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Studies on the interactions of some small biomolecules with antibacterial drug benzethonium chloride and its active pharmaceutical ingredient ionic liquid (API-IL) benzethonium L-proline at varying temperatures

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ABSTRACT

From the conductivity, density and spectroscopy measurements, the interactions of benzethonium chloride ([BTC]) and its active pharmaceutical ingredient in ionic liquid format benzethonium L-proline ([BT][PRO]) with some amino acids and glycyl dipeptides have been examined. A number of useful parameters, such as standard partial molar volume ($V^{o}_{2,\phi}$), partial molar transfer volume ($\Delta_t V^{o}$), standard partial molar expansibility (E^{o}_{ϕ}) of these small biomolecules, critical micelle concentration (*cmc*), aggregation number (N_{agg}), thermodynamic micellization parameters of [BTC]/[BT][PRO], as well as binding constant for small biomolecule-drug complex have been evaluated. Group contributions of charged end group (NH_3^+, COO^-), CH_2 and (CH_2CONH) groups of amino acids/dipeptides to $V^{o}_{2,\phi}$ values have also been perceived. These parameters are helpful to study the structure making capacity of small biomolecules, solvation behavior of [BTC]/[BT][PRO] and various interactions present in the studied ternary solution. The interaction difference of [BTC] and [BT][PRO] with small biomolecules was studied.

1. Introduction

In recent years, the design of active pharmaceutical ingredients in ionic liquid form (API-ILs) leads to the appearance of 3rd generation of ionic liquids. These pharmaceutically active ILs provide enhanced properties in comparison with the relative solid pharmaceutical forms, such as controlled solubility, increased stability, permeability and drug delivery [1,2]. Due to their simple preparation and the wide range of possible ILs [3], these new ILs open a large field of applications in drug delivery. Many API-ILs have been synthesized for specific applications [4,5] and were found that they can bring increasing bioactivity of the involved active pharmaceutical ingredients (APIs) [1,6,7]. For example, Pinto et al. [8] reported the protein binding affinity and partition coefficients of three salicylate API-ILs. The obtained results show that three studied API-ILs can bind to human serum albumin strongly and display improved bioavailability. The obtained partition coefficient was up to six times bigger than APIs (sodium salicylate). These results were evidenced that the studied API-ILs can exert greater direct effects on cell membranes than the inorganic salt form of salicylate. As another example, benzalkanium ibuprofenate [BA][ibu] first synthesized by Rogers' group was found as an anti-inflammatory drug against various types of bacteria [9].

Drug-macromolecular interactions are important subject in biophysical chemistry. Understanding of the interactions can be helpful in the discovery of modern drug for identification target proteins via which bioactive molecules manifest their pharmacological action [10,11]. At present, the unique effect of active pharmaceutical ingredient ionic liquids (API-ILs) on the biomolecules in aqueous medium is only at the beginning of studies. Thus the physicochemical properties of aqueous individual small biomolecules with API-ILs will be necessary and useful to understand various interactions between API-ILs and biomacromolecules. Although the thermodynamic properties of the aqueous solutions containing small biomolecules and some drugs have been investigated [12–20], there are only few studies on API-ILs-biomolecule interactions. Shekaari and his co-workers [21,22] determined volumetric, refractometric, ultrasonic and viscometric parameters of aqueous solutions of amino acids with 1-butyl-3-methylimidazolium salicylate [BMIM][SAL] and 1-butyl-3-methylimidazolium ibuprofenate [BMIM][Ibu]. Their results indicate that the dominance of ion-hydrophilic and hydrophilic-hydrophilic interactions between (glycine, L-alanine) and two API-ILs. They also have been reported the electrical conductivities of [BMIM][Ibu] in the aqueous solutions of amino acids [23], and found that the limiting values of molar conductivities of [BMIM][Ibu] decrease with increase in the amino acid concentration, glycine exhibits stronger solvation to [BMIM][Ibu] ions compared with alanine.

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As an effort to get more information about the mixtures containing drug or its API-IL with small biomolecules, in this work, we carried out the density, conductivity, fluorescence and UV–vis absorption spectroscopy measurements for the ternary systems of an antibacterial drug benzethonium chloride [BTC] and its API-ILs benzethonium L-proline ([BT][PRO]) with simple biomolecules like alkyl amino acids and glycyl dipeptides at different temperatures. Benzethonium chloride is a quaternary ammonium drug having anti-infective and antiseptic activities. It is also an anticancer agent against head and neck cancer [24]. L-proline is an osmoprotectant and therefore is used in many pharmaceutical and biotechnological applications. Non-availability of any reported literature on such systems supports the novelty of the present work. This work aims to experimentally determining standard partial molar volumes, standard partial molar transfer volumes, partial molar expansions of amino acids/dipeptides, critical micellar concentration (*cmc*), aggregation number, thermodynamic parameters for the formation of [BT][PRO]/[BTC] micelles, and binding constant of small biomolecules with [BT][PRO]/[BTC]. Through analyzing these parameters, the recognizing the different types of possible interactions in considered systems and then interpreting of drug action, predicting the drugs' absorption and transport across the biological membranes would be very promising. The results obtained in [BT][PRO]-small biomolecules-water system were also compared with the properties in [BTC]-small biomolecules-water system. The comparison of the API-ILs' property with the relative drug will highlight the characteristics of active pharmaceutical ingredients in IL format.

