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Electronic Supplementary Information

Effective separation of aromatic and aliphatic amino acids mixtures using ionic-liquid-based aqueous biphasic systems

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Combinations of ionic liquids and amino acids tested

Table S1. Identification of the systems able (✓) and not able (×) to form ABS at 25ºC.

Amino acid	IL		Amino acid	IL	
L-Lys HCl	$[P_{444(14)}]Cl$	✓	L-Lys	$[P_{444(14)}]Cl$	✓
	$[P_{4444}]Br$	✓		$[P_{4444}]Br$	✓
	$[N_{4444}]Br$	✓		$[P_{4441}][MeSO_4]$	✓
	$[P_{4441}][MeSO_4]$	×		$[N_{4444}]Br$	×
	$[P_{4442}][Et_2PO_4]$	×		$[P_{4444}][EtSO_4]$	×
	$[P_{i(444)1}][TOS]$	×		$[P_{i(444)1}][TOS]$	×
	$[P_{4444}]Cl$	×		$[P_{4444}]Cl$	×
	$[N_{4444}]Cl$	×		$[N_{4444}]Cl$	×
	$[N_{3333}]Br$	×		$[N_{3333}]Br$	×
L-Pro	$[P_{444(14)}]Cl$	✓	L-Arg	$[P_{4444}]Br$	×
	$[P_{4444}]Br$	✓	DL-Asp		×
	$[P_{4441}][MeSO_4]$	✓	L-Asn		×
	$[N_{4444}]Br$	×	L-Val		×
	$[P_{4444}][EtSO_4]$	×	L-Ile		×
	$[P_{i(444)1}][TOS]$	×	L-Ala		×
	$[P_{4444}]Cl$	×	L-Cys		×
	$[N_{4444}]Cl$	×			
	$[N_{3333}]Br$	×			

Materials

The phosphonium-based ILs investigated were the following: ethyl(tributyl)phosphonium diethylphosphate, $[P_{4442}][Et_2PO_4]$ (purity > 95.0 wt %), tetrabutylphosphonium bromide, $[P_{4444}]Br$ (purity > 96.0 wt %), tetrabutylphosphonium chloride, $[P_{4444}]Cl$ (purity > 96.0 wt %), tri(isobutyl)methylphosphonium tosylate, $[P_{i(444)1}][TOS]$ (purity > 99.0 wt %), tributyl(methyl)phosphonium methylsulfate, $[P_{4441}][MeSO_4]$ (purity > 98.6 wt %), and tributyl(tetradecyl)phosphonium chloride, $[P_{444(14)}]Cl$ (purity > 97.0 wt %). All the phosphonium-based ILs were kindly provided by CYTEC Industries, Inc. The ammonium-based ILs investigated were tetrapropylammonium bromide, $[N_{3333}]Br$ (purity > 98.0 wt %), tetrabutlyammonium bromide, $[N_{4444}]Br$ (purity > 98.0 wt %), and tetrabutylammonium chloride, $[N_{4444}]Cl$ (purity > 97.0 wt %). $[N_{4444}]Br$ was supplied by Fluka, while $[N_{3333}]Br$ and $[N_{4444}]Cl$ were obtained from Sigma-Aldrich. For the extraction studies, 1-butyl-3-methylimidazolium trifluoromethanesulfonate, $[C_4mim][CF_3SO_3]$ (purity ≥ 99 wt %), and 1-butyl-3-methylimidazolium dicyanamide, $[C_4mim][N(CN)_2]$ (purity > 98 wt %), both purchased from Iolitec, were also used. Ammonia aqueous solutions at *ca.* 25 wt% were from CHEM-LAB.

The following aliphatic amino acids were used: L-Proline (L-Pro) (Acros, purity > 99 w/w %), L-Lysine monohydrated (L-Lys) (Acros, purity > 99 w/w %), L-Lysine hydrochloride (L-Lys HCl) (Sigma, purity > 99 w/w %), L-Arginine (L-Arg) (Merck, purity > 99 w/w %), DL-Aspartic Acid (DL-Asp) (Fluka, purity > 99 w/w %), L-Asparagine monohydrated (L-Asn) (Fluka, purity > 99 w/w %), L-Valine (L-Val) (Fluka, purity > 99 w/w %), L-Isoleucine (L-Ile) (Merck, purity > 99 w/w %), L-Alanine (L-Ala) (Biochemical, > 99 w/w %) and L-Cysteine (L-Cys) (Biochemicals, > 99 w/w %). The studied aromatic amino acids were the following: L-Tryptophan (L-Trp) (Sigma, > 99 w/w %), L-Tyrosine (L-Tyr) (Fluka, > 99 w/w %) and L-Phenylalanine (L-Phe) (Sigma, > 99 w/w %). A Dowex 50W X8 (20 to 50 mesh) cation exchange resin was purchased from DOW. Figures S1 and S2 depict the chemical structures of the aliphatic and aromatic amino acids investigated in this work.

