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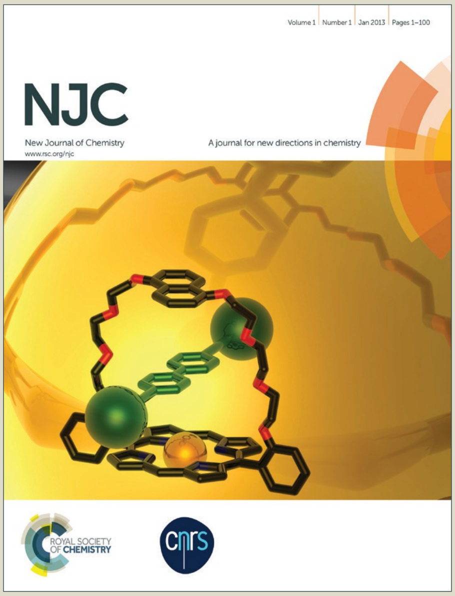

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ARTICLE

New Low Viscous Cholinium-based Magnetic Ionic Liquids

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Magnetic Ionic Liquids (MILs) are a type of Ionic Liquids (ILs) that have a paramagnetic metal based anions, and so can response to an external magnetic field. Due to the high propensity to crystalize of these anions, there are only few reported MILs that are liquid at room temperature. In this work is described a new family of cholinium based ILs, containing two and three ethanol side chains that are liquid at room temperature, even in combination with paramagnetic anions. Additionally, these new family of ILs are prone to generate low toxicities on human cell lines.

Introduction

Ionic Liquids (ILs) have been attracted the scientific community due to several advantages compared with traditional solvents, such as their unique structures and solubility properties, low volatility and ease of recyclability. [1-5] These solvents have been assigned as intrinsically green solvents, although important environment concerns have to be taken into account, such as toxicity and biodegradability. Recently has been reported ILs based on paramagnetic metal as anions that combines the desired properties of ILs with their paramagnetic properties, such as response to an external magnetic field, and catalytic properties.[6, 7] Several metals have been reported as MILs anions, such as Fe(III)[8, 9], Co(II)[10, 11], Mn(II)[12, 13], and also lanthanides based ILs [14-16] that have been known for their strong response to an applied external magnetic field. Choline (Chol) based ILs have been reported as more biocompatible and biodegradable[17] than imidazolium based ILs. In addition, they can also be intrinsically cheaper [18] due to the lower price of choline precursor. A drawback of these ILs is the high ability to crystalize, even in the presence of anions known being less prone to crystalize and to decrease the viscosity of some ILs, leading to high viscous ILs at room temperature. In this work different cholinium derivatives were prepared in combination with various anions, including paramagnetic anions (based on metals such as gadolinium (III), cobalt(II), iron(III), and manganese (II)), resulting in ILs that are liquid at room temperature, and present low toxicity to human cell lines.

Results and discussion

Cholinium (Chol) based ILs

A range of known cations [19-28] based on the cholinium with different alkyl side chains were synthetized (Table 1). From this collection can concluded that cholinium derivatives with longer *n*-alkyl chains (up to *n*-hexyl) had a tendency to decrease the melting point (Table 1, entries 1 to 4). When the anion bromide was replaced by tetrachloroferrate, this tendency was more evident, and for alkyl chains higher than six carbons (C6), the resulting ILs were liquid at room temperature as described by us[28] (Table 1, entries 10-12). Based on those observations, it was explored the effect of introduction of ethanol chains on the cation in the viscosity of the IL (Table 3). We selected ILs containing an alkyl chain of C6 due to the lower melting point identified in the first series (Table 1). Salts with two (DHEA) and three (THEA) ethanol chains were synthetized, and both were liquid having chloride or bromide as counter ions. Interestingly, [THEA]Cl was less viscous than its pair [DHEA]Cl (Figure 1).

