Supporting Information

Valorization of expired energy drinks by designed and integrated ionic-liquid-based aqueous biphasic systems

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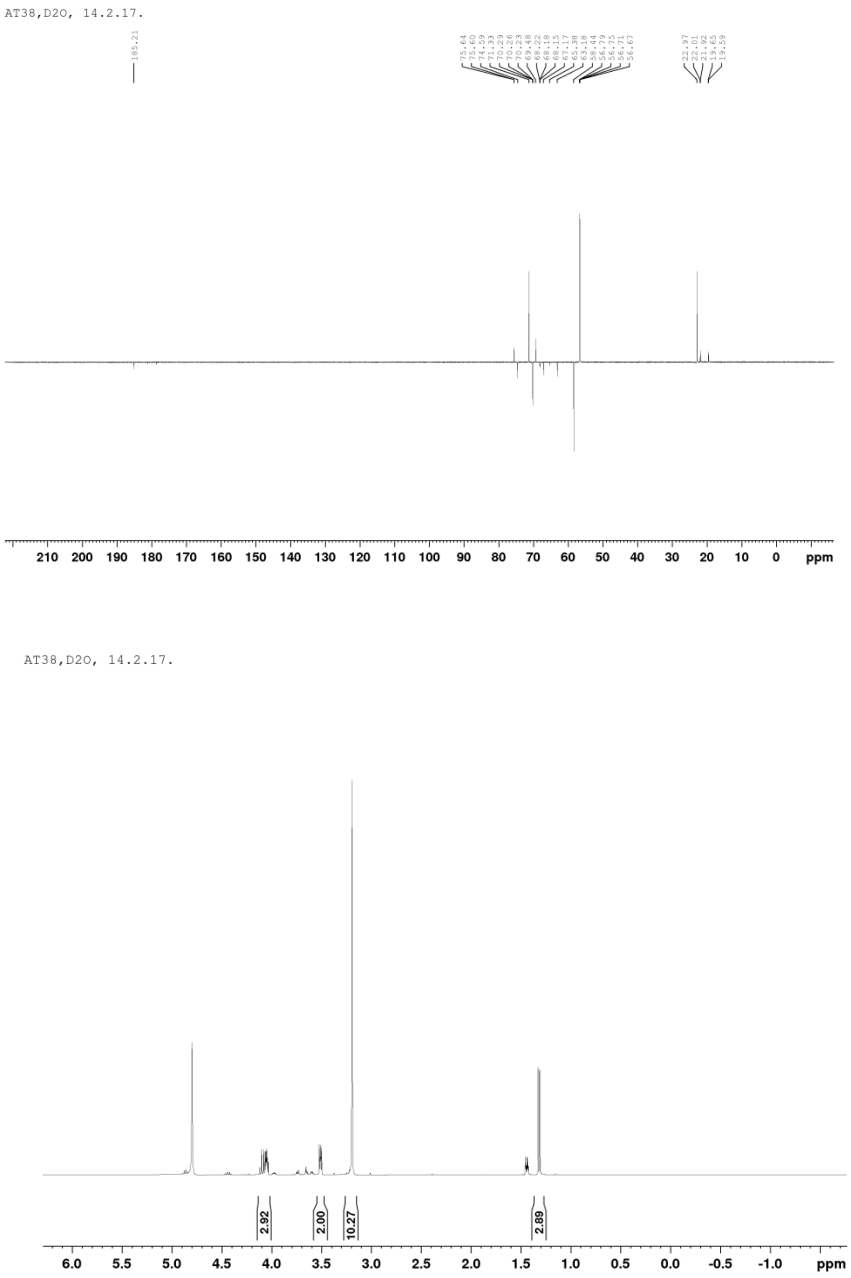


Figure S1. ^{1}H NMR and ^{13}C NMR spectra for [Ch][Lac].

^{1}H NMR (D_2O): 1.32 (d, 3H, $J_{CH2,CH3}$= 7,0 Hz, $CO_2CHOHC\mathit{H}_3$); 3.19 (s, 9H, $N(C\mathit{H}_3)_3$); 3.51 (m, 2H, $C\mathit{H}_2N(CH_3)_3$); 4.05 (m, 2H, $C\mathit{H}_2OH$); 4.09 (q, 1H, $J_{CH2,CH3}$= 7,0 Hz, $CO_2C\mathit{H}OHCH_3$)

^{13}C NMR (D2O): 22.97 ($CO_2CHOH\mathit{C}H_3$); 56.67, 56.71 and 56.75 ($N(\mathit{C}H_3)_{3,}$); 58.44 ($\mathit{C}H_2OH$); 70.23,70.26 and 70.29 ($\mathit{C}H_2N(CH_3)_3$); 71.33 ($CO_2\mathit{C}HOHCH_3$); 185.21 (C=O).

