

A polar liquid zwitterion does not critically destruct cytochrome c at high concentration: an initial comparative study with a polar ionic liquid

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Supplementary Material

A polar liquid zwitterion does not critically destruct cytochrome *c* at high concentration: an initial comparative study with a polar ionic liquid

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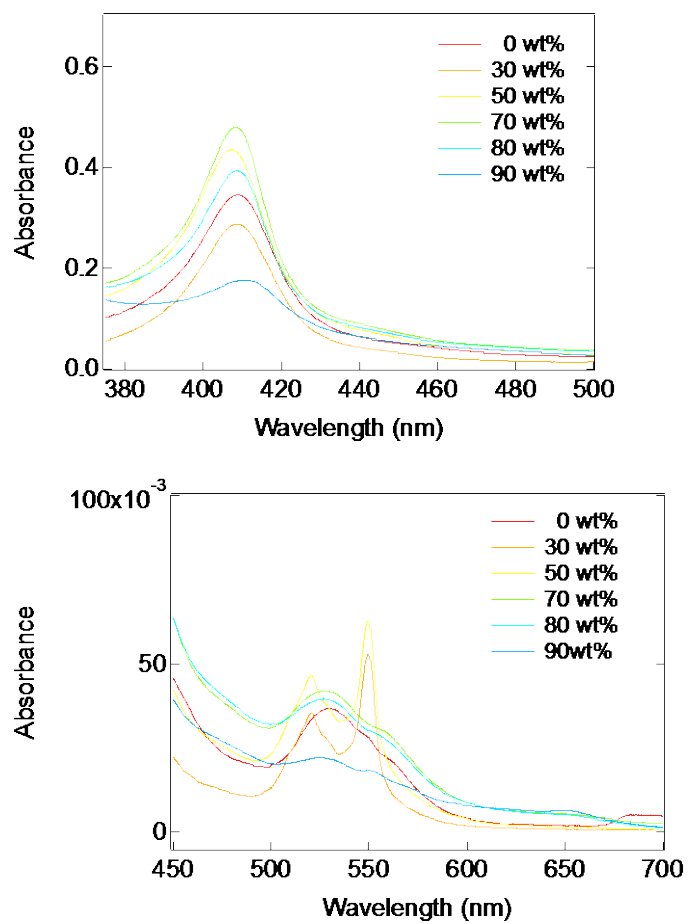


Figure S1. Spectra of cyt. *c* in solutions of OE₂imC₃C at various concentrations at room temperature.

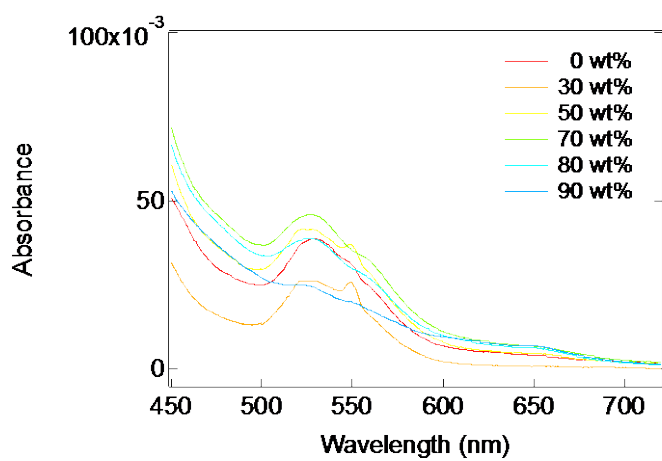
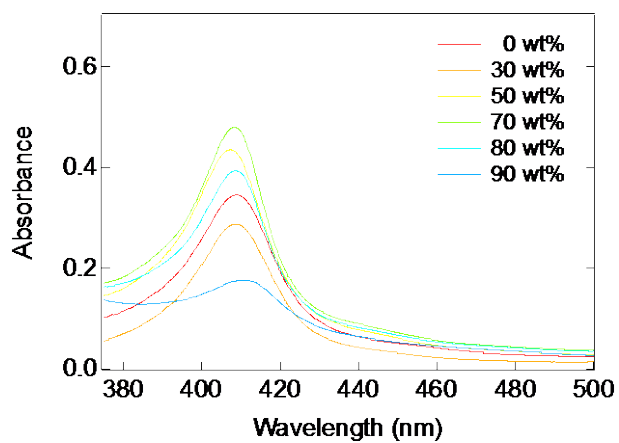


Figure S2. Spectra of cyt. *c* in solutions of OE₂imC₃C at various concentrations at room temperature after heat shock.