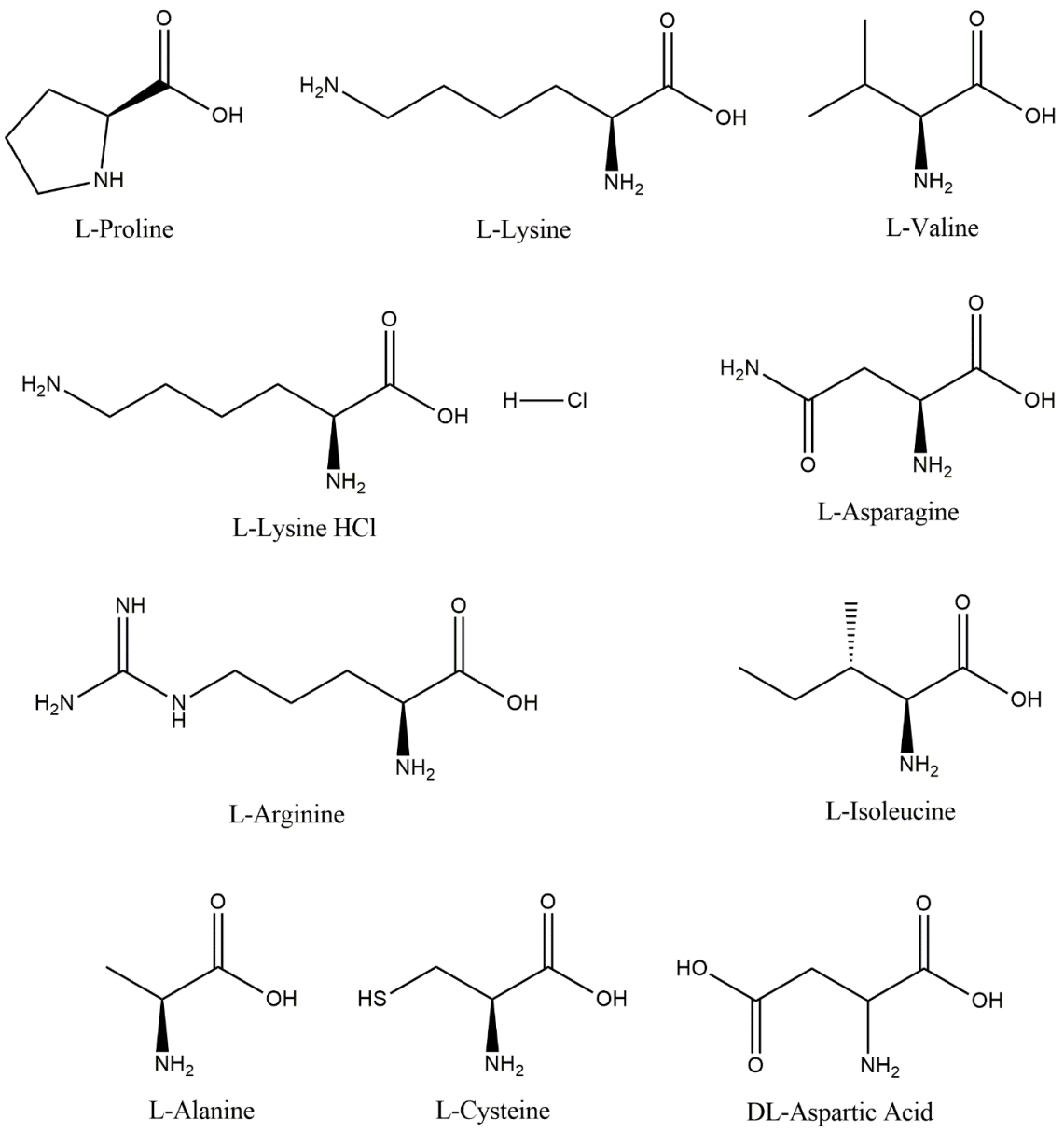

Figure S1. Chemical structure of the studied aliphatic amino acids.

