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Benzoate-based room temperature ionic liquids—thermal properties and glycosaminoglycan dissolution

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Abstract

Benzoate-based room temperature ionic liquids (RTILs) have been synthesized with cations of varying structures. The thermal properties of these RTILs have been characterized to understand their structure–property relationship. A subset of these benzoate-RTILs was found to be capable of dissolving highly charged polysaccharides, glycosaminoglycans (GAGs) to an extent of 10 mg/ml while PF_6 and BF_4 RTILs were not able to dissolve GAGs in significant quantities. The impact of the nature of the GAG counter-ion on GAG solubility is also addressed. The imidazolium salts of GAGs dissolved easier when compared to the sodium salts.

Keywords: RTILs; Glycosaminoglycans

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1. Introduction

Ionic liquids are beginning to replace conventional, volatile, organic solvents in many applications. A subset of these neoteric solvents, called room temperature ionic liquids (RTILs), remains liquid at room temperature. RTILs can be used as solvents, catalysts and solvent/catalysts in many organic reactions that conventionally use organic solvents (Seddon, 1997; Sheldon, 2001). The favorable properties of RTILs include: a near zero vapor pressure resulting in low volatility, a recyclable nature and a large liquidus range.

Since the properties of an RTIL depend on the structure of its cation and anion, adjusting the substituents they carry can result in an RTIL with specific desired properties. The hydrophobicity/hydrophilicity, organic solvent miscibility, basicity, hydrogen bonding ability and melting point of an RTIL can be tuned to specific values appropriate for a particular application. For example, when RTILs are used as solvent/medium for enzymatic reactions, specific properties are required to enhance reaction rate while not disturbing the three dimensional structure of the enzyme catalyst, thus, not all RTILs maintain enzyme activity (Kaar, Jesionowski, Berberich, Moulton, & Russel, 2003). In general, enzymes show greatest activity in RTILs having anions with low hydrogen bonding basicity—BF_4^-, PF_6^- and triflimide (Park & Kazlauskas, 2003). Carbohydrates, particularly sulfated ones, including glycosaminoglycans (GAGs), are intractable solutes that can only be dissolved in water and a few highly polar organic solvents with poor properties, such as formamide and pyridine. Recent studies in our laboratory suggested different properties were important for RTILs as enzyme solvents than those used in synthetic carbohydrate chemistry (Murugesan, Karst, Islam, Wiencek, & Linhardt, 2003), here the hydrogen bonding of the weakly basic benzoate anion was required. Since there is an incomplete understanding of the structure–property relationship of RTILs for dissolution of the polar, oxygen-rich carbohydrates, it is important to characterize the physical properties of RTILs to select the ideal RTIL for specific applications in carbohydrate chemistry. For example, we have previously shown that the imidazolium benzoate family of RTILs is of particularly useful for the peracetylation and perbenzoylation of carbohydrates (Murugesan et al., 2003). In this report we examine the synthesis and properties of RTILs

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having benzoate as the anion and a cation comprised of alkyl substituted imidazolium, pyridinium or phosphonium moieties.

2. Materials and methods

The synthesis of RTIL by different methods can afford products having different properties. This is the result of the presence of different levels of impurities. In solvent-free synthesis (Fig. 1, method-(a)), 1-ethyl-3-methyl imidazolium chloride (1 mmol) and ammonium benzoate (1 mmol) were irradiated in a test tube with microwaves for 45 s with 20 s of intermittent vortex mixing (20 s + 10 s (mix) + 20 s + 10 s (mix) + 5 s). The ammonium chloride by-product precipitates on the sides of the test tube resulting in the recovery of RTIL as a clear liquid (Namboodiri & Varma, 2002). RTIL was then washed with ether, and dried under vacuum at 70 °C. RTILs prepared by this method showed the presence of residual Cl^- by electrospray ionization-mass spectrometry (ESI-MS).