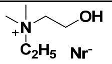
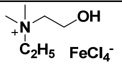
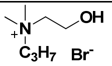
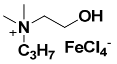
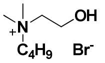
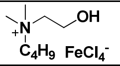
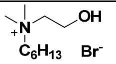
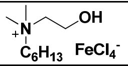
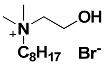
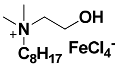
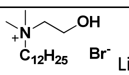
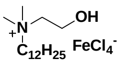
Entry	Salt (Chol$^+$Br$^-$)	m.p.(ºC)	Ref	Entry	Salt (Chol$^+$FeCl$_4^-$)	m.p.(ºC)	Ref
1		280-282 Lit 282-284	19, 21	7		254-256	28
2		101-105 Lit 107	21	8		35-37	28
3		110-113	20	9		hygros- copic	28
4		67	22	10		Viscous liquid	28
5		107-100 Lit 116–117	23, 24	11		Viscous liquid	28
6		190-193 Lit 193-196	25-27	12		Viscous liquid	28

Table 1: Melting point of cholinium (Chol) based ILs, with different alkyl chains.

ILs characterization

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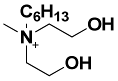
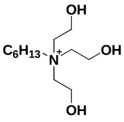
ILs based on DHEA cation in combination with dicyanamide (DCA), tetrafluoroborate (BF_4), bistrifliimide (NTf_2) and acetate (Acet) presented Tg values between -81ºC and -93ºC (Table 2). However, when the cation was changed to THEA, Tg values increased to values around -60ºC. For anions based on paramagnetic metals the Tg follow the same pattern, DHEA based ILs have Tg values lower than THEA based ILs (Table 1). Paramagnetic materials presents a response to an applied magnetic field, being the magnetic susceptibility an indicator of the magnetization degree of the material to the external field. Magnetic susceptibilities of the synthetized MILs were measured (Table 2), and for iron, gadolinium and manganese based ILs the values were lower than expected, when compared with other reported MILs. [12, 29-31] For example, butylmethylimidazolium tretrachloroferrate ([Bmim]Fe) have a molar susceptibility of 4.11 emu K·mol^{-1}, higher than 3.57 or 3.54 emuKmol^{-1} found for [DHEA]Fe and [THEA]Fe, respectively (Table 2).[32] The magnetic susceptibilities obtained for cobalt based ILs ([DHEA]Co and [THEA]Co, Table 2) were in agreement with the reported values (2.1-2.5 emuKmol^{-1}). [31]

Table 2: ILs characterization by DSC, ICP, and magnetic susceptibility based on [DHEA] and [THEA] cations.

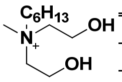
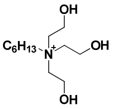
Entry	ILs		Tg (ºC)	ICP		χ_{mT} (emuKmol^{-1}) 298K
	Cations	Anions		% Metal Predicted	% Metal Observed	
1	 [DHEA]	Co	-65,99	9,70	9,54	2,30
2		Mn	-51,17	9,10	8,84	3,63
3		Fe	-64,87	13,97	14,08	3,57
4		Gd	-36,69	16,11	14,22	5,57
5		DCA	-93,34	-	-	
6		Acet	-92,49	-	-	
7		BF4	-93,73	-	-	
8		NTf_2	-81,19	-	-	
9	 [THEA]	Co	-43,68	8,83	8,64	2,39
10		Mn	-41,18	8,28	8,04	nd
11		Fe	-44,17	13,00	12,50	3,54
12		Gd	-62,71	14,75	12,05	5,80
13		DCA	-69,91	-	-	-
14		Acet	-65,7	-	-	-
15		BF4	-83,01	-	-	-
16		NTf2	-58,82	-	-	-

nd – not determined due to experimental difficulties.

ILs viscosity

Viscosity is an important physical property that is highly dependent of the temperature, and also of the water content of the IL. Being water an IL impurity difficult to remove, all the synthetized ILs were kept under vacuum (1-4x10^{-2} mbar), at 50ºC for at least 48 h prior to their use. Karl-Fischer Coulometric titration shown water contents lower than 1 wt.%, while for gadolinium based MILs (highly hygroscopic) the water content was always lower than 5 wt.%. All ILs based on the cations [DHEA] and [THEA] were liquid at room temperature, although in some cases the viscosity was so high, that it was not possible to measure it (Table 3, entries 4, 10 and 13).

Table 3 : Viscosity results for the studied ILs Based on [DHEA] and [THEA] cations.