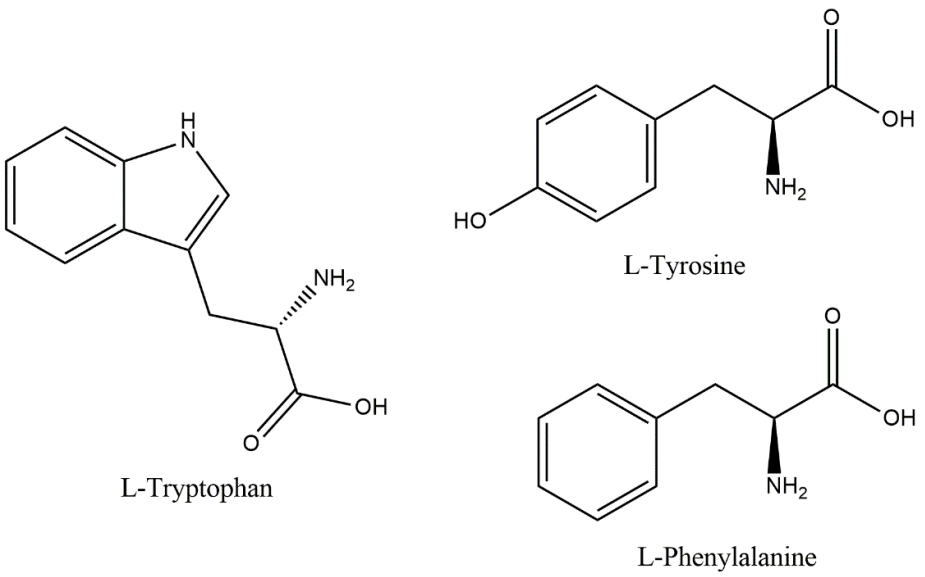

Figure S2. Chemical structure of the studied aromatic amino acids.

Experimental Procedure

The ternary phase diagrams (IL + amino acid + water) were determined with the following ILs: $[P_{4442}][Et_2PO_4]$, $[P_{4444}]Br$, $[P_{4444}]Cl$, $[P_{i(444)1}][TOS]$, $[P_{4441}][MeSO_4]$, $[P_{444(14)}]Cl$, $[N_{3333}]Br$, $[N_{4444}]Br$ and $[N_{4444}]Cl$, combined with the aliphatic amino acids L-Pro, L-Lys and L-Lys HCl. The IL with the higher ability to be salted-out, was also tested with the remaining aliphatic amino acids, namely L-Arg, DL-Asp, L-Asn, L-Val, L-Ile, L-Ala and L-Cys. The binodal curves of each ABS were determined by the cloud point titration method at (25 ± 1)°C and at atmospheric pressure. The experimental procedure adopted has been validated in previous works[1, 2]. Aqueous solutions of aliphatic amino acids at ≈ 50 wt% and aqueous solutions of the different hydrophilic ILs at variable concentrations (from 50 wt% to 100 wt%) were gravimetrically prepared and used for the determination of the binodal curves. The drop-wise addition of each aqueous amino acid solution to each IL aqueous solution was carried out until the detection of a cloudy solution (biphasic region), followed by the drop-wise addition of ultra-pure water until the detection of a clear and limpid solution (monophasic region). In some cases, the inverse procedure was also performed to complete the phase diagrams. Each mixture composition was determined by the weight quantification of all components added within an uncertainty of ± 10^{-4} g (using an analytical balance, Mettler Toledo Excellence XS205 DualRange).

The tie-lines (TLs) of each phase diagram, and at the mixtures compositions for which the extraction of aromatic amino acids was carried out, were determined by a gravimetric method originally described by Merchuk et al.[3]. The selected mixture, at the biphasic region, was prepared by weighting the appropriate amount of IL + amino acid + water, vigorously stirred, and further submitted to centrifugation for 30 min and at controlled temperature (25 ± 1)°C. After centrifugation, the sample was left in equilibrium for more 5 min at (25 ± 1)°C to guarantee the equilibration of the coexisting phases at the target temperature. After this period, each phase was carefully separated and weighted. Each individual TL was determined by the application of the lever-arm rule to the relationship between the weight of the top and bottom phases and the overall system composition. Previously to this approach, each experimental binodal curve was properly fitted as described elsewhere[3].