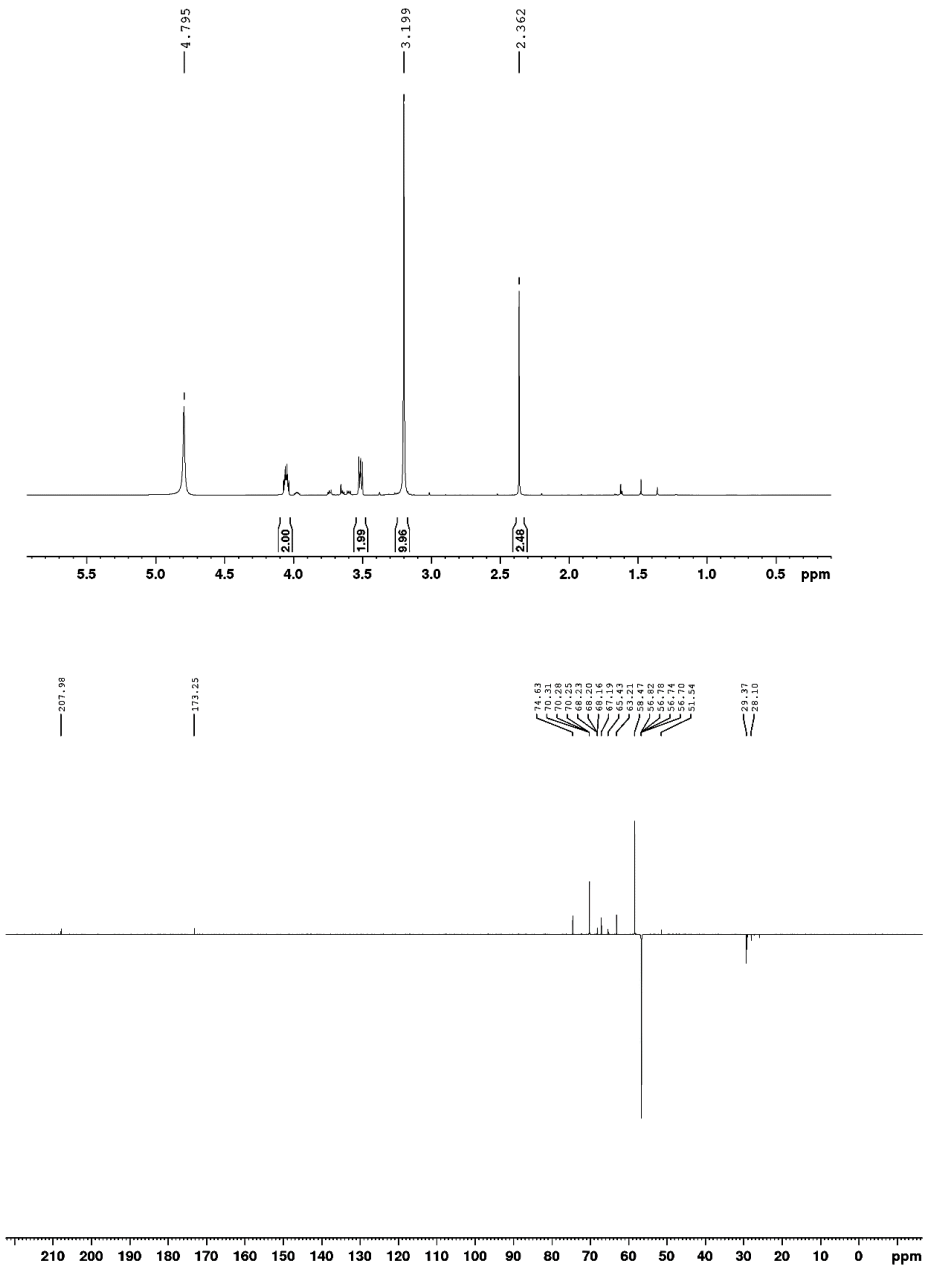


Figure S2. ^{1}H NMR and ^{13}C NMR spectra for [Ch][Pyr].

^{1}HNMR (D_2O): 2.36 (s, CO_2COCH_3); 3.20 (s, 9H, $N(CH_3)_3$,); 3.52 (m, 2H, $CH_2N(CH_3)_3$); 4.06 (m, 2H, CH_2OH).

^{13}CNMR (D_2O): 29.37 (CO_2COCH_3); 56.70, 56.74, and 56.78 ($N(CH_3)_3$,); 58.47 (CH_2OH); 70.25,70.28 and 70.31 ($CH_2N(CH_3)_3$); 173.25 (CO_2COCH_3); 207.98 (CO_2COCH_3).

