

Supplementary Material

Glycine-betaine ionic liquid analogues as novel phase-forming components of aqueous biphasic systems

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Experimental section

The determination of the TLs was accomplished by applying the following system of four equations (Equations (S1) to (S4)) to determine four unknown values ($[IL]_{IL}$, $[IL]_{salt}$, $[salt]_{IL}$ and $[salt]_{salt}$):

$$[IL]_{IL} = A \exp[(B[salt]_{IL}^{0.5}) - (C[salt]_{IL}^3)] \quad (S1)$$

$$[IL]_{salt} = A \exp[(B[salt]_{salt}^{0.5}) - (C[salt]_{salt}^3)] \quad (S2)$$

$$[IL]_{IL} = \frac{[IL]_M}{\alpha} - \left(\frac{1-\alpha}{\alpha}\right) [IL]_{salt} \quad (S3)$$

$$[salt]_{IL} = \frac{[salt]_M}{\alpha} - \left(\frac{1-\alpha}{\alpha}\right) [salt]_{salt} \quad (S4)$$

where the subscripts "IL", "salt" and "M" represent the top and the bottom phases and the mixture composition, respectively. The parameter α is the ratio between the weight of the top phase and the weight of the overall mixture. For the calculation of each tie-line length (TLL), Equation S5 was used:

$$TLL = \sqrt{([salt]_{IL} - [salt]_{salt})^2 + ([IL]_{IL} - [IL]_{salt})^2} \quad (S5)$$

Results

Characterization of the synthesized AGB-ILs

- Triethyl[2-ethoxy-2-oxoethyl]ammonium bromide ([Et₃NC₂]Br)

¹H NMR (250 MHz, DMSO-d₆): 1.28 (m, 12 H), 3.53 (q, 6 H), 4.16 (s, 2 H), and 4.27 (q, 2 H);

- Tri(n-propyl)[2-ethoxy-2-oxoethyl]ammonium bromide ([Pr₃NC₂]Br)

¹H NMR (250 MHz, DMSO-d₆): 0.7 (t, 9H), 1.1 (t, 3H), 1.55 (m, 6H), 4.00 (s, 2H) and 4.41 (q, 2H);

- Tri(n-butyl)[2-ethoxy-2-oxoethyl]ammonium bromide ([Bu₃NC₂]Br)

¹H NMR (250 MHz, DMSO-d₆): 0.93 (t, 9 H), 1.25 (t, 3 H), 1.30 (q, 6 H), 1.62 (m, 6 H), 3.43 (m, 6 H), 4.23 (s, 2 H), and 4.38 (q, 2 H);

- N-(1-methylpyrrolidyl)-2-ethoxy-2-oxoethyl]ammonium bromide ([MepyrNC₂]Br)

¹H NMR(250MHz, DMSO-d₆): 1.20 (t, 3H), 2.10 (s, 4 H), 3.14 (s, 3 H), 3.60 (m, 4 H), 4.20 (s, 2 H), and 4.50 (q, 2 H);

- Tri(n-butyl)[2-ethoxy-2-oxoethyl]phosphonium bromide ([Bu₃PC₂]Br)

¹H NMR (300 MHz, DMSO-d₆): 0.93 (t, 9H), 1.25 (t, 3H), 1.30 (q, 6H), 1.62 (m, 6H), 3.43 (m, 6H), 4.23 (s, 2H), 4.38 (q, 2H).

Table S1. EC₅₀ values (mg·L⁻¹) with the respective 95% confidence limits (lower limit; upper limit) of the studied ILs after 5 and 30 minutes of exposure of the marine bacterium *Vibrio fischeri*.