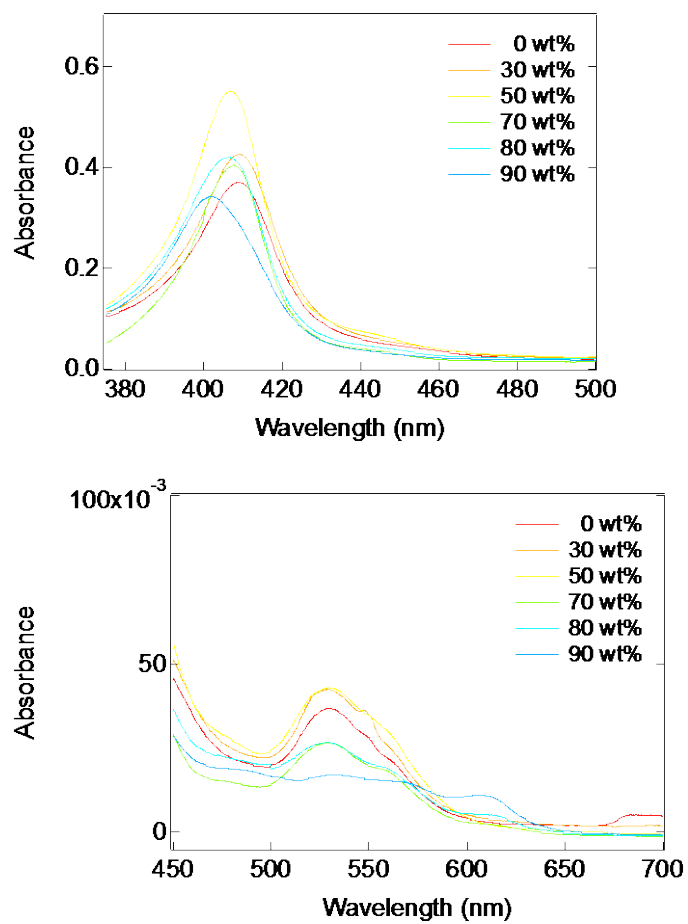


Figure S3. Spectra of *cyt. c* in solutions of [C₂mim]OAc at various concentrations at room temperature.

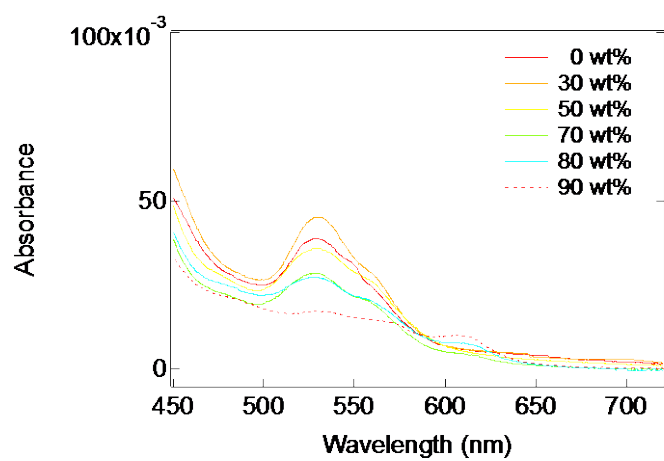
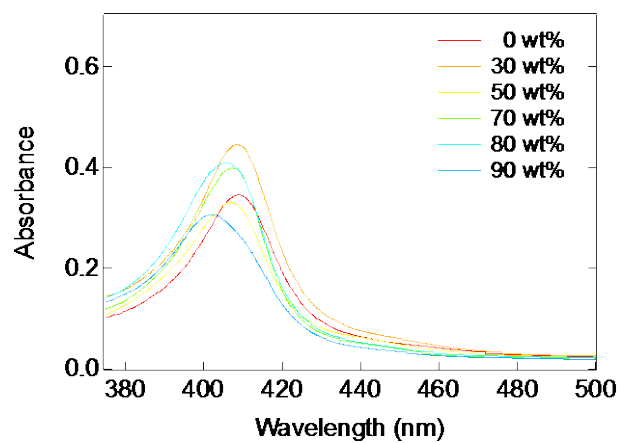


Figure S4. Spectra of *cyt. c* in solutions of [C₂mim]OAc at various concentrations at room temperature after heat shock.