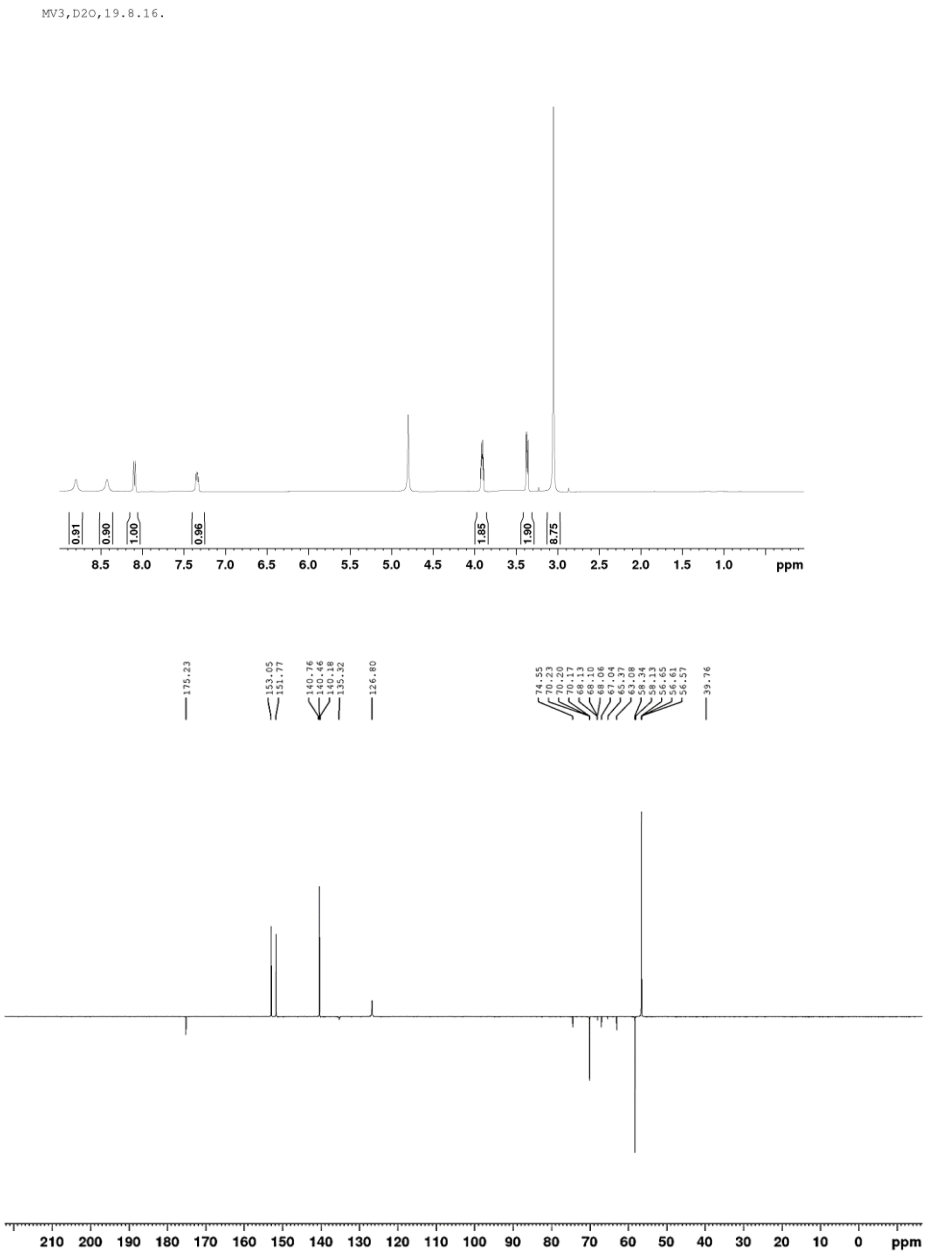


Figure S3. ^{1}H NMR and ^{13}C NMR spectra for [Ch][Nia].

^{1}HNMR (D_2O): 3.05 (*s*, 9H, N(CH_3)$_3$); 3.37 (*m*, 2H, CH_2N(CH_3)$_3$); 3.91 (*m*, 2H, CH_2OH); 7.34 (*dd*, 1H, $J_{4,5}$=7.7 Hz, $J_{5,6}$=5.0 Hz, H-5); 8.09 (*bd*, 1H, $J_{4,5}$=7.9 Hz, H-4); 8.43 (*bs*, 1H, H-6); 8.80 (*bs*, 1H, H-2)

^{13}CNMR (D_2O): 56.57, 56.61 and 56.65 (N(CH_3)$_3$); 58.34 (OHCH_2CH_2N(CH_3)$_3$); 70.17, 70.20 and 70.23 (OHCH_2CH_2N(CH_3)$_3$); 126.80 (C-5); 135.32 (C-3); 140.46 (C-4); 151.77 (C-2); 153.05 (C-6); 175.23 (C=O)

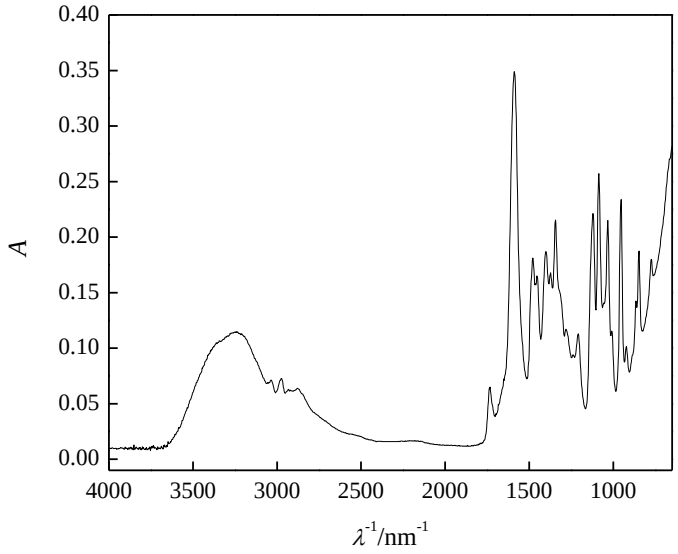


Figure S4. FTIR spectrum of [Ch][Lac].

IR (neat): 3243 (stretching OH); 2974 (sym. stretching CH, ($N(CH_3)_3$)); 1589 (asym. stretching COO^-); 1479 (CH_3 scissor); 1400 (bending –OH); 1344 (sym. stretching CH_3); 1121 (stretching CH_3); 1032 (stretching C-CH_3); 953 (asym. streching C-C-O); 847 (C-COO^- stretching);773 (C-H out plane bending).

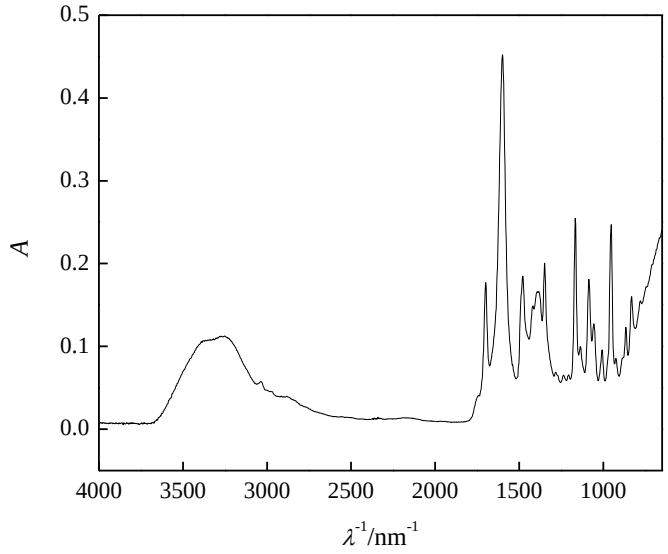


Figure S5. FTIR spectrum of [Ch][Pyr].

IR (neat): 3372 (stretching OH); 1600 (asym. stretching NH); 1481 (CH_3 scissor); 1373 and 1342 (stretching C=O) (CH_2 waging); 1138 (stretching C-O); 1110 (stretching C-O-C); 1087 and 1058 (twisted CH_2 and NH_2); 1008 (stretching C-C) 953 (asym. stretching C-C-O); 790 (C-H out of plane bending).