IL	EC₅₀ (mg·L⁻¹) at 5 min	(lower limit; upper limit)
[MepyrNC ₂]Br	1750.9	1326.29; 2175.59
[Et ₃ NC ₂]Br	7112.7	6873.45; 7551.94
[Pr ₃ NC ₂]Br	4277.3	3361.29; 5193.38
[Bu ₃ NC ₂]Br	1270.6	1229.82; 1311.45
[Bu ₃ PC ₂]Br	2034.6	1412.68; 2656.59
[P ₄₄₄₄]Br*	216.0	21.60; 1382.40
[N ₄₄₄₄]Br	233.3	223.36; 243.15
[C ₄ mim]Br*	1651.7	1228.03; 2221.48
IL	EC₅₀ (mg·L⁻¹) at 15 min	(lower limit; upper limit)
[MepyrNC ₂]Br	1593.4	906.39; 2280.51
[Et ₃ NC ₂]Br	6475.6	969.92; 1311.63
[Pr ₃ NC ₂]Br	2449.8	828.40; 4071.29
[Bu ₃ NC ₂]Br	513.5	367.38; 659.59
[Bu ₃ PC ₂]Br	2113.6	1322.66; 2904.51
[P ₄₄₄₄]Br*	172.80	0.00; 3218.40
[N ₄₄₄₄]Br	160.2	143.55; 176.87
[C ₄ mim]Br*	735.93	494.69; 1094.83
IL	EC₅₀ (mg·L⁻¹) at 30 min	(lower limit; upper limit)
[MepyrNC ₂]Br	1590.0	1165.35; 2014.71
[Et ₃ NC ₂]Br	4634.8	3823.66; 5502.15
[Pr ₃ NC ₂]Br	1589.8	673.78; 2505.87
[Bu ₃ NC ₂]Br	340.0	299.20; 380.83
[Bu ₃ PC ₂]Br	2141.9	1519.93; 2763.84
[P ₄₄₄₄]Br*	-	-
[N ₄₄₄₄]Br	128.6	
[C ₄ mim]Br*	-	-

*Values taken from Ventura SPM, Marques CS, Rosatella AA, Afonso CAM, Gonçalves F, Coutinho JAP. Toxicity assessment of various ionic liquid families towards *Vibrio fischeri* marine bacteria. *Ecotoxicol Environ Saf.* 2012;76 (1):162-168.

Table S2. Experimental weight fraction data for the systems composed of IL (1) + Na₂SO₄ (2) + H₂O (3) at 25°C.

[MepyrNC ₂]Br		[Et ₃ NC ₂]Br		[Pr ₃ NC ₂]Br		[Bu ₃ NC ₂]Br	
100 w ₁	100 w ₁	100 w ₂	100 w ₂	100 w ₁	100 w ₂	100 w ₁	100 w ₂
50.3730	2.7154	49.1203	2.3680	42.7144	2.2365	13.6713	12.0942
46.9720	3.3929	46.7899	2.8111	41.4645	2.4928	15.7194	11.2106
45.2233	3.8143	44.8294	3.2435	40.0246	2.7756	17.6766	10.4290
44.0663	4.1646	43.3685	3.5852	39.1167	3.0236	19.2316	9.8090
42.3150	4.6771	40.9810	4.1975	37.7549	3.3447	20.2670	9.0821
40.1111	5.4507	38.8827	4.8770	36.4336	3.6577	21.2023	8.6881
38.4450	6.0902	36.9211	5.5339	34.9715	4.0738	21.6827	8.4006
36.6415	6.7211	34.9656	6.2954	33.5973	4.4774	22.3581	8.0770
34.4708	7.6320	33.5676	6.9598	32.2404	4.9226	23.4242	7.6979
33.1378	8.3559	31.6004	7.8192	30.7546	5.4067	23.7964	7.4287
		29.1670	8.9985	29.7898	5.7869	24.9692	7.0591
				28.7305	6.1966	25.7420	6.7887
				27.6955	6.6495	26.4669	6.4900
				26.5713	7.1643	27.0642	6.2875
				25.1633	7.7507	27.7519	6.0275
				24.0998	8.3795	28.3946	5.7589
				23.1188	8.8211	29.3150	5.5259
				22.4079	9.2350	31.1238	4.7654
				21.3152	10.0477	31.9612	4.5799
				20.3311	10.9583	33.0661	4.3011
						34.3332	4.0105
						35.2187	3.7924
						36.3686	3.5531
						37.4250	3.3252

Table S3. Experimental weight fraction data for the systems composed of IL (1) + Na₂SO₄ (2) + H₂O (3) at 25°C.