In order to avoid discrepancies in the results which could arise from the different compositions of the phases, all the partitioning studies were performed at a constant TLL (≈80). The mixture compositions which correspond to a TLL of ≈ 80 are the following:

- 42.81 wt% of $[P_{4444}]Br$ + 19.78 wt% of L-Lys + 37.41 wt% of H_2O;
- 39.17 wt% of $[P_{4441}][MeSO_4]$ + 29.12 wt% of L-Lys + 31.71 wt% of H_2O;
- 36.53 wt% of $[P_{4444}]Br$ + 31.73 wt% of L-Pro + 31.74 wt% of H_2O;
- 40.03 wt% of $[C_4mim][CF_3SO_3]$+ 20.15 wt % of L-Lys + 39.82 wt% of H_2O;
- 51.50 wt% of $[C_4mim][DCA]$ + 20.80 wt % of L-Lys + 27.7 wt% of H_2O.

For the $[P_{4444}]Br$ + L-Lys·HCl ABS a different TLL (17) was used due to the smaller liquid-liquid region of this system, which corresponds to the following initial ternary mixture composition: 42.09 wt% of $[P_{4444}]Br$ + 11.40 wt% of L-Lys·HCl + 46.01 wt% of H_2O.

For the amino acids separation studies, instead of water, the systems were loaded with aqueous solutions containing the aromatic amino acids. Each mixture was vigorously stirred, centrifuged for 30 min, and left to equilibrate for at least 5 min at 25 (± 1) °C to achieve the complete partitioning between the two phases. After a careful separation of both phases, the quantification of each amino acid in the two phases was carried by UV-spectroscopy, using a SYNERGY|HT microplate reader, BioTek, at a wavelength of 275 nm (for L-Trp and L-Tyr) or 255 nm (for L-Phe). At least three individual experiments were performed in order to determine the average in extraction efficiency, as well as the respective standard deviations. The interference of the amino acids and ILs with the quantification method was also ascertained and blank control samples were always used. The pH of each aqueous phase was determined at (25±1)°C using an HI 9321 Microprocessor pH meter (HANNA instruments).

The partition coefficients (K_{aa}) of each amino acid were determined by the ratio of concentrations of each amino acid in the IL-rich phase to that in the opposite phase, and the selectivity was determined as the ratio between the K_{aa} values for aromatic and aliphatic amino acids, as described in a previous work.[4]

The separation of the aromatic amino acids from the ionic liquid was performed by solid phase extraction, by cation exchange, with a Dowex-50 X8 (20 to 50 mesh) resin. The resin was initially washed with methanol (8 volumes), followed by 8 volumes of an ammonia aqueous solution at 4 wt% (pH *ca.* 11-12). Then, the IL-amino acid aqueous mixtures

(corresponding to the IL-rich phase) were passed through the column. The column was finally regenerated with methanol. All fractions were collected, and the amino acid quantified by UV-Vis spectroscopy using calibration curves previously established and the IL quantified by ^{1}H NMR spectroscopy (Bruker AMX 300) operating at 300 MHz, using benzene as an internal reference.