RTIL synthesis could also be accomplished using an equimolar amounts (1 mmol) of ethanol, benzoic acid and cation (chloride salt), stirred rapidly for 4–5 h as shown in Fig. 1, method-(b) (Ren et al., 2002). The reaction mixture was washed five times with hexanes, to remove by-products affording a clear solution of RTIL, which was then similarly dried. (The reaction mixture affording the trihexyltetradecylphosphonium benzoate was washed with water instead of hexanes).

RTILs produced (Fig. 2) by this method-(b) were dissolved in perdeuterated dimethyl sulfoxide and analyzed using either 300 or 500 MHz ^1H NMR spectrometry. The [bp_3‘ph’][ba] and [h_3C_{14}‘ph’][ba] RTILs were also characterized by 162 MHz ^{31}P NMR. ESI-MS analysis was performed in both positive- and negative-ion modes to assess the purity of each RTIL. Differential scanning calorimetry (DSC) was used to measure the glass transition temperature (Tg) and melting point (Tm) of each RTIL using liquid N_2 coolant and helium purge and cover gas in the laminar regime. A cooling rate of 1 °C/min was used to ensure the crystallization (if any) of the RTIL. Thermogravimetric analysis (TGA) was used to measure the decay temperature (Td) of the RTIL with N_2 protective gas at a flow rate of 50 cc/min and the heating range was from 50 to 500 °C (10 °C/min).

GAGs, heparin (MWav = 12,500), heparan sulfate (MWav = 14,800), chondroitin 6-sulfate and hyaluronic acid (MWav = 1,00,000) were next used in dissolution studies in these imidazolium benzoate RTILs. GAGs are isolated from tissues as sodium salts. GAGs having imidazolium counterions were prepared by ion exchange chromatography using Dowex cationic resin. Two benzoate based RTILs ([emIm][ba] and [bmIm][ba]) and two commercially available RTILs ([bmIm][PF_6] and [bmIm][BF_4]) were selected to study GAG solubility. Among the synthesized benzoate RTILs, [emIm][ba] and [bmIm][ba] were chosen for GAG solubility study based on their least viscosity values (data not shown). To further evaluate the effect of GAG MWav on their dissolution in RTILs, hyaluronic acid was examined from two different sources, rooster comb (MWav = 1,200,000) (Shen & Zhang, 1986) and Streptococcus zooepidemicus (MWav = 1,00,000)

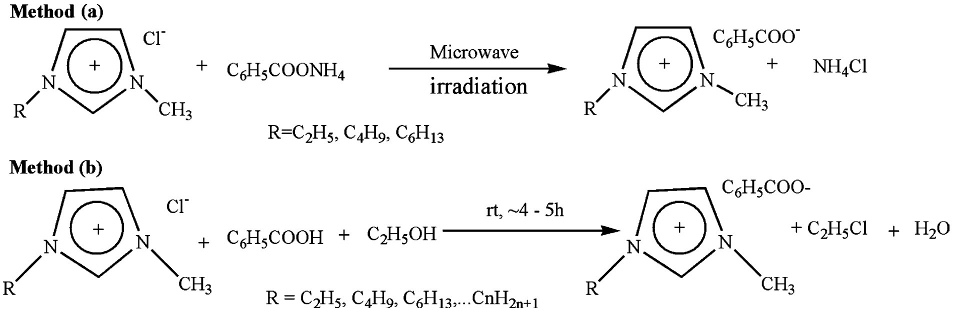

Fig. 1. Synthesis of RTILs (a) with Microwave (b) in ethanol.