[Bu ₃ PC ₂]Br		[C ₄ mim]Br		[N ₄₄₄₄]Br		[P ₄₄₄₄]Br	
100 w ₁	100 w ₁	100 w ₂	100 w ₂	100 w ₂	100 w ₂	100 w ₂	100 w ₂
31.0361	4.2511	62.4113	1.0174	50.2958	0.8363	30.0017	4.4432
29.7960	4.3385	56.5756	1.4512	48.4898	1.0909	27.9674	5.0055
28.4314	4.6051	47.7396	2.4727	46.5244	1.3171	25.6062	5.5881
26.8843	5.0331	42.7477	3.6575	44.8226	1.4892	24.3047	5.9098
25.1688	5.4887	45.1938	2.9366	39.4603	2.3631	22.7542	6.4117
23.9059	5.8683	41.5077	3.7749	37.5272	2.6499	21.1874	6.8870
22.4596	6.2975	40.1937	4.1412	31.2435	4.7123	20.1487	7.1607
20.6246	6.8615	39.8571	4.3160	29.6738	5.1157	19.4578	7.3901
18.5541	7.5297	38.7764	4.5713	28.2428	5.5901	18.7170	7.7043
16.6607	8.1858	37.1573	5.1101	26.8206	6.2750	17.7847	8.0131
14.4647	8.9694	36.2823	5.4377	24.8826	6.8159	16.9717	8.3237
11.6045	10.1799	34.9923	5.8486	22.2513	7.7082	15.7334	8.7593
		33.4746	6.4890	20.5019	8.3928	15.0418	8.9604
		33.3805	6.5190	18.4613	9.2310	14.2612	9.2652
		32.0494	7.1121	16.9185	9.9135	13.2128	9.7471
		30.5096	7.8114	15.0941	10.7357	12.5679	9.9820
		29.5487	8.3329	14.2588	11.1167	11.9578	10.2655
		27.9840	9.0948	13.4593	11.4959	11.3610	10.5312
		26.6234	9.8307	12.9936	11.7361	10.7083	10.8800
		25.5277	10.5116	12.3809	12.0523	9.8111	11.3371
		24.2651	11.2708	11.9972	12.2391		
		21.9512	12.5731	11.5267	12.4887		
		19.6937	14.1217	11.0448	12.7539		

Table S4. Correlation parameters of Eq. (1) used to describe the experimental binodal data at 25 °C.

IL	$A \pm \sigma$	$B \pm \sigma$	$10^5 (C \pm \sigma)$	R^2
IL + Na₂SO₄ + water				
[MepyrNC ₂]Br	87.01 ± 10.21	-0.340 ± 0.064	0.67 ± 14.17	0.9843
[Et ₃ NC ₂]Br	82.73 ± 9.33	-0.347 ± 0.065	4.75 ± 8.60	0.98196
[Pr ₃ NC ₂]Br	77.21 ± 4.41	-0.402 ± 0.031	3.92 ± 19.91	0.9995
[Bu ₃ NC ₂]Br	79.41 ± 6.04	-0.421 ± 0.037	16.29 ± 4.79	0.9920
[Bu ₃ PC ₂]Br	99.21 ± 16.00	-0.542 ± 0.083	36.27 ± 12.31	0.9956
[C ₄ mim]Br	91.01 ± 2.58	-0.396 ± 0.015	0.26 ± 1.74	0.9972
[N ₄₄₄₄]Br	68.19 ± 1.91	-0.358 ± 0.018	27.27 ± 3.12	0.9963
[P ₄₄₄₄]Br	99.88 ± 12.38	-0.565 ± 0.08	29.71 ± 18.63	0.9918

Table S5. Experimental TLs and TLLs of the ABS composed of IL + Na₂SO₄ + H₂O at 25°C.