2. Experimental

2.1. Chemicals

The provenances of the chemicals used in this study along with their source, mass fraction purity, etc. are given in Table 1. The studied amino acids and glycyl dipeptides were used after twice recrystallization from ethanol + water mixtures and dried under vacuum for 48 h over P_2O_5 at room temperature. Potassium chloride was dried at $T = 373$ K at least 48 h before use. The other chemicals were used as received. The physicochemical properties, such as density, standard partial molar volume, *cmc* of the new synthesized ionic liquid benzethonium L-proline in water were measured and listed in Table S1 in supplementary material and Table 2. However, there are no literature data with which we could compare our experimental values. Doubly distilled and deionized water (specific conductivity = 0.8–1.0 × 10^{-4} S·m^{-1}) was used to prepare the solutions.

Table 2
Standard partial molar volumes, critical micellar concentration for [BT][PRO] in water at experimental temperatures and pressure $P = 101$ kPa[a].

T/K	$V^o_{2,\phi}$/cm^3·mol^{-1}	*cmc*/mmol·kg^{-1}
293.15	491.71	3.10
298.15	493.66	2.95
303.15	495.34	2.90
308.15	497.31	2.99
313.15	–	3.19

[a] The solvent is water. Standard uncertainties u are: $u(T) = 0.01$ K, $u(P) = 5$ kPa, $u(V_{2,\phi}^o) = 0.02$ cm^3·mol^{-1}, $u(cmc) = 0.05$ mmol·kg^{-1}.

2.2. Synthesis of [BT][PRO]

The benzethonium L-proline ([BT][PRO]) was synthesized by the reaction of benzethonium chloride with L-proline according to the procedure described previously in the literature [25]. Briefly, 20 mL 0.02 mol aqueous L-proline and potassium hydroxide mixed solution was added into 16 mL 0.02 mol aqueous benzethonium chloride solution with stirring at $T = 353$ K for 12 h. Then dichloromethane was added leading to the precipitation of KCl, which was removed by filtration, and the solvent was removed under vacuum. This procedure was repeated several times until no further precipitation of KCl occurred. The obtained API-IL was dried under vacuum for 8 h at $T = 343$ K. Water content in API-IL was found to be approximately 350 ppm by Karl–Fischer measurement. The synthesized API-IL was characterized by ^{1}H NMR (Bruker DPX), IR (Nicolet IR-470) and elemental analysis (Thermo Flash EA 1112).

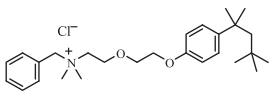
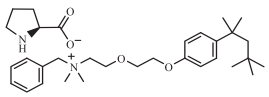
For [BT][PRO], IR (KBr, cm^{-1}): 3451(s), 3033(w), 2953(s), 2889(s), 1579(w), 1512(s), 1483(s), 1458(s), 1289(m), 1243(s), 1190(m), 1125(s), 1064(s), 828(m), 768(m); elemental analysis found (%):C 72.95, H 9.60, N 5.30; calc. for $C_{32}H_{50}N_2O_4$ (526.76):C 72.97, H 9.57, N 5.32. ^{1}H NMR spectra of [BT][PRO] is presented in Fig. S1 of supplementary material.

2.3. Apparatus and procedure

Densities measurements of the solutions were carried out by means of an automated vibrating tube digital densimeter DMA 4500 M purchased from Anton Paar, Austria. The calibration and other procedures have been given in our earlier paper [26]. The uncertainty of the density was estimated to be 1 × 10^{-4} g·cm^{-3}.

Conductivity measurements of each sample were taken using a 145A + model Thermo Orion conductivity meter with 011510 model Thermo Orion conductivity cell. The electrode was calibrated by a standard KCl solution at different temperatures. The temperature was kept constant

Table 1
Specification of studied chemicals.

Chemical	CAS No.	Source	Purity (mass fraction)	Structure	Purification method	Analysis method
Glycine	56–40-6	J&K Chemicals	>0.99		Recrystallization	As stated by the supplier
L-alanine	56–41-7	J&K Chemicals	>0.99		Recrystallization	As stated by the supplier
L-valine	72–18-4	J&K Chemicals	>0.99		Recrystallization	As stated by the supplier
L-leucine	61–90-5	J&K Chemicals	>0.99		Recrystallization	As stated by the supplier
Glycylglycine	556–50-3	Sigma	>0.99		Recrystallization	As stated by the supplier
Glycyl-L-valine	1963–21-9	Sigma	0.99		Recrystallization	As stated by the supplier
Glycyl-L-leucine	869–19-2	Sigma	0.99		Recrystallization	As stated by the supplier
Benzethonium chloride	121–54-0	Sigma	0.98		None	
L-proline	147–85-3	J&K Chemicals	>0.99		None	As stated by the supplier
[BT][PRO]	–	Synthesized	0.98		Rotary/evaporator and vacuum	^{1}H NMR and FT-IR
Dichloromethane	75–09-2	J&K Chemicals	0.999		None	As stated by the supplier
Potassium chloride	7447–40-7	Aldrich	0.99999		None	As stated by the supplier