Experimental Results

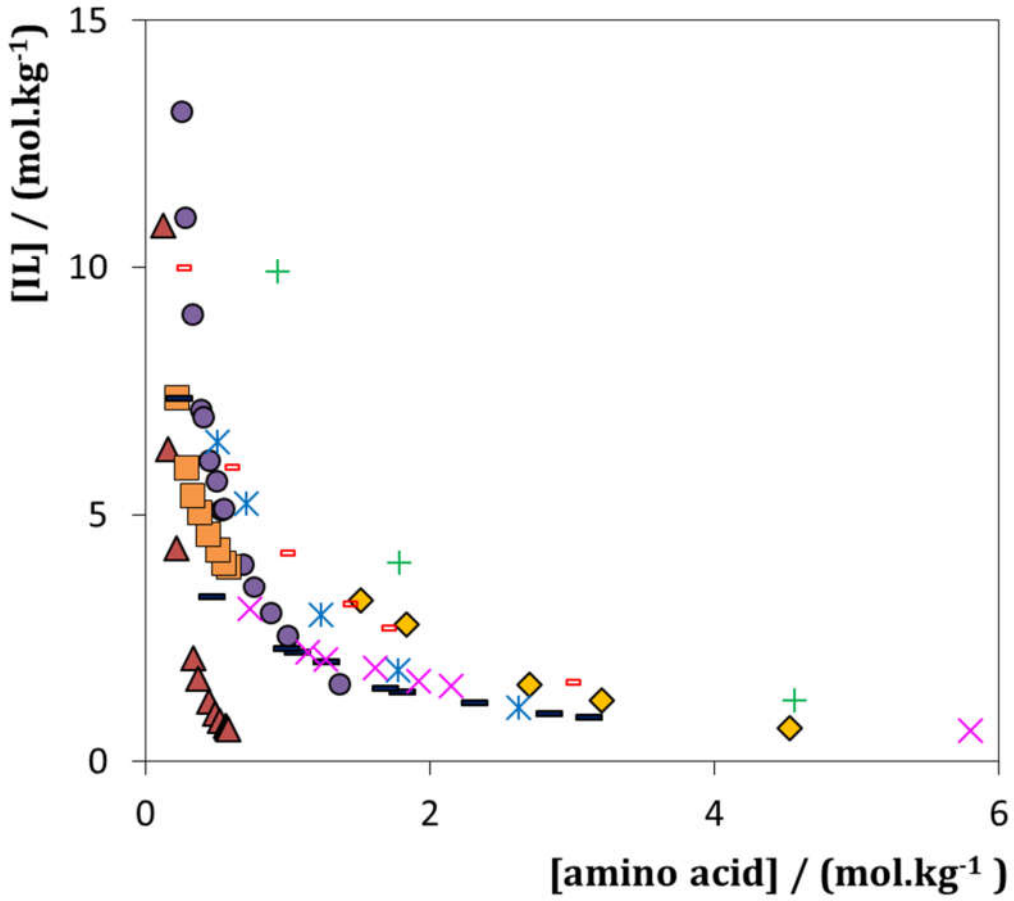

Figure S3. Phase diagrams for ABS composed of IL + amino acid + H_2O, in molality units: [$P_{444(14)}$]Cl + L-Lys HCl (▲); [P_{4444}]Br + L-Lys HCl (●); [N_{4444}]Br + L-Lys HCl (■); [P_{4444}]Br + L-Pro (♦); [$P_{444(14)}$]Cl + L-Pro (-); [P_{4441}][$MeSO_4$] + L-Pro (+); [$P_{444(14)}$]Cl + L-Lys (—); [P_{4444}]Br + L-Lys (✳); [P_{4441}][$MeSO_4$] + L-Lys (✕).

Table S2. Experimental weight fraction data for the ABS composed of IL (1) + L-Pro (2) + H_2O (3) at (25 ± 1)°C and atmospheric pressure.

$[P_{4444}]Br$		$[P_{444(14)}]Cl$		$[P_{4441}][MeSO_4]$	
100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
52.4469	14.8037	81.2828	2.5436	76.5021	9.6749
48.4375	17.4572	72.1773	6.1021	57.0012	17.0449
34.3810	23.7189	64.8412	9.8817	28.9556	34.4505
29.5149	26.9483	58.0551	13.8495		
18.5569	34.2650	53.9099	16.1209		
12.6129	43.6532	41.1791	25.4198		

Table S3. Experimental weight fraction data for the system composed of IL (1) + L-Lys (2) + H_2O (3) at (25 ± 1)°C and atmospheric pressure.

$[P_{4444}]Br$		$[P_{4441}][MeSO_4]$		$[P_{444(14)}]Cl$	
100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
48.5787	6.9086	50.3553	9.7081	70.6953	3.3043
43.2679	9.3753	42.0748	14.2591	52.2885	6.3741
21.1127	20.5862	40.3529	15.6073	42.8826	12.6564
30.2762	15.2376	38.2926	19.0634	42.0802	13.4776
13.7898	27.7359	34.7101	21.9222	39.8599	15.6188
		33.3449	23.9293	32.6468	19.7220
		16.8827	45.8624	31.5743	20.8495
				27.8538	25.2479
				24.1209	29.2933
				22.8034	31.3063
				21.3934	33.0705
				19.6582	34.9982
				14.9842	37.9356
				10.1466	42.2832
				7.3510	45.4719
				4.3395	55.2285

Table S4. Experimental weight fraction data for the system composed of IL (1) + L-Lys HCl (2) + H_2O (3) at (25 ± 1)°C and atmospheric pressure.

