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Synthesis, Characterization, Physical Properties, and Cytotoxicities of 1-(6-Hydroxyhexyl)-3-alkylimidazolium Chloride Ionic Liquids

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ABSTRACT: Four hydroxyl-functionalized ionic liquids, 1-(6-hydroxyhexyl)-3-alkylimidazolium chlorides [6OHimC_n][Cl]; (n = 0, 1, 2, 4), have been synthesized from the appropriate imidazole precursors and characterized by IR and NMR spectroscopies and elemental analysis. Densities, dynamic viscosities, refractive indices, and surface tensions have been measured for each liquid as a function of temperature at atmospheric pressure and fitted using simple empirical correlation functions. Consistent with related imidazolium-based ionic liquids, an increase in the carbon side-chain length of the imidazolium cation was found to decrease the density and surface tension but increase the viscosity and refractive index. Cytotoxicities of the present liquids were found to decrease with increasing carbon side chain length and were broadly similar to those of other ionic liquids.

INTRODUCTION

Room temperature ionic liquids (ILs) are nonvolatile, low-melting-point organic salts that due to their characteristic properties, such as their high chemical, electrochemical, and thermal stabilities and solvent abilities, show potential for many industrial applications.[1,2] ILs present a low hazard with regard to atmospheric pollution due to their nonvolatile characteristic and thus offer potential as green substitutes for volatile organic compounds in numerous chemical industries. The wide range of possible cation−anion combinations allows ILs to be designed for particular purposes or to show a specific set of physicochemical properties.[3] For many ILs, the choice of anion exerts a strong influence on their hydrophilicity (aqueous solubility) and electrical conductivity, whereas the cation often has a greater influence on their lipophilicity, viscosity, density, refractive index, and melting point.[4]

Of the wide range of possible ILs, those based on imidazolium cations have been the most widely studied to date.[5] Typical examples include 1-alkyl-3-methylimidazolium, with anions Cl^-, I^-, BF_4^-, PF_6^-, and Tf_2N^-, and 1-(2-hydroxyethyl)-3-methyl imidazolium, with anions Cl^-, Br^-, and BF_4^-.[4,6] Such cations have a rich chemistry, which can be utilized to further "fine-tune" the IL properties.

In this study, a series of 1-(6-hydroxyhexyl)-3-alkylimidazolium chloride salts, [6OHimC_n][Cl] with n = 0, 1, 2, or 4, have been synthesized and their densities, dynamic viscosities, refractive indices, and surface tensions measured as a function of temperature at atmospheric pressure. The cytotoxicities of these ILs toward a representative human cell line were also investigated.

EXPERIMENTAL SECTION

Synthesis of 1-(6-Hydroxyhexyl)-3-alkylimidazolium Chlorides. All ILs were synthesized according to established methods.[6,7] By way of example, ∼ 0.14 mol of 1-methylimidazole (Sigma-Aldrich, U.S.A., AR grade, 99 %) and an excess (0.2 mol) of 6-chlorohexanol (Sigma-Aldrich, 96 %) were added to a round-bottomed flask containing 200 mL of acetonitrile (Sigma-Aldrich, 98 %). After fitting a reflux condenser, the flask was maintained at 343 K under nitrogen for 24 h with magnetic stirring. Reaction progress was monitored by thin layer chromatography using aluminum sheets coated with silica gel, with methanol as the mobile phase. The product (1-(6-hydroxyhexyl)-3-methylimidazolium chloride, [6OHimC][Cl]) was obtained (78 %) as a colorless liquid. This material was kept at ∼ 353 K under vacuum (∼ 1 Pa) overnight to remove volatiles and moisture.