Entry	IL		Viscosity (mPa.s)			
	Cations	Anions	298K	303K	308K	313K
1	[DHEA] 	Co	7744	4989	3311	2261
2		Mn	18084	10757	6628	4225
3		Fe	349	248	182	138
4		Gd	nd	nd	nd	nd
5		DCA[a]	251	188	143	112
6		Acet	660	466	336	248
7		BF4	122	95	74	60
8		NTf2	296	216	161	123
9		Cl[a]	8157	5438	3708	2577
10	[THEA] 	Co	nd	nd	nd	nd
11		Mn	2006	1226	777	510
12		Fe	142	103	77	-
13		Gd	nd	nd	nd	nd
14		DCA	561	402	294	220
15		Acet	solid	solid	solid	solid
16		BF4	158	120	92	73
17		NTf2	1038	700	487	349
18		Cl[a]	4547	3002	2036	1413

[a] Some iodide may be present due to the use of NaI as promoter in the alkylation reaction. nd – not determined due to experimental difficulties (viscosity very high).

Inorganic anions such as tetrafluorborate (BF_4), bistriflimide (NTf_2) and dicyanamide (DCA) decreased the viscosity of the respective ILs when compared with the chloride precursor (Table 3, entries 5-9 and entries 14-18). It is interesting to observe that ILs based on acetate with one ethanol chain (cholinium acetate) is solid at room temperature[33], although when two ethanol chains are present ([DHEA]Acetate), the salt is liquid at room temperature with low viscosity (Table 3, entry 6), and when the cation have three ethanol chains ([THEA]Acetate) become again solid (Table 3, entry 15).

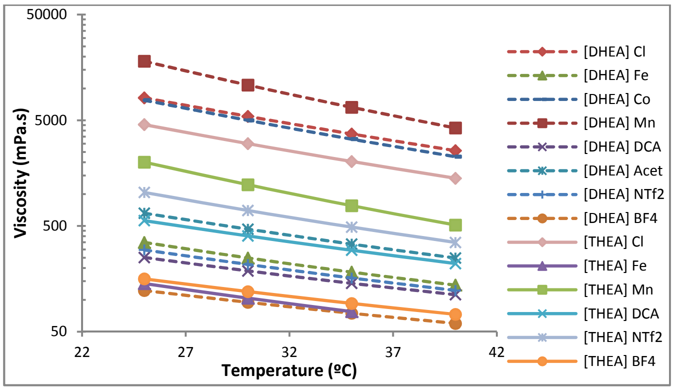


Figure 1: Dependence of viscosity with temperature for ILs based on DHEA] and [THEA] cations.

In addition, Figure 1 shows that DHEA based ILs, are less viscous than the respective THEA ILs when non-magnetic anions are present. In opposition, when paramagnetic anions are present, DHEA based ILs are more viscous than THEA. In spite of cholinium tretrachloroferrate being solid at room temperature[34], we observed that this anion largely decrease the viscosity of the MILs synthetized [DHEA] and [THEA]. Indeed at 298K [THEA]Fe presents an viscosity of 142 mPa.s that is lower than the reported value of [$P_{6,6,6,14}$] at the same temperature (trihexyltetradecylphosphonium tetrachloroferrate, 650 mPa.s at 298K[27]). The viscosity of manganese MILs can be up to thirty times lower than the reported viscosity of [$P_{6,6,6,14}$]Mn[31] ([DHEA]Mn, 18084mPas, [THEA]Mn 2006 mPas, [$P_{6,6,6,14}$] Mn, 75230 mPas). Although [DHEA]Gd, [THEA]Gd and [THEA]Co are liquids at room temperature, it was not possible to measure the viscosity due to experimental difficulties. In addition, [DHEA]Co have a viscosity slightly lower than [$P_{6,6,6,14}$]Co [31] ([DHEA]Co 7744 mPas vs. [$P_{6,6,6,14}$]Co 83450 mPas).

ILs Toxicity

Human epithelial colorectal adenocarcinoma cell line CaCo-2 is an interesting model for testing toxicity since these cells can resemble absorptive cells of the small intestine after monolayer differentiation.[35] ILs toxicity was assessed in this model and determined cytotoxicities were low. In general, calculated IL concentration necessary to reduce in 50% cellular viability (IC_{50}) is above 1000 μM, with the exception of [THEA] Acetate which is below that value but still higher than 500 μM.