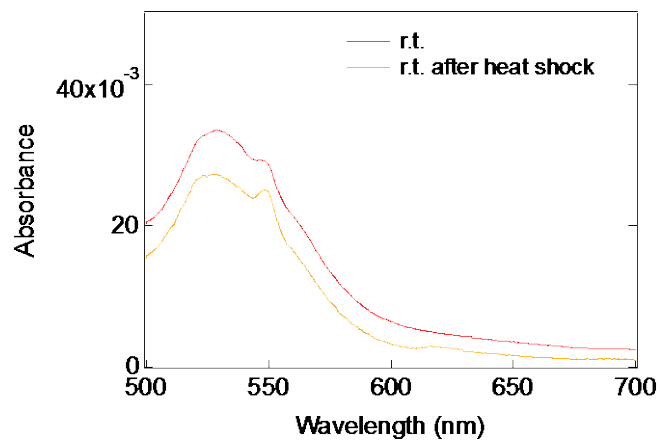


Figure S5. Spectrum (around 615 nm) of cyt. *c* in pure water at room temperature before and after heat shock.

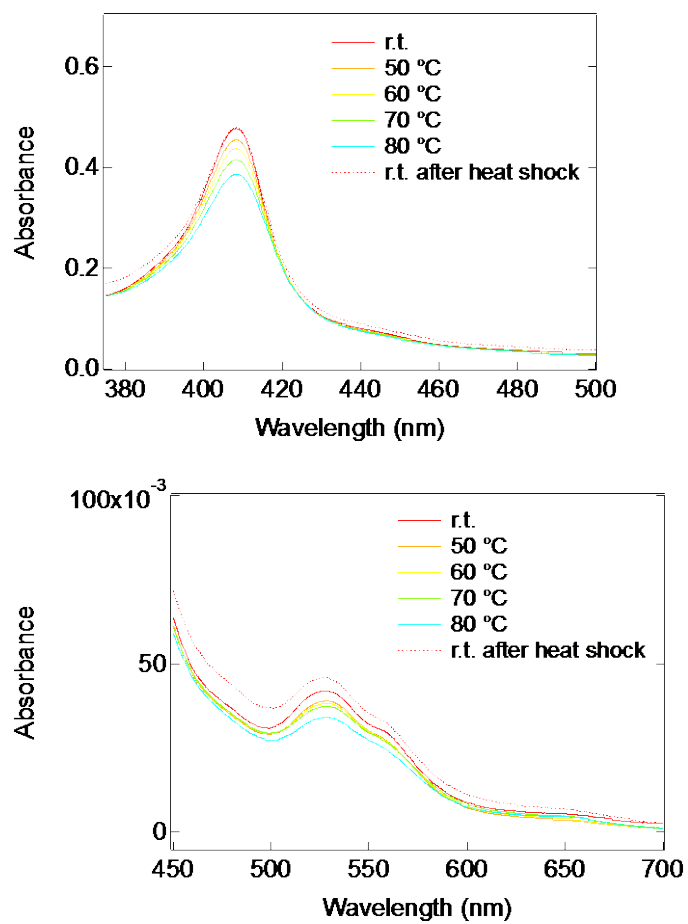


Figure S6. Spectra of cyt. *c* in 70 wt% solution of OE₂imC₃C at various temperatures.

