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Water-immiscible room temperature ionic liquids (RTILs) as drug reservoirs for controlled release

Vikas Jaitely [1], Aysegül Karatas [2], Alexander T. Florence *

Centre for Drug Delivery Research, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK

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Abstract

Water-immiscible room temperature ionic liquids (RTILs) have a largely unexplored potential as pharmaceutical solvents and reservoirs. This paper explores some relevant properties of the hexafluorophosphate (PF_6^-) salts of butyl, hexyl and octyl-3-methylimidazolium cations (BMIM, HMIM, OMIM, respectively). The dodecyl analogue is solid at room temperature, but its melting point can be lowered by addition of the lower homologues. Although water-immiscible, the liquids absorb water to an extent depending on their structure, the higher alkyl analogues having a lower affinity for absorbed water. The RTIL/water partition coefficients of sucrose, penicillin V potassium, dexametasone, progesterone and dehydro-epiandrosterone have been compared with octanol–water coefficients. The viscosities of the salts were measured in anhydrous, water-saturated and intermediate states. The PF_6^- ionic liquids display a low and decreasing aqueous solubility as the alkyl chain length is increased: 0.035 mol l^{-1} (BMIM), 0.032 mol l^{-1} (HMIM) and 0.09 mol l^{-1} (OMIM). The release of sucrose and dexametasone from RTIL reservoirs into water can be prolonged over 48 h. Saturated solutions of these RTILs show little toxicity towards Caco-2 cells, although the OMIM derivative, which is more surface-active, has a small effect on cell viability.

Keywords: Room temperature ionic liquids (RTILs); 1-Alkyl-3-methylimidazolium salts; Controlled release; Partitioning; Reservoir

1. Introduction

Room temperature ionic liquids (RTILs) are salts whose melting points lie below room temperature. They comprise bulky organic cations such as the 1-butyl-3-methylimidazolium ion or the 1-butylpyridinium ion with a variety of anions such as PF_6^-, NO_3^- or $AlCl_4^-$ (Welton, 1999). These liquids have been referred to as green solvents as they are claimed to cause less environmental pollution than organic solvents because of their very low vapour pressure even at high temperatures (Seddon, 1997; Wasserscheid and Keim, 2000; Olivier-Bourbigou and Magna, 2002; Swatloski et al., 2001). Their properties and potential as a solvent class have been actively investigated since the early 1990s but there has been little attention paid to their possible use in pharmaceutical formulation and processing, perhaps because of a lack of information on their toxicity. RTILs are capable of dissolving high concentrations of a wide range of organic and inorganic molecules, making them efficient reaction media for complex organic reactions. Their good solvent properties and low vapour pressure make them useful in liquid/liquid extraction processes as alternatives to organic solvents (Swatloski et al., 2001; Wilkes, 2004).

The capability to engineer the properties of RTILs by manipulating their structure is potentially valuable. A large number of cation-anion combinations have been studied. Of particular interest are the salts based on the *N*/*N*-dialkylimidazolium cations because of their wide spectrum of physico-chemical properties. RTILs with the imidazolium cation and hexafluorophosphate anion are water-immiscible, although they do possess a degree of water solubility. The tetrafluoroborate $(BF_4)^-$ salts of the imidazolium cation are, on the other hand, water-miscible. Elsewhere, we have reported on the solvent properties of mixtures of the hexafluorophosphate and the tetrafluoroborate salts (Mizuuchi et al., in press).

In this paper we have investigated some properties – such as water-uptake, viscosity and surface tension – of three alkyl-

* Corresponding author at: 23 North Esk Road, Edzell, Angus DD9 7TW, UK.
E-mail address: ataylorflorence@aol.com (A.T. Florence).
[1] Present address: MHRA, Market Towers, Nine Elms Lane, London, UK.
[2] Present address: Department of Pharmaceutical Technology, School of Pharmacy, University of Ankara, Ankara, Turkey.