However, it is interesting that most of the ILs based on DHEA (with two ethanol chains) generated higher IC_{50}. For instance, the [DHEA]Cl was not able to vary significantly the cellular viability up to 5000 μM, but its correspondent [THEA] Cl has its IC_{50} in the concentration interval [1000-2000] μM. Identical behavior was obtain for [DHEA]Mn/[THEA]Mn, [DHEA]NTf_2/[THEA]NTf_2, [DHEA]DCA/[THEA]DCA and [DHEA]Acetate/[THEA]Acetate ILs pairs. Previous work from our group[28] have shown that C6-cholinium based IL (with only one ethanol chain) combined with $FeCl_4$ did not decrease CaCo-2 viability up to 1000 μM which agrees with the work described here. Also in this published work, it was evident the effect of the alkyl chain length on cytotoxicity. Here this effect was not explored, but the number of alcohol chains seems to influence slightly toxicity as mentioned before.

Based on results from Table 4 it is not possible to link this behavior with the determined viscosities.

Table 4: Determined IC_{50} interval range for the studied ILs. Toxicity was assessed in the human epithelial colorectal adenocarcinoma cell line (CaCo-2) for an incubation period of 24 hours.

ILs Cation	ILs Anion	IC_{50} (mM) (CaCo-2)	ILs Cation	ILs Anion	IC_{50} (mM) (CaCo-2)
[DHEA]	Cl	>5	**[THEA]**	Cl	$1<IC_{50}<2$
	$MnCl_4$	$2<IC_{50}<5$		$MnCl_4$	$1<IC_{50}<2$
	$FeCl_4$	$1<IC_{50}<2$		$FeCl_4$	$1<IC_{50}<2$
	$CoCl_4$	$1<IC_{50}<2$		$CoCl_4$	$1<IC_{50}<2$
	NTf_2	$2<IC_{50}<5$		NTf_2	$1<IC_{50}<2$
	$GdCl_6$	$1<IC_{50}<2$		$GdCl_6$	$1<IC_{50}<2$
	BF_4	$2<IC_{50}<5$		BF_4	$2<IC_{50}<5$
	DCA	>5		DCA	$1<IC_{50}<2$
	Acetate	$1<IC_{50}<2$		Acetate	$0.5<IC_{50}<1$

The obtained data is useful for predicting ILs impact in humans and based on Table 4, we can conclude that [DHEA] cation is safer than [THEA] cation and the most human friendly studied ILs are [DHEA] Cl and [DHEA] DCA.

Conclusions

In summary, it is reported a new family of Ionic Liquids (ILs), including magnetic ILs (MILs) based on cholinium like cations that are liquid at room temperature. With the introduction of an alkyl chain of six carbons and one or two ethanol chains into the cholinium like cation, it was possible to obtain liquid salts at room temperature, keeping a low toxicity for human cells. These new MILs were characterized and the magnetic susceptibilities were lower than for reported MILs, although it was possible to observe a decrease on the viscosity when compared with reported MILs.

Experimental

Materials and methods

All the solvents were distilled prior use. All chemicals were purchased from Aldrich. Magnetic Moment and Magnetic Susceptibility were determined by using a Magnetic Susceptibility Balance, from Sherwood Scientific. ^1H and ^{13}C-NMR spectra were recorded in CDC$_{l3}$ or D_2O on a Bruker ARX 400 spectrometer, at 400 and 100.62 MHz respectively. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling constants (J) are reported in Hertz (Hz). Measurements of viscosity were performed in the temperature range of 288–313 K at atmospheric pressure using an automated SVM 3000 Anton Paar rotational Stabinger viscometer–densimeter. The precision of the dynamic viscosity measurements is ±0.5%. In order to reduce water and volatile organic content, vacuum ($1\text{-}4 \times 10^{-2}$ mbar) at 50 º C was applied to all ILs for at least 48 h always prior to their use. Karl-Fischer Coulometric titration (Metrohm 831 KF Coulometer) was employed to measure the water content of ILs. ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometer) and DSC (differential scanning calorimetry) analysis were performed by Laboratório de Análises at REQUIMTE, Portugal. Elemental analysis (Flash2000 CHNS-O analyzer (ThermoScientific, UK), and ESI-MS analysis (Micromass Quattro Micro triple quadrupole (Waters, Ireland) with anelectrospray (ESI) ion source) were performed at Mass Spectrometry Service in Faculdade de Farmácia (Universidade de Lisboa,Portugal).

Synthetic procedures

General method for chloride based ILs

In flask at room temperature, was added 1-chlorohexane (17.2 g, 143 mmol), *N*-methyldiethanolamine (17.0 g, 143 mmol), or triethanolamine (21.4 g, 143 mmol), sodium iodide (2.1 g, 0.143 mmol) and MeCN (12 mL). The reaction mixture was refluxed for four days. The solvent was evaporated and the sodium salt removed by adding dichloromethane followed by filtration.

