ORIGINAL ARTICLE



Synthesis, Characterization and Evaluation of Surface and Thermal Properties of 3-Cyclohexyloxy-2-Hydroxypropyl Pyridinium and Imidazolium Surface-Active Ionic Liquids

Rajni Aggarwal¹ · Sukhprit Singh¹

Received: 21 July 2017 / Revised: 2 September 2017 / Accepted: 6 September 2017 © 2018 AOCS

Abstract In this work, 1-halo-3-(cyclohexyloxy)propan-2-ol (3a/3b) were reacted with N-methylimidazole (4) or pyridine (5) to yield the respective 3-(3-(cyclohexyloxy)-2hydroxypropyl)-1-methyl-1*H*-imidazol-3-ium (**6a/6b**) or pyridinium (7a/7b) surface-active ionic liquids (SAIL). The self-aggregation behavior of these ionic liquids (IL) was evaluated by conductometric and tensiometric methods. The thermal stability and size of the micelles were determined by thermogravimetric analysis and dynamic light scattering studies, respectively. The investigated IL were found to exhibit very low cytotoxicity as evaluated by MTT (3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay on the C6 glioma cell line, indicating that the investigated SAIL can be considered for biological applications like drug and gene delivery. The conventional IL 3-methyl-1-octyl imidazolium bromide (C8mimBr) was used for comparison in property evaluations.

Keywords Cationic surfactants · Interfacial science

J Surfact Deterg (2018) **21:** 43–52.

Introduction

Environmental concerns have compelled the search for the synthesis of the molecules that can replace hazardous and

Rajni Aggarwal rajni.aggarwal33@yahoo.com polluting organic solvents. Ionic liquids (IL) contain only ions and exhibit melting points up to 100 °C. Pyridinium and imidazolium are important classes of IL. IL have recently gained much attention as benign solvents because of their unique properties such as lower melting points, nonvolatility, nonflammability, and the wide range of the liquid state with negligible vapor pressure (Cadena et al., 2004; Welton, 1999). In the past few years, different types of IL have been synthesized and applied in different chemical fields such as synthesis (Howarth, 2000; Kitazume, Zulfiqar, & Tanaka, 2000), biochemical reactions (McClements, 2004), catalysis (Peng & Deng, 2001), and electrochemistry (Hanabusa, Fukui, Suzuki, & Shirai, 2005; Sanders, Ward, & Hussey, 1986). Because of their unique properties, IL attract much attention from both academia and chemical industries. IL cover applications in chemical industries such as plasticizers, lubricants, electrolyte in batteries (He, Li, Simone, & Lodge, 2006; Huddleston et al., 2001; Lu et al., 2002), catalysts in synthesis (Suarez, Dullius, Einloft, de Souza, & Dupont, 1996; Zhao & Malhotra, 2002) solvents to manufacture nanomaterials (Antonietti, Kuang, Smarsly, & Yong, 2004; Javadian et al., 2013; Zhou, 2005), stationary phases for chromatography (Poole, 2004), and selective extraction of metals (Wellens, Thijs, & Binnemans, 2012). IL have the capability of being reprocessed; therefore they can easily replace traditional organic solvents (Seth, Chakraborty, Setua, & Sarkar, 2006). Owing to their nonvolatility and nonflammability, IL offer great potential for applications in organic synthesis (Avery, Jenkins, Kimber, Lupton, & Taylor, 2002; Huddleston, Willauer, Swatloski, Visser, & Rogers, 1998), electrochemistry (Fukumoto, Yoshizawa, & Ohno, 2005; Leone, Weatherly, Williams, Thorp, & Murray, 2001), and material preparation (Brezesinski, Erpen, Iimura, & Smarsly, 2005; Taubert, 2004; Zhou & Antonietti, 2003). By

Electronic supplementary material The online version of this article (doi:10.1002/jsde.12002) contains supplementary material, which is available to authorized users.

¹ Department of Chemistry, UGC Sponsored Centre for Advanced Studies-I, Guru Nanak Dev University, Amritsar 143005, India

changing the cation and anion combinations and inserting a suitable functionality at the carbon chain, many new IL with distinct physicochemical properties can be synthesized and investigated for different industrial and biomedical applications (Scheeren, Machado, Dupont, Fichtner, & Texeira, 2003; Wang & Voth, 2005).

On going through the literature, we found very few publications reporting the preparation, self-assembly, and cytotoxicity of functionalized and flexible IL. Recently, our group had synthesized ester-based (Aggarwal, Singh, & Hundal, 2013) and alcohol-based (Chauhan, Singh, & Bhadani, 2012) single-tailed and gemini amphiphiles (Aggarwal & Singh, 2014) and evaluated their surface and other physical properties.

In continuation of this work, we were curious to look at the effect of the presence of a cycloalkyl group with the ether and alcohol functional groups in the hydrophobic chain. Hence, in this work, our group has synthesized cyclohexyloxy-2-hydroxypropyl containing SAIL and evaluated them for their micellar, thermal, and cytotoxic properties.

Experimental Section

Materials

Cyclohexanol ($\geq 98\%$), epichlorohydrin ($\geq 98\%$), and epibromohydrin ($\geq 98\%$) were purchased from Central Drug House, Mumbai, India, and were used without further refinement. Zinc perchlorate hexahydrate ($\geq 98\%$) and pyridine ($\geq 99.5\%$) were purchased from Sigma-Aldrich, St. Louis, Missouri, United States. *N*-Methylimidazole ($\geq 99\%$) was an Acros product, Janssen-Pharmaceuticalaan 3, 2440 Geel, Belgium. The conventional IL **C₈mimBr** was synthesized by the quaternization of *n*-octyl bromide ($\geq 98\%$) with *n*-methyl imidazole ($\geq 99\%$) and reference IL by an earlier reported procedure (Chauhan, Singh, & Kamboj, 2014). Chemical structures of conventional IL **C₈mimBr** and reference IL are shown in Fig. S2 in File S1 (Supporting Information).