Weight fraction composition / (wt %)							
IL + Na ₂ SO ₄ + water							
IL	[IL] _{IL}	[salt] _{IL}	[IL] _M	[salt] _M	[IL] _{salt}	[salt] _{salt}	TLL
[MepyrNC ₂]Br	34.46	7.38	30.94	10.04	18.62	19.31	19.83
	37.43	6.14	32.44	10.07	15.33	23.54	28.12
	39.09	5.53	34.86	8.78	14.96	24.08	30.44
	41.10	4.86	38.55	6.90	12.70	27.57	36.36
	41.58	4.71	35.11	10.10	11.34	29.91	39.36
[Et ₃ NC ₂]Br	33.73	6.49	28.21	10.01	10.63	21.21	27.40
	38.60	4.76	32.35	8.69	7.67	24.17	36.52
	40.06	4.32	30.95	10.04	6.72	25.26	39.37
	42.93	3.54	32.22	10.29	5.09	27.36	44.72
	44.81	3.10	36.03	9.20	3.15	32.73	51.95
[Pr ₃ NC ₂]Br	33.81	4.48	24.90	10.07	8.62	20.84	30.20
	38.85	2.91	28.02	10.10	6.96	23.00	39.89
	42.61	2.18	32.81	8.21	5.61	24.97	43.45
	46.39	1.60	39.20	6.51	2.30	31.70	53.39
	50.04	1.16	42.21	6.67	1.22	35.49	59.68
[Bu ₃ NC ₂]Br	41.83	2.30	29.93	7.76	3.30	19.97	42.39
	50.58	1.15	40.36	5.57	1.78	22.30	53.18
	55.73	0.71	39.65	7.43	1.24	23.52	59.08
	62.58	0.32	39.90	10.05	0.36	27.03	67.71
	68.05	0.13	49.79	7.65	0.23	28.05	73.33
[Bu ₃ PC ₂]Br	52.89	1.13	30.19	7.56	3.17	15.21	51.68
	58.01	0.80	39.77	5.77	2.50	15.93	57.54
	54.76	0.48	39.46	7.76	0.76	18.91	66.59
	73.42	0.21	39.79	9.88	0.24	21.26	76.15
	74.63	0.18	50.05	7.76	0.08	23.17	78.02

Table S5. (cont.) Experimental TLs and TLLs of the ABS composed of IL + Na₂SO₄ + H₂O at 25°C.

Weight fraction composition / (wt %)							
IL + Na ₂ SO ₄ + water							
IL	[IL] _{IL}	[salt] _{IL}	[IL] _M	[salt] _M	[IL] _{salt}	[salt] _{salt}	TLL
[C ₄ mim]Br	30.14	7.78	27.82	10.03	11.84	25.48	25.46
	34.83	5.89	30.04	9.96	10.65	27.90	30.22
	37.49	5.02	35.21	7.05	9.92	29.54	36.89
	38.60	4.70	36.99	6.37	7.44	36.26	44.35
	40.80	4.11	34.81	9.91	7.37	37.10	46.53
[N ₄₄₄₄]Br	44.04	1.48	29.83	7.68	1.55	20.00	46.35
	51.20	0.64	39.62	5.51	0.72	21.92	54.77
	56.54	0.27	37.74	7.52	0.20	24.56	62.35
	63.40	0.04	39.40	10.18	0.06	26.81	68.78
	68.24	0.01	49.76	7.64	0.03	28.27	73.86
[P ₄₄₄₄]Br	53.87	1.19	30.51	7.73	3.94	15.16	51.85
	56.31	1.03	39.58	5.69	3.27	15.81	55.06
	66.06	0.54	39.25	8.45	0.81	19.79	68.03
	67.38	0.49	34.93	9.99	0.26	20.95	73.30
	70.25	0.38	48.00	7.34	0.26	22.30	75.16

Table S6. Extraction efficiencies (*EE%*) of the studied ABS for L-tryptophan, L-phenylalanine, D-phenylalanine, L-tyrosine, and L-dopa, and respective standard deviation (σ), and weight fraction compositions of the initial mixtures at 25 °C.