General method for bis(trifluoromethane)sulfonimide based ILs

Lithium bis(trifluoromethane)sulfonimide salt (1.1 equiv.) was added to an aqueous solution of chloride salt ([DHEA]Cl or [THEA]Cl; 10 mmol). Almost immediately were formed two phases, and the mixture was stirred at room temperature overnight. The two phases were separated, and the aqueous phase was washed with dichloromethane. The organic phases were mixed, and the solvent was removed on a rotary evaporator, and then kept under vacuum for 48 h at 1-4x10^{-2} mbar (rotatory pump) under stirring.

General method for acetate based ILs

A solution of the chloride salt ([DHEA]Cl or [THEA]Cl; 10 mmol) in methanol (30 mL) was passed through a column with Amberlite IRA-400 (OH) resin. A solution of acetic acid (1 equiv.) in methanol was slowly added to the hydroxide salt obtained from the column and the mixture stirred at room temperature for 30 minutes. The solvent and the residual acetic acid were evaporated on a rotary evaporator at 50ºC, and then kept under vacuum for 48 h at 1-4x10^{-2} mbar (rotatory pump) and 48 h to 6x10^{-5} mbar under stirring at 50ºC.

General method for dicyanamide based ILs

To a solution of the chloride salt ([DHEA]Cl or [THEA]Cl; 10 mmol) in dichloromethane/methanol 1:1 mixture (20 mL) was added sodium dicyanamide and the mixture stirred at room temperature overnight. The remain solid was filtered and the solvent was removed on a rotary evaporator, and then kept under vacuum for 48 h at 1-4x10^{-2} mbar (rotatory pump) and 48 h to 6x10^{-5} mbar under stirring at 50ºC.

General method for tetrafluorborate based ILs

A solution of chloride salt ([DHEA]Cl or [THEA]Cl; 10 mmol), in acetone (20 mL) was mixed with sodium tetrafluorborate (1.2 equiv.). The reaction mixture was stirred at room temperature overnight. The solid was filtered and the solvent was removed on a rotary evaporator, and then kept under vacuum for 48 h at 1-4x10^{-2} mbar (rotatory pump) and 48 h to 6x10^{-5} mbar under stirring at 50ºC.

General method for magnetic ILs

The MILs were prepared using reported procedures, with some modifications.[12] To a solution of chloride salt ([DHEA]Cl or [THEA]Cl; 10mmol) in methanol (20 mL) was added the metal chloride hydrated salt MCln.xH_2O (1 equiv. for $FeCl_3{\cdot}6H_2O$; 0.5 equiv for $CoCl_2{\cdot}6H_2O$ and $MnCl_2{\cdot}4H_2O$, and 0.3 equiv. for $GdCl_3{\cdot}6H_2O$). The reaction mixture was stirred overnight at room temperature. The solvent was evaporated on a rotary evaporator at 50ºC, and then kept under vacuum for 48 h at 1-4x10^{-2} mbar (rotatory pump) and 48 h to 6x10^{-5} mbar under stirring at 50ºC.

***N*-hexyl-*N,N*-2-dihydroxyethyl-N-methyl ammonium chloride – [DHEA]Cl:** quantitative; yellow liquid; ^{1}H-NMR δ (400 MHz, D_2O): 3.96 – 3.93 (m, 4H), 3.49 – 3.32 (m, 6H), 3.08 (s, 3H), 1.70 – 1.66 (m, 3H), 1.29 – 1.20 (m, 6H), 0.79 (t, J = 7.1 Hz, 3H). ^{13}C-NMR δ (101 MHz, D_2O): 63.59, 63.38, 55.19, 49.49, 30.44, 25.18, 21.76, 21.65, 13.34. Elemental Anal. Calc. ($C_{11}H_{26}ClNO_2{\cdot}0.8\ H_2O$): C, 51.97; H, 10.94, N, 5.51. Found: C,51.57; H, 10.51; N, 6.00.