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3-methyl-imidazole hexafluorophosphate RTILs as potential reservoirs for hydrophilic and hydrophobic drugs. They solubilise a range of polar and non-polar solutes/drugs. The release of two model drugs from their RTIL reservoirs into aqueous media has been studied. In addition to the shorter butyl, hexyl and octyl alkyl analogues, the dodecyl derivative was also prepared. While it is solid at room temperature, with a melting point of 43 °C, in admixture with the butyl and octyl analogues the melting point could be lowered to below room temperature, thus providing preliminary insight into the utility of RTIL mixtures to adjust critical parameters.

2. Material and methods

2.1. Materials

Chlorobutane, chlorohexane, chlorooctane, 1-methylimidazole and hexafluorophosphoric acid (HPF_6) were obtained from Lancaster Chemicals, UK. ^{3}H-Sucrose, ^{3}H-dexametasone, ^{3}H-progesterone and ^{3}H-penicillin V potassium were obtained from Amersham Ltd., UK. Dehydroepiandrosterone (DHEA) was purchased from DuPont Chemicals, USA. Methanol-free Karl-Fischer reagent and dried methanol were procured from BDH Ltd., UK. Dialysis membranes (Snakeskin®, molecular weight cut-off 3500) were purchased from Perbio Science, UK and Caco-2 cells were supplied by ECACC. DMEM (Dulbecco's Modified Eagle's Medium) was obtained from Life Technologies, Gibco, UK.

2.2. Synthesis of ionic liquids

Salts of 1-alkyl-3-methyl-imidazolium cations and hexafluorophosphate anions were synthesized as described by Cull et al. (2000). The butyl, hexyl and octyl substituted 3-methyl imidazolium halides were prepared by refluxing equimolar amounts of 1-methylimidazole and chlorobutane, chlorohexane or chlorooctane, respectively, for 72 h at 70 °C under an argon atmosphere. After reaction the lower layer was collected and washed with ethyl acetate (200× 3 ml), to remove unreacted starting material. Excess ethyl acetate was removed under high vacuum. An aqueous solution of hexafluorophosphoric acid (65% (w/v) solution; 1.3 mol) was added slowly to 1-alkyl-3-methylimidazolium chloride (1 mol) in 500 ml of water with continuous stirring in an ice bath. After 12 h the upper acidic layer was discarded. The lower layer was collected and washed with water until the washings were no longer acidic. Contaminants originating from unreacted starting material were removed after repeated washing with water (Seddon et al., 2000). The product was washed with water until no trace of halide was detected. Excess water was removed by heating the ionic liquid layer to ∼90 °C under high vacuum for 2 h.

2.3. Molecular weight, density and surface tension

The molecular weight of each compound was determined using FAB mass spectroscopy and structures confirmed by NMR and IR analysis. The density of each RTIL was measured using 10 ml density bottles. Viscosities were determined using a U-tube viscometer at 25, 40, 50, 60 and 70 °C (±0.5 °C).

The surface tension of each RTIL in its pure state was measured using the Wilhelmy plate method (DCA meter, Cahn Instruments, Germany) calibrated by measuring the surface tension of double distilled deionised water (72 mN m^{-1}). Each measurement was made at 25 °C (±0.5 °C) using a flame cleaned glass plate. The surface tension of aqueous solutions of RTILs up to their saturation concentrations were determined for BMIM (saturation 0.035 mol l^{-1}), HMIM (saturation 0.032 mol l^{-1}) and OMIM (saturation 0.009 mol l^{-1}) with a Delta-8 microtensiometer (Kibron), based on the Wilhelmy plate method modified to accommodate small sample sizes and multiple measurements.

2.4. Water uptake

Each RTIL was equilibrated with water for 24 h to reach saturation. The aqueous layer was separated carefully. The amount of water dissolved in the ionic liquid layer was determined by thermogravimetic analysis (Perkin-Elmer TGA, UK). Anhydrous RTILs unexposed to water were used as controls. Known concentrations of water were added to the anhydrous ionic liquids to measure the effect of water content on viscosity and density.

