# ELECTRONIC SUPPLEMENTARY INFORMATION

## Suitability of bio-based ionic liquids for the extraction and purification of IgG antibodies

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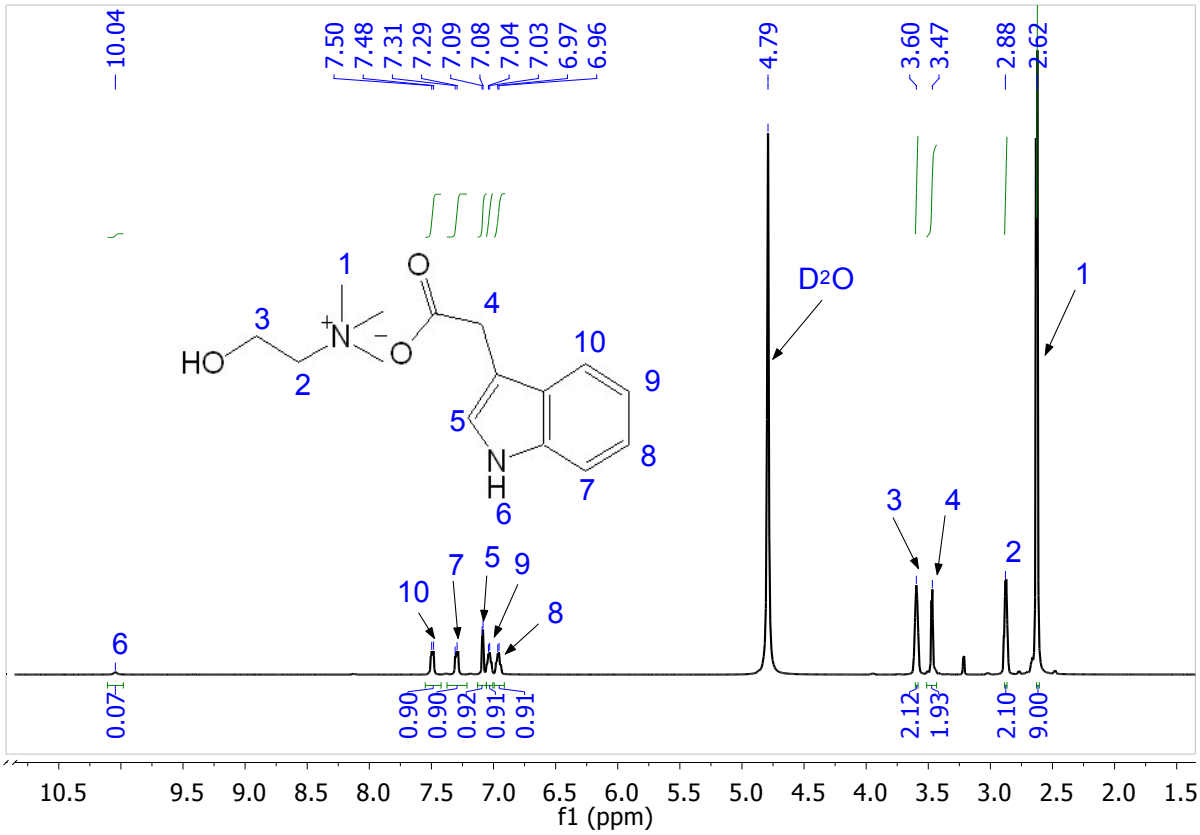


**Figure S1**: $^{1}$H NMR spectra of IL-1 (cholinium indole-3-acetate).

$^{1}$H NMR ($D_2O$, 500 MHz, δ/ppm relative to TMS): 2.62 (s, 9H, -$CH_3$), 2.88 (t, 2H, -$CH_2$-N-), 3.47 (s, 2H, -$CH_2$-CO-), 3.60 (t, 2H, -O-$CH_2$-), 7.08 (d, 1H, =CH-N-), 6.96-7.51 (m, 4H, aromatic protons), 10.04 (s, 1H, -HN-). ESI-MS: Calculated for $C_{15}H_{22}N_2O_3$ $[M]^+$: *m/z* = 104.11; Found: 104.17, $[M]^-$: *m/z* = 174.06; Found: 174.29.