***N*-hexyl-*N,N*-2-dihydroxyethyl-N-methyl ammonium bis(trifluoromethane)sulfonimide [DHEA]NTf$_2$:** yield 81.27%; yellow liquid; ^{1}H NMR δ (400 MHz, DMSO) 3.82 (m, 4H), 3.43 (m, 4H), 3.40 – 3.29 (m, 2H), 3.06 (s, 3H), 1.67 (s, 2H), 1.28 (m, 6H), 0.86 (m, 3H). ^{13}C NMR δ (101 MHz, DMSO): 122.05, 117.79, 63.68, 63.01, 55.26, 49.42, 31.04, 25.82, 22.26, 21.94, 14.09. Elemental Anal. Calc. ($C_{13}H_{26}F_6N_2O_6S_2{\cdot}0.8H_2O$): C, 31.30; H, 5.58; N, 5.62; Found: C, 31.68; H, 5.77; N, 5.66. T_g -81.19ºC.

***N*-hexyl-*N,N*-2-dihydroxyethyl-N-methyl ammonium acetate [DHEA]Acet**: yield 74.91%; brown liquid; ^{1}H NMR δ (400 MHz, D_2O): 3.89 (t, J = 4.5 Hz, 4H), 3.46 – 3.36 (m, 4H), 3.34 – 3.24 (m, 2H), 3.03 (s, 3H), 1.82 (s, 3H), 1.64 (m, 2H), 1.18 (m, 6H), 0.74 (t, J = 6.9 Hz, 3H). ^{13}C NMR δ (101 MHz, D_2O): 179.84, 63.50, 63.34, 55.08, 49.38, 30.40, 25.15, 22.68, 21.74, 21.58, 13.30. Elemental Anal. Calc. ($C_{13}H_{29}NO_4{\cdot}1{,}5H_2O$): C, 53.77; H, 11.11; N, 4.82; Found: C, 53.62; H, 10.35; N, 5.10. T_g -92.49ºC.

***N*-hexyl-*N,N*-2-dihydroxyethyl-N-methyl ammonium dicyanamide [DHEA]DCA**: yield 60.74%; yellow liquid; ^{1}H NMR δ (400 MHz, D_2O): 4.00 (t, J = 4.9 Hz, 4H), 3.55 – 3.52 (m, 4H), 3.45 – 3.39 (m, 2H), 3.14 (s, 3H), 1.77 – 1.73 (m, 2H), 1.37 – 1.29 (m, 6H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ (101 MHz, D_2O): 119.84, 63.68, 63.49, 55.24, 49.53, 30.62, 25.35, 21.95, 21.79, 13.47. Elemental Anal. Calc. ($C_{63}H_{130}IN_{17}O_{10}{\cdot}H_2O$): C, 50.96; H, 9.87; N, 18.29; Found: C, 53.39; H, 9.19; N, 16.25; T_g -93.34ºC.

***N*-hexyl-*N,N*-2-dihydroxyethyl-N-methyl ammonium tetrafluorborate [DHEA]BF$_4$**: yield 82.82%; brown, viscous liquid; ^{1}H NMR δ (400 MHz, D_2O): 4.00 (t, J = 4.8 Hz, 4H), 3.52 (td, J = 4.6, 1.9 Hz, 4H), 3.43 – 3.33 (m, 2H), 3.12 (s, 3H), 1.76 – 1.72 (m, 2H), 1.35 – 1.29 (m, 6H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ (101 MHz, D_2O): 63.62, 63.39, 55.20, 49.40, 30.49, 25.21, 21.81, 21.65, 13.31. Elemental Anal. Calc. ($C_{11}H_{26}BF_4NO_2{\cdot}H_2O$): C, 42.74; H, 9.13; N, 4.53; Found: C, 42.56; H, 8.51; N, 4,65. T_g -93.73ºC.

***N*-hexyl-*N,N*-2-dihydroxyethyl-N-methyl ammonium tetrachlorocobaltate (II) [DHEA]Co**: yield 91,56%; dark blue, viscous liquid; Elemental Anal. Calc. ($C_{22}H_{52}Cl_4CoN_2O_4{\cdot}2{,}6H_2O$): C, 40.26; H, 8.79; N, 4.27; Found: C, 40.66; H, 8.19; N, 4,66. ICP-AES (Co): Calc. 9.7%, Found 9.54%. T_g -65.9ºC.

