Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Title: Cold crystallisation behaviour of water molecules in ionic liquids as a screening method to evaluate biocompatibility of the hydrated ionic liquids

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Materials and Methods.

Cholinium dihydrogen phosphate ([ch][dhp]), cholinium dibuthyl phosphate ([ch][dBp]), 1-butyl-3-methylimidazolium dihydrogen phosphate ([C4mim][dhp]) and cholinium dihydrogen citrate ([ch][dhC]) were synthesised by a modified method.[1] Phosphocholine was synthesised similar procedure by using ion exchange resin. Phosphocholine chloride calcium solution was treated on an anion exchange resin (Amberlite IRN77), then treated on an cation exchange resin (Tulsion-93). The solvent evaporated and the product was dried *in vacuo*. Synthesised materials were identified using ^{1}H NMR, DSC and Electrospray mass spectrometry. The melting point of [ch][dhp], [C4mim][dhp], [ch][dBp] were observed at 119, 73 and 66^{o}C, respectively. PC and [ch][dhC] did not show the melting peak by DSC measurement. Guanidine hydrochloride and other chemicals were purchased from Kanto Chemical Co. Ltd and Sigma-Aldrich Co. LLC.

All salts used in this study were solid at room temperature. No melting point was observed in neat [ch][dhp], PC and [ch][dhC] below 80 ^{o}C. Different amount of water were added to all salts to prepare the hydrated state having molar ratio from 3:1 to 20:1 (water molecules: ion pair). Thermal behaviors of these HyILs were investigated with DSC measurement (SII DSC6220).

The Raman spectra were obtained using a JASCO NRS-1000 spectrometer with a Kaiser Optical holographic super-notch filter and a liquid N_2-cooled CCD detector. Data were accumulated for 200 s with the spectral resolution of 0.6 cm^{-1}. The excitation source was a Coherent Innova 90C Kr laser with a 20 mW beam at a 413.1 nm excitation wavelength. Spectra were collected on samples in bulk condition at room temperature using a backscattering geometry. The peak frequencies were calibrated relative to an indene standard and are accurate to ±1 cm^{-1}.

References

[1] K. Fujita, D. R. MacFarlane, M. Forsyth, M. Yoshizawa-Fujita, K. Murata, N. Nakamura, H. Ohno, *Biomacromolecules* **2007**, *8*, 2080.

