ATCC 885653 (fungus). The data obtained have shown that among AAILS the [C_{10}mim][Ser] is more effective against this bacteria.

***Key words**: amino acid ionic liquid, 1-alkyl-3-methylimidazolium cation, electrical conductivity, micellization, antibacterial effect.*

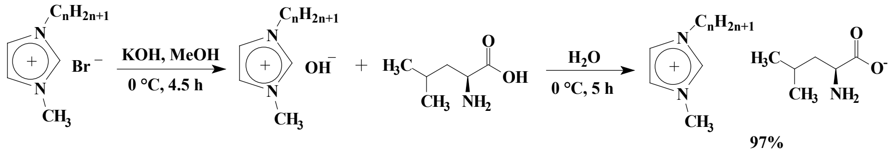
INTRODUCTION

Imidazolium-based Ionic Liquids (ILs) form a class of compounds with the number of specific physicochemical properties – such as the thermal stability, negligible vapor pressure, non-flammability. They are wildly used in «green chemistry» as solvents in synthesis and as catalysts (they can also combine these two properties), in electrochemical and extraction methods. Imidazolium ionic liquids with alkyl side chains longer than four are generally regarded as amphiphilic compounds and behave as typical cationic surfactants in aqueous solution [1-3]. The self-organization of ionic liquids in aqueous solution may have significant effects on a variety of processes such as the extraction of compounds from IL-containing systems, the synthesis and purification of bulk ILs, the formation of dispersed or phase separated systems, etc. [4]. Long-chain imidazolium-based ionic liquids can exhibit, besides to interfacial activity, significant biological activity against bacteria and fungi [4, 5]. Cornellas et al. [5] found that antimicrobial activity of amphiphilic imidazolium ILs is closely related to their surfactant properties. Likewise, toxicology data indicate a strong correlation between the length of the alkyl chain attached to the IL cation and the resulting toxicity [6]. In the present work, a series of 1-alkyl-3-methylimidazolium serinate [$C_n$mim][Ser], where n = 8, 10, 12, in aqueous solutions have been synthesized and the effect of the length of the alkyl chain on their aggregation behavior and antimicrobial activity investigated.

MATERIAL

Commercially available 1-octyl-3-methylimidazolium bromide [C_8mim]Br (97%), 1-decyl-3-methylimidazolium bromide [C_{10}mim]Br (99 %) and 1-dodecyl-3-methylimidazolium bromide [C_{12}mim]Br (98%) were purchased from Sigma-Aldrich Company and it were used without any additional purification. L- Serinate ($C_3H_7NO_3$) was purchased from Sigma-Aldrich Company (≥ 98.5 %) and it was used also without any additional purification. Acetonitrile and Methanol from Vekton (≥ 99.8 %) were used without further purification. Distilled water was used in the experiments.

Synthesis of [$C_n$mim][Ser]. 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).



$C_nH_{2n+1} = C_8H_{17}, C_{10}H_{21}, C_{12}H_{25}$

Figure 1. Synthesis route of AAIL [$C_n$mim][Ser]

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 serinate [$C_n$mim][Ser] using the continuous gas extraction method [10] and methanol solution of potassium hydroxide KOH at the first stage , allowed to obtain during a day about 10 g of [$C_n$mim][Ser] (n = 8, 10, 12) with the purity ≥ 97 % (as it concerns organic constituents) and with the water content of 3-4 %.

Figure 1 presents the steps along the route of preparation of the investigated AAILs [$C_n$mim][Ser]: 1-2) Potassium hydroxide KOH (1.05 mol) in MeOH (20 mL) at 0 °C dropwise added to a stirred solution of 1-alkyl-3-methylimidazolium bromide [$C_n$mim]Br (1.0 mol) in MeOH (20 mL) and stirred for 4.5 h. 2-3) Then the prepared [$C_n$mim]OH separated from KBr or KCl. The methanol solution of [$C_n$mim]OH is added drop wise to the equimolar amount of the amino acid aqueous solution cooled down to 0 °C. The mixture is stirred under cooling and then it is heated up to 40 °C for 5 h. The excess residual water was removed from the filtrate under reduced pressure using the continuous gas extraction method [5]. The residual Br^- were below the detection limit of approximately 50 ppm under a test of $AgNO_3$/ HNO_3 solution. The AAIL solution has been separated from the excess amino acid using acetonitrile ACN. The products, [$C_n$mim][Ser] with the water content of 3-4 %, have been dried during two hours in the vacuum at the room temperature. [$C_n$mim][Ser] products are viscous yellow-brown liquids.