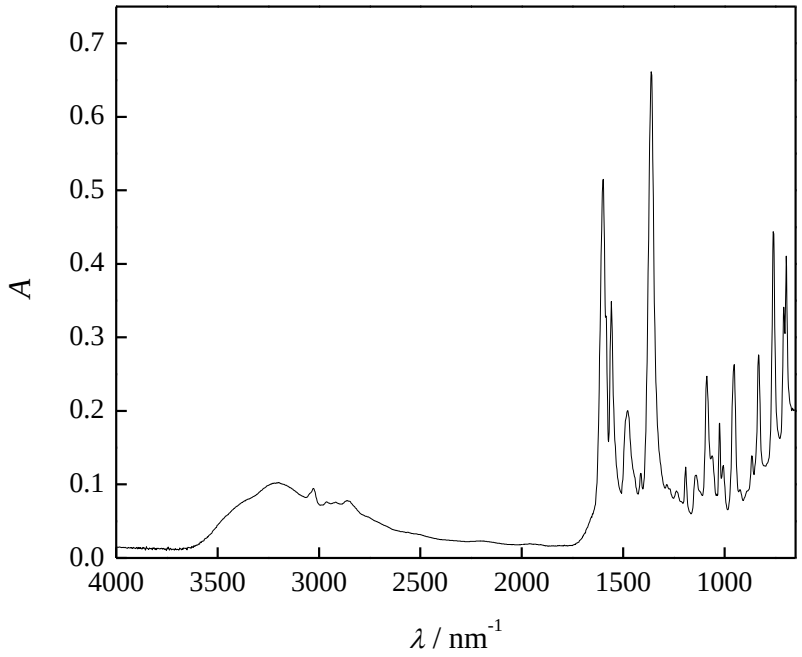


Figure S6. FTIR spectrum of [Ch][Nia].

IR (neat): 3201 (stretching OH); 1598 (stretching C=C); 1558 (asym. stretching NH); 1478 (CH3 scissor); 1361 (sym. stretching C=O from COO¬); 1192 (stretching C-OH, from Ph-OH); 1088 (stretching C-C-N); 1025; 953 (asym. streching C-C-O); 832 (C-C stretching); 759, 708 and 696 (C-H out plane bending).

Determination of the phase diagrams of ABS

The phase diagrams of the ABS composed of each IL, PPG 400 and water were determined through the cloud point titration method at (25 ± 1) °C at atmospheric pressure. The experimental binodal curves of the ternary phase diagrams were fitted by least squares regression to an empirical relationship developed by Merchuk et al.[1], as described by Eqn (S1),

$$Y = A\ exp(B\ X^{0.5} + C\ X^3) \qquad \text{(S1)}$$

where *Y* and *X* correspond to the weight fractions percentages of PPG 400 and IL, respectively, and *A*, *B* and *C* are fitting parameters obtained by least-squares regression. These parameters were determined using the OriginPro 8.5 software.

Tie-lines (TLs) of each system, which give the composition of each phase at a given mixture point, were determined by the lever–arm rule according to Merchuk et al.[1] For the determination of the TLs, the following system of four equations with four unknown values was applied, using the MathCad 15.0 software:

$$Y_{PPG} = A\ exp\left[\left(BX_{PPG}^{0,5}\right) - \left(CX_{PPG}^{3}\right)\right] \qquad \text{(S2)}$$

$$Y_{IL} = A\ exp\left[\left(BX_{IL}^{0,5}\right) - \left(CX_{IL}^{3}\right)\right] \qquad \text{(S3)}$$

$$Y_{PPG} = \left(\frac{Y_M}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right) Y_{IL} \qquad \text{(S4)}$$

$$X_{PPG} = \left(\frac{X_M}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right) X_{IL} \qquad \text{(S5)}$$

where subscripts *PPG*, *IL*, and *M* correspond to the PPG400-rich phase, the IL-rich phase and the mixture, respectively; and α is the ratio between the mass of the top phase and the total mass of the mixture. The system solution results in the composition (wt%) of the PPG400 and IL in the top and bottom phases. For the calculation of each tie-line length (TLL), the following equation was used:

$$TLL = \sqrt{(X_{PPG} - X_{IL})^2 + (Y_{PPG} - Y_{IL})^2} \qquad \text{(S6)}$$

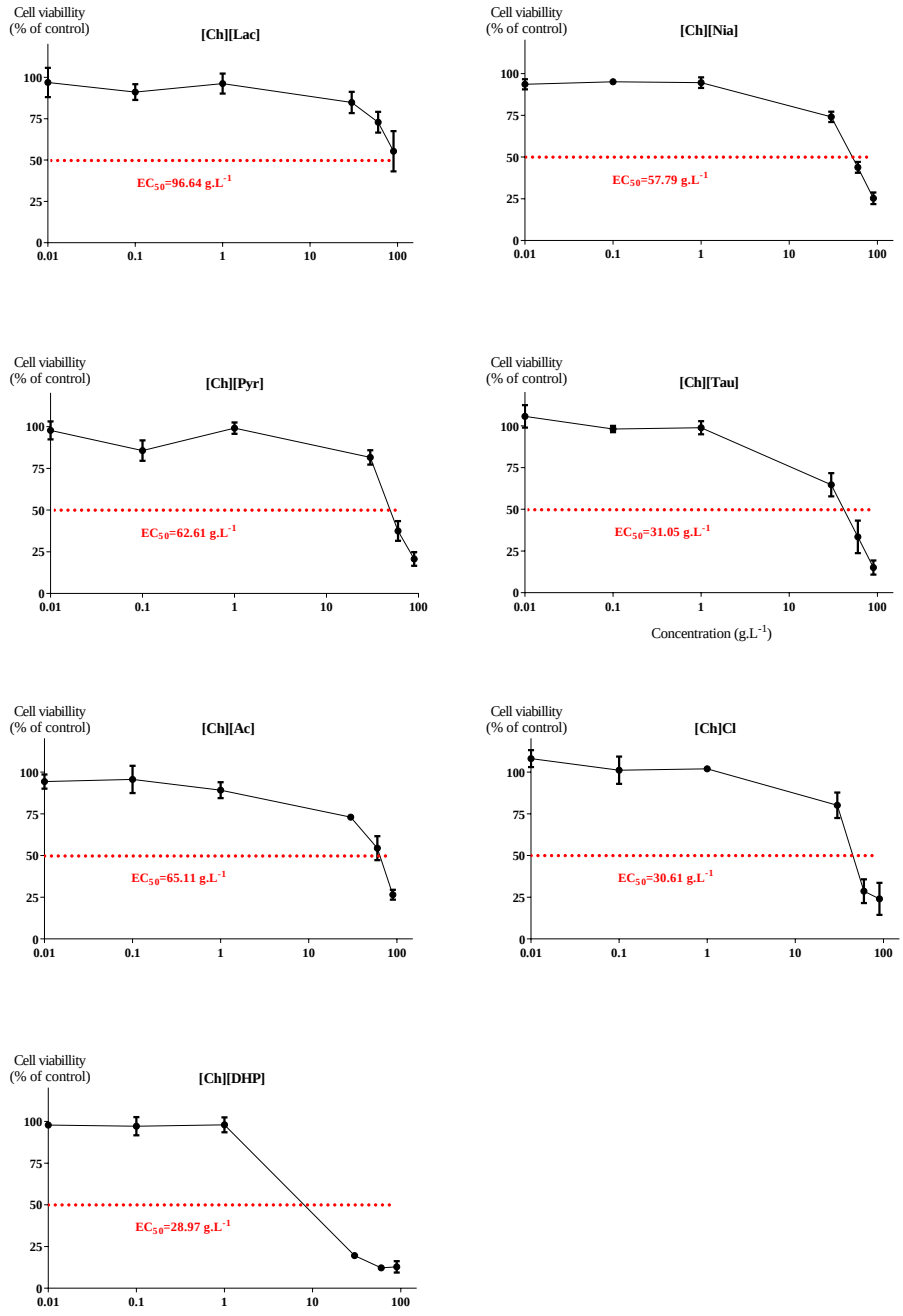


Figure S7. Caco-2 cell viability after 24 h exposure to [Ch][Lac], [Ch][Nia], [Ch][Pyr], [Ch][Tau], [Ch][Ace], [Ch]Cl, [Ch][DHP]. The experimental points, expressed as percentage of the control, correspond to the average of five replicates of three independent experiments (*n* = 3), except for [Ch]Cl that corresponds to two independent experiments (*n* = 2). The lines correspond to the respective standard error of the mean (SEM). The control corresponds to untreated cells. The dose response curves and median effective concentration (EC_{50}) calculations were performed using the GraphPad PRISM Software (version 8.01). Values of cell viability higher than 100% are indicative of cell proliferation. The dash line indicates the EC_{50} values for each IL.

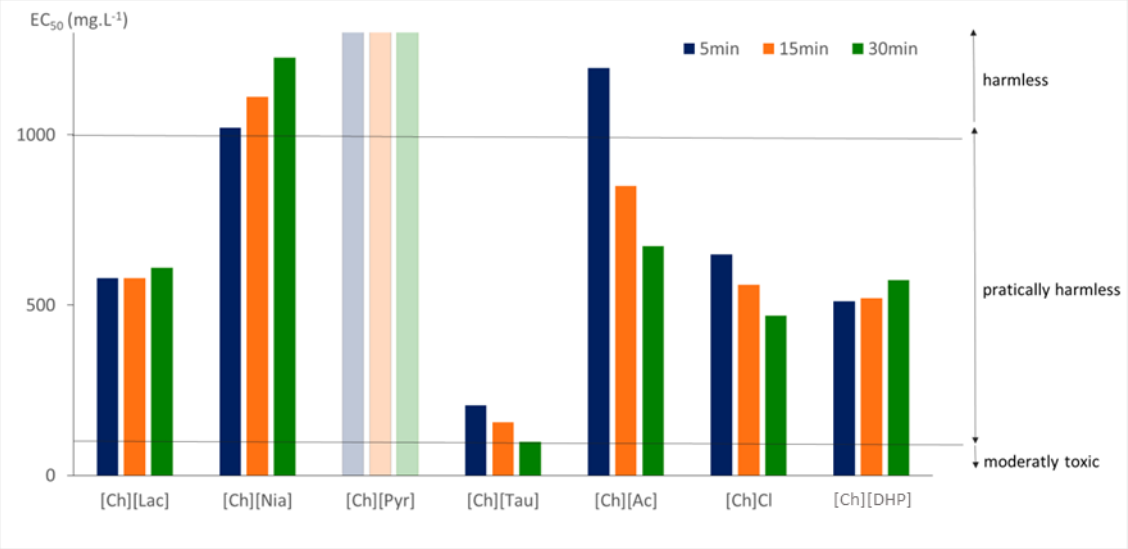


Figure S8. Values of average effective concentration (EC_{50}), in $mg.L^{-1}$, obtained after 5, 15, and 30 min of exposure of the marine bacteria *V. fischeri* (Microtox toxicity test) to the different cholinium-based ionic liquids. For [Ch][Pyr] it was not possible to calculate the EC_{50} value, meaning that this IL is not toxic for the bacteria. Values for [Ch][Ac], [Ch]Cl and [Ch][DHP] were obtained from Ventura et al. [2]