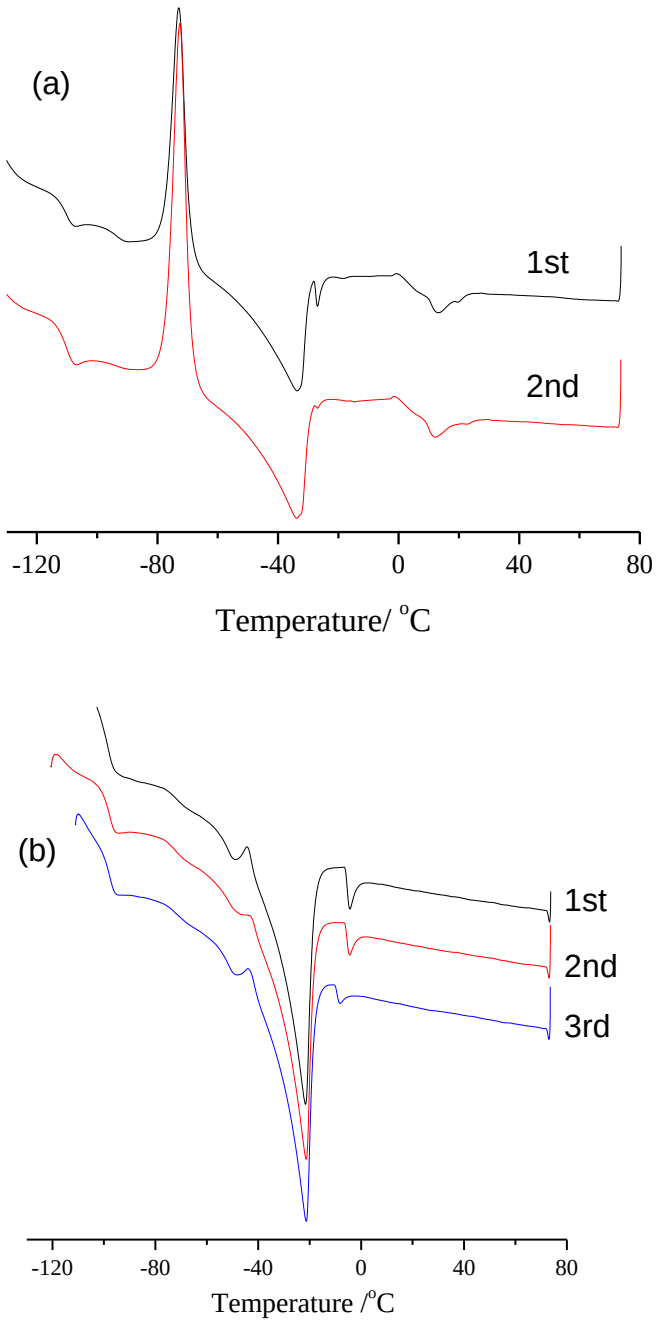


Figure S1. *Effect of cycle time on DSC charts of Hy[ch][dhp] with water contents of 7:1 (a) and Hy[ch][dBp] with water contents of 12:1 (b).*

We observed DSC charts of 1st and 2nd scan for all sample to confirm reliability of the CC behavior. When 3rd scan was observed there was almost same that of 1st and 2nd scan as shown (b). We choose 2nd cycle for the figure in this paper.