An analogous procedure was used to synthesize the other three ILs, using 0.11 mol imidazole (Merck, synthesis grade, ≥ 99 %), 0.1 mol 1-ethylimidazole (Arcos Organics, 98 %), and 0.6 mol 1-butylimidazole (Merck, synthesis grade, ≥ 98 %). The reaction employed and the structure of the ILs so obtained are represented in Figure 1. Altogether, four ILs were synthesized and characterized: 1-(6-hydroxylhexyl)imidazolium chloride, [6OHim][Cl], 1-(6-hydroxylhexyl)-3-methylimidazolium chloride, [6OHimC][Cl], 1-(6-hydroxylhexyl)-3-ethylimidazolium chloride, [6OHimC_2][Cl], and 1-(6-hydroxylhexyl)-3-butylimidazolium chloride, [6OHimC_4][Cl].

IR, NMR, and Elemental Analysis. The synthesized ILs were characterized using Fourier transform infrared (FTIR) spectroscopy (Shimadzu 8400S), ^{1}H-nuclear magnetic resonance (NMR) spectroscopy (Bruker Avance, 400 MHz), and elemental analysis (Leco 932). The FTIR spectra (Figure 2) were obtained by dripping the IL onto the surface of the "MIRacle" single-reflection attenuated total reflectance (ATR) accessory. For NMR, 5 mg of sample was dissolved in 0.7 cm^3 of deuterated methanol (CD_3OD). The observed peaks, which are summarized in Table 1, are abbreviated as s (singlet), d (doublet), t (triplet), and m (multiplet). Elemental analyses were performed according to standard procedures (ASTM D-5291). Solid samples (< 2 mg) were enclosed in a silver capsule while liquid samples

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were analyzed in a silver capsule containing a sorbit pad. Analyses (Table 1) were performed in triplicate and averaged.

Water Content. The presence of water can cause significant discrepancies in the physical properties of ILs, especially for viscosities, densities, and conductivities,[8] which has created serious concerns about many of the data in the published literature.[9,10] Accordingly, the water content of the synthesized ILs was determined using a coulometric Karl Fischer titrator (Mettler Toledo DL 39) with CombiCoulomat Karl Fischer reagent (Merck). Measurements were performed in triplicate immediately after drying under vacuum. The average values so obtained are given in Table 1; all four ILs had water contents less than 900 ppm with most < 600 ppm.

Physical Properties. All instruments used for physical property measurements were calibrated using Millipore-quality water. Densities were determined using an Anton Paar vibrating-tube densimeter (DMA 5000) over the temperature range (303.15 to 353.15) K at atmospheric pressure with a precision of ± 10 $\mu g \cdot cm^{-3}$ and a temperature control precision of ± 0.01 K. Dried and degassed ILs were injected into the densimeter using nonlubricated disposable polycarbonate syringes.

Viscosities were measured in triplicate (with a reproducibility of ± 0.1 mPa·s and an overall estimated uncertainty of ± 0.5 % relative) at temperatures (293.15 to 353.15) K using a rotating automated Anton Paar viscometer (SVM 3000) with a temperature control precision of ± 0.01 K. Refractive indices were determined at temperatures from (293.15 to 333.15) K using an ATAGO programmable digital refractometer (RX-5000 Alpha) with a measuring accuracy of ± 4 × 10^{-5}, with a temperature control precision of ± 0.05 K. Surface tensions were measured with an estimated overall accuracy of ± 0.2 $mN \cdot m^{-1}$ using a pendant drop shape analysis which was compared with the sessile drop method (contact angle system OTA, Dataphysics GmbH) over the temperature range (298.15 to 323.15) K, with a temperature control precision of ± 0.05 K. All measurements were performed in triplicate immediately after drying under vacuum.

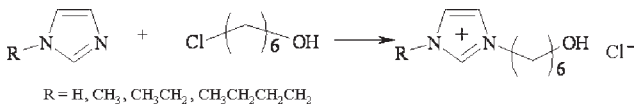

Figure 1. Synthesis reaction and structure of the present 1-(6-hydroxyhexyl)-3-alkylimidazolium chloride ILs.