$[N_{4444}]Br$		$[P_{4444}]Br$		$[P_{444(14)}]Cl$	
100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_2
67.1662	3.8223	77.1337	3.9021	76.2195	3.6485
62.3434	5.0246	70.6170	4.3317	65.1042	4.3658
59.9824	5.6475	66.8310	4.8076	56.0457	5.9213
58.3972	6.4817	62.3276	5.6670	38.1548	9.0186
56.1905	7.5090	56.5949	6.5720	33.0996	9.9123
54.3407	8.4925	52.7577	7.5527	26.2961	11.5972
52.8286	9.1652	48.2322	8.7993	22.0848	12.6596
52.2874	9.6303	56.0101	6.8766	19.4538	13.4409
		50.9561	8.2614	17.3705	14.2045
		48.3445	9.1401	16.5245	14.4793
		42.2101	11.1236	15.8981	14.6396
		39.3458	12.1806	12.8999	15.9903
		35.5196	13.7702		
		31.7553	15.4322		

Table S5. Correlation parameters used to describe the experimental binodal data, determined by the method described by Merchuk et al.[3], and respective standard deviations (σ) and correlation coefficients (R^2).

IL	Amino Acid	$A \pm \sigma$	$B \pm \sigma$	10^{-5} ($C \pm \sigma$)	R^2
$[P_{4444}]Br$	L-Pro	227.8 ± 0.6	-0.37 ± 0.07	0.64 ± 0.33	0.994
$[P_{4441}][MeSO_4]$		175.5 ± 0.0	-0.27 ± 0.00	0.60 ± 0.00	1.000
$[P_{444(14)}]Cl$		103.1 ± 1.7	-0.15 ± 0.01	1.14 ± 0.16	0.999
$[P_{4444}]Br$	L-Lys	117.6 ± 15.3	-0.33 ± 0.05	2.11 ± 0.78	0.997
$[P_{4441}][MeSO_4]$		95.6 ± 6.8	-0.21 ± 0.02	0.32 ± 0.09	0.995
$[P_{444(14)}]Cl$		105.2 ± 5.6	-0.24 ± 0.02	0.79 ± 0.15	0.989
$[P_{4444}]Br$	L-Lys HCl	181.1 ± 9.0	-0.45 ± 0.02	1.10 ± 1.48	0.995
$[P_{444(14)}]Cl$		205.3 ± 14.1	-0.53 ± 0.03	17.2 ± 2.35	0.998
$[N_{4444}]Br$		110.7 ± 4.2	-0.26 ± 0.02	6.19 ± 2.48	0.997

Table S6. Log K_{ow}[5] and solubility in water[6] of aliphatic amino acids.

Amino acid	Log K_{ow}	Solubility in water (100 g^{-1})
L-Pro	-3.05	162.3
L-Lys	-2.54	Very soluble

Table S7. Experimental TLs and TLLs of the ABS investigated, where: $[IL]_{IL}$ and $[aa]_{IL}$ are, respectively, the IL and amino acid weight percentages in the IL-rich phase; $[IL]_{aa}$ and $[aa]_{aa}$ are, respectively, the IL and amino acid weight percentages in amino-acid-rich phase; $[IL]_M$ and $[aa]_M$ are, respectively, the IL and amino acid weight percentages in the initial mixture point; and pH_{IL} and pH_{aa} represent the pH value of the IL-rich phase and amino-acid-rich phase, respectively.