Methods

Conductivity Measurements

The instrument used for conductivity measurements was a conductivity meter (model EQ661; Equip-Tronics, Mumbai, Maharastra, India). A conductivity cell of cell constant K = 1.00 was used for all measurements. The aqueous solutions prepared for the experiments were thermostated in the cell at 25.0 \pm 0.1 °C. For the measurement of the critical micelle concentration (CMC), double-distilled water of specific conductivity in the range 1–2 μ S cm⁻¹ was used. The

WILEY ACCS*

average of three measurements was taken for each solution. The degree of counterion binding (β) was determined from the ratio of the linear slopes before and after CMC, given by ($\beta = 1 - \alpha$), where α is the slope of micellar region/premicellar region (Bordes, Tropsch, & Holmberg, 2010).

Surface Tension Measurements

A du Nöuy interfacial tensiometer (Central Scientific Co., Inc., Chicago, USA) equipped with a platinum–iridium ring and of circumference 5.992 cm was used for the measurement of surface tensions to obtain the CMC and other surface properties. Double-distilled water of surface tension 72.6 mN m⁻¹ at 25.0 \pm 0.1 °C was used for the calibration of the instrument.

Dynamic Light Scattering

A light-scattering apparatus (Zeta-sizer Nanoseries, nano-ZS; Malvern Instrument Ltd., Malvern, UK) was used for the dynamic light scattering (DLS) measurements at 298.15 K. For the measurement of dimension of aggregates of pure IL, a 20 mM aqueous solution was prepared. A Millipore membrane filter of pore size 0.45 μ m was used for the filtration of all solutions prior to measurements. The average of 10 measurements was taken for each solution. The temperature was maintained with the help of an in-built Peltier device with an accuracy of ± 0.1 K, and the scattering angle was 173°. The data were analyzed using standard algorithms, and are reported with an uncertainty of less than 8%.

Thermal Stability Measurements

A Perkin Elmer Pyris 1 thermogravimetric analyzer (TGA) was used for the measurement of thermal stability of the synthesized IL. All the experiments were run in aluminum pans under nitrogen atmosphere at the heating rate of 10 °C/min.

Cytotoxicity Analysis

Cytotoxicity tests were carried out on C6 glioma (cancerous brain cell line, passage number 65) by using MTT (3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) to evaluate the IC₅₀ values of all the synthesized IL. Cells were seeded at a density of 10×10^3 cells/mL in 96-well microtiter plates. After 24 h of seeding, cells were treated with the test compounds in the concentration range 20–500 μ M. After 24 h of treatment with the test compounds, the cells were incubated at 37 °C with MTT containing 10 mg/10 mL of serum-free medium. The blue formazan crystals formed by viable cells were solubilized by 100 μ L DMSO, and absorbance was read at 595 nm using a



Scheme 1 Synthesis of ionic liquids

Multiskan PLUS plate reader (Thermo Scientific, Waltham, Massachusetts, USA). The conventional IL C_8 mimBr was also tested for cytotoxicity as a reference.

Synthesis and Analysis of Surface-Active IL

A two-step methodology was used for the synthesis of hydroxyl group-functionalized SAIL. The first step included the synthesis of the intermediates 1-halo-3-(cyclohexyloxy) propan-2-ol (3a/3b). Cyclohexanol (1; 2 g, 20 mmol) was stirred magnetically with epichlorohydrin (2a; 3.68 g, 40 mmol) or epibromohydrin (2b; 5.44 g, 40 mmol) at 80 °C for 1 h under solvent-free conditions. Zinc perchlorate hexahydrate was used as catalyst in this reaction. The progress of the reaction was measured by thin-layer chromatography using silica gel G-coated 0.25-mm-thick glass plates with hexane/ethyl acetate (90:10) as the mobile phase. Then 1-halo-3-(cyclohexyloxy)propan-2-ol (3a/3b) were separated by stepwise fractionation by column chromatography using silica gel (60-120 mesh) and hexane/ethyl acetate (95:5) as the solvent system. In the next step, 1-chloro-3-(cyclohexyloxy)propan-2-ol (3a; 3.09 g, 15 mmol) and 1-bromo-3-(cyclohexyloxy)propan-2-ol (3b; 3.75 g, 15 mmol) were quaternized with N-methylimidazole (4; 1.23 g, 15 mmol) or respective cationic imidazolium and pyridinium amphiphiles (Scheme 1). These amphiphiles were purified by recrystallization from ethyl acetate and acetone and then dried under vacuum to get the final product **6a/6b** or **7a/7b**. The characterization of IL **6a/6b** and **7a/7b** was done by using various spectroscopic techniques. IR spectra were recorded in chloroform on a Perkin Elmer FT-IR spectrometer. A Bruker LC-MS system MicroTOF-Q II 10356 was used for mass spectrometry measurements. An FT-NMR spectrometer (Bruker, Avance II, Billerica, Masachusetts, USA) was used for ¹H and ¹³C/DEPT NMR analysis. Samples for the NMR analysis were prepared in CDCl₃ using the internal standard tetramethylsilane (TMS). CHNS analyses were carried out by a Flash EA 1112 series elemental analyzer (Thermo Electron Corporation, Waltham, Massachusetts, USA).

pyridine (5; 1.18 g, 15 mmol) at 80 °C for 4 h to give the

Results and Discussion

Analysis

The infrared spectra of these IL exhibited the OH stretching band at 3435 cm^{-1} . The C–H stretching frequencies were