2.5. Partition coefficients of solutes between RTILs and water

Partition coefficients of ^{3}H-sucrose, ^{3}H-penicillin V potassium, ^{3}H-dexametasone, ^{3}H-progesterone and ^{3}H-dehydroxyepiandosterone were measured between equal volumes of BMIM, HMIM and OMIM PF_6^- salts and a pure water phase using the shake-flask method. 1 ml of RTIL containing drug was equilibrated with 1 ml of deionised water by shaking for 24 h. The RTIL and the water phase were carefully separated and the radioactivity determined in each phase (Beckman LS 6500 scintillation counter). The apparent partition coefficient (P) and log P values for each drug were calculated.

2.6. Drug release profiles

BMIM, HMIM or OMIM salts (2 g) containing equal quantities of ^{3}H-sucrose (100 mg; ∼0.5 μCi) or ^{3}H-dexametasone (100 mg; ∼0.2 μCi) were placed in a dialysis bag (SnakeSkin® Perbio Science, UK), suspended in 100 ml of water as the external medium and maintained at room temperature under continual stirring. Samples (1 ml) were withdrawn periodically and the radioactivity determined using a Beckmann LS 6500 scintillation counter. An equivalent volume of water (1 ml) was replaced in the release medium to maintain sink conditions. Experiments were run in triplicate. Similar techniques were applied to determine the release of ^{3}H-dexametasone from the dodecyl salt (DDMIM), with and without added BMIM and OMIM, noting the melting points of the mixed systems.

2.7. Interaction with Caco-2 cells

The effect of saturated aqueous solutions of the three RTILs (BMIM, HMIM and OMIM) on Caco-2 cells was determined using the MTT assay (Mossman, 1983). Aqueous solutions of BMIM (0.1–1%, w/v), HMIM (0.1–1%, w/v) and OMIM (0.1–0.3%, w/v) were incubated for 24 h with cell lines maintained under standard conditions. Caco-2 cells were seeded onto 96 well plates in DMEM culture media, cultured in an atmosphere of 95% air and 5% CO_2 at 37 °C and 90% humidity for 48 h. Subsequently, the culture medium was replaced with fresh 80 μl of fresh medium and 20 μl of each RTIL solution. Negative controls contained no RTILs. After adding the RTIL solution, the Caco-2 cells were incubated at 37 °C for 4 h. The RTIL solutions were removed and 100 μl MTT solution (5 mg/ml in PBS) was added to each well and the cells incubated for 4 h at 37 °C. The reaction product was quantified using a Bio-Rad plate reader (Bio-Rad, Herts, UK).

3. Results and discussion

Table 1 summarises the data obtained for the liquids studied. Literature values for the viscosity and density of these RTILs, where available, are in general agreement with our data except for BMIM, where our value is lower (Huddleston et al., 2001; Marsh et al., 2004; Wilkes, 2004). Differences are often due to the presence of low amounts of impurities in the RTILs, or due to differences in the degree of water saturation as the data show. Complete removal of water from synthetic RTILs is not readily achieved due to significant hydrogen-bonding, which contributes to the equilibrium water values, found to be 1.53%, 0.92% and 0.22% (w/w) for BMIM, HMIM and OMIM, respectively. Ohno and Yoshizawa (2002) compared the viscosity of RTILs with different anions and suggested that the size of component ions bore little relation to viscosity, but it is clear that increasing the alkyl chain length results in increased viscosity of the pure liquids, until the systems are solid with the introduction of the dodecyl analogue. This is perhaps less due to shape (asymmetry) than to interactions between the alkyl groups. The surface tension of the pure liquids (Table 1) decreases from 48 mN m^{-1} to 35.7 mN m^{-1} with increase in alkyl chain length.