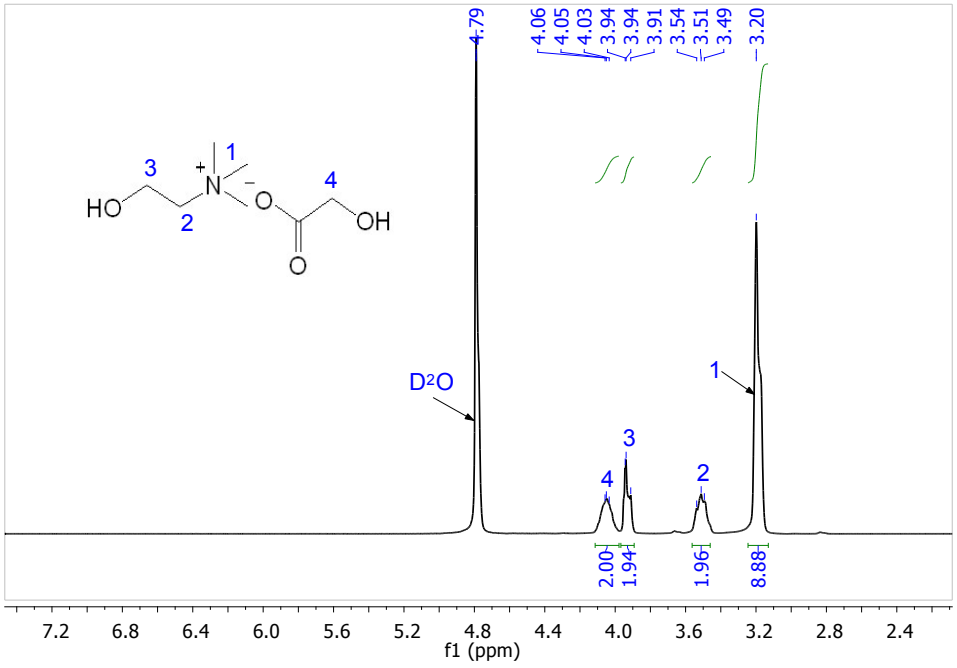


**Figure S2**: $^{1}$H NMR spectra of IL-2 (choline glycolate).

$^{1}$H NMR ($D_2O$, 500 MHz, δ/ppm relative to TMS): 3.20 (s, 9H, -N-$CH_3$), 3.51 (t, 2H, -$CH_2$-N-), 3.94 (t, 2H, -O-$CH_2$-), 4.05 (t, 1H, -$CH_2$-O-). ESI-MS: Calculated for $C_7H_{17}NO_4$ $[M]^+$: *m/z* = 104.11; Found: 103.97, $[M]^-$: *m/z* = 75.01; Found: 75.05.