IL	CMC (mM)		β	γcmc	$\Gamma_{\rm max} \times 10^6$	A_{\min}	$\pi_{\rm CMC}$	$\Delta G^{\circ}{}_{ m m}$	$\Delta G^_{ m ad}$
	Conductance ^a	Surface tension ^b		$(mN m^{-1})$	$(\text{mol } \text{m}^{-2})$	(nm ²)	(mN m ⁻¹)	(kJ/mol)	
6a	7.98	7.62	0.30	51.87 ± 0.01	1.56	1.04	20.33	-21.55	-34.10
6b	6.86	7.33	0.38	49.01 ± 0.01	2.01	0.82	23.19	-28.77	-42.50
7a	8.43	8.27	0.28	45.12 ± 0.01	2.05	0.79	27.08	-27.94	-38.00
7b	7.00	7.16	0.37	44.91 ± 0.01	2.69	0.61	27.29	-28.18	-41.58
C ₈ mimBr	137.36	119.29	0.49	29.33 ± 0.01	3.065	0.53	42.87	-22.5	-36.5

Table 1 Surface and micellar parameters of IL in aqueous systems

^a Electrical conductivity measurements with error estimate ± 0.1 mM

^b Surface tension measurements with error estimate ± 0.1

observed at 2858 and 2934 cm⁻¹. The C=N stretching frequency was observed in the range 1633-1635 cm⁻¹. The C=C stretching frequency for the aromatic imidazolium and pyridinium ring was observed at 1452 cm^{-1} . The stretching frequency of C-O was observed in the range 1085–1093 cm⁻¹. The structures of these IL were further been confirmed by ¹H, ¹³C, DEPT (distortionless enhanced polarization transfer) and 2D HETCOR (heteronuclear chemical shift correlation) experiments. The methylene $(-CH_2)$ protons of cyclohexyl ring appeared as a multiplet in the range 1.22-1.89 ppm in all IL. The methylene protons H-4', H-3', and H-5' of cyclohexyl ring of the IL 6a (Fig. S1 in File S1) appeared in the range δ 1.22–1.53 ppm, whereas H-2' and H-6' appeared in the range δ 1.71–1.88 ppm. However, these signals were observed in the range δ 1.22–1.89 ppm for all the other IL. The chemical shift for the heterocyclic protons $-N^+CHN-$ was observed at δ 9.60 ppm for **6a** and δ 9.52 ppm for **6b**, and the heterocyclic protons -N⁺CHCHNwere observed as a doublet at δ 7.43 ppm and δ 7.55 ppm for **6a** and δ 7.47 ppm and δ 7.53 ppm for **6b**, respectively. All the pyridinium ring protons were observed in the range δ 8.10-9.20 ppm. The methyl (-CH₃) group directly attached to the heterocyclic moiety was observed as a singlet at δ 4.05

for **6a** and δ 4.07 ppm for **6b**. The methylene (–CH₂) protons directly attached to the positively charged quaternized nitrogen are diastereotopic in nature and were observed as two multiplets at δ 4.33–4.37 ppm and δ 4.51–4.55 ppm for **6a**; δ 4.34–4.38 ppm and δ 4.53–4.58 ppm for **6b**; δ 4.31–4.34 ppm and δ 4.83–4.90 ppm for **7a**; and δ 5.12–5.15 ppm and δ 4.84–4.89 ppm for **7b**. The signal for –CHOH protons was observed at δ 4.14 for **6a**, δ 4.04 for **6b**, δ 4.31 for **7a**, and δ 4.34 for **7b**. Further, the methylene protons –OCH₂CHOH were observed as multiplets at δ 3.33–3.61 ppm for **6a**, at δ 3.39–3.65 ppm for **6b**, and at δ 3.37–3.71 ppm for **7a/7b**. The (–CH) proton of the cyclohexyl ring carbon directly attached to the ether oxygen was observed as a multiplet at δ 3.26–3.33 ppm and δ 3.28 ppm for **6(a/b)** and **7(a/b)**, respectively.

¹³C DEPT spectra also helped in assigning the sp³ ring methylene carbons at δ 23.98–32.16 for all the amphiphiles. The carbon directly attached to the quaternized nitrogen atom was observed at δ 52.98 ppm for both **6a** and **6b**. The heterocyclic carbon –N⁺CHN– was observed at δ 137.77 ppm and δ 137.43 ppm for **6a** and **6b**, respectively. The resonance for the heterocyclic carbon –N⁺CHCHN– was observed in the range δ 123.06–123.21 ppm for **6a** and **6b**. The methyl



Fig. 1 (a) Variation of specific conductivity as a function of the concentration of ionic liquids 6a/6b and 7a/7b. (b) Variation of surface tension as a function of the log of concentration of ionic liquids 6a/6b and 7a/7b

WILEY ACCS*

carbon directly attached to the imidazolium moiety was observed at δ 36.65 ppm for **6a** and δ 36.93 for **6b** ppm. The signal for the carbon of the cyclohexyl moiety directly attached to the ether oxygen was observed at δ 78.21–78.22 ppm and δ 78.30–78.41 ppm for **6a/6b** and **7a**/7**b**, respectively.

ESI-MS (positive ion) mass spectroscopy was found to be a very valuable technique for the elucidation of the structures of the synthesized compounds. The parent ion peaks were observed for the imidazolium (**6a/6b**) and pyridinium (**7a/7b**) IL at m/z 239.15 and 236.17, respectively. These intensities were due to the loss of the counterions, i.e., the chloride ion (Cl⁻) and bromide ion (Br⁻) from the molecule, i.e, the formation of the positively charged parent ion [M⁺- Cl or M⁺- Br].