***N*-hexyl-*N,N*-2-dihydroxyethyl-N-methyl ammonium tetrachloromanganate (II) [DHEA]Mn**: yield 92,8%; brown, viscous liquid; Elemental Anal. Calc. ($C_{22}H_{52}Cl_4MnN_2O_4{\cdot}2{,}5H_2O$): C, 40.62; H, 8.83; N, 4.31; Found: C, 40.64; H, 8.15; N, 4,68. ICP-AES (Mn) Calc. 9.10%, Found 8.84%. T_g -51.2ºC.

***N*-hexyl-*N,N*-2-dihydroxyethyl-N-methyl ammonium tretrachloroferrate (III) [DHEA]Fe**: yield 82.0%; dark brown, liquid; Elemental Anal. Calc. ($C_{11}H_{26}Cl_4FeNO_2$): C, 32.87; H,6.56; N, 3.48; Found: C, 32.97; H, 6.65; N, 3.78. ICP-AES (Fe) Calc. 13.97%, Found 14.08%. T_g -64.8ºC

***N*-hexyl-*N,N*-2-dihydroxyethyl-N-methyl ammonium hexachlorogadolinate (III) [DHEA]Gd**: yield 87.37%; brown very viscous liquid; Elemental Anal. Calc. ($C_{33}H_{78}Cl_6GdN_3O_6{\cdot}6H_2O$): C, 36.33; H, 8.31; N, 3.85; Found: C, 36.45; H, 8.02; N, 4,21. ICP-AES (Gd) Calc. 16.1%, Found 14.2%. T_g -36.7ºC.

***N*-hexyl-*N,N,N*-2-trihydroxyethyl ammonium chloride - [THEA]Cl:** 74% yield; yellow liquid; ^{1}H-NMR δ (400 MHz, D_2O): 3.96 (t, J = 4.4 Hz, 6H), 3.59 – 3.56 (m, 6H), 3.43 – 3.39 (m, 2H), 1.69 (s, 2H), 1.27 – 1.24 (m, 6H), 0.80 (t, J = 6.8 Hz, 3H). ^{13}C-NMR δ (101 MHz, D_2O): 61.06, 60.92, 55.62, 54.98, 30.38, 25.08, 21.71, 21.35, 13.23. Elemental Anal. Calc. ($C_{36}H_{84}Cl_2IN_3O_9$·1.7 H_2O): C, 46.42; H, 9.46; N, 4.51. Found: C, 46.59; H, 9.05; N, 4.55.

***N*-hexyl-*N,N,N*-2-trihydroxyethyl ammonium bis(trifluoromethane) sulfonimide [THEA]NTf$_2$**: yield 73.89%; brown viscous liquid; ^{1}H NMR δ (300 MHz, DMSO): 3.82 (m, 6H), 3.50-3.36 (m, 8H), 1.68 (m, 2H), 1.28 (m, 6H), 0.98 – 0.77 (m, 3H). ^{13}C NMR δ (101 MHz, DMSO): 126.33, 122.07, 117.80, 113.54, 61.29, 60.75, 55.12, 30.97, 25.77, 22.27, 21.73, 14.05. Elemental Anal. Calc. ($C_{14}H_{28}F_6N_2O_7S_2$): C, 32.68; H, 5.49; N, 5.44; S, 12.46; Found: C, 32.49; H, 5.61; N, 5.37; S, 11.98. T_g -58.82ºC.

***N*-hexyl-*N,N,N*-2-trihydroxyethyl ammonium acetate [THEA]Acet**: yield 87,79%; dark brown solid; ^{1}H NMR δ (400 MHz, D_2O): 3.94 – 3.86 (m, 6H), 3.54 – 3.48 (m, 6H), 3.40 – 3.29 (m, 3H), 1.81 (s, 3H), 1.64 (m, 2H), 1.25 – 1.17 (m, 6H), 0.75 (t, J = 5.8 Hz, 3H). ^{13}C NMR δ (101 MHz, D_2O): 180.80, 60.96, 60.81, 54.86, 30.31, 25.00, 23.04, 21.65, 21.23, 13.14. Elemental Anal. Calc. ($C_{14}H_{31}NO_5$·0,5H_2O): C, 55.60; H, 10.67; N, 4.63. Found: C, 55.21; H, 10.51; N, 4.99. T_g -65.70ºC.