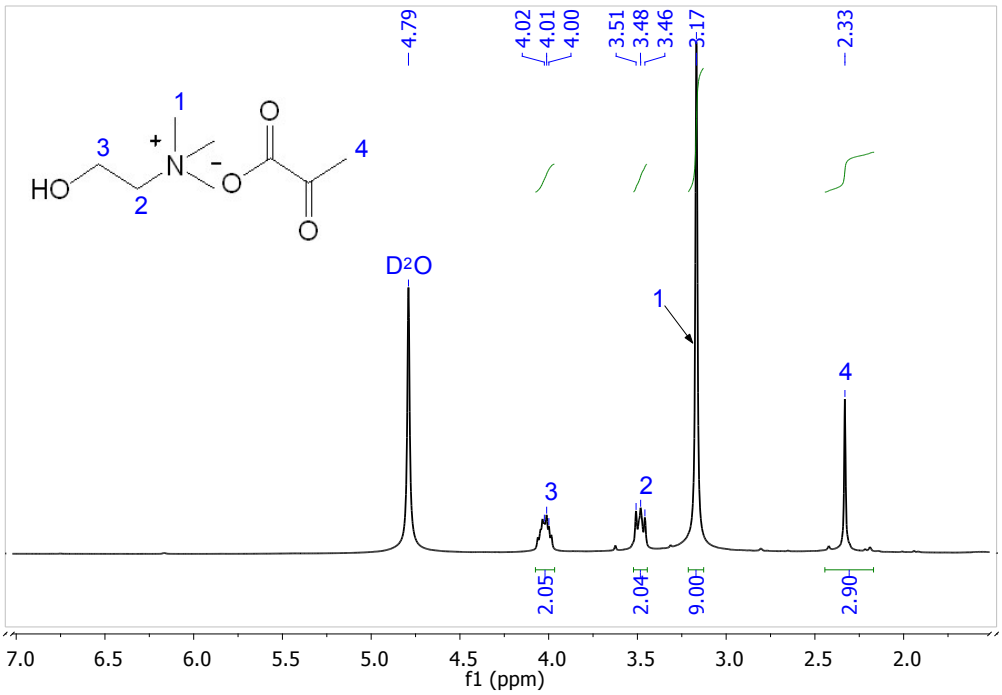


**Figure S3**: $^{1}$H NMR spectra of IL-3 (cholinium pyruvate).

$^{1}$H NMR ($D_2O$, 500 MHz, δ/ppm relative to TMS): ): 2.33 (s, 3H, -$CH_3$), 3.17 (s, 9H, -N-$CH_3$), 3.48 (t, 2H, -$CH_2$-N-), 4.01 (t, 2H, -O-$CH_2$-). ESI-MS: Calculated for $C_8H_{17}NO_4$ $[M]^+$: *m/z* = 104.11; Found: 104.00, $[M]^-$: *m/z* = 87.01; Found: 87.07.