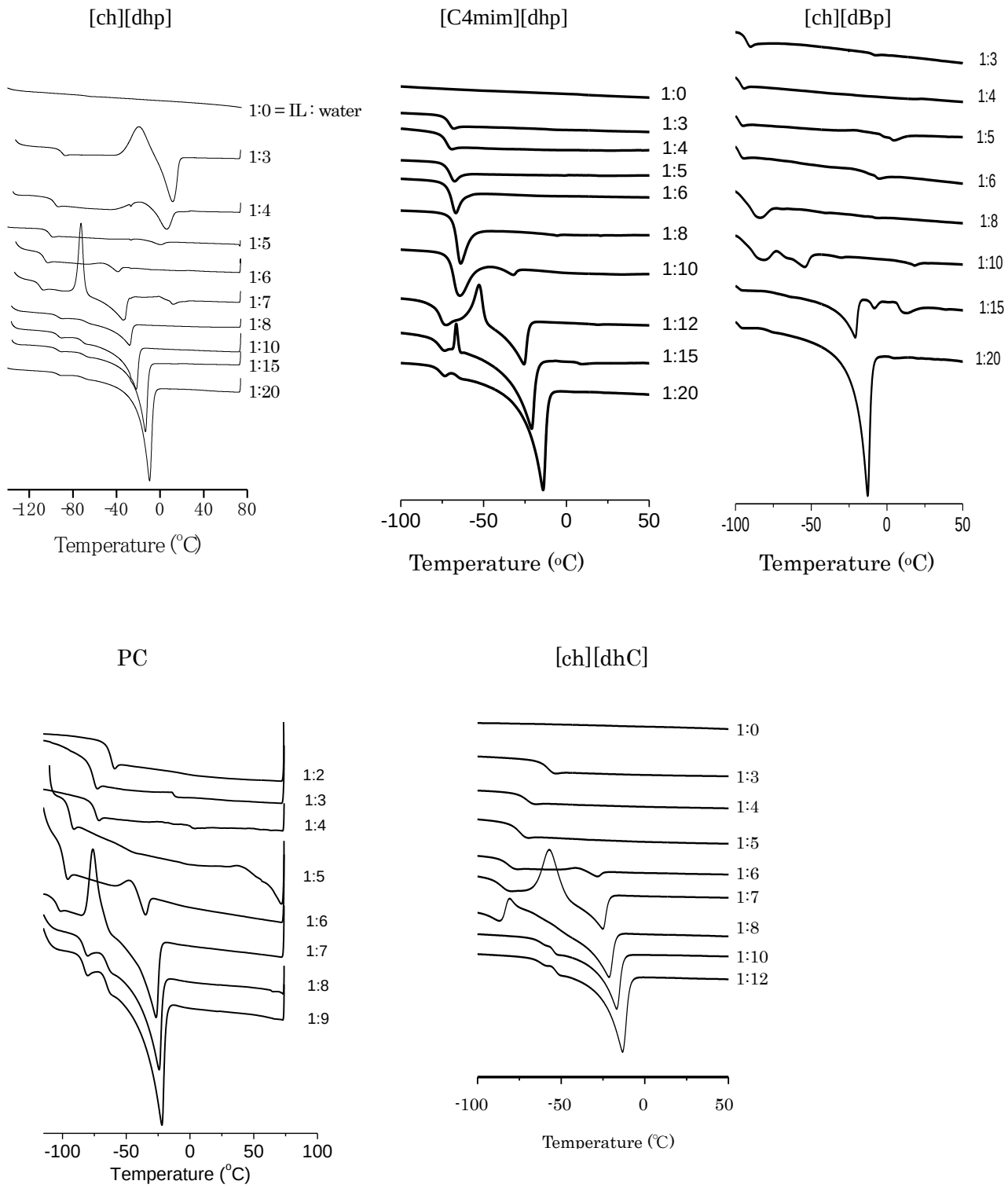


Figure S2. *DSC hearting curves of hydrated ILs with different water content.*

Samples were prepared with different amount of water. In DSC analysis, samples were heated from -150 to 80°C at a rate of 10°C/min. The change of heat quantity was recorded in the 2nd cycle. The curves of [ch][dhp], [ch][dhC] and PC shown exothermic peak based on the cold crystallization (CC) at water contents of 1:7, though no such exothermic peak at other water contents. In the case of [C4mim][dhp], similar CC behavior was observed at water contents of 1:12. On the other hand, no CC was observed in [ch][dBp] through the examined water concentration.