Micellization and Surface Activity

The CMC values and other surface properties of the IL in an aqueous system were determined from the conductivity and surface-tension measurements and are summarized in Table 1. Figure 1a shows the change of specific conductance of the aqueous solution *versus* the concentration of the IL in the aqueous system. The specific conductivity values fall into two linear regions of different slopes. The position of the abrupt changes of the slopes was taken as the CMC.

Figure 1b shows the surface tension (γ) *versus* the logarithm of concentration plots of the IL in the aqueous system at 298 K. At low concentration, the surface tension gradually decreased on incremental addition of IL to the aqueous system, but after some additions, the surface tension (γ) reached a point near the CMC value where the adsorption of IL tends to a limiting value, so the surface tension curve appears to be linear. This region indicates the formation of micelles. The concentration corresponding to the break point was taken as CMC.

The CMC values obtained from the specific conductivity (κ) *versus* concentration plots are in good agreement with the values obtained from surface tension (γ) *versus* log concentration plots. From Fig. 1a, it can be clearly observed that the slope of the linear region below the CMC is greater than the linear region above the CMC. The values of the counter-ion binding, β , of the IL are listed in Table 1. Further, the CMC values of the IL with chloride (Cl⁻) as the counter-ion are more than with Br⁻ as the counterion; it is well known that the affinity of Br⁻ is stronger for the air/water interface. As a result, the respective hydrophobic tail adsorbed more densely at the air/water interface and the value of γ_{CMC} decreased at the interface. The above results established that the counterion affects the γ_{CMC} values of SAIL with the same hydrophobic tail.

Surface tension measurement provides essential information about the adsorption of IL at the air/water interface; results for these IL are shown in Fig. 1. According to the Gibbs adsorption isotherm, Γ_{max} (maximum surface excess concentration) was estimated by using the following equation (Rosen, 2004):

$$\Gamma_{\max} = -1/2.303 nRT \left(\frac{\partial \gamma}{\partial lnC}\right)_T \tag{1}$$

where *R*, *T*, and *C* are the gas constant, absolute temperature, and the IL concentration, respectively. The value of *n* here is 2, because there is one counterion linked to a single cationic head group. Γ_{max} is the amount of IL adsorbed per unit area at the air/water interface after the development of an absolute monolayer and is determined from the slope of the log of CMC *versus* surface tension plot when the concentration is approaching the CMC. The minimum area engaged by a single IL molecule at the air/water interface, A_{\min} , was also determined via the following equation:

$$A_{\min} = (10^{23}) / N_{\rm A} \times \Gamma_{\max} \tag{2}$$

where N_A is the Avogadro number and A_{\min} is in nm². The micellization parameters, i.e, CMC, $\gamma_{\rm CMC}$, $\Gamma_{\rm max}$, and $A_{\rm min}$, for the IL 6a/6b and 7a/7b are listed in Table 1. However, according to the studies of Menger and Rizvi (2011), Menger, Rizvi, and Shi (2011), the determination of area from the surface-tension curve is limited by the effects of the finite width of micellization. With the start of micelle formation, the surfactant molecules prefer to go in to the micelle rather than for the saturation of the interface. Therefore, the decrease in the surface tension value ceases at the CMC. A detailed survey of the literature suggests that adsorption is a cooperative and spontaneous process, and the applicability of the Gibbs adsorption equations is a topic of debate (Li, Thomas, & Penfold, 2014; Mukherjee, Moulik, & Rakshit, 2013). A comprehensive investigation of the state of adsorption is beyond this work.

The reduction in surface tension at the CMC was determined by the equation

$$\pi_{\rm CMC} = \gamma_{\rm o} - \gamma_{\rm CMC} \tag{3}$$

where π is the reduction in the value of surface tension, γ_{o} is the surface tension of pure solvent, and γ_{CMC} is the measured surface tension at the CMC.

The micellization parameter, i.e., CMC, of the bromidecontaining IL, was less than that of IL with the chloride counterion, and the degree of counterion binding (β) was found to be higher for IL containing the Br⁻ counterion. The repulsion in case of chloride ions from the interface is higher than that with the bromide ions due to their higher polarizability and van der Waals interaction with the interface. The value of Γ_{max} is higher in case of bromidecontaining IL, which is ascribed to the weak hydration of Br⁻. In addition, A_{min} is lower for the bromide-containing



Fig. 2 Thermal decomposition curves of ionic liquids: (a) 6a/6b and (b) 7a/7b, showing the start (T_{start}) and the onset (T_{onset}) temperature of degradation

IL, suggesting that the Br⁻ counterion assembles more densely at the air/water interface compared to Cl⁻- containing IL.

On comparing the CMC values of the cyclohexyloxy IL with the earlier synthesized alkyloxy IL (Bordes et al., 2010), the CMC value of the former is lower. This proves that the modification of the structure of hydrophobic tail from open chain to cyclic structure induces more hydrophobicity to the moiety and affects the interfacial and micellar parameters (Mukherjee, 1967). Therefore, the cyclohexy-loxy IL are more surface active compared to open-chain alkyloxy IL.