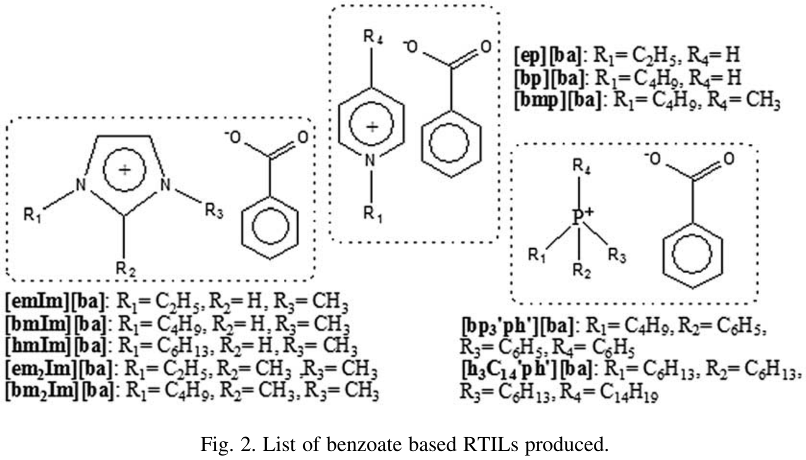

Fig. 2. List of benzoate based RTILs produced.

Table 1
DSC and TGA results

RTIL	Method	Tg (°C)	Tm (°C)	Td (°C)
[emIm][ba]	a	−83.64	–	–
[emIm][ba]	b	−61.72	–	295.00
[bmIm][ba]	a	−62.36	–	–
[bmIm][ba]	b	−72.08	–	279.60
[hmIm][ba]	b	−74.96	–	269.50
[em$_2$Im][ba]	b	−65.38	15.76	269.00
[bm$_2$Im][ba]	b	−70.46	–	255.60
[ep][ba]	b	−64.41	–	227.20
[bp][ba]	b	−73.24	10.70	233.90
[bmp][ba]	b	−64.73	10.46	229.40
[bp$_3$‘ph’][ba]	b	−61.76	18.56	235.80
[h$_3$C$_{14}$‘ph’][ba]	b	−75.45	–	217.50

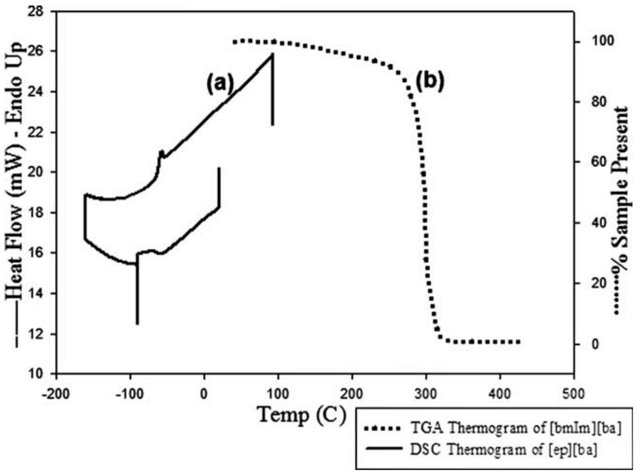

Fig. 3. (a) DSC thermogram of [ep][ba]; (b) TGA thermogram of [bmIm][ba].

(Park, Jang, & Kang, 1994). GAG (10 mg) was added to each RTIL (1 ml). The mixtures were then vortexed and heated for 20 min to 35 °C. Before the analysis, the samples were centrifuged (9230 rpm) to remove undissolved GAG. Aliquots were withdrawn from the homogenous top layer of the ionic liquid solution containing the dissolved GAGs and subjected to carbazole assay (Bitter & Muir, 1962) and compared to calibration curves using known concentrations of the GAGs.

3. Results and discussion

The values of *T*g, *T*m, *T*d for the benzoate family of RTILs are given in Table 1. All RTILs showed a *T*g. A typical DSC thermogram corresponding to [ep][ba] is given in Fig. 3a. Both the cooling and the heating curves for [ep][ba] fail to show a clear *T*m. The RTILs prepared by method (a) and (b) showed differences in their *T*g presumably due to the residual chloride observed in RTILs prepared using method-(a).

The glass transition temperature (*T*g) of an RTIL decreased with an increasing alkyl chain length. This is consistent with the molecular weight dependence of melting points. This trend has been attributed to the degree of packing of the RTIL during the cooling phase. On the basis of these data, it should be possible to design a task specific benzoate RTIL with a specific *T*m and/or *T*g.