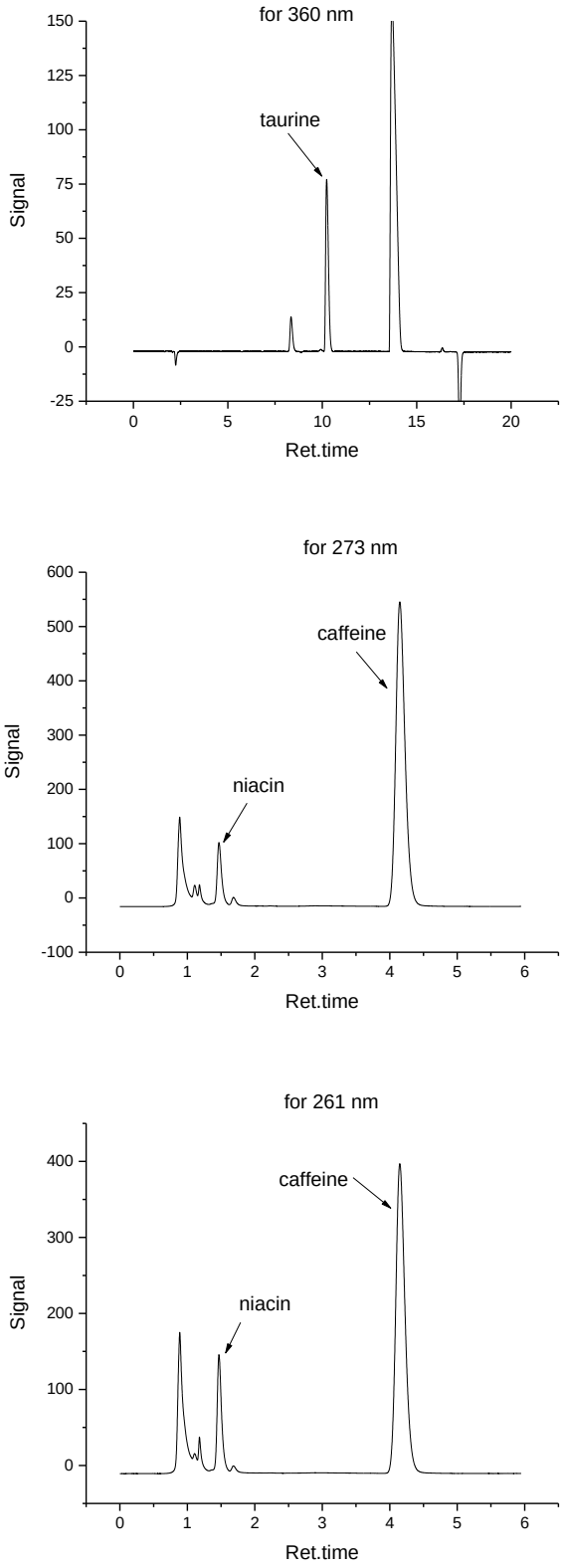


Figure S9. HPLC chromatograms of diluted energy drink samples and target compounds identification and quantification.

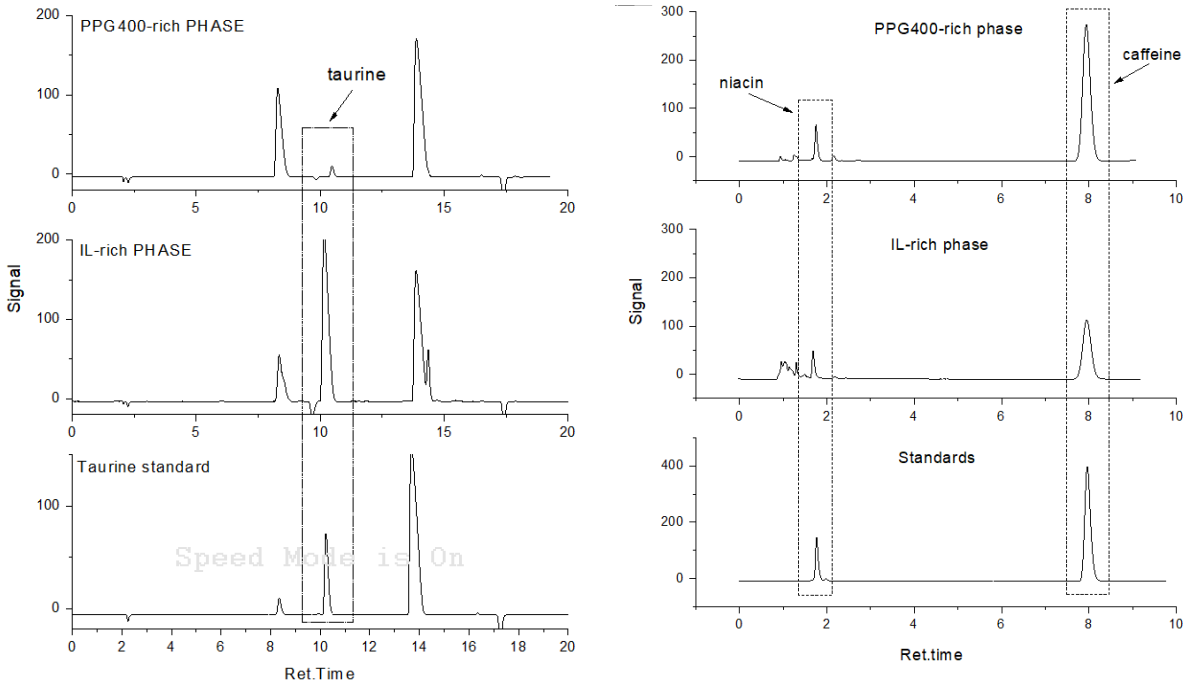


Figure S10. HPLC chromatograms of the ABS phases after the extractions carried out from the energy drink and standard aqueous solutions of caffeine, niacin and taurine.

Table S1. Experimental binodal mass fraction data for the ABS (IL (X) + PPG400 (Y) + H_2O) based on the newly synthetized ILs at 25°C and 0.1 MPa.