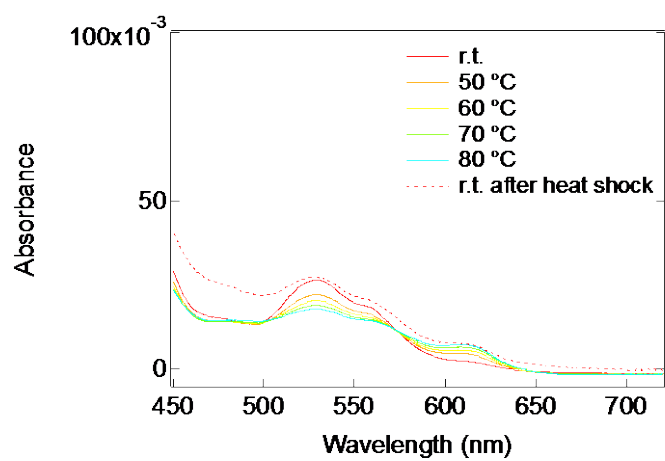
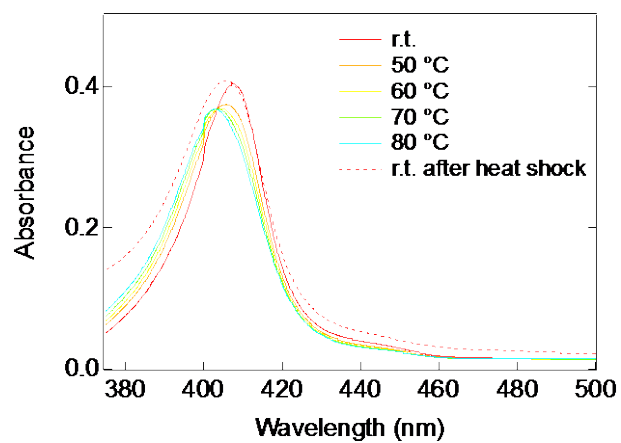


Figure S7. Spectra of *cyt. c* in 70 wt% solution of [C₂mim]OAc at various temperatures.

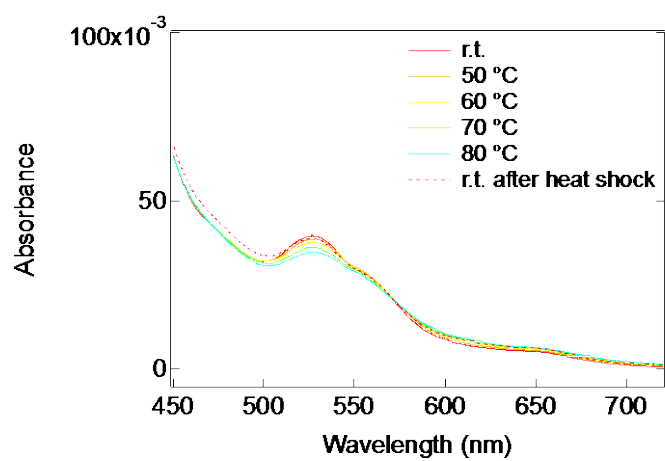
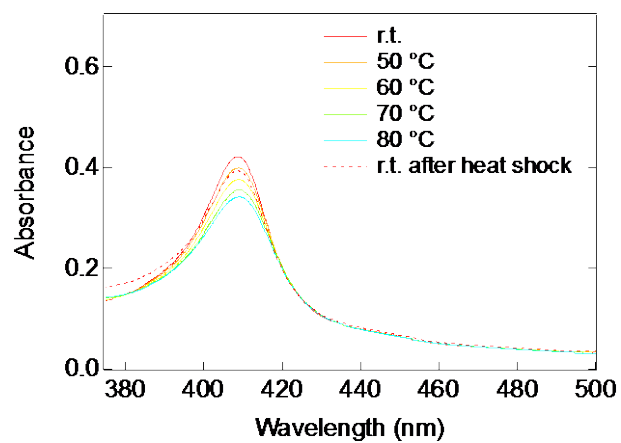


Figure S8. Spectra of *cyt. c* in 80 wt% solution of OE₂imC₃C at various temperatures.