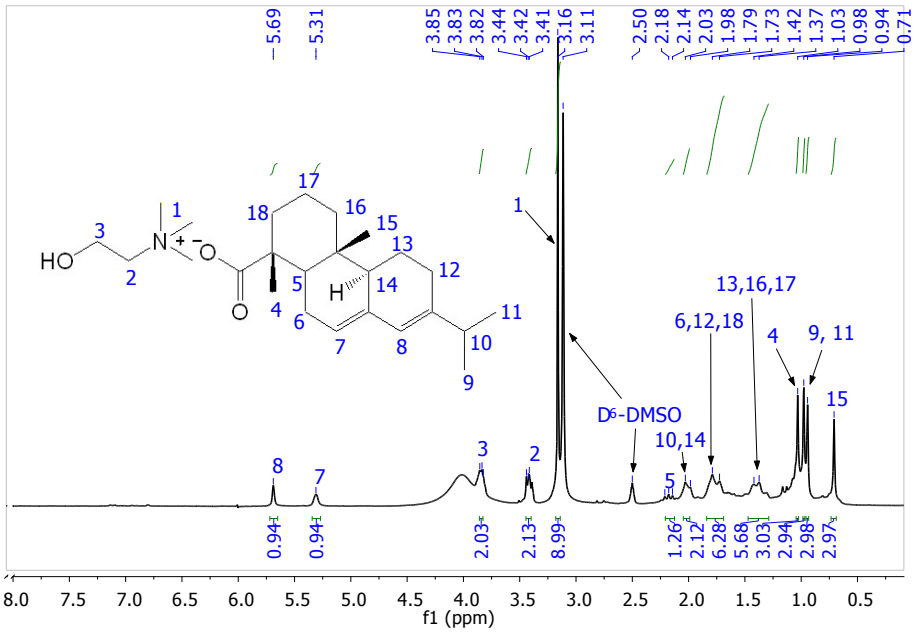


**Figure S4**: [1]H NMR spectra of IL-4 (cholinium abeitate).

[1]H NMR ($d_6$-DMSO, 500 MHz, δ/ppm relative to TMS): 0.71 (s, 3H, -$CH_3$), 0.94 (d, 3H, -$CH_3$), 0.98 (d, 3H, -$CH_3$), 1.03 (m, 3H, -$CH_3$), 1.37 (m, 6H, -$CH_2$-), 1.79 (m, 6H, -$CH_2$-), 2.03 (m, 2H, -$CH_2$-), 2.18 (m, 2H, -CH-), 3.16 (s, 9H, -N-$CH_3$), 3.42 (t, 2H, -$CH_2$-N-), 3.83 (t, 2H, -O-$CH_2$-), 5.31 (s, 1H, -HC=C-), 5.69 (s, 1H, -HC=C-). ESI-MS: Calculated for $C_{25}H_{43}NO_3$ $[M]^+$: *m/z* = 104.11; Found: 104.17, $[M]^-$: *m/z* = 301.22; Found: 301.28.

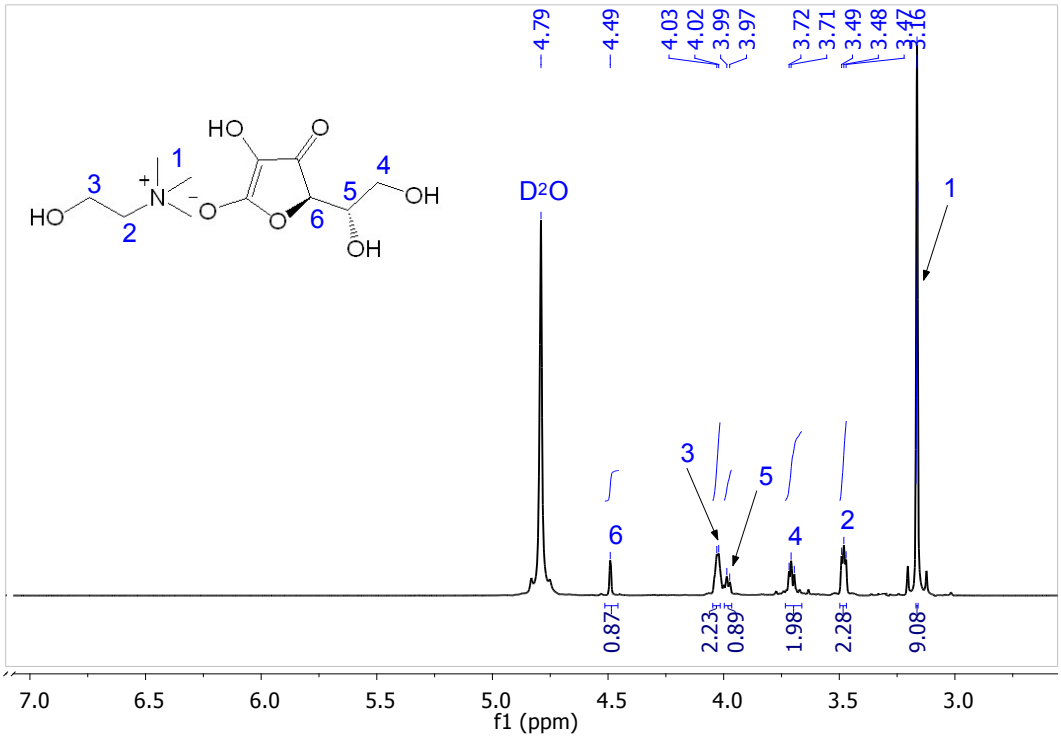


**Figure S5**: [1]H NMR spectra of IL-5 (cholinium-L-ascorbate).

[1]H NMR ($D_2O$, 500 MHz, δ/ppm relative to TMS): 3.16 (s, 9H, -N-$CH_3$), 3.48 (t, 2H, -N-$CH_2$-), 3.71 (dt, 2H, -$CH_2$-OH), 3.97 (m, 1H, -CH-OH), 4.02 (t, 2H, -$H_2C$-OH), 4.49 (s, 1H, -O-CH-). ESI-MS: Calculated for $C_{11}H_{21}NO_7$ $[M]^+$: m/z = 104.11; Found: 104.20, $[M]^-$: m/z = 175.02; Found: 175.13.