IL	Weight fraction composition / (wt %)		<i>EE%</i> _{TRP} ± σ	<i>EE%</i> _{L-PHE} ± σ	<i>EE%</i> _{D-PHE} ± σ	<i>EE%</i> _{TYR} ± σ	<i>EE%</i> _{L-DOPA} ± σ	pH	
	IL	Na ₂ SO ₄	pKa = 2.54, 9.38	pKa = 2.47, 9.45	pKa = 2.47, 9.45	pKa = 2.00, 9.19	pKa = 1.65, 9.06	IL-rich phase	NaSO ₄ -rich phase
			pI = 5.96*	pI = 5.96*	pI = 5.96*	pI = 5.60*	pI = 5.36*		
[MepyrNC ₂]Br	39.87 ± 0.02	7.53 ± 0.03	98.91 ± 0.05	96.95 ± 0.67	97.40 ± 0.42	96.70 ± 0.62	94.37 ± 0.19	3.31 ± 0.01	3.14 ± 0.01
[Et ₃ NC ₂]Br	39.67 ± 0.05	7.53 ± 0.01	98.86 ± 0.20	93.83 ± 0.13	96.21 ± 0.62	97.06 ± 1.29	91.74 ± 0.52	4.76 ± 0.05	4.84 ± 0.08
[Pr ₃ NC ₂]Br	39.95 ± 0.01	7.51 ± 0.02	98.87 ± 0.09	93.76 ± 0.15	93.86 ± 0.41	94.70 ± 0.31	89.88 ± 0.42	4.40 ± 0.01	4.23 ± 0.07
[Bu ₃ NC ₂]Br	40.09 ± 0.10	7.57 ± 0.04	97.45 ± 0.18	84.02 ± 0.07	83.64 ± 0.07	88.59 ± 1.73	79.26 ± 1.21	5.12 ± 0.16	6.24 ± 0.05
[Bu ₃ PC ₂]Br	39.94 ± 0.13	7.55 ± 0.04	93.66 ± 0.01	79.42 ± 1.25	72.05 ± 0.18	79.42 ± 1.25	64.66 ± 0.60	3.61 ± 0.05	3.24 ± 0.04
[C ₄ mim]Br	37.07 ± 0.02	6.36 ± 0.01	99.44 ± 0.04	99.57 ± 0.01	98.70 ± 0.04	99.15 ± 0.03	98.00 ± 0.01	4.96 ± 0.01	4.21 ± 0.06
[N ₄₄₄₄]Br	39.48 ± 0.29	7.24 ± 0.01	98.35 ± 0.14	88.95 ± 0.03	89.22 ± 0.12	90.77 ± 0.79	83.57 ± 0.24	5.15 ± 0.20	5.22 ± 0.05
[P ₄₄₄₄]Br	29.53 ± 0.18	7.38 ± 0.27	96.02 ± 0.15	76.88 ± 0.19	76.06 ± 0.30	82.03 ± 0.01	68.16 ± 0.21	3.45 ± 0.03	3.19 ± 0.01

* ChemSpider – The Free Chemical Database. <www.chemspider.com>

Table S7. Extraction efficiencies (*EE%*) of the studied ABS for L-tryptophan and L-dopa, and respective standard deviation (σ), and weight fraction compositions of the initial mixtures at 25 °C.

IL	Weight fraction		<i>EE%</i>_{TRP} ± σ	<i>EE%</i>_{L-DOPA} ± σ
	composition / (wt %)			
	IL	Na₂SO₄		
	49.95 ± 0.01	7.70 ± 0.01	99.11 ± 0.04	81.84 ± 0.14
	40.09 ± 0.10	7.57 ± 0.04	97.45 ± 0.18	79.26 ± 0.80
[Bu ₃ NC ₂]Br	29.90 ± 0.01	7.61 ± 0.01	94.83 ± 0.41	74.85 ± 0.01
	39.50 ± 0.01	5.63 ± 0.01	98.17 ± 0.15	83.67 ± 0.52
	39.81 ± 0.01	9.99 ± 0.01	98.06 ± 0.05	69.56 ± 0.54