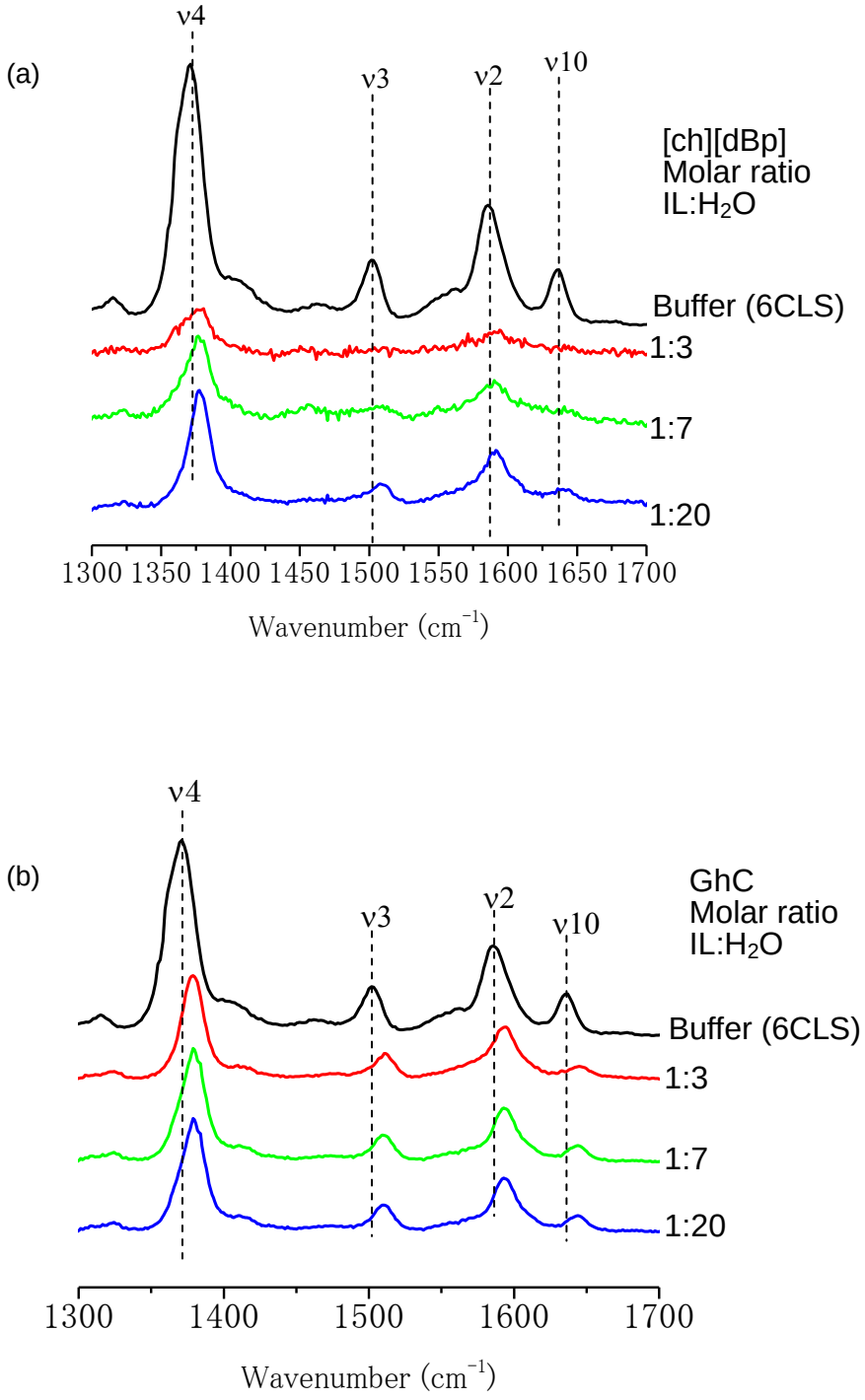


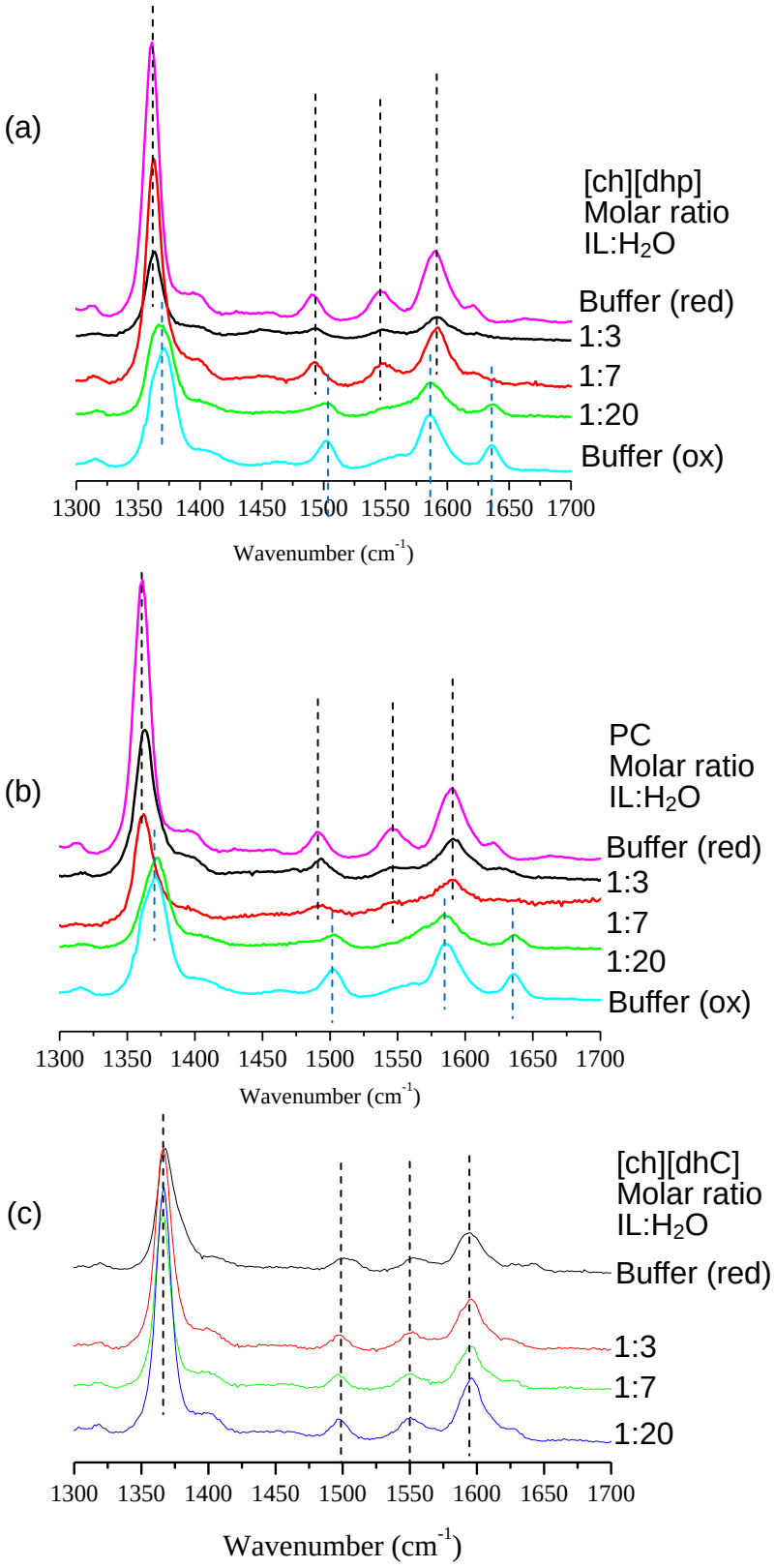
Figure S3. *RR spectra of cytochrome c dissolved in [ch][dBp](a) and GhC (b) with different water contents.*