***N*-hexyl-*N,N,N*-2-trihydroxyethyl ammonium dicyanamide [THEA]DCA**: yield 84.98%; dark brown liquid; ^{1}H NMR δ (400 MHz, D_2O): 4.08 – 3.86 (m, 6H), 3.75 – 3.55 (m, 6H), 3.52 – 3.38 (m, 2H), 1.74 (m, 2H), 1.30 (m, 6H), 0.86 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ (101 MHz, D_2O): 119.83, 61.04, 61.13, 55.05, 30.60, 25.29, 21.95, 21.53, 13.47. Elemental Anal. Calc. ($C_{14}H_{28}N_4O_3$·H_2O): C, 52.81; H, 9.50; N, 17.60; Found: C, 52.94; H, 9.17; N, 18.12. T_g -69.91ºC.

***N*-hexyl-*N,N,N*-2-trihydroxyethyl ammonium tetrafluorborate [THEA]BF$_4$**: yield 82,29%; dark brown liquid; ^{1}H NMR δ (400 MHz, D_2O): 3.95 (m, 6H), 3.57 (m, 6H), 3.47 – 3.33 (m, 3H), 1.69 (m, 2H), 1.27 (m, 6H), 0.82 (s, 2H). ^{13}C NMR δ (101 MHz, D_2O): 61.03, 60.91, 54.96, 30.41, 25.10, 21.74, 21.34, 13.26. Elemental Anal. Calc. for ($C_{12}H_{28}BF_4NO_3$·0.5H_2O): C, 43.65; N, 4.24; H, 8.85; Found: C, 43.98; N, 4.47; H, 8.58. T_g -83.01ºC.

***N*-hexyl-*N,N,N*-2-trihydroxyethyl ammonium tetrachlorocobaltate (II) [THEA]Co**: yield 89.65%; dark blue, very viscous liquid; Elemental Anal. Calc. ($C_{24}H_{56}Cl_3CoIN_2O_6$·4$H_2O$): C, 34.61; H, 7.74; N, 3.36. Found: C, 34.50; H, 7.22; N, 3.74. ICP-AES (Co) Calc. 8.83%, Found 8.64%. T_g -43.68ºC.

***N*-hexyl-*N,N,N*-2-trihydroxyethyl ammonium tetrachloromanganate (II) [THEA]Mn**: yield 90.04%; dark brown, very viscous liquid; Elemental Anal. Calc. ($C_{24}H_{56}Cl_3IMnN_2O_6$·3$H_2O$): C, 35.55; H, 7,71; N, 3.45. Found: C, 35.18; H, 7,46; N, 3.74. ICP-AES (Mn) Calc. 8.28%, Found 8.04%. T_g -41.18ºC.

***N*-hexyl-*N,N,N*-2-trihydroxyethyl ammonium tretrachloroferrate (III) [THEA]Fe**: yield 90,79%; dark brown liquid; Elemental Anal. Calc. ($C_{12}H_{28}Cl_4FeNO_3$): C, 33.36; H, 6.53; N, 3.24; Found: C, 33.90; H, 6.78; N, 3.58. ICP-AES (Fe) Calc. 13.00%, Found 12.50%. T_g -44.17ºC.

***N*-hexyl-*N,N,N*-2-trihydroxyethyl ammonium hexachlorogadolinate (III) [THEA]Gd**:yield 98,64%; dark brown liquid; Elemental Anal. Calc. ($C_{36}H_{84}Cl_5GdIN_3O_9$·12$H_2O$): C, 31.32; H, 7.88; N, 3.04. Found: C, 31.32; H, 7.12; N, 3.32. ICP-AES (Gd) Calc. 14.75%, Found 12.05%. T_g -62.71 ºC.

Cell Culture

Human colorectal adenocarcinoma cells (CaCo-2) were purchased from the American Type Culture Collection (ATCC). They were cultured in RPMI-1640 supplemented with 10% FBS and antibiotic antimyco tic solution in 75 cm^2 tissue culture flasks and in an incubator with a humidified 5% CO_2 atmosphere and at37 ºC.

Toxicity assay

CaCo-2 cells were grown until confluency in 96-well-plates and differentiated cell monolayers with 3-4 days of post-confluency were incubated with the ionic liquids at concentrations 0.5, 1, 2 and 5 mM. After an incubation period of 24 hours, cells were washed and viability was determined using the neutral red dye uptake assay, as described elsewhere.[36]

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Is described the synthesis of cholinium based Magnetic Ionic Liquids that are LIQUID AT ROOM TEMPERATURE, and are LOW-TOXIC for human cells.