The miscibility of the RTILs with water depends both on the nature of the relevant anion and on temperature. The BF_4^-, $CH_3SO_3^-$, $CF_3CO_2^-$ or NO_3^- salts of the 1-butyl-3-methylimidazolium ions are miscible with water at 25 °C whereas the PF_6^- and the SbF_6^- salts have a low aqueous miscibility. As stated, although immiscible with water, the hexafluorophosphate liquids can absorb water or dissolve water. TGA analysis of liquids exposed to water for 24 h reveals that BMIM dissolves almost 10% water, whereas HMIM accommodates around 5% and OMIM 3%. Thermograms show two peaks one at 90 °C indicating dissolved water, and the other close to 250 °C due to bound water. Both the anion and the cation contribute to the affinity of the salts for water. With the compounds investigated here, the effect of alkyl chain length is intuitive, the octyl compound having a lower affinity for water than any of its shorter alkyl chain homologues. The presence of water in the liquids significantly decreases their viscosity: increase in the water content of BMIM results, for example, in a 60% reduction in viscosity and OMIM experiences a similar – 64% – decrease (Fig. 1).

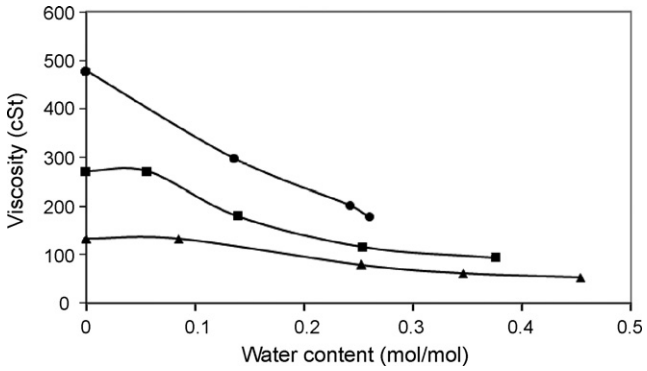

Fig. 1. The effect of the concentration of water on the viscosity (cSt) of RTILs. A maximal 60% decrease in viscosity of OMIM, for example, can be seen on addition of water.

Table 2
log *P* values for drugs in RTIL-water systems*

Drug	BMIM PF_6	HMIM PF_6	OMIM PF_6
Progesterone	2.35 ± 0.13	2.14 ± 0.35	2.01 ± 0.14
DHEA	0.98 ± 0.09	0.91 ± 0.14	0.87 ± 0.077
Dexametasone	0.011 ± 0.008	0.29 ± 0.02	0.32 ± 0.08
Sucrose	−2.36 ± 0.18	−2.52 ± 0.39	−1.81 ± 0.16
Penicillin V (K salt)	−0.86 ± 0.06	−0.95 ± 0.19	−0.75 ± 0.07

* log *P* = drug concentration in RTIL phase/drug concentration in water phase.

Table 2 presents the partition coefficients (as log *P* values) of a number of hydrophilic and hydrophobic drugs or solutes in RTIL/water systems. The log *P* values were found to be related to their octanol/water partition coefficient values, although not in a linear fashion (Fig. 2). The polarities of RTILs have been investi-

Table 1
Some physicochemical properties of 1-alkyl-3-methyl imidazolium hexafluorophosphates at 25 °C

Alkyl chain	RTIL	Molecular weight (Da)	Density[a] (g cm^{-3})	Viscosity[b] (cSt)	Surface tension (mN m^{-1})	Refractive[c] index
C_4H_9	BMIM	284	1.3824	133.19	48.6	1.4079
C_6H_{13}	HMIM	312	1.2837	271.57	41.2	1.4231
C_8H_{17}	OMIM	340	1.2343	478.12	35.7	1.4235

[a] Literature values for density: 1.36, 1.29, 1.22 g cm^{-3} for BMIM, HMIM and OMIM, respectively (Marsh et al., 2004).
[b] Literature values for viscosity: 215, 253, 450 cSt for BMIM, HMIM and OMIM, respectively (Huddleston et al., 2001).
[c] Literature values for refractive index: 1.409, 1.423 for BMIM and OMIM, respectively (Huddleston et al., 2001).