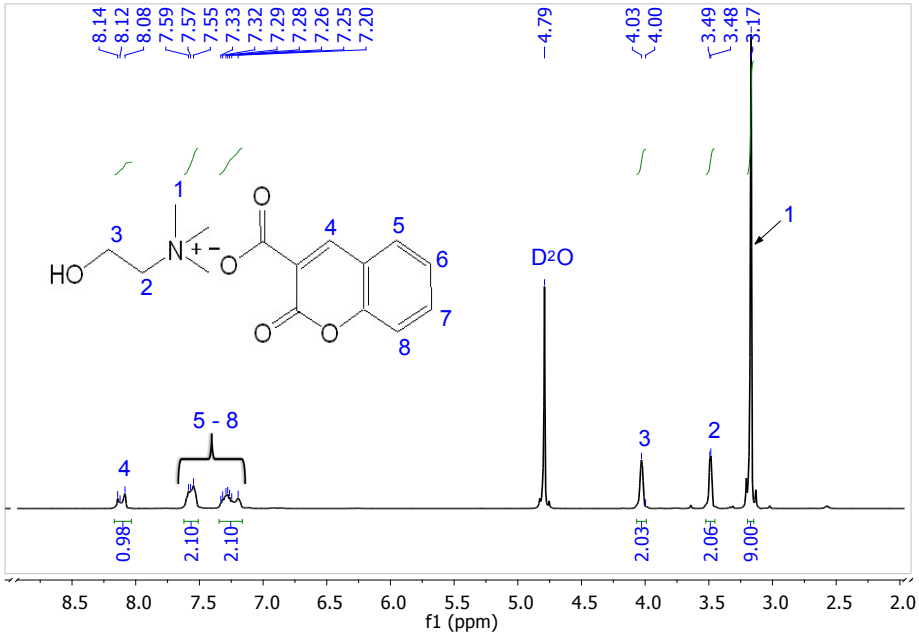


**Figure S6**: [1]H NMR spectra of IL-6 (cholinium coumarine-3-carboxylate).

[1]H NMR ($D_2O$, 500 MHz, δ/ppm relative to TMS): 3.17 (s, 9H, -N-$CH_3$), 3.498(t, 2H, -$CH_2$-N-), 4.00 (t, 2H, -$CH_2$-OH), 7.20-7.59 (m, 4H, aromatic protons), 8.12 (t, 1H, =CH-Ar). ESI-MS: Calculated for $C_{15}H_{19}NO_5$ $[M]^+$: m/z = 104.11; Found: 104.18, $[M]^-$: m/z = 189.02; Found: 189.15.