Cytotoxicity Determination. The cytotoxicities of the synthesized ILs were determined for the human breast cancer cell line, MCF-7. Closely following the procedure of Kumar et al.,[11] the MCF-7 cells, originally purchased from the American type culture collection (ATCC), were cultured in an RPMI 1640 medium (without $NaHCO_3$ but supplemented with 1 % penicillin−streptomycin and 1% L-glutamine, pH 7) with 10 % horse serum at 37 °C (5 % CO_2). After tripsinization, most of the cells are removed from contact with the plate and then centrifuged (1000 rpm for 10 s). The plates were resuspended with phosphate buffer saline (PBS) solution (with 5−10 % dimethylsulfoxide, DMSO) and cell counting was done in hemocytometer via a microscope. Cell concentration was maintained at a density of ~ 10^6 cells·mL^{-1}. To each well was added freshly prepared media (100 μL) followed by the test-IL solution (100 μL) using serial dilution. The plates were then transferred to the incubator after adding 100 μL cell suspension to each well. After 48 h of growth, cell viability was measured with the MTT (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan, Sigma-Aldrich) assay[12] as follows: to each well was added 20 μL of 2.5 mg·mL^{-1} MTT in PBS followed by incubation for 3 h at 37 °C. The resulting liquid (170 μL) was then aspirated and the purple formazan crystals were dissolved in DMSO (100 μL). Absorbance was measured at 570 nm using an ELISA (enzyme-linked immunosorbent assay) microplate

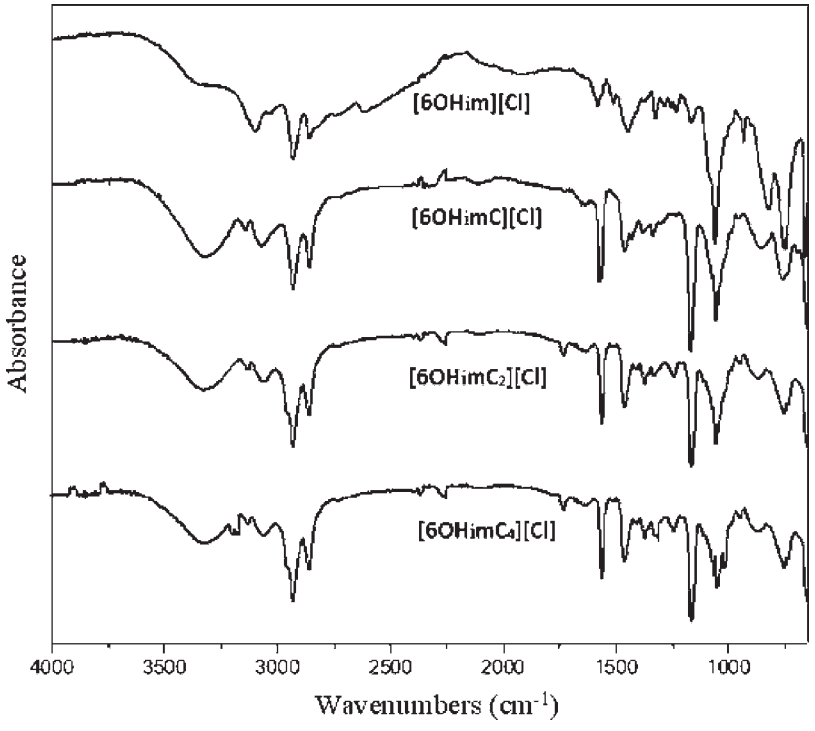

Figure 2. FTIR spectra of the present 1-(6-hydroxyhexyl)-3-alkylimidazolium chloride ILs at room temperature.