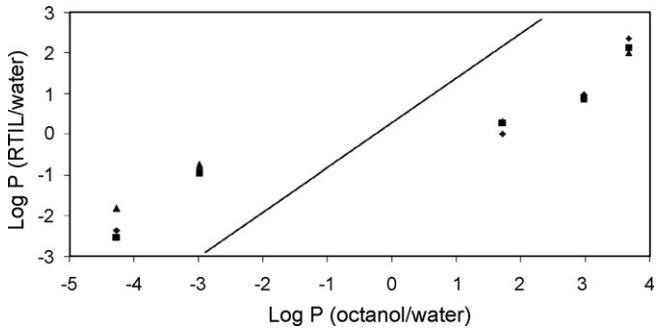

Fig. 2. Correlation between the log (partition coefficient) between ionic liquid/water and octanol/water systems (▲, BMIM; ■, HMIM; ●, OMIM). The line shows the 1:1 correlation and that the lipidic molecules, dexametasone, progesterone and DHEA display a lower affinity for the RTILs than for octanol while the polar molecules have a greater affinity for the RTILs than for octanol.

gated by different groups (Park and Kazlauskas, 2001; Bonhote et al., 1996; Carmichael and Seddon, 2000). The polarity of BMIM PF_6^- is similar to that of conventional polar solvents such as ethanol or *N*-methylformamide. On Reichardt's normalised polarity scale, which ranges from 0 for tetramethylsilane to 1 for water, RTILs have values around 0.6 (Carmichael and Seddon, 2000). Octanol is less polar with a value of 0.35. The lipidic drugs have a lower affinity for RTILs than for octanol while the polar solutes display the opposite trend.

These properties are all relevant to the consideration of room temperature ionic liquid as pharmaceutical solvents. We envisage their use as drug reservoirs or depots for controlled drug release. Fig. 3 shows the results of release experiments for sucrose and dexametasone. Sucrose release increases with increase in the alkyl chain length of the RTIL used as the reservoir, in agreement with the order of its log *P* values of −2.36 (BMIM–water) and −1.81 (OMIM–water). There was little dependency, on the other hand, of dexametasone release on the structure of the RTIL. Delayed release profiles were observed for both of these solutes. Inhibition of water through the dialysis bag within the time frame of the experiment may result in some decrease of ionic liquid viscosity causing some of the biphasic patterns of release which can be seen in Fig. 3a.

Of key importance is, of course, how safe these systems are for use in pharmaceutical products or processes. LD_{50} values of 1400 mg kg^{-1} have been reported for 3-hexoyloxymethyl-1-methylimidazolium BF_4^- in female Wistar rats. Ranke et al. (2004) conducted some ecotoxicology studies and reported on the safety of RTIL chloride salts as compared to conventional organic solvents. However, as far as we are aware, published toxicity studies have not addressed RTILs possessing the PF_6^- anion. Our studies using Caco-2 cell lines with saturated aqueous solutions of these RTILs suggest that the compounds studied are largely non-toxic: cell lines remained 90% viable after being exposed to saturated aqueous solutions of the liquids (Fig. 4). The alkyl chain length of the cation has a modest effect on toxicity of the RTILs, OMIM registering the largest, but still modest, effect on cell viability. As shown in Fig. 5, aqueous solutions of RTILs are quite surface-active even in dilute solution as might be expected from their amphipathic structure. Hence, one would