RR spectra of cytochrome c dissolved in hydrated [ch][dBp] and hydrated *GhC* indicated different coordination state compared with native state regardless the water contents. Observed spectra were suggested ligand exchange from Met to His reported in literature (Oellerich et al., *J. Phys. Chem. B*, 2002, 6566, 106).

Table S1 Summarised wavenumbers of dissolved cytochrome c in buffer, [ch][dBp] and GhC shown in Figure S3.

	v4 /cm^{-1}	v3 /cm^{-1}	v2 /cm^{-1}	v10 /cm^{-1}
Buffer (6CLS)[a]	1373	1502	1585	1635
Buffer (5CHS) [a]	1355	1469	1592	
[ch][dBp]	1380	1504	1590	1640
GhC	1379	1511	1594	1645

[a] Scott, R. A.; Mauk, A. G. Eds. *Cytochrome c: A Multidisciplinary Approach*; University Science Press: Menlo Park, 1996.



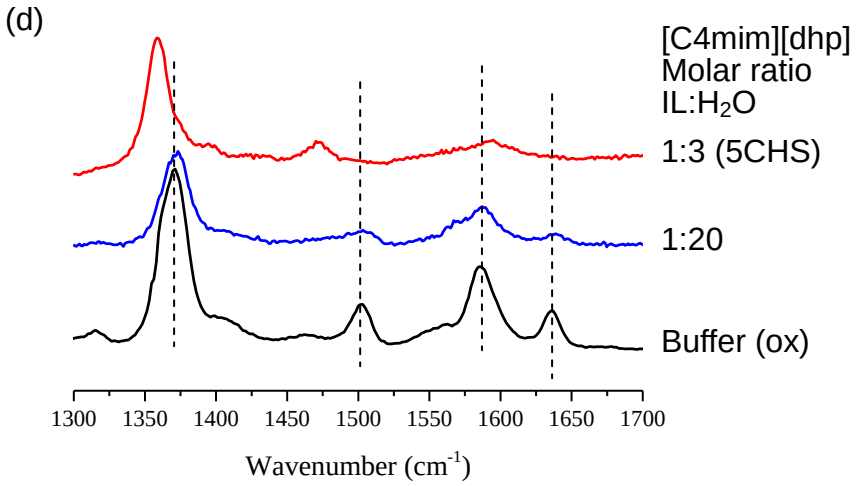
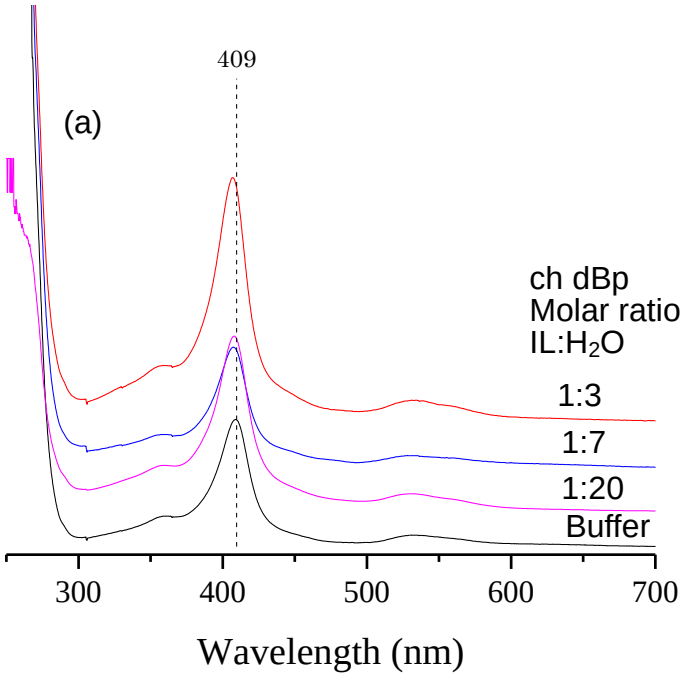
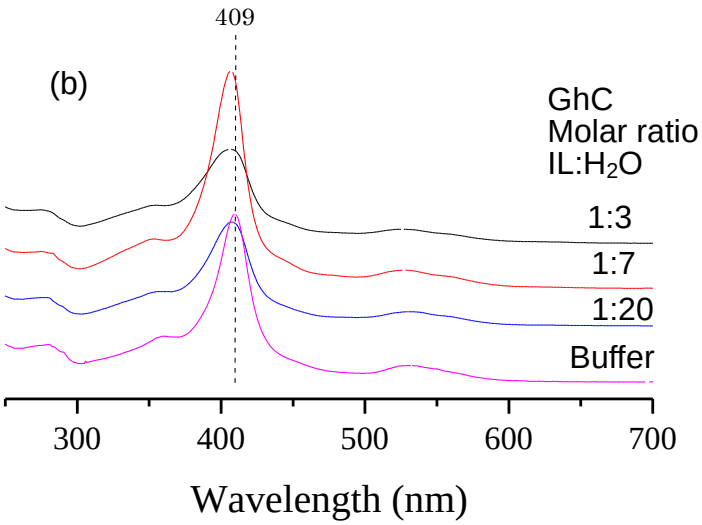