Table 1. FTIR and NMR Spectroscopic and Analytical Data for the Present ILs

ionic liquid	MW (g/mol)	yield (%)	water content (ppm)	FTIR (cm^{-1})	^{1}H NMR (δ ppm)	elemental analysis (%) experimental (calculated)
[6OHim][Cl]	204.70	86	590	662, 750, 824, 1061, 1445, 2932, 3100	1.41 (m, 4H, 2 × CH_2), 1.55 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 3.56 (t, 2H, OCH_2), 4.19 (t, 2H, NCH_2), 7.40 (s, 1H, CH), 7.71 (s, 1H, CH), 8.40, 8.45 (s, 1H, 2-CH), 9.15 (s, 1H, NH)	C 53.20 (52.81) H 8.52 (8.31) N 13.41 (13.69)
[6OHimC_1][Cl]	218.72	78	447	760, 1055, 1169, 1462, 1570, 2858, 2932, 3070, 3329	1.42 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 1.94 (m, 2H, CH_2), 3.55 (t, 2H, NCH_2), 3.98 (s, 3H, NCH_3), 4.27 (t, 2H, OCH_2), 7.63 (s, 1H, CH), 7.70 (s, 1H, CH), 9.06 (s, 1H, 2-CH)	C 55.47 (54.92) H 8.49 (8.70) N 12.55 (12.81)
[6OHimC_2][Cl]	232.75	90	472	757, 1055, 1170, 1460, 1590, 2837, 2956, 3066, 3341	1.23 (t, 3H, CH_3), 1.43 (m, 4H, 2 × CH_2), 1.64 (m, 2H, CH_2), 2.12 (m, 2H, CH_2), 3.76 (t, 2H, OCH_2), 4.27 (t, 4H, 2 × NCH_2), 7.63 (s, 1H, 2 × CH), 9.08 (s, 1H, 2-CH)	C 57.25 (56.77) H 8.77 (9.03) N 11.30 (12.04)
[6OHimC_4][Cl]	260.80	88	890	752, 1057, 1165, 1462, 1562, 2860, 2932, 3063, 3331	1.01 (t, 3H, CH_3), 1.39 (m, 6H, 3 × CH_2), 1.58 (m, 2H, CH_2), 1.89 (m, 2H, CH_2), 3.55 (m, 2H, OCH_2), 4.27 (t, 4H, 2 × NCH_2), 7.71 (s, 2H, 2 × CH), 9.14 (s, 1H, 2-CH)	C 60.31 (59.88) H 9.84 (9.60) N 10.37 (10.75)

Table 2. Experimental Densities (ρ), Dynamic Viscosities (η), Refractive Indices (n_D), and Surface Tensions (σ) of the Present ILs as a Function of Temperature

T/K	[6OHim][Cl]	[6OHimC_1][Cl]	[6OHimC_2][Cl]	[6OHimC_4][Cl]
		$\rho/(g \cdot cm^{-3})$		
303.15	1.136588	1.119488	1.101742	1.063326
308.15	1.133516	1.116646	1.098913	1.060599
313.15	1.130533	1.113908	1.096234	1.057845
318.15	1.127576	1.111275	1.093528	1.055251
323.15	1.124629	1.108627	1.090952	1.052519
328.15	1.121672	1.105916	1.088273	1.049889
333.15	1.118718	1.103398	1.085626	1.047183
338.15	1.115771	1.100778	1.083071	1.044457
343.15	1.112836	1.098144	1.080431	1.041726
348.15	1.109919	1.095590	1.077842	1.039051
353.15	1.107032	1.092916	1.075273	1.036320
		$\eta/(mPa \cdot s)$		
293.15	587.2	1025.9	1348.1	1489.7
298.15	420.0	679.8	861.7	983.3
303.15	320.1	489.7	604.5	707.0
308.15	245.6	357.6	431.3	515.8
313.15	180.5	249.7	294.0	360.3
318.15	140.6	188.0	217.7	271.5
323.15	110.4	143.4	163.9	207.7
328.15	91.4	116.6	132.2	169.4
333.15	72.6	91.2	102.6	133.0
338.15	60.7	75.7	84.7	110.8
343.15	48.9	60.6	67.8	89.4
348.15	41.3	51.3	57.4	76.1
353.15	35.0	43.8	49.1	65.3
		n_D		
293.15	1.5093	1.5106	1.5115	1.5132
298.15	1.5080	1.5093	1.5102	1.5119
303.15	1.5067	1.5080	1.5089	1.5106
308.15	1.5054	1.5067	1.5076	1.5093
313.15	1.5041	1.5054	1.5063	1.5080
318.15	1.5028	1.5041	1.5050	1.5067
323.15	1.5015	1.5028	1.5037	1.5054
328.15	1.5002	1.5015	1.5024	1.5041
333.15	1.4989	1.5002	1.5011	1.5028
		$\sigma/(mN \cdot m^{-1})$		
298.15	51.3	50.0	42.3	32.1
303.15	50.1	48.8	41.3	30.8
308.15	48.9	47.8	40.0	29.7
313.15	47.8	46.7	38.8	28.4
318.15	46.4	45.4	37.4	27.3
323.15	45.0	44.0	36.3	25.9