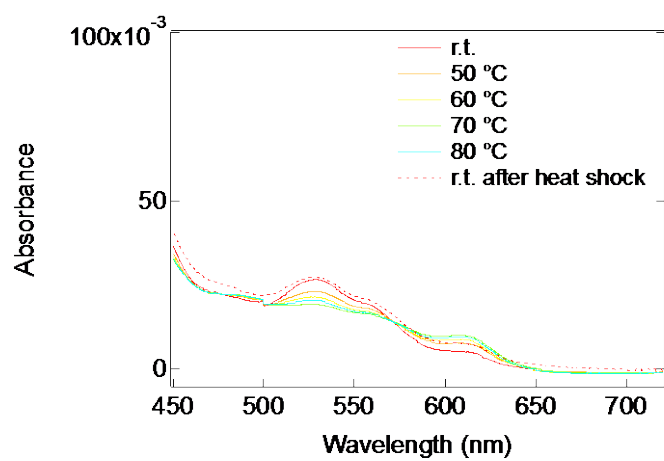
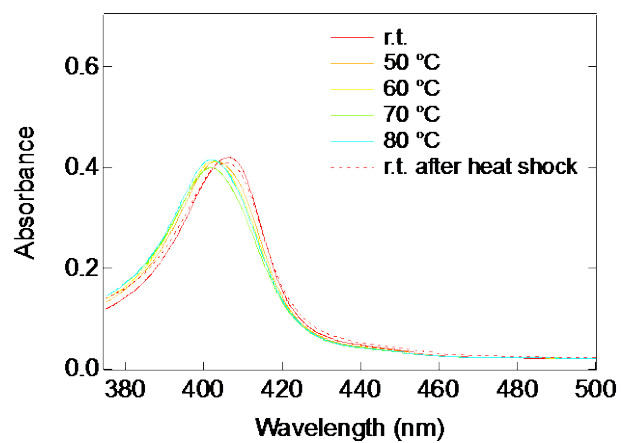


Figure S9. Spectra of *cyt.c* in 80 wt% solution of [C₂mim]OAc at various temperatures.

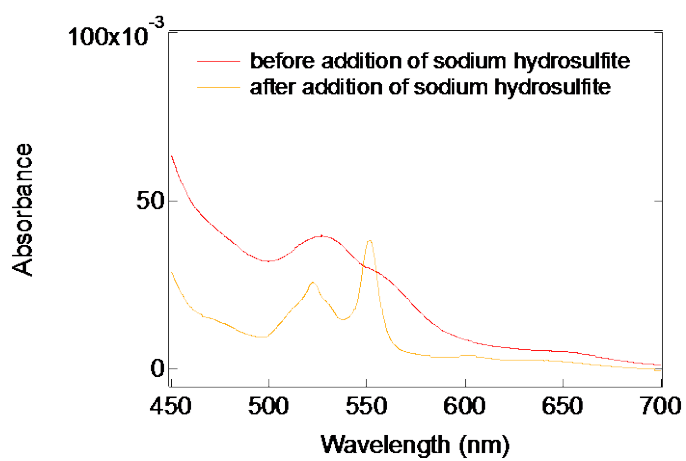
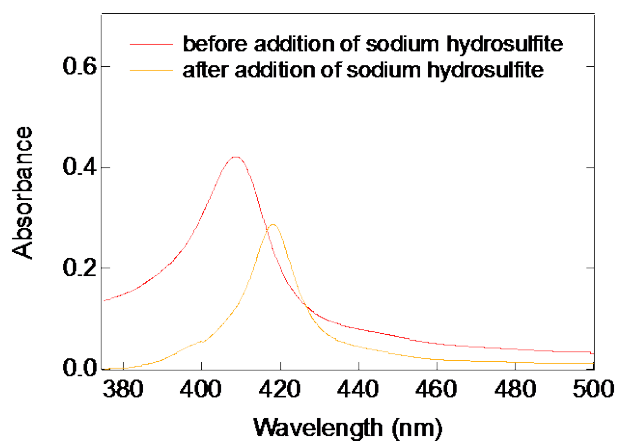


Figure S10. Spectra of cyt. *c* in 80 wt% solution of OE₂imC₃C at room temperature before and after addition of sodium hydrosulfite.

Table S1. Wavelength at the maximum absorbance (λ_{\max}) and the absorbance of the Soret-, Q-, 695 nm-, 615 nm bands of cyt.c in solutions of OE₂imC₃C or [C₂mim]OAc at various concentrations at room temperature before and after heat shock.