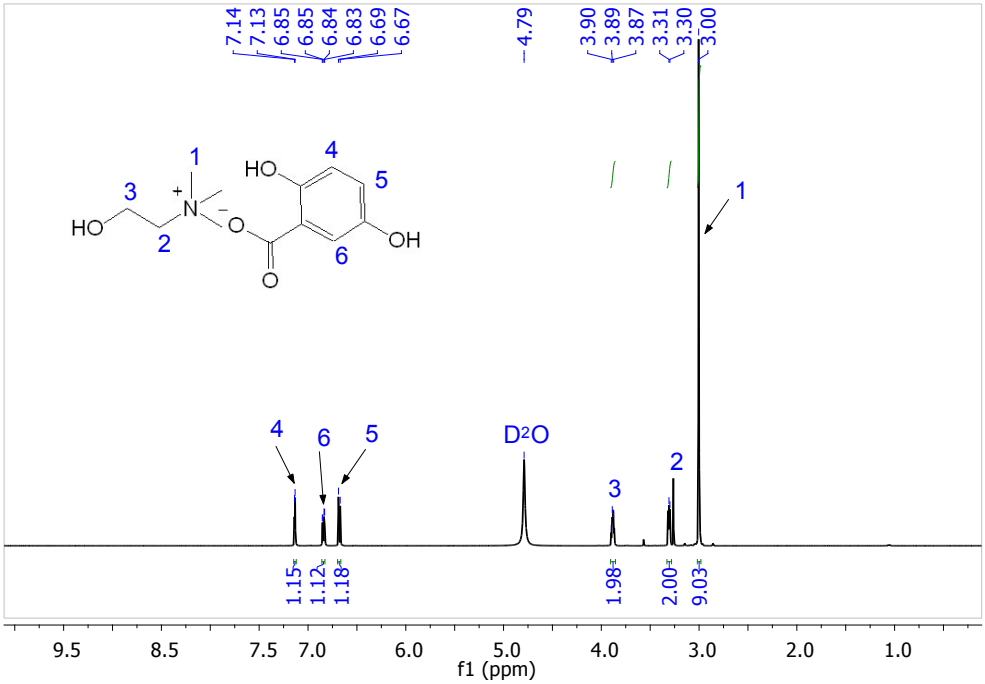


**Figure S7**: [1]H NMR spectra of IL-7 (cholinium-2,5-dihydroxybenzoate).

[1]H NMR ($D_2O$, 500 MHz, δ/ppm relative to TMS): 3.00 (s, 9H, -N-$CH_3$), 3.30 (t, 2H, -$CH_2$-N-), 3.87 (t, 2H, -O-$CH_2$-), 6.67 (d, 1H, =CH- aromatic), 6.84 (dd, 1H, =CH- aromatic), 7.13 (d, 1H, =CH- aromatic). ESI-MS: Calculated for $C_{12}H_{19}NO_5$ $[M]^+$: *m/z* = 104.11; Found: 104.13, $[M]^-$: *m/z* = 153.02; Found: 153.06.