reader (MQX2000, JICA Technical Corporation, Japan). The experiments were performed in triplicate at each IL concentration; typically seven concentrations were tested for each IL. The dose−response curves were plotted and the IC_{50} values, the test-substance concentration that resulted in 50 % growth inhibition, were determined.

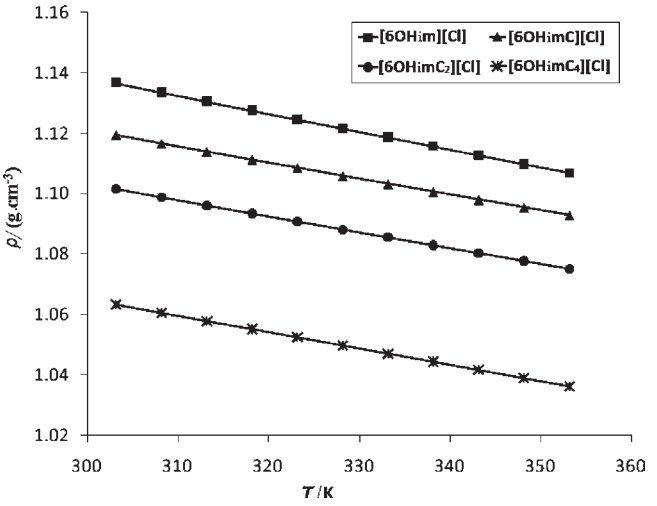

Figure 3. Densities (ρ) of the present ILs as a function of temperature.

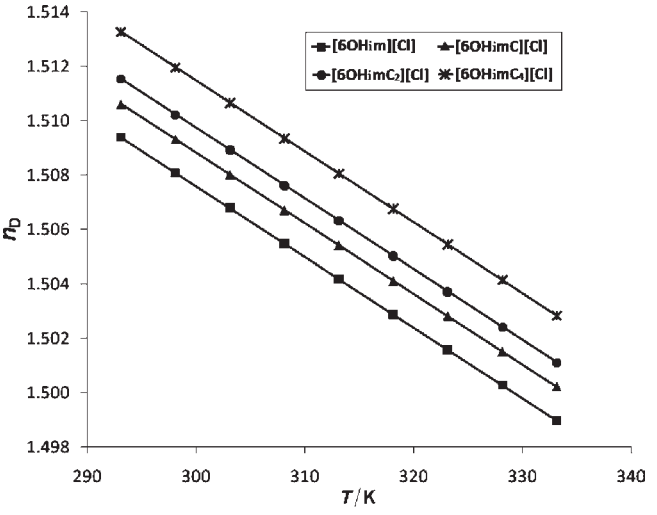

Figure 4. Refractive indices (n_D) of the present ILs as a function of temperature.

RESULTS AND DISCUSSION

Characterization. Product yields ranged from 70 % to 90 % as shown in Table 1, which also includes the IR, ^{1}H NMR, and elemental analysis data. The IR spectra for the present ILs are shown in Figure 2 and imply the presence of the hydroxyl (−OH) group from the broad absorption between (3250 and 3600) cm^{-1}. Spectral analysis of this broad absorption range is complicated due to signals rising from antisymetric and symmetric stretching modes of water together with the signal rising from hydroxyl groups in ILs. Consistent with the observations of Chowdhury and Thynell,[13] the −C═C−, −C═N−, and C−N stretching frequencies within the imidazolium ring were observed around (1565, 1475, and 1230) cm^{-1}, respectively. The C−H and C−N(1)−C bending-in-plane (bip) modes appear around 1060 cm^{-1}, with the C−H bending-out-of-plane (bop) modes occurring at (780 and 740) cm^{-1}. The intense peaks at (2900 and 2870) cm^{-1} can be assigned to −C−H in-plane stretching of the alkyl groups.[14] The low-intensity band at 3080 cm^{-1} can be attributed to ═C−H stretching.[15]