IL	Weight fraction composition / wt %								TLL
	$[IL]_{IL}$	$[aa]_{IL}$	pH_{IL}	$[IL]_M$	$[aa]_M$	$[IL]_{aa}$	$[aa]_{aa}$	pH_{aa}	
IL + L-Pro + H_2O									
$[P_{4444}]Br$	53.60	14.80	-	29.60	27.66	25.10	30.07	-	32.33
	77.75	8.35	5.31	36.53	31.73	9.08	47.31	4.69	78.95
$[P_{444(14)}]Cl$	74.64	4.86	-	59.53	17.93	0.77	68.73	-	97.65
	78.19	3.57	-	59.18	20.13	0.50	71.25	-	98.55
IL + L-Lys + H_2O									
$[P_{4444}]Br$	65.49	3.16	-	30.89	19.82	12.11	28.86	-	59.24
	77.96	1.56	10.16	42.81	19.78	3.83	39.97	10.2	83.49
$[P_{4441}][MeSO_4]$	55.17	6.87	-	33.50	28.50	18.44	43.53	-	51.90
	63.36	3.86	10.71	39.17	29.12	9.30	60.33	10.4	78.17
$[P_{444(14)}]Cl$	56.40	6.50	-	29.92	30.29	6.24	51.56	-	67.43
	77.54	1.57	-	47.29	25.21	2.97	59.86	-	94.65
$[C_4mim][CF_3SO_3]$	68.92	3.27	10.6	40.03	20.15	42.96	1.96	10.7	77.80
$[C_4mim][DCA]$	91.35	1.70	10.3	51.50	20.80	35.61	20.49	10.4	78.45
IL + L-Lys HCl + H_2O									
$[P_{4444}]Br$	47.32	9.12	2.94	42.09	11.40	31.88	15.86	3.55	16.84

Table S8. Extraction efficiencies of the aromatic amino acids to the IL-rich phase (EE_{AR}%) and extraction efficiencies of aliphatic amino acids to the opposite phase (EE_{AL} %).

Amino acid	**ABS**	L-Pro + $[P_{4444}]Br$	L-Lys + $[P_{4444}]Br$	L-Lys + $[P_{4441}][MeSO_4]$	L-Lys·HCl + $[P_{4444}][Br]$	L-Lys + $[C_4mim][CF_3SO_3]$	L-Lys + $[C_4mim][DCA]$
L-Trp	EE_{AR} **(%)**	26.26 ± 0.20	85.31 ±0.09	76.63 ± 0.45	71.62 ± 1.27	29.42 ± 1.60	18.98 ± 1.12
	EE_{AL} **(%)**	93.83 ± 1.12	94.63 ± 0.41	97.36 ± 0.51	39.24 ± 1.66	90.79 ± 0.81	96.40 ± 0.80
L-Phe	EE_{AR} **(%)**	11.83 ± 2.30	60.20 ± 3.08	60.31 ± 1.11	67.29 ± 1.51	20.98 ± 0.86	36.44 ± 0.33
	EE_{AL} **(%)**	92.22 ± 0.61	94.54 ± 0.87	97.23 ± 0.87	34.50 ± 2.66	91.65 ± 0.080	95.85 ± 0.45
L-Tyr	EE_{AR} **(%)**	30.32 ± 0.61	45.07 ± 2.60	40.34 ± 1.50	60.87 ± 0.28	10.81 ± 0.93	35.77 ± 2.50
	EE_{AL} **(%)**	91.40 ± 0.72	95.26 ± 1.47	97.80 ± 0.040	34.64 ± 3.03	91.60 ± 0.025	95.85 ± 0.34

Table S9. Partition coefficients (K_{aa}) of amino acids between the IL-rich and the opposite phase, and selectivity of aromatic amino acids over aliphatic ones towards the IL-rich phase.

Amino Acid		L-Pro + $[P_{4444}]Br$	L-Lys + $[P_{4444}]Br$	L-Lys + $[P_{4441}][MeSO_4]$	L-Lys.HCl + $[P_{4441}][MeSO_4]$	L-Lys + $[C_4mim][CF_3SO_3]$	L-Lys + $[C_4mim][DCA]$
L-Trp	K_{aa}	0.570	4.546	3.220	0.220	0.626	0.603
	Selectivity	4.144	120.892	121.279	2.396	0.048	0.029
L-Phe	K_{aa}	0.230	1.256	1.540	1.540	0.320	1.430
	Selectivity	1.672	29.930	59.795	1.513	0.024	0.069
L-Tyr	K_{aa}	0.655	2.714	0.750	0.910	0.160	1.500
	Selectivity	4.763	69.368	28.248	1.583	0.012	0.072