Figure S4. *RR spectra of cytochrome c dissolved in hydrated [ch][dhp](a), PC (b), [ch][dhC] (c) and [C4mim][dhp] (d) with different water contents.*

RR spectra of cytochrome c dissolved in hydrated [ch][dhp], PC, and [ch][dhC] maintained the native six coordination state with that in buffer solution regardless the water contents, though iron ion was changed from oxidised state (ox) to reduced state (red) in some IL rich mixtures (water to IL ratio of 3:1 and 7:1) during laser irradiation (413 nm) of RR measurement. RR spectrum of cytochrome c dissolved in [C4mim][dhp] with water to IL ratio of 3:1 indicated five coordinate high spin state (5CHS) of active site.





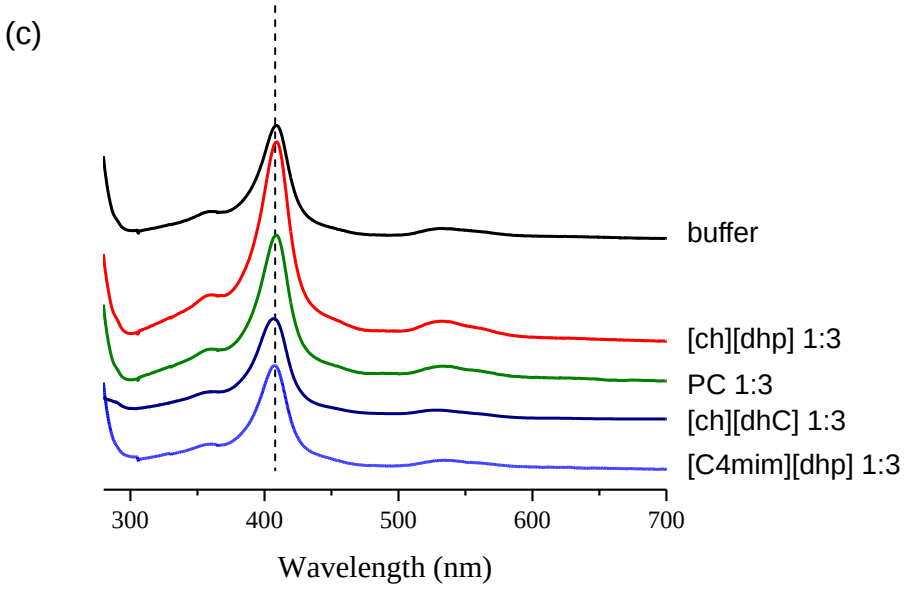


Figure S5. *UV-Vis spectra of cytochrome c dissolved in [ch][dBp](a), GhC (b) with different water contents and other hydrated ILs with water to IL ratio of 3:1 (c).*

Soret band of cytochrome c dissolved in hydrated [ch][dBp] and hydrated GhC showed blue shift regardless of water contents compared with that in phosphate buffer (409 nm). In other hydrated ILs, no shift was observed at Soret band.

Table S2 Comparison of the λ_{max} at the Soret band of cytochrome c dissolved in [ch][dBp] and GhC as shown in Figure S5.