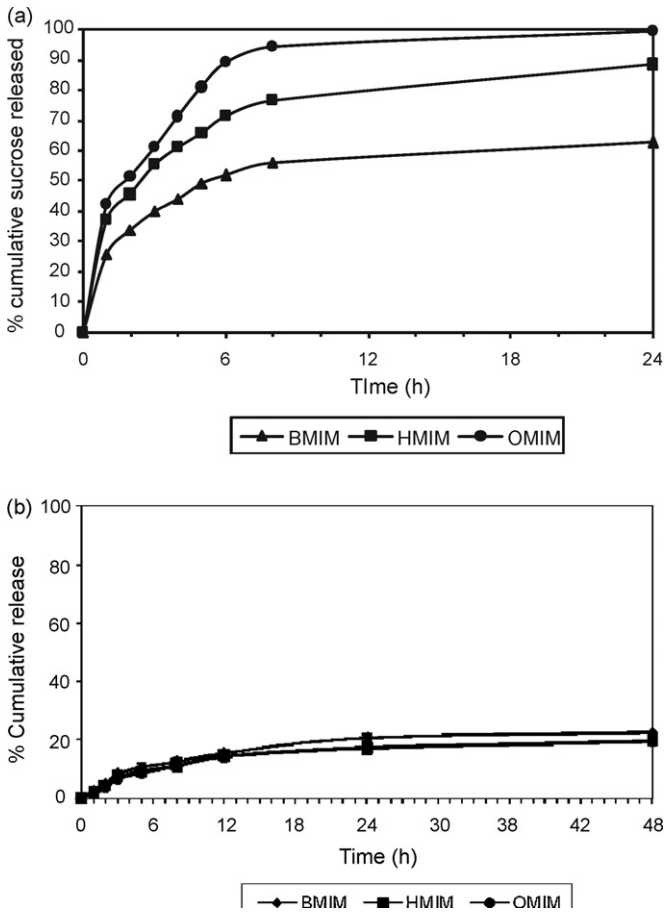

Fig. 3. Release of (a) sucrose and (b) dexametasone from RTILs across a dialysis membrane into an aqueous recipient phase at 37 ± 5 °C. Release of sucrose is most rapid from the OMIM system. The rate of sucrose release decreases from the octyl to the butyl homologue.

expect a degree of membrane activity, especially of the octyl derivative, and some toxicity in cell culture systems. In terms of the amphipathic nature of these materials, it is interesting to note that 1-decylimidazoliumbromide spontaneously forms a liquid crystalline gel on addition of water (Firestone et al., 2002).

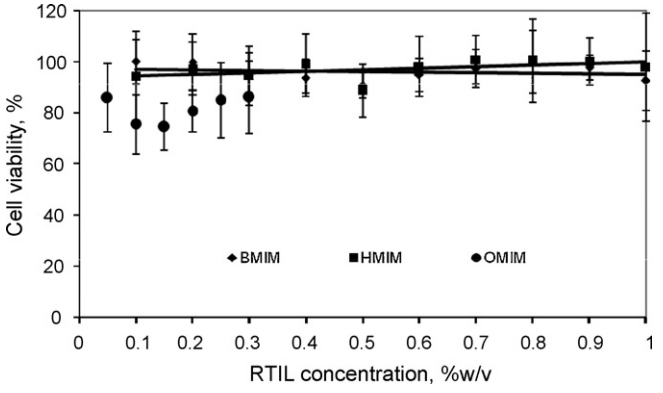

Fig. 4. Caco-2 cell viability as determined by the MTT test after incubation with RTIL aqueous solutions up to saturation solubility. Only the OMIM analogue causes any loss of viability but this is low, and is possibly due to the membrane activity of the solutions, as seen in Fig. 5.

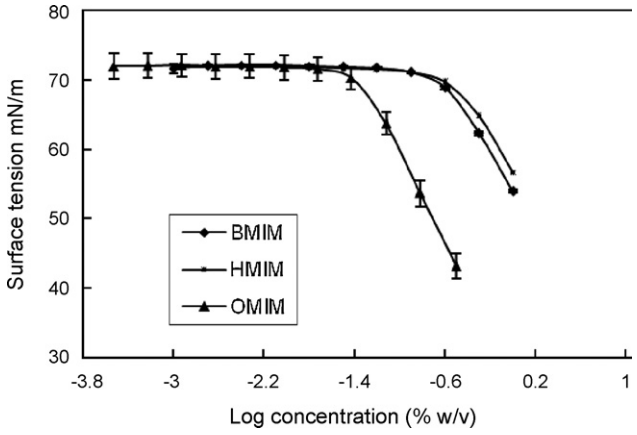

Fig. 5. Change in surface tension of three RTILs up to saturation levels in aqueous solution. Limiting surface tensions are seen to be ~57 mN m^{-1} for the BMIM, 53 mN m^{-1} for the HMIM salt and 42 mN m^{-1} for the OMIM member of the series.