A typical TGA thermogram corresponding to [bmIm][ba] is given in Fig. 3b. Onset of mass loss associated with the evaporation of the moisture present in the RTIL occurs at 100 °C and onset of decomposition occurs at 279 °C. The imidazolium RTILs were found to be more thermally stable than the pyridinium RTILs. In contrast to a previous report (Ngo, LeCompte, Hargens, & McEwen, 2000), the longer the alkyl chain at the 1-position of the imidazolium, the lower the thermal stability of the RTIL. Imidazolium and pyridinium RTILs with methyl substitution at the 2- and 4- position of their cations have a significantly lower *T*g than the corresponding unsubstituted RTILs. The liquidus range of an RTIL (*T*d−*T*g) decreased with longer alkyl chain size.

Solid symbols in Fig. 4 give the dissolution data of imidazolium salts of GAGs in four different RTILs. [EmIm][ba] has the highest dissolution of GAGs. [BmIm][PF$_6$] and [bmIm][BF$_4$] failed to dissolve GAGs. These data serve as yet another testimony of the tunable properties of RTILs. The empty symbols in the Fig. 4 show the dissolution data of the sodium salts of GAGs in these RTILs. As expected the imidazolium salts of GAGs were more soluble than their

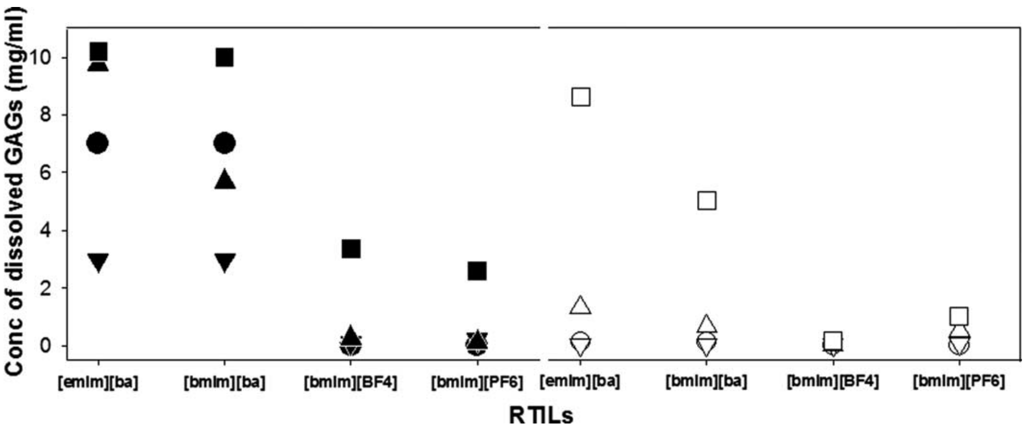

Fig. 4. Dissolution of imidazolium (solid symbols) and sodium (empty symbols) salts of GAGs in RTILs (○/●, heparin; ▲/△, chondroitin 6-sulfate; ▼/▽, heparan sulfate; ■/□, hyaluronic acid).

corresponding sodium salts. The HA having a MWav of 1,00,000 (Fig. 4) was soluble both as the sodium and imidazolium salt while the HA of MWav 1,200,000 was not.

4. Conclusions

The structure dependent variation of properties of benzoate RTILs, useful in carbohydrate synthesis, is clearly demonstrated. Benzoate-based RTILs, having a significant basicity and capable of entering into H-bonds with GAGs, were synthesized and characterized by using various analytical techniques. The presence of Cl^- impurities significantly affected these physical properties of the RTILs. While all the RTILs examined had a very large liquidus range, their thermal stability decreased with increased length of the alkyl chain of the cation. Furthermore, imidazolium based RTILs were found to be thermally more stable than the pyridinium or phosphonium based RTILs. In addition, these benzoate based RTILs were also found capable of dissolving a variety of GAGs. Studies are now focused on the application of RTILs for the chemoenzymatic synthesis of GAG oligosaccharides.

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