Thermodynamic Studies

The standard Gibbs free energy of micellization (ΔG°_{m}) and the standard Gibbs free energy of adsorption (ΔG°_{ad}) (Callaghan, Doyle, Alexander, & Palepu, 1993; Rosen, 2004) were evaluated by using the following equation:

$$\Delta G^{\circ}_{m} = (1+\beta) RT \ln X_{\text{CMC}} = \Delta G^{\circ}_{ad} + (\pi_{\text{CMC}}/\Gamma_{\text{max}})$$
(4)

where X_{CMC} is the mole fraction at CMC and β is the counterion binding. The free energy of adsorption (ΔG°_{ad}) signifies the free energy change when 1 mol of the surfactant in solution is transferred to the surface, and the free energy of micellization (ΔG°_{m}) is defined as the amount of work done when the surfactant molecules from the monomeric form are transferred to the micellar phase from the surface (Wang, Du, Li, & Zhang, 2010). The values of ΔG°_{m} and ΔG°_{ad} determine the affinity to form micelles in solution and to adsorb at the air/water interface (Yoshimura, Bong, Matsuoka, Honda, & Endo, 2009). If ΔG°_{ad} , the adsorption of surfactant molecule at the air/water interface becomes more favorable than in the interior of the micelle. However, a smaller energy difference

WILEY ACCS*

between ΔG°_{ad} and ΔG°_{m} means a more favorable transfer of the surfactant molecules from the surface to the micellar phase and that the surfactant undergoes aggregation more readily in water (Rebelo, Lopes, Esperança, & Filipe, 2005). Our experimental results show a smaller free energy gap between ΔG°_{ad} and ΔG°_{m} , and therefore these IL have a greater tendency to aggregate in solution. The low values of A_{min} also indicate the formation of premicellar aggregates more readily in water. As the value of free energy of adsorption (ΔG°_{ad}) is more negative for bromide ions compared to chloride ions, adsorption is more favorable in case of 6b and 7b. But the difference in the values of free energies of adsorption (ΔG°_{ad}) and free energy of micellization (ΔG°_{m}) is also small in case of **6b** and **7b**. Therefore, **6b** and 7b require less amount of work to transfer to bulk solution from the air/water interface to form premicellar aggregates. The lower A_{\min} values of **6b** and **7b** compared to **6a** and 7a are also in concurrence with the above outcomes.

Thermal Stability Assessment by TGA

IL possess negligible vapor pressure and have high thermal stability (Monteiro, Camilo, Ribeiro, & Torresi, 2010). Therefore, the thermal stability of the synthesized IL was analyzed by using a thermogravimetric analyzer. All these new IL possess reasonably good thermal stability. The decomposition range of these IL was found to be between 215 and 242 °C. Figure 2 shows the T_{onset} and T_{start} of the IL **6a/6b** and **7a/7b**. The start temperature (T_{start}) is the

 Table 2 Onset and start temperatures for thermal decomposition of ionic liquids

IL	6a	6b	7a	7b
T_{start} (°C)	213.81	226.60	201.81	221.52
$T_{\text{onset}} (^{\circ} \text{C})$	220.82	242.53	215.72	236.01

Table 3 Melting points of ionic liquids

IL	6a	6b	7a	7b
Mp (°C)	84.79	69.38	67.93	64.31

temperature at which the degradation of the compound starts, while the onset temperature (T_{onset}) is the point of intersection of baseline weight from the start of the experiment and the tangent of the weight *versus* temperature curve where the disintegration of the compound takes place.

Among these IL (Table 2), 6b possesses the highest thermal stability whereas 7a has the least. Furthermore, these IL show better thermal stability than the earlier synthesized ester-based IL (Mukherjee, 1967), likely because of the higher thermal stability of the ether linkage (Gusain, Gupta, Saran, & Khatri, 2014). The corresponding differential thermogravimetric analysis (DTA) profiles are also shown in Fig. S3 in File S1. All these IL show a two-step weight loss in the range 240–265 °C ($\approx 25\%$ weight loss) and 320–366 °C (\approx 56% weight loss). The thermal degradation of the investigated compounds was observed as a large weight loss (50%) at 366.50 °C for 6a, (55%) at 346.20 °C for **6b**, (56%) at 320.81 °C for **7a**, and (59%) at 345.43 °C for 7b, due to loss of the ether-linked group $(C_6H_{11}O)^{-1}$ along with counterion. The weight loss (13.76%) at 139 °C for 6a, (25.06%) at 243.2 °C for 6b, (13.04%) at 197.58 °C for 7a, and (25.26%) at 242 °C for 7b is due to the loss of counterions (Cl⁻ in 6a and 7a and Br^{-} in **6b** and **7b**).

Further, the IL with bromide as the counterion has higher thermal stability than those with chloride as the counterion. Our experimental results also show that the IL with imidazolium head group are more thermally stable than pyridinium core-containing IL. This is due to the more packed structure of the imidazolium ring, higher intermolecular interactions, and low steric hindrance (Ise, 2010).

Table 4 Hydrodynamic diameter (in nm) of ionic liquids (6a/6b and 7a/7b)

Sample	6a	6b	7a	7b
Pure IL	1.72	1.45	0.65	0.62

These results are in agreement with the earlier reported results (Gusain et al., 2014; Ise, 2010; Monteiro et al., 2010).

Melting Point

Melting point is the temperature at which there is equilibrium between the solid and liquid state at a fixed pressure. There are many factors on which the melting point of any compound depends, like symmetry of the hydrophobic chain or cation (Wasserscheid & Welton, 2003), or intermolecular forces like hydrogen bonding, electrostatic or van der Waals forces, distribution of charge, and size of the anion (Elaiwi et al., 1995). With increase in the size of the anion, the melting point decreases. The weaker electrostatic interaction is stimulated by larger anions with heterocyclic moieties like pyridine and imidazolium. The melting points of the present IL were found to be in the range 70–80 °C (Table 3).