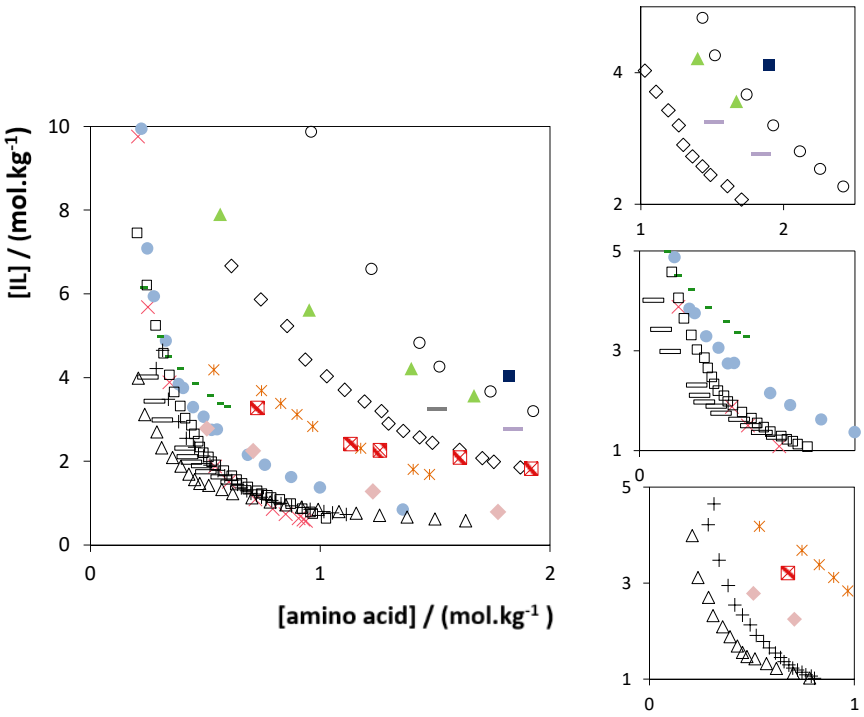

Figure S4. Ternary phase diagrams determined in this work compared to those in the literature (amino acid + $[C_4mim][CF_3SO_3]$+ H_2O and amino acid + $[C_4mim][BF_4]$+ H_2O) at 25 °C and atmospheric pressure[5]: L-Pro-based ABS ($[C_4mim][CF_3SO_3]$ (○); $[P_{4441}][MeSO_4]$ (■); $[P_{4444}]Br$ ( ); $[P_{44414}]Cl$ (▲); $[C_4mim][BF_4]$ (☐); L-Lys HCl-based ABS ($[N_{4444}]Br$ ( ); $[P_{4444}]Br$ (●); $[C_4mim][CF_3SO_3]$ (☐); $[C_4mim][BF_4]$ (═); $[P_{44414}]Cl$ (×)); L-Lys-based ABS ($[P_{4441}][MeSO_4]$ (☒); $[P_{4444}]Br$ (◆); $[P_{44414}]Cl$ (*); $[C_4mim][BF_4]$ ( ); $[C_4mim][CF_3SO_3]$ (+)).

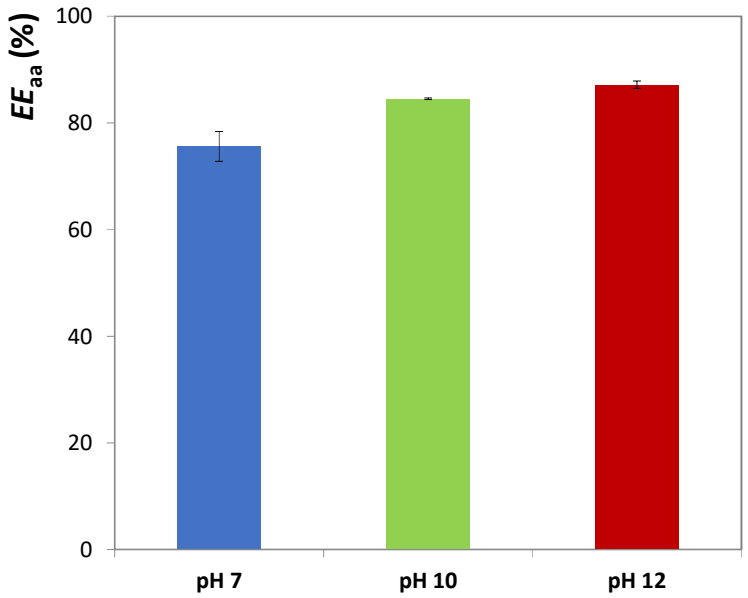

Figure S5. Extraction efficiency (%EE_{aa}) of L-Trp in the system formed by [P_{4444}]Br + L-Lys + H_2O (TLL ≈ 80), at different pH values (7, 10 and 12).

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