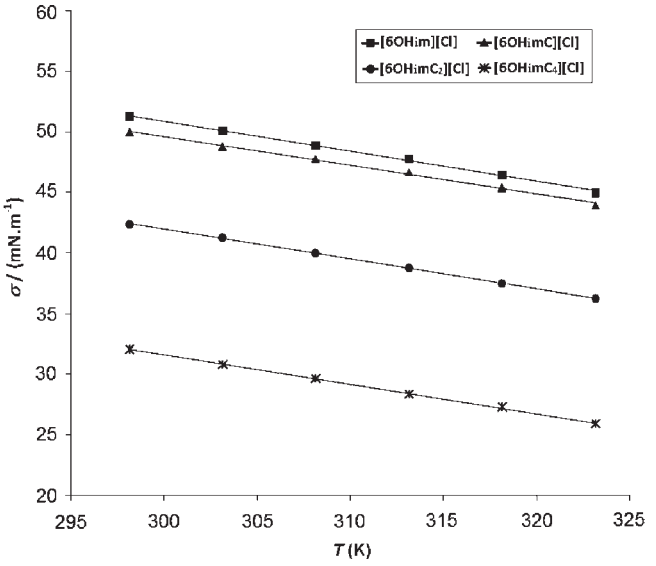

Figure 5. Surface tensions (σ) of the present ILs as a function of temperature.

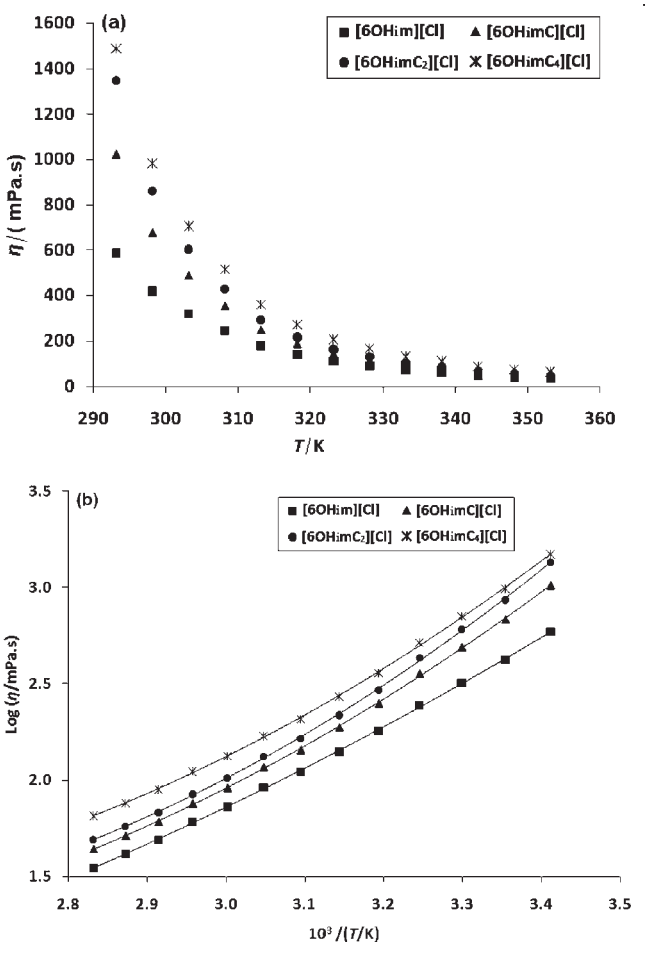

Figure 6. Dynamic viscosities (η) of the present ILs as (a) a function of temperature and (b) a function of reciprocal temperature.