DLS Experiments

DLS provides useful information on the size distribution of the micelles of amphiphiles in an aqueous solution. The above physicochemical studies, i.e., surface tension and conductometry, indicate that these hydroxyl groupfunctionalized pyridinium/imidazolium cationic IL have the ability to form aggregates above the CMC. The aggregate size of the investigated IL in the aqueous system was measured by DLS experiments. Intensity-weighted light scattering graphs (Fig. 3) show two size distributions, with



Fig. 3 (a) Intensity-weighted and (b) number-weighted size distribution in aqueous solution of different ionic liquids (6a/6b and 7a/7b) from DLS measurement



Fig. 4 (a) Absorbance (nm) *versus* concentration (μ M) plot of IL **6a/6b**, **7a/7b**, C₈mimBr, and reference IL for determination of IC₅₀. The results shown in the figure represent the average IC₅₀ value of three different trials done in triplicate. (b) Percent cell viability at different concentrations of IL **6a/6b** and **7a/7b**

smaller aggregates having an average hydrodynamic diameter in the range 0.62-1.45 nm, and the larger aggregates with hydrodynamic radius ranging between 123.61 and 215.19 nm, whereas the number-weighted size distribution graphs show the presence of aggregate with a size 0.62-1.45 nm only. It has been assumed that the collision of smaller colloidal particles with each other will result in the larger aggregates (Bohren & Huffman, 1983). According to Rayleigh's approximation, the intensity of scattered light varies directly as the sixth power of particle size; therefore it is considered that larger particles scatter much more light than the smaller ones (Kamboj, Singh, Bhadani, Kataria, & Kaur, 2012). The outcomes from Fig. 3 clearly indicate a higher hydrodynamic diameter (D_h) of the IL containing the chloro (Cl⁻) counterion compared to their bromo (Br⁻)-containing counterparts. From the data (Table 4), it can also be seen that the hydrodynamic diameter of pure imidazolium IL (6a/6b) is slightly larger than that of the pure pyridinium IL (7a/7b).

Cytotoxicity Assessment

In the present research work, C6 glioblastoma cells were treated with 20–500 μ M concentrations of the IL by performing MTT assay to evaluate cytotoxicity or cell viability after 24 h of treatment. The histograms in Fig. S4 in File S1 represent the percentage of viable cells compared to control after 24 h of treatment. To determine significance of results, one-way analysis of variance with Holm–Sidak *post hoc* test was used (difference between control and cells treated with IL). Cells were healthy up to 250 μ M concentration of the test compounds. Retraction of processes and rounding of cells were observed in a culture treated with higher than 250 μ M concentration of IL. The experimental results clearly show that the ether- and alcohol-

functionalized IL possess low toxicity and can be used as carriers in many biomedical applications like gene and drug delivery. The cytotoxicity of the SAIL was expressed as percentage of cell viability in terms of the IC₅₀ value (Fig. 4b), defined as the concentration of the test compound (in μ M) that causes the death of 50% of the living cells. The conventional IL C8mimBr and the reference IL (3-(2-hydroxy-3-(pentyloxy)propyl)-1-methyl-1*H*-imidazol-3-ium chloride) (ref) were also tested along with the other amphiphiles. The experimental results are shown in Figs. 4a, b, which shows that the investigated cyclohexyloxy SAIL are not cytotoxic up to a concentration of 250 µM, while at all concentrations the conventional IL C₈mimBr and the reference compound (ref) were toxic to the cells. From the above results, it can be concluded that the modification in the hydrophobic tail from the open chain to cyclic affects the cytotoxicity value. The compound becomes less toxic by inducing the cyclohexyloxy group into the hydrophobic tail. Furthermore, the present compounds have also found to be less toxic compared to the earlier synthesized imidazolium and pyridinium amphiphiles (Chauhan, Singh, Kamboj, Mishra, & Kaur, 2014).

Hence, the introduction of ether and alcohol functional groups and the cyclohexyl moiety in SAIL affects the physicochemical properties in a positive way for potential delivery applications in biological systems.

Conclusion

Four new cyclohexyloxy-based SAIL were synthesized and evaluated for their self-aggregation behavior, thermal stability, and cytotoxicity. The introduction of the cyclohexyloxy moiety and the coexistence of an ether or a hydroxyl group in these IL affect the physicochemical properties positively by decreasing the CMC values of the investigated IL compared to earlier reported conventional (Cornellas et al., 2011) and alkyloxy IL (Bordes et al., 2010). Thermal degradation temperatures of investigated IL were determined by TGA, which showed a dependence on the size of the counterion and cationic moiety (Mukherjee, 1967). The investigated IL containing the bromo counterion possessed higher thermal stability than the corresponding chloride counterioncontaining ones. Similarly, the imidazolium cations were found to be more thermally stable, due to their compact structure and lesser free volume, than the pyridinium analogs. The MTT assay was performed to deduce the cytotoxicity of the investigated IL. The results showed that the studied IL were noncytotoxic in the prescribed range below 250 µM. The cytotoxicity of these SAIL was found to be much lower than that of the conventional IL (C₈mimBr) and some earlier reported cationics (Chauhan, Singh, Kamboj, Mishra, & Kaur, 2014; Cornellas et al., 2011). DLS experiments indicated that these new IL form aggregates above the CMC with $D_{\rm h}$ in the range 0.62–1.45 nm.

Hence, tailoring of the SAIL structure with suitable functional groups and cyclohexyl ring affects the surface, thermal, and cytotoxicity positively, and these IL can find a better future in scientific applications such as gene/drug delivery, medicine, and other areas.

Acknowledgements The author thanks the UGC BSR for providing a Research Fellowship. He also thanks the Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh, India, for providing the NMR facilities.