The degree of control over the release profiles and properties of RTILs can be extended by using RTIL mixtures. DDMIM PF_6^- samples spiked with low concentrations of BMIM PF_6^- or OMIM PF_6^-, were heated to 50 °C and allowed to cool to room temperature. Melting points of a range of mixtures are shown in Fig. 6a. Release from pure DDMIM PF_6^- was virtually zero.

^{3}H-Dexametasone was solubilised in a mixture of BMIM (0.05%) or OMIM (0.075%) in DDMIM PF_6. Release was monitored at 25° and 37 °C and shows a significant increase suggesting that such RTIL mixtures could be used in the design of controlled delivery systems.

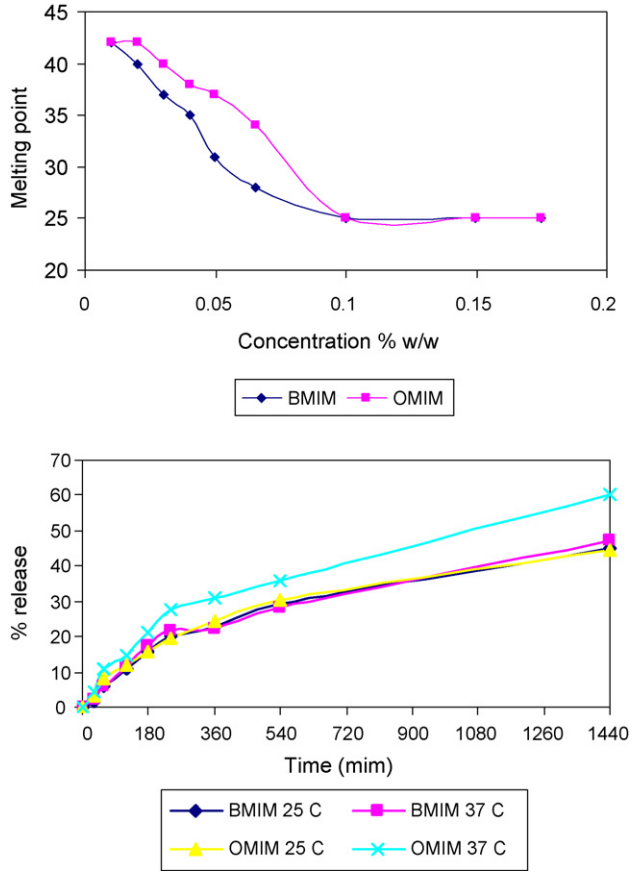

Fig. 6. (a) The melting points of DDMIM as a function of the concentration of two added RTILs, BMIM and OMIM PF_6^- salts. The melting points reduce to a minimum around 25 °C. (b) The release of dexametasone from mixtures of DDMIM showing the increased release (dexametasone release from pure DDMIM approximates to zero) as a function of time at 25 °C and 37 °C.

4. Conclusions

RTILs might be useful as versatile solvents in the design of controlled release drug delivery systems. Not only is there a wide variety of basic structures and counter ion combinations from which to choose, but various RTIL mixtures can extend the range of properties at our disposal. The association of water in otherwise water immiscible ionic liquids make them versatile materials for the containment of entrapped/solubilised drug, thus making them interesting reservoirs for controlled release. The hydrophobic RTILs studied here are predominantly non-toxic and offer potential as pharmaceutical excipients in a variety of scenarios. We have been concurrently exploring a range of RTIL mixtures as solvents for poorly water-soluble drugs (Mizuuchi et al., in press). There is evidence from our laboratory of release of polar solutes being enhanced by the passage of electric current (Jaitely et al., unpublished), hence, we feel that these materials should be the subject of some interest in the years ahead. Clearly the choice of individual RTILs or their mixtures will depend on their toxicity. It goes without saying that good measures of toxicity have to be carefully examined and evaluated.

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