The alkyl (−CH_3 and −CH_2−), imidazolium-(NH), aromatic (CH), and hydroxyl (OH) proton structures detected via

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Table 3. Values of the Empirical Coefficients, A_i in eqs 1 to 4 and the Overall Standard Deviation of Fit (SD)

	A_0	A_1	SD
[6OHim][Cl]	1.31548	−0.00059	0.0098
[6OHimC_1][Cl]	1.27939	−0.00053	0.0088
[6OHimC_2][Cl]	1.26170	−0.00053	0.0088
[6OHimC_4][Cl]	1.22657	−0.00054	0.0089
	A_2	A_3	SD
[6OHim][Cl]	−4.4454	2106.05	0.40
[6OHimC_1][Cl]	−5.0722	2351.77	0.44
[6OHimC_2][Cl]	−5.3645	2467.96	0.47
[6OHimC_4][Cl]	−4.8420	2330.30	0.44
	A_4	A_5	SD
[6OHim][Cl]	1.58561	−0.00026	0.0036
[6OHimC_1][Cl]	1.58684	−0.00026	0.0036
[6OHimC_2][Cl]	1.58775	−0.00026	0.0036
[6OHimC_4][Cl]	1.58948	−0.00026	0.0036
	A_6	A_7	SD
[6OHim][Cl]	125.5158	−0.2489	2.33
[6OHimC_1][Cl]	120.3801	−0.2360	2.21
[6OHimC_2][Cl]	116.1033	−0.2471	2.31
[6OHimC_4][Cl]	104.5407	−0.2431	2.28

^{1}H NMR, which are detailed in Table 1, are consistent with the IR data. The observed carbon, hydrogen and nitrogen percentages are in good agreement with the calculated values (Table 1).

Physical Properties. The experimental densities (ρ), viscosities (η), refractive indices (n_D), and surface tensions (σ) for all four ILs at (depending on the property) $293.15 \lesssim T/K \lesssim 353.15$ and atmospheric pressure are presented in Table 2. The densities of all four ILs are plotted as a function of T in Figure 3; over the present temperature range, they decrease linearly with increasing T. As has been found for other series of ILs containing alkyl-substituted imidazolium ions,[4,16] the densities of the present ILs decrease with increasing alkyl side-chain length of the imidazolium cation. The refractive indices (Figure 4) and surface tensions (Figure 5) of the ILs also decrease linearly with increasing T, while the dynamic viscosities (Figure 6) decrease exponentially. At a given temperature, both n_D and η increase with increasing alkyl chain length of the cation.

On the basis of the above observations, the investigated physical properties for the present ILs were fitted to the following equations:

$$\rho = A_0 + A_1 T \quad (1)$$

$$\log \eta = A_2 + \frac{A_3}{T} \quad (2)$$

$$n_D = A_4 + A_5 T \quad (3)$$

$$\sigma = A_6 + A_7 T \quad (4)$$

where T is the temperature (in K) and A_i ($0 \le i \le 7$) are empirical correlation coefficients established from the data using the method of least-squares. The standard deviations (SD) of the fits were calculated as

$$SD = \sqrt{\frac{\sum_i^N (Z_{exp} - Z_{calc})^2}{N}} \quad (5)$$

where N is the number of experimental points and Z_{exp} and Z_{calc} are the individual experimental and calculated values, respectively. The correlation coefficients and standard deviations so determined are listed in Table 3 for each of the present ILs. Equation 2, which corresponds to Arrhenius behavior for η (T), requires a

Table 4. Thermal Expansion Coefficients (α_p) of the Present ILs at $303.15 \le T/K \le 353.15$ and Atmospheric Pressure

	$10^4\ \alpha_p/K^{-1}$			
T/K	[6OHim][Cl]	[6OHimC_1][Cl]	[6OHimC_2][Cl]	[6OHimC_4][Cl]
303.15	5.19	4.74	4.81	5.08
308.15	5.20	4.75	4.83	5.09
313.15	5.22	4.76	4.84	5.11
318.15	5.23	4.77	4.85	5.12
323.15	5.25	4.78	4.86	5.13
328.15	5.26	4.79	4.87	5.15
333.15	5.27	4.81	4.88	5.16
338.15	5.29	4.82	4.90	5.17
343.15	5.30	4.83	4.91	5.19
348.15	5.31	4.84	4.92	5.20
353.15	5.33	4.85	4.93	5.21