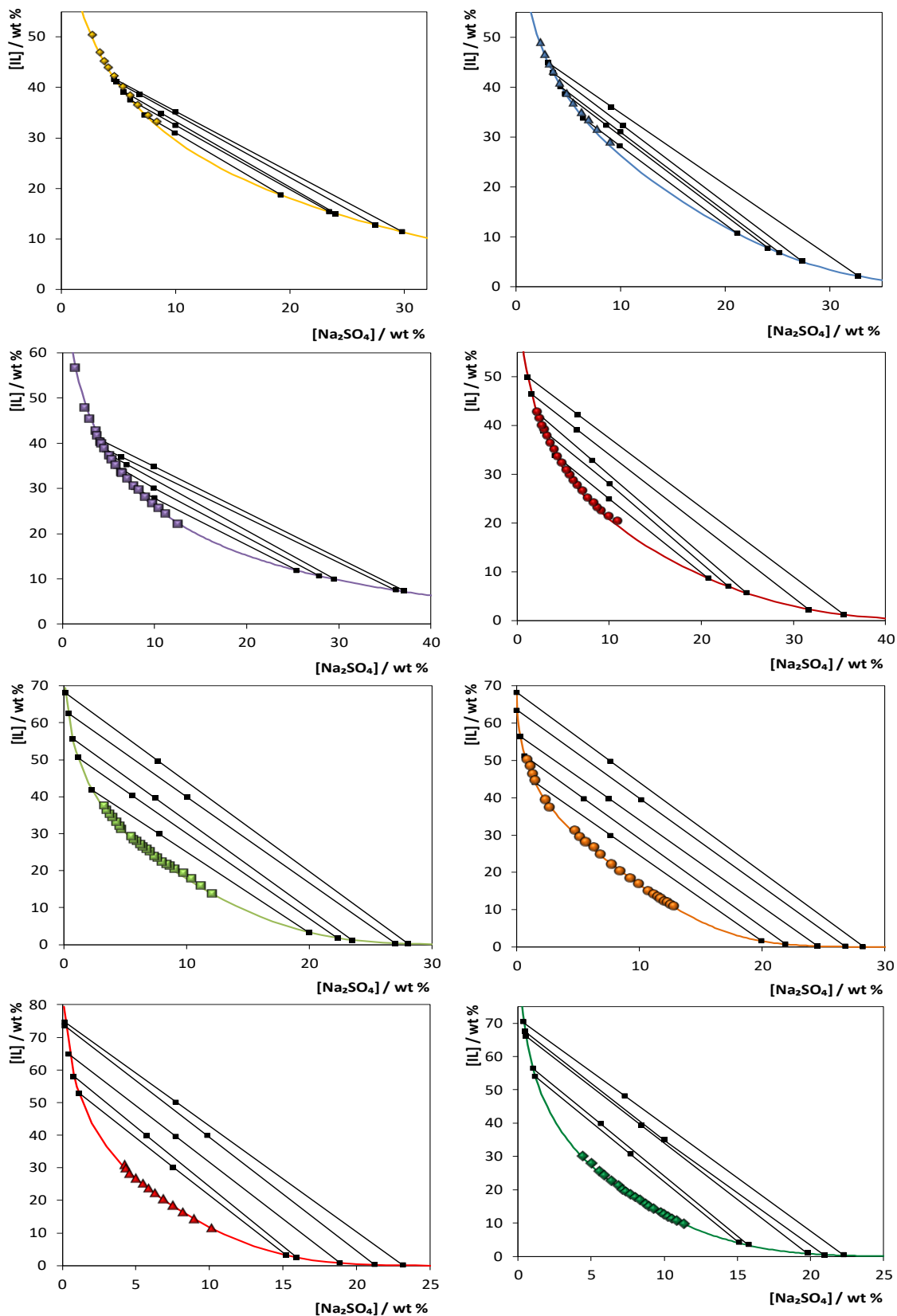


Figure S1. Phase diagrams for the systems composed of IL + Na₂SO₄ + H₂O at 25°C. Binodal curves data: [MepyrNC₂]Br (◆); [Et₃NC₂]Br (▲); [C₄mim]Br (■); [Pr₃NC₂]Br (●); [Bu₃NC₂]Br (■); [N₄₄₄₄]Br (○); [Bu₃PC₂]Br (▲); [P₄₄₄₄]Br (◆); TL data (■); The lines correspond to the fitting by Equation 1.