The results of ^{1}H NMR and ^{13}C NMR analysis of [C_8mim][Ser] 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 97% (Table 1). The water content in the AAILs, determined by a volumetric Karl Fisher titration (V20 METTLER TOLEDO), was lower than 0.1 wt%.

Conductivity data. The results of the conductivity measurements for [$C_n$mim][Ser] aqueous solutions are shown in Figures 2, 3 and in Table 2, where the specific conductivity χ in dependence on the AAIL concentration is presented. A characteristic shape of the curves has been observed. They exhibit typical behavior with two linear fragments, and the concentration at which the two linear fragments intersect is assigned to the critical micelle concentration (CMC). The ratio of the slope of the linear fragments above and below the CMC gives an estimate of the degree of ionization (α) of the aggregates. The counterions binding parameter (β) gives the extent of the counterions association with the micelles and it can be estimated as $\beta = (1 - \alpha)$ [12].

The cmc values determined for [$C_n$mim][Ser] (n = 8, 10, 12) and [C_8mim]Br aqueous solutions from the electrical conductivity versus the concentration data (as the intersection points), the β value and the change of the standard Gibbs free energy ($\Delta G°_m$) in the micellization process at 298.15 K are presented in Table 2. The values of the CMC and β for [C_8mim]Br reported by Cornellas and co-workers [5] from conductivity measurements is also included in this table.

For all AAILs [$C_n$mim][Ser] (n = 8, 10, 12) the micellar self-organization in the aqueous solutions takes place. The results of the conductivity measurements obtained for [C_8mim]Br in the present study (Table 2) are in a good agreement with those reported in literature [13]. The values of the counterion binding parameter β (Table 2) increase with the length of the alkyl side chain similarly as reported for non-functionalized ILs [13]. Thus, the counter ion is stronger bonded to the aggregate as the alkyl chain becomes longer. Applying the phase separation model to the monomer-micelle equilibrium for cationic amphiphiles, one can calculate the standard Gibbs energy of micellization ($\Delta G°_m$) from the following equation [14]:

$$\Delta G°_m = (1+\beta)\, RT \ln x_{cmc} = (1+\beta)\, RT \ln \frac{Ccmc}{55.4} \cdot \qquad (1)$$

Table 1. Specification of the synthesized compounds

Chemical Name	Molar Mass, g·mol^{-1}	Purification Method	Purity, Mole Fraction	Analysis Method
[C_8mim][Ser]	299.4	reprecipitation	0.975	^{1}H NMR, ^{13}C NMR
[C_{10}mim][Ser]	328.4	reprecipitation	0.973	^{1}H NMR, ^{13}C NMR
[C_{12}mim][Ser]	356.4	reprecipitation	0.978	^{1}H NMR, ^{13}C NMR

Table 2. Cmc values, degree of counter ions binding (*β*) and Standard Gibbs Energy of Aggregation ($\Delta G°_m$) for the ILs Aqueous Solutions at 298.15 K determined from the electric conductivity measurements in our study

IL	cmc, mmol·kg^{-1}	*β*	$\Delta G°_m$, kJ/mol
[C$_8$mim][Ser]	187	0.58	-22.45
[C$_{10}$mim][Ser]	68	0.63	-27.22
[C$_{12}$mim][Ser]	13	0.77	-33.14
[C$_8$mim]Br	160	0.57	-22.83

The average values obtained from two repeated experimental runs are presented. The estimated errors for cmc and *β* obtained from conductivity are less than 5%.

In the equation *β* is the fraction of counterions condensed in the micellar interface and x_{cmc} is the cmc expressed as the mole fraction unit, c_{cmc} is the cmc value in mol·dm^{-3}, and 55.4 comes from that 1 dm^3 of water corresponds to 55.4 moles of water at 298.15 K. $\Delta G°_m$ indicates the free energy difference per IL molecule between molecules in water and in micelles and also the free energy of transfer of 1 mol of IL from the aqueous pseudophase to the micellar pseudophase. The values of $\Delta G°_m$ calculated according to Eq. (1) for the ILs are presented in Table 2. These values are negative and give evidence that the micelle formation in aqueous solutions of the AAILs investigated is the spontaneous process.

Antibacterial Activity. Antimicrobial tests were carried out using Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 25922 and Candida albicans ATCC 885653 cultures. The Ionic Liquids tested were dissolved in Mueller-Hinton broth (MBH) in the concentration range of 0.0005-0.0035 g/mL at 37°C and no precipitate was observed at the highest concentration of the ILs. From these qualitative experiments, all AAILs exhibited measurable antimicrobial activity (Table 3). For comparison with the literature data, the activity of bromides of 1-octyl-3-methylimidazolium was determined.