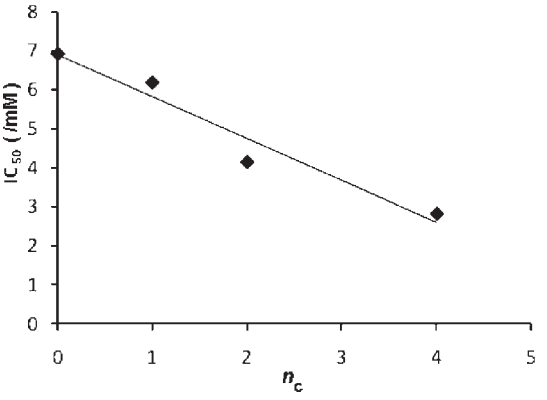

Figure 7. IC_{50} values of the present ILs as a function of the number of carbon atoms (n_c).

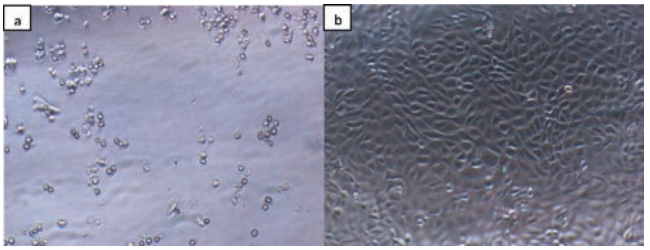

Figure 8. Microscopic images of MCF-7 cell viability after 48 h: (a) with [6OHimC_4][Cl] at IC_{50} and (b) without the IL.

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linear relationship between log η and $1/T$. However, the data in Figure 6b are slightly curved which imply Vogel−Fulcher−Tammann (VFT) behavior for these systems. Such VFT behavior is typical of ionic liquid systems.[17,18]

The isobaric coefficient of thermal expansion (α_p) for the ILs can also be calculated from experimental densities using the temperature derivative of eq 1

$$\alpha_p = -\frac{1}{\rho}\left(\frac{\delta\rho}{\delta T}\right)_p = -\frac{A_1}{A_0 + A_1 T} \quad (6)$$

The values of α_p for the present ILs (Table 4) do not change appreciably with temperature ($\lesssim$ 2.5 % over the 50 K interval studied) as found previously for other imidazolium-based ILs.[19] It is noteworthy that α_p increases with increasing alkyl side-chain length on the imidazolium cation, which may reflect coiling of the side-chain.

Cytotoxicities. The human breast cancer cell line, MCF-7, is well-characterized and has been widely used in toxicity and safety studies as an alternative to in vitro experiments.[20,21] The IC_{50} values of the present ILs were: 2.8 ± 0.6, 4.1 ± 0.1, 6.2 ± 0.2, and 6.9 ± 0.5 mM for [6OHimC_4][Cl], [6OHimC_2][Cl], [6OHimC][Cl], and [6OHim][Cl], respectively (Figure 7), indicating that IC_{50} increased with decreasing alkyl side-chain length on the imidazolium cation. Figure 8 shows the cell response when treated with [6OHimC_4][Cl] at the IC_{50} value compared with untreated cells. These IC_{50} values indicate that the present ILs have intermediate cytotoxicities compared with ILs containing pyridinium, pyrrolidinium, piperidinium, or imidazolium cations with various alkyl chain lengths, which have IC_{50} values ranging from (0.01 to 43.4) mM.[11]

■ CONCLUSIONS

The physicochemical properties (densities, viscosities, refractive indices, and surface tensions) of a series of four hydroxyl-functionalized alkyl-imidazolium chloride ionic liquids show simple systematic variations (linear or, for viscosities, exponential) with temperature. These properties and the cytotoxicities of these ionic liquids also show a straightforward dependence on the carbon side-chain length of the cation.

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