		Temperature & Ion concentration (wt%)												
		r.t.						r. t. ^a						
		0 % ^b	30 %	50 %	70 %	80 %	90 %	0 % ^b	30 %	50 %	70 %	80 %	90 %	
OE ₂ imC ₃ C	Soret band	λ_{\max}	408.9	412.6	410.4	408.4	408.7	411.2	409.0	408.7	407.4	408.3	408.7	410.6
		Abs	0.370	0.318	0.424	0.476	0.419	0.159	0.345	0.285	0.434	0.482	0.391	0.175
	Q band	λ_{\max}	529.0	520.7, 549.5	520.4, 549.4	527.7	527	524.6	528.7	528.0, 549.0	528.0, 548.5	526.2	526.7	522.4
		Abs	0.037	0.036, 0.053	0.046, 0.063	0.042	0.039	0.021	0.039	0.026, 0.026	0.041 0.037	0.046	0.039	0.024
	695 nm ^c	Abs	0.005	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>
	~615 nm ^c	Abs	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>
	Presumed state		III	IV	IV	III-3.5	III-3.5	III-3.5	III-3.5	3.5	3.5	III-3.5	III-3.5	III-3.5
	pH		7.0	7.7	8.2	10.1	10.9	11.5	-	-	-	-	-	-
[C ₂ mim]OAc	Soret band	λ_{\max}	408.9	409.0	406.8	407.6	406.3	401.9	409.0	408.5	407.02	407.2	406.4	401.9
		Abs	0.370	0.425	0.550	0.402	0.420	0.340	0.345	0.444	0.328	0.398	0.386	0.304
	Q band	λ_{\max}	529.0	528.7	529.3	528.5	528.3	533.2	528.7	528.9	529.3	527.7	526.5	529.8
		Abs	0.037	0.042	0.043	0.027	0.027	0.017	0.039	0.045	0.036	0.028	0.033	0.017
	695 nm ^c	Abs	0.005	0.001	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>	0.001	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>
	~615 nm ^c		<i>-d</i>	<i>-d</i>	<i>-d</i>	0.001	0.002	0.004	<i>-d</i>	<i>-d</i>	<i>-d</i>	<0.001	0.001	0.003
	Presumed state		III	III (- 3.5)	III-3.5	III-3.5	III-3.5	III-3.5	III-3.5	III-3.5	III-3.5	III-3.5	III-3.5	III-3.5
	pH		7.0	6.7	8.0	9.7	11.0	12.1	-	-	-	-	-	-

^aMeasured at room temperature after heated at 80 °C for 1min. ^bMeasured in pH 7.0 phosphate buffer. Other samples

were prepared by dilution of OE₂imC₃C or [C₂mim]OAc with pure water. ^c Calculated the difference from the

estimated baseline. ^dNot detected.

Table S2. Wavelength at the maximum absorbance (λ_{\max}) and the absorbance of the Soret-, Q-, 695 nm-, 615 nm bands of *cyt.c* in 70 and 80 wt% solutions of OE₂imC₃C or [C₂mim]OAc at various temperatures.

		Ion concentration (wt%) & Temperature (°C)												
		70 wt%						80 wt%						
		r. t.	50	60	70	80	r. t. ^a	r. t.	50	60	70	80	r. t. ^a	
OE ₂ imC ₃ C	Soret band	λ_{\max}	408.4	408.2	408.2	408.4	408.6	408.3	408.7	408.9	409.1	409.1	409.1	408.7
		x												
	Abs	0.476	0.455	0.437	0.414	0.386	0.482	0.419	0.398	0.376	0.354	0.340	0.391	
	Q band	λ_{\max}	527.7	528.3	527.9	528.8	528.7	526.2	527.0	528.5	528.5	527.8	528.3	526.7
		x												
	Abs	0.042	0.039	0.038	0.037	0.034	0.046	0.039	0.039	0.038	0.036	0.035	0.039	
	695 nm ^b	Abs	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c
~615 nm ^b	Abs	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	
Presumed state		III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	
[C ₂ mim]OAc	Soret band	λ_{\max}	407.6	405.9	404.4	403.6	403.0	407.2	406.3	403.2	402.4	401.8	402.2	406.4
		x												
	Abs	0.402	0.374	0.368	0.368	0.367	0.398	0.420	0.410	0.411	0.399	0.414	0.386	
	Q band	λ_{\max}	528.5	528.4	528.4	528.4	528.1	527.7	528.3	528.6	526.1	526.5	526.4	526.5
		x												
	Abs	0.027	0.022	0.020	0.019	0.018	0.028	0.027	0.023	0.021	0.019	0.020	0.033	
	695 nm ^b	Abs	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c	– ^c
~615 nm ^b	Abs	0.001	0.001	0.002	0.002	0.003	<0.001	0.002	0.002	0.003	0.004	0.004	0.001	
Presumed state		III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	III–3.5	

^aMeasured at room temperature after heated at 80 °C for 1min. ^bCalculated the difference from the estimated baseline.

^cNot detected.