References

- Aggarwal, R., & Singh, S. (2014) Synthesis, characterization, and evaluation of surface properties of cyclohexyloxyoxoethylbipyridinium gemini amphiphiles and a comparison with single-tailed amphiphiles. *Industrial and Engineering Chemistry Research*, **53**:2549–2557.
- Aggarwal, R., Singh, S., & Hundal, G. (2013) Synthesis, characterization and evaluation of surface properties of cyclohexanoxycarbonylmethyl pyridinium and cyclohexanoxycarbonyl methyl imidazolium ionic liquids. *Industrial and Engineering Chemistry Research*, **52**:1179–1189.
- Antonietti, M., Kuang, D., Smarsly, B., & Yong, Z. (2004) Ionic liquids for the convenient synthesis of functional nanoparticles and other inorganic nanostructures. *Angewandte Chemie, International Edition*, 43:4988–4992.
- Avery, T. D., Jenkins, N. F., Kimber, M. C., Lupton, D. W., & Taylor, D. K. (2002) First examples of the catalytic asymmetric ring-opening of *meso* 1,2-dioxines utilising cobalt(II) complexes with optically active tetradentate Schiff base ligands: Formation of enantio-enriched cyclopropanes. *Chemical Communications*, 1: 28–29.
- Bohren, C. F., & Huffman, D. R. (1983) Absorption and scattering of light by small particles. New York, NY: Wiley.
- Bordes, R., Tropsch, J., & Holmberg, K. (2010) Role of an amide bond for self-assembly of surfactants. *Langmuir*, 26:3077–3083.
- Brezesinski, T., Erpen, C., Iimura, K., & Smarsly, B. (2005) Mesostructured crystalline ceria with a bimodal pore system using block

copolymers and ionic liquids as rational templates. *Chemistry of Materials*, **17**:1683–1690.

- Cadena, C., Anthony, J. L., Shah, J. K., Morrow, T. I., Brennecke, J. F., & Maginn, E. J. (2004) Why is CO₂ so soluble in imidazolium-based ionic liquids? *Journal of the American Chemi*cal Society, **126**:5300–5308.
- Callaghan, A., Doyle, R., Alexander, E., & Palepu, R. (1993) Thermodynamic properties of micellization and adsorption and electrochemical studies of hexadecylpyridinium bromide in binary mixtures of 1,2-ethanediol with water. *Langmuir*, 9:3422–3426.
- Chauhan, V., Singh, S., & Bhadani, A. (2012) Synthesis, characterization and surface properties of long chain β-hydroxy-γ-alkyloxy-N-methylimidazolium surfactants. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **395**:1–9.
- Chauhan, V., Singh, S., & Kamboj, R. (2014) Self-assembly and thermal stability of ether functionalized imidazolium ionic liquids. *Industrial and Engineering Chemistry Research*, 53:13247–13255.
- Chauhan, V., Singh, S., Kamboj, R., Mishra, R., & Kaur, G. (2014) Self-assembly, DNA binding and cytotoxicity trends of ether functionalized gemini pyridinium amphiphiles. *Journal of Colloid and Interface Science*, 417:385–395.
- Cornellas, A., Perez, L., Cornelles, F., Ribosa, I., Manresa, A., & Garcia, M. T. (2011) Self-aggregation and antimicrobial activity of imidazolium and pyridinium based ionic liquids in aqueous solution. *Journal of Colloid and Interface Science*, 355:164–171.
- Elaiwi, A., Hitchcock, P. B., Seddon, K. R., Srinivasan, N., Tan, Y. M., Welton, T., & Zora, J. A. (1995) Hydrogen bonding in imidazolium salts and its implications for ambient-temperature halogenoaluminate (III) ionic liquids. *Journal of the Chemical Soci*ety Dalton Transactions, 21:3467–3472.
- Fukumoto, K., Yoshizawa, M., & Ohno, H. (2005) Room temperature ionic liquids from 20 natural amino acids. *Journal of the American Chemical Society*, **127**:2398–2399.
- Gusain, R., Gupta, P., Saran, S., & Khatri, O. P. (2014) Halogen free bis(imidazolium)/bis (ammonium)-di[bis(salicylato)borate] ionic liquids as energy efficient and environmentally friendly lubricant additives. ACS Applied Materials & Interfaces, 6:15318–15328.
- Hanabusa, K., Fukui, H., Suzuki, M., & Shirai, H. (2005) Specialist gelator for ionic liquids. *Langmuir*, 21:10383–10390.
- He, Y., Li, Z., Simone, P., & Lodge, T. P. (2006) Self-assembly of block copolymer micelles in an ionic liquid. *Journal of the American Chemical Society*, **128**:2745–2750.
- Howarth, J. (2000) Oxidation of aromatic aldehydes in the ionic liquid [bmim]PF₆. *Tetrahedron Letters*, **41**:6627–6629.
- Huddleston, J. G., Visser, A. E., Reichert, W. M., Willauer, H. D., Broker, G. A., & Rogers, R. D. (2001) Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. *Green Chemistry*, 4: 156–164.
- Huddleston, J. G., Willauer, H. D., Swatloski, R. P., Visser, A. E., & Rogers, R. D. (1998) Room temperature ionic liquids as novel media for 'clean' liquid–liquid extraction. *Chemical Communications*, 16:1765–1766.
- Ise, N. (2010) Like likes like: Counterion mediated attraction in macroionic and colloidal interaction. *Physical Chemistry Chemical Physics*, 12:10279–10287.
- Javadian, S., Ruhi, V., Heydari, A., Shahir, A. S., Yousefi, A., & Akbari, J. (2013) Self-assembled CTAB nanostructures in aqueous/ionic liquid systems: Effects of hydrogen bonding. *Industrial and Engineering Chemistry Research*, **52**:4517–4526.
- Kamboj, R., Singh, S., Bhadani, A., Kataria, H., & Kaur, G. (2012) Gemini imidazolium surfactants: Synthesis and their bio-physiochemical study. *Langmuir*, 28:11969–11978.
- Kitazume, T., Zulfiqar, F., & Tanaka, G. (2000) Molten salts as a reusable medium for the preparation of heterocyclic compounds. *Green Chemistry*, 2:133–136.