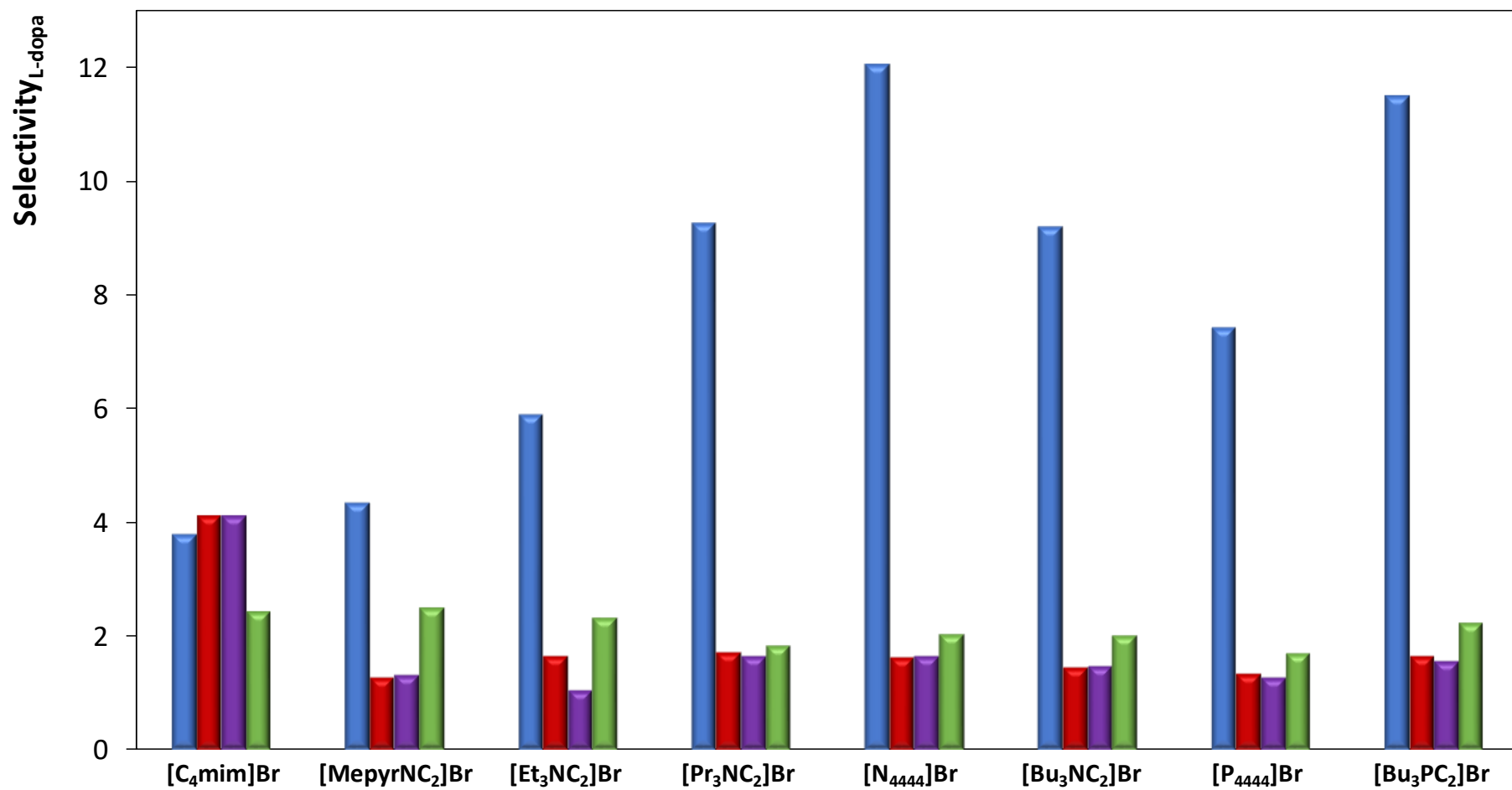


Figure S2. Selectivity of the amino acids L-tryptophan (■), D-phenylalanine (■), L-phenylalanine (■), L-tyrosine (■) over L-dopa in the IL-rich phase.