[Ch][Tau]		[Ch][Lac]		[Ch][Pyr]		[Ch][Nia]	
100·Y	100·X	100·Y	100·X	100·Y	100·X	100·Y	100·X
65.79	2.75	63.00	4.77	62.11	4.81	54.35	10.44
61.96	3.19	58.34	5.68	57.66	5.68	50.34	11.76
58.82	3.51	53.89	6.51	53.89	6.66	48.10	12.51
55.88	4.05	49.43	6.86	50.64	7.52	45.33	13.08
53.54	4.41	45.49	7.45	48.05	8.17	42.52	14.21
52.11	4.73	42.75	8.26	45.31	8.61	40.02	15.24
50.72	4.99	38.98	9.21	43.26	9.26	37.72	16.15
48.74	5.28	36.58	9.68	40.35	9.59	35.77	17.00
47.15	5.66	33.94	10.30	37.30	10.30	33.64	18.03
45.57	5.96	31.95	10.74	35.59	10.76	31.71	19.30
44.01	6.28	29.73	11.43	34.01	11.23	29.75	20.66
42.84	6.55	28.06	11.64	32.69	11.74	28.23	21.77
41.92	6.75	26.92	12.31	31.48	11.96	26.43	23.27
40.49	7.03	25.89	12.63	30.29	12.39	25.51	24.11
39.57	7.27	25.19	13.19	29.10	12.90	24.57	25.24
38.41	7.49	24.17	13.77	28.21	13.21	23.90	26.03
37.03	7.81	22.68	14.15	26.95	13.84	23.20	26.79
35.44	8.28	21.31	15.21	25.87	14.32	22.54	27.36
34.20	8.63	19.85	16.12	24.78	14.95	21.69	28.14
32.63	8.95	18.23	17.12	23.86	15.46	20.99	28.98
31.55	9.30	17.25	18.09	23.13	15.71	10.34	48.75
30.67	9.50	16.38	18.89	22.17	16.47	12.31	42.41
29.60	9.97	3.32	59.08	21.08	17.83	14.62	38.49
28.59	10.34	5.56	34.23	20.45	18.11	17.58	34.36
27.52	10.62	8.28	27.67	19.77	18.49	20.15	30.94
26.83	10.94	10.54	24.18	19.14	18.85		
25.94	11.32	13.26	21.45	18.42	19.42		
25.07	11.82			17.91	19.69		
23.59	12.54			17.42	20.00		
22.64	13.10			16.86	20.55		
21.55	14.09			16.42	20.85		
20.68	14.68			15.97	21.23		
19.84	15.43			15.49	21.71		
19.07	16.03			15.00	22.30		
18.43	16.45			14.64	22.69		
17.43	17.06			6.27	54.95		
17.19	17.53			7.58	45.22		
5.02	60.26			8.91	36.48		
8.79	34.62			10.35	31.19		
10.66	28.62			11.60	28.39		
12.26	24.98			12.05	26.24		
13.96	21.88			13.09	24.25		
21.21	14.97						

Standard uncertainties (u): $u(w) = 0.01$; $u(T) = 1$ K. Relative standard uncertainty: u_r (p) = 1.5%

Table S2. Experimental binodal mass fraction data for the system (IL (X) + PPG400 (Y) + H_2O) ABS based on commercial ILs at 25°C and 0.1 MPa.

[Ch][DHP]		**[Ch][Ac]**		**[Ch][Cl]**	
100·Y	100·X	100·Y	100·X	100·Y	100·X
55.43	2.79	62.24	2.57	39.12	7.46
49.36	3.27	53.30	3.64	36.28	8.05
45.50	3.74	45.02	4.28	33.56	8.44
36.53	4.75	40.24	4.82	31.32	8.92
34.78	4.92	35.75	5.58	29.62	9.24
34.08	4.94	32.32	5.99	26.22	10.62
33.50	5.04	30.31	6.38	25.46	11.22
33.14	5.20	28.04	6.87	24.24	11.56
32.68	5.27	23.88	7.89	23.57	12.01
32.44	5.39	22.41	8.49	20.50	14.02
31.11	5.44	20.71	9.21	19.97	14.34
31.10	5.54	19.28	10.00	19.33	14.57
30.70	5.73	16.08	11.18	18.89	14.90
29.92	5.89	15.49	11.49	18.46	15.24
29.44	5.96	14.99	11.85	17.95	15.43
29.41	6.02	14.43	12.38	17.22	16.01
28.70	6.17	13.90	12.66	16.79	16.68
27.93	6.30	13.39	12.94	16.46	16.88
25.87	6.70			15.98	17.55
23.56	7.26			15.48	17.53
21.82	7.65				
20.35	8.28				
19.30	8.56				
18.49	9.01				
17.71	9.25				
16.68	9.92				
10.39	13.93				
9.82	14.39				
8.87	14.69				
8.69	15.34				
8.23	16.05				
7.82	16.60				
7.78	17.42				
6.95	18.51				

Standard uncertainties (u): $u(w)$ = 0.01; $u(T)$ = 1 K. Relative standard uncertainty: u_r (p) = 1.5%

Table S3. Fitting parameters *A*, *B* and *C* obtained by the experimental data of the ternary phase diagrams of the studied (IL + PPG400 + H_2O) systems obtained by application of Eq. S1 and respective standard error (σ) and correlation coefficients (R^2).

IL	A±σ	B±σ	C±σ	R^2
[Ch][Tau]	165.88±2.57	-0.541±0.006	(-3.37 ± 0.78) × 10^{-6}	0.9967
[Ch][Lac]	276.57±8.96	-0.658±0.011	(-1.69±1.79) × 10^{-6}	0.9962
[Ch][Pyr]	239.52±7.96	-0.585±0.011	(-3.78±1.17) × 10^{-6}	0.9922
[Ch][Nia]	235.17±6.55	-0.454±0.007	(-6.88±4.44) × 10^{-7}	0.9984
[Ch][DHP]	226.29±4.70	-0.841±0.009	(-1.67±0.71) × 10^{-5}	0.9988
[Ch][Ac]	224.72±12.73	-0.784±0.028	(-6.04±0.44) × 10^{-6}	0.9950
[Ch]Cl	305.09±35.50	-0.766±0.042	(-4.93±1.34) × 10^{-5}	0.9944

Table S4. Partition coefficients and respective properties of the organic acids which are the anion precursors of the synthetized ILs.