	λ_{max} at the Soret band / nm	
	ch dBp	GhC
1:20	408	408
1:7	408	407
1:3	407	406

λ_{max} at the Soret band of cytochrome c dissolved in buffer is 409 nm.

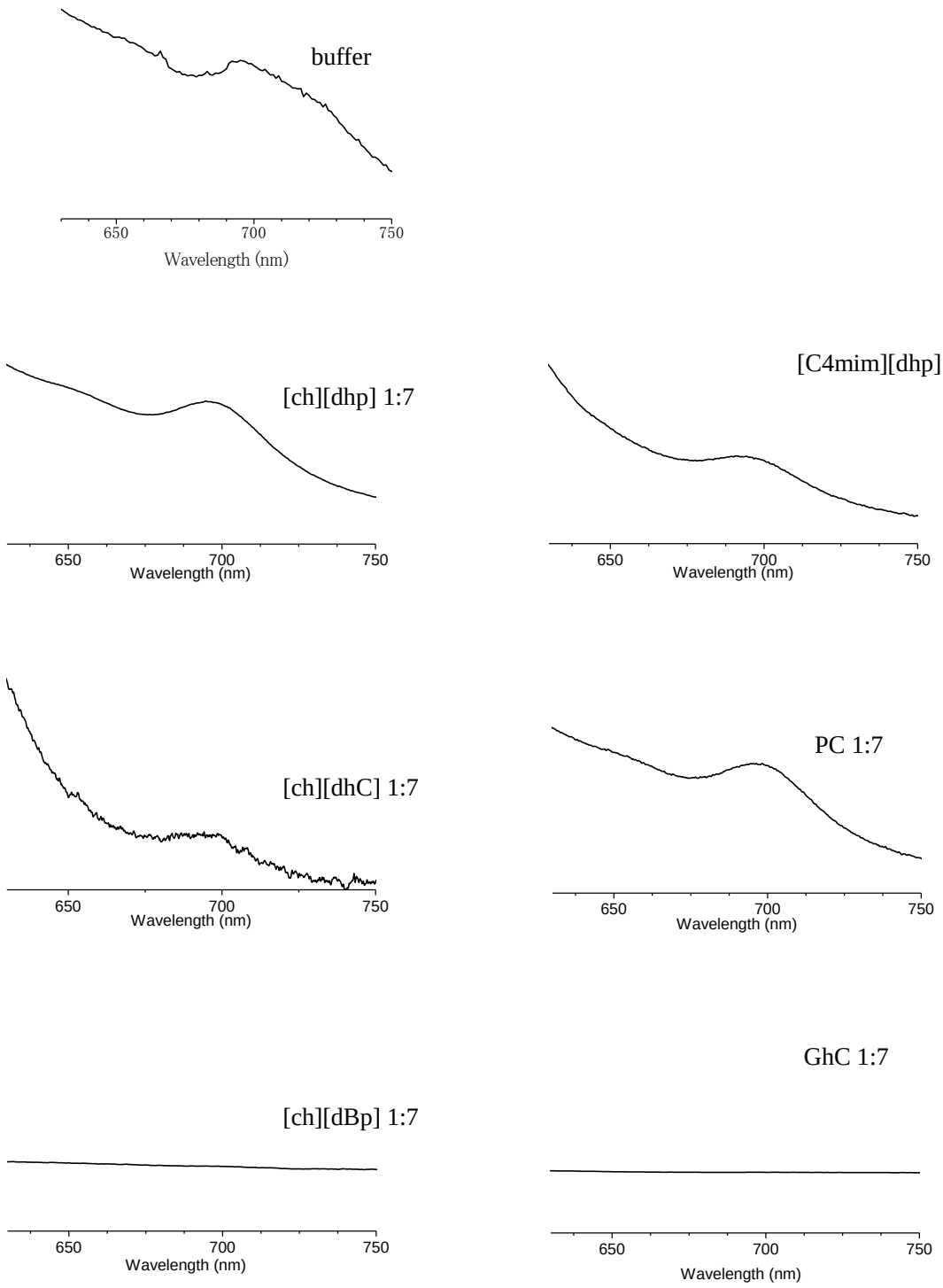


Figure S6. *UV-Vis spectra indicating charge transfer band of cytochrome c dissolved in buffer and hydrated ILs.*

Charge transfer band at 695 nm was investigated and compared with IL species. Charge transfer band was based on a native ligand in which heme is axially ligated with His 18 and Met 80 yielding a six coordinated low spin form.