- Leone, A. M., Weatherly, S. C., Williams, M. E., Thorp, H. H., & Murray, R. W. (2001) An ionic liquid form of DNA: Redox active molten salts of nucleic acids. *Journal of the American Chemical Society*, **123**:218–222.
- Li, P. X., Thomas, R. K., & Penfold, J. (2014) Limitations in the use of surface tension and the Gibbs equation to determine surface excesses of cationic surfactants. *Langmuir*, **30**:6739–6747.
- Lu, W., Fadeev, A. G., Qi, B. H., Smela, E., Mattes, B. R., Ding, J., ... Forsyth, M. (2002) Use of ionic liquids for π-conjugated polymer electrochemical devices. *Science*, **297**:983–987.
- McClements, D. J. (2004) *Food emulsions: Principles, practice and techniques* (2nd ed.). Boca Raton, FL: CRC Press.
- Menger, F. M., & Rizvi, S. A. A. (2011) Relationship between surface tension and surface coverage. *Langmuir*, 27:13975–13977.
- Menger, F. M., Rizvi, S. A. A., & Shi, L. (2011) Reply to "Should the Gibbs analysis be revised?". *Langmuir*, 27:7963–7965.
- Monteiro, M. J., Camilo, F. F., Ribeiro, M. C. C., & Torresi, R. M. (2010) Ether bond containing ionic liquids and the relevance of the ether bond position to transport properties. *The Journal of Physical Chemistry. B*, **114**:12488–12494.
- Mukherjee, I., Moulik, S. P., & Rakshit, A. K. (2013) Tensiometric determination of Gibbs surface excess and micelle point: A critical revisit. *Journal of Colloid and Interface Science*, **394**:329–336.
- Mukherjee, P. (1967) The nature of association equilibria and hydrophobic bonding in aqueous solutions of association colloids. *Advances in Colloid and Interface Science*, 1:242–275.
- Peng, J. J., & Deng, Y. Q. (2001) Ionic liquids catalyzed Biginelli reaction under solvent-free conditions. *Tetrahedron Letters*, 42: 5917–5919.
- Poole, C. F. (2004) Chromatographic and spectroscopic methods for the determination of solvent properties of room temperature ionic liquids. *Journal of Chromatography. A*, **1037**:49–82.
- Rebelo, L. P. N., Lopes, J. N. C., Esperança, J. M. S. S., & Filipe, E. (2005) On the critical temperature, normal boiling point, and vapor pressure of ionic liquids. *The Journal of Physical Chemistry. B*, 109:6040–6043.
- Rosen, M. J. (2004) *Surfactants and interfacial phenomenon* (3rd ed.). New York, NY: John Wiley & Sons.
- Sanders, J. R., Ward, E. H., & Hussey, C. L. (1986) Aluminum bromide-1-methyl-3-ethyl imidazolium bromide ionic liquids: Densities, viscosities, electrical conductivities, and phase transitions. *Journal of the Electrochemical Society*, **133**:325–330.
- Scheeren, C. W., Machado, G., Dupont, J., Fichtner, P. F. P., & Texeira, S. G. (2003) Nanoscale Pt(0) particles prepared in

imidazolium room temperature ionic liquids: Synthesis from an organometallic precursor, characterization, and catalytic properties in hydrogenation reactions. *Inorganic Chemistry*, **42**: 4738–4742.

- Seth, D., Chakraborty, A., Setua, P., & Sarkar, N. (2006) Interaction of ionic liquid with water in ternary microemulsions (Triton X-100/water/1-butyl-3-methylimidazolium hexafluorophosphate) probed by solvent and rotational relaxation of coumarin 153 and coumarin 151. *Langmuir*, 22:7768–7775.
- Suarez, P. A. Z., Dullius, J. E. L., Einloft, S., de Souza, R. F., & Dupont, J. (1996) The use of new ionic liquids in two-phase catalytic hydrogenation reaction by rhodium complexes. *Polyhedron*, 15:1217–1219.
- Taubert, A. (2004) CuCl nanoplatelets from an ionic liquid-crystal precursor. Angewandte Chemie, International Edition, 43: 5380–5382.
- Wang, G. Y., Du, Z. P., Li, Q. X., & Zhang, W. (2010) Carbohydrate modified-siloxane surfactants and their adsorption and aggregation behavior in aqueous solution. *The Journal of Physical Chemistry*. *B*, **114**:6872–6877.
- Wang, Y. T., & Voth, G. A. (2005) Unique spatial heterogeneity in ionic liquids. *Journal of the American Chemical Society*, **127**: 12192–12193.
- Wasserscheid, P., & Welton, T. (Eds.) (2003) Ionic liquids in synthesis (pp. 41–55). Weinheim, Germany: Wiley-VCH.
- Wellens, S., Thijs, B., & Binnemans, K. (2012) An environmentally friendlier approach to hydrometallurgy: Highly selective separation of cobalt from nickel by solvent extraction with undiluted phosphonium ionic liquids. *Green Chemistry*, 14:1657–1665.
- Welton, T. (1999) Room temperature ionic liquids: Solvents for synthesis and catalysis. *Chemical Reviews*, 99:2071–2083.
- Yoshimura, T., Bong, M., Matsuoka, K., Honda, C., & Endo, K. (2009) Surface properties and aggregate morphology of partially fluorinated carboxylate type anionic gemini surfactants. *Journal of Colloid and Interface Science*, **339**:230–235.
- Zhao, H., & Malhotra, S. V. (2002) Application of ionic liquids in organic synthesis. *Aldrichimica Acta*, 35:75–83.
- Zhou, Y. (2005) Recent advances in ionic liquids for synthesis of inorganic nanomaterials. *Current Nanoscience*, 1:35–42.
- Zhou, Y., & Antonietti, M. (2003) Synthesis of very small TiO₂ nanocrystals in a room-temperature ionic liquid and their self-assembly toward mesoporous spherical aggregates. *Journal of the American Chemical Society*, **125**:14960–14961.