Organic acid	[1]$logP_{o/w}$	[2]HBD	[3]HBA	[4]Anion polar surface / (A^2)
Taurine (2-aminoethanesulfonic acid)	-2.77	3	4	80.39
Lactic acid	-0.85	2	3	60.36
Pyruvic acid	-1.24	1	3	57.20
Nicotinic acid	0.22	1	3	50.19

[1] Partition coefficient in octanol/water system calculated by ACD/Labs PhysChem program

[2] HBD (hydrogen bond donor) = the sum of the atoms in the molecule which have H donor property

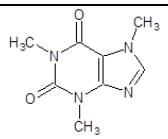
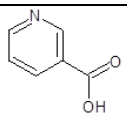
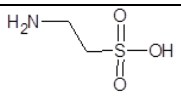
[3] HBA (hydrogen bond acceptor) = the sum of the acceptor atoms

[4] The anion polar surface –the surface sum over all polar atoms

Table S5. Experimental tie-lines data in percentage weight fraction for the ABS composed of (IL (*X*) + PPG 400 (*Y*) + H_2O) at 25°C and 0.1 MPa.

IL	Total composition		Top phase		Bottom phase		TLL	Slope
	100X	100Y	100X	100Y	100X	100Y		
[Ch][Tau]	25.01	24.94	1.95	77.98	32.09	8.66	75.59	-2.30
	13.80	40.00	3.22	62.99	27.38	10.51	57.77	-2.17
[Ch][Lac]	24.86	24.86	2.56	96.41	32.09	7.78	92.83	-3.21
	15.60	40.01	3.85	76.13	25.37	10.31	69.25	-3.06
[Ch][Pyr]	25.02	25.11	2.53	94.34	29.63	10.91	87.72	-3.08
	15.40	45.04	3.88	75.60	23.64	14.60	64.12	-3.10
[Ch][Nia]	30.10	30.00	4.12	94.03	35.74	16.12	84.08	-2.20
	21.04	45.04	5.92	78.23	33.67	17.28	66.97	-2.46

Table S6. Chemical structure and properties of the targeted biomolecules.

Compound	Chemical structure	[1]pK_{a1}/pK_{a2}	[2]$logP_{ow}$ at pH (5.8-10.2)	[3]**HBD**	[4]**HBA**
Caffeine		0.12 / 10.5	-0.63	0	3
Niacin		4.80	-2.03 – -2.93	1	3
Taurine		-1.49 / 9.06	-5.27 – -6.23	2	4

[1] Calculated by ACD/Labs PhysChem program

[2] Partition coefficient in octanol/water system calculated by ACD/Labs PhysChem program

[3] HBD (hydrogen bond donor) = the sum of the atoms in the molecule which have H donor property

[4] HBA (hydrogen bond acceptor) = the sum of the acceptor atoms

Table S7. Recovery efficiencies (*RE* %) plus the corresponding standard deviations (σ) of the targeted compounds for corresponding phases (IL-rich or PPG-rich) at two ternary ABS compositions: (A) 15 wt% of IL and 40 wt% of PPG 400, and (B) 30 wt% of IL and 30 wt% of PPG 400.

Phase	IL	Caffeine (*RE ± σ, %*)		Niacin (*RE ± σ, %*)		Taurin (*RE ± σ, %*)	
		A	**B**	**A**	**B**	**A**	**B**
IL	[Ch][DHP]	16.19±1.28	24.51±1.12	53.49±1.24	68.43±1.83	71.68±2.74	88.18±2.48
	[Ch][Ac]	31.16±1.65	39.24±1.22	47.89±1.65	69.47±3.63	98.98±2.22	99.86±1.21
	[Ch]Cl	53.59±2.22	55.38±1.91	73.30±2.68	83.36±2.74	99.41±3.02	99.87±2.03
	[Ch][Tau]	46.78±2.50	46.22±1.84	59.15±3.41	69.16±4.11	98.27±3.57	98.46±3.00
	[Ch][Lac]	48.17±1.52	59.69±3.72	61.10±2.52	75.33±2.63	98.17±1.77	96.11±3.14
	[Ch][Pyr]	60.37±2.49	64.18±3.28	72.49±3.09	79.00±2.41	97.77±2.30	96.20±2.23
	[Ch][Nia]	87.72±3.17	94.88±3.67	82.32±2.88	92.55±1.82	97.06±2.76	98.91±3.76
PPG	[Ch][DHP]	83.81±2.83	75.49±1.85	46.51±1.65	31.57±2.51	28.32±1.57	11.82±1.86
	[Ch][Ac]	68.84±3.44	60.76±2.46	52.11±1.84	30.53±1.95	1.02±0.43	0.14±0.12
	[Ch]Cl	46.41±1.30	44.62±2.09	26.70±1.56	16.64±1.30	0.59±0.32	0.13±0.01
	[Ch][Tau]	53.22±2.68	53.78±2.34	40.85±1.43	30.84±2.99	1.73±0.26	1.54±0.62
	[Ch][Lac]	51.83±2.96	40.31±1.93	38.90±1.29	24.67±2.35	1.83±0.41	3.89±1.53
	[Ch][Pyr]	39.63±2.52	35.82±1.82	27.51±1.41	21.00±4.35	2.23±0.26	3.80±1.11
	[Ch][Nia]	12.28±0.54	5.12±1.05	17.68±0.71	7.45±0.96	2.94±0.35	1.09±0.81

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