The present study confirms the existence of strong relationships between the structure, surface activity and the biological action of imidazolium based ILs on bacteria and fungi. Overall, the diameter of inhibition zone for all the tested compounds fell in the range of 27.9 mm to 35.2 mm. Among AAILs, [C$_{10}$mim][Ser] exhibits the highest susceptibility with inhibition zone of 39.5 ± 0.5 mm against *S. aureus*.

CONCLUSION

The successful modification of the method has been proposed by us for the synthesis of AAIL [C$_n$mim][AA] using the continuous gas extraction in metathesis reactions. We have come to the conclusion that the use of alkali instead of the anion exchange resin in the exchange reactions significantly reduces the synthesis time and the loss of the initial reagents. We found that specific solvents are needed to separate various amino acids from AAILs. The method used here allows synthesizing ionic liquids in a short time with a large amount. The present study has clarified some features of the aggregation behavior of [C$_n$mim][Ser] (n=8, 10, 12) in dilute aqueous solutions, what is of interest for colloid science. The results obtained can be helpful in the search of potential applications of [C$_n$mim][Ser] with various n as new surfactants. This study shows that amphiphilic imidazolium AAILs have antimicrobial activity against Gram-positive and Gram-negative microorganisms and fungi. The results showed that [C$_{10}$mim][Ser] is more effective against tested bacteria.

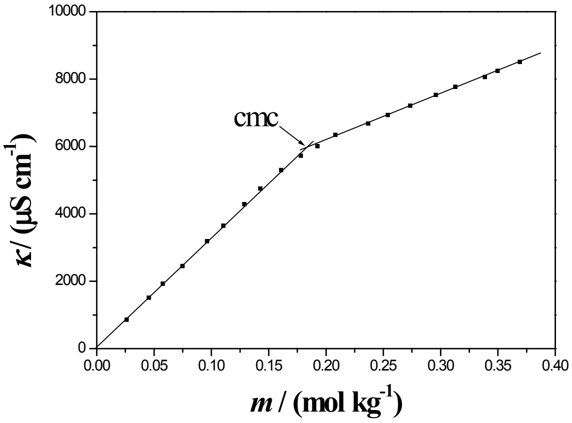


Figure 2. Specific conductivity values (*κ*) for aqueous solutions of [C$_8$mim][Ser] at various AAIL molal concentrations; T = 298.15 K

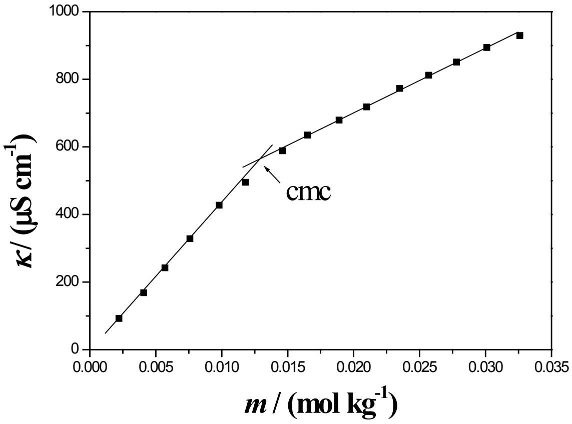


Figure 3. Specific conductivity values (*κ*) for aqueous solutions of [C$_{12}$mim][Ser] at various AAIL molal concentrations; T = 298.15 K

Table 3. Antibacterial properties of ionic liquids tested on three strains of clinical human pathogens

Antibacterial Properties	Compounds	SA	EC	CA
DIZ (mm) (mean±SD)	[C_8mim][Ser]	29.8±0.5	29.0±1.0	27.9±0.5
	[C_{10}mim][Ser]	39.5±0.5	38.4±0.5	37.7±0.5
	[C_{12}mim][Ser]	31.2±0.5	30.4±0.5	29.4±0.5
	[C_8mim][Br]	31.7±0.5	31.8±0.5	28.0±1.0

Note: SA – *Staphylococcus aureus* (ATCC 6538); EC – *Escherichia coli* (ATCC 25922); CA – *Candida albicans* (ATCC 885653). The DIZ value of negative control for each bacterium was 4.9 mm (bored well diameter in the agar plates). The concentration of DIZ test was 10 mg/mL.

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