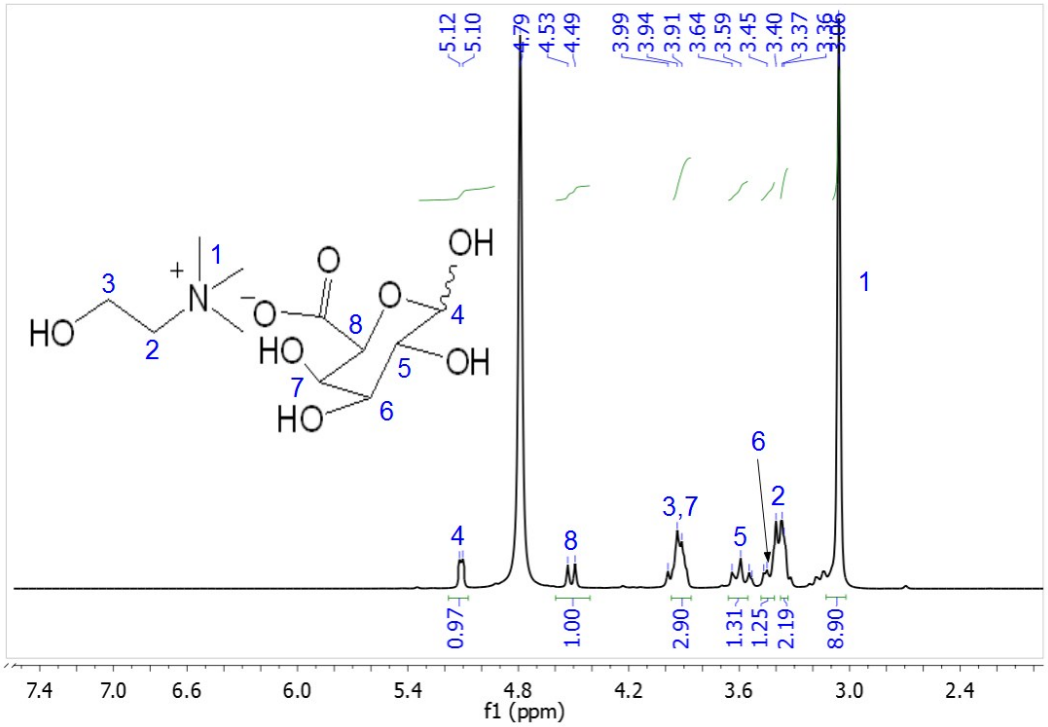


**Figure S8**: $^{1}$H NMR spectra of IL-8 (cholinium-D-(+)-galactouronate).

$^{1}$H NMR ($D_2O$, 500 MHz, δ/ppm relative to TMS): 3.06 (s, 9H, -N-$CH_3$), 3.36 (t, 2H, -$CH_2$-N-), 3.45 (d, 1H, -C**H**-OH), 3.59 (t, 1H, -C**H**-OH), 3.91 (m, 2H, -O-$CH_2$-), 3.99 (m, 1H, -C**H**-OH), 4.49 (d, 1H, -C**H**-$COO^-$), 5.10 (d, 1H, -C**H**-OH). ESI-MS: Calculated for $C_{11}H_{23}NO_8$ $[M]^+$: *m/z* = 104.11; Found: 104.17, $[M]^-$: *m/z* = 193.03; Found: 193.14.

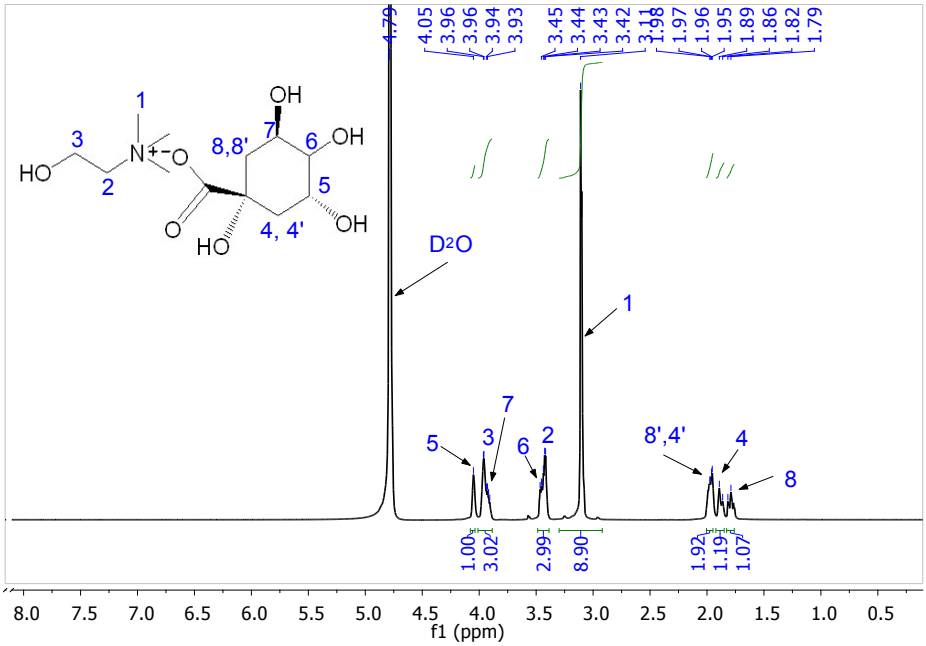


**Figure S9**: $^{1}$H NMR spectra of IL-9 (cholinium-D-(–)-quinate).

$^{1}$H NMR ($D_2O$, 500 MHz, δ/ppm relative to TMS): 1.79 (m, 1H, -$CH_2$-), 1.86 (m, 1H, -$CH_2$), 1.89 (m, 1H, -$CH_2$-), 1.96 (m, 2H, -$CH_2$-), 3.11 (s, 9H, -N-$CH_3$), 3.43 (t, 2H, -$CH_2$-N-), 3.45 (m, 1H, -CH-OH), 3.93 (m, 1H, -CH-OH), 3.96 (t, 2H, -O-$CH_2$-), 4.05 (m, 1H, -**H**C-OH). ESI-MS: Calculated for $C_{12}H_{25}NO_7$ $[M]^+$: *m/z* = 104.11; Found: 104.17, $[M]^-$: *m/z* = 191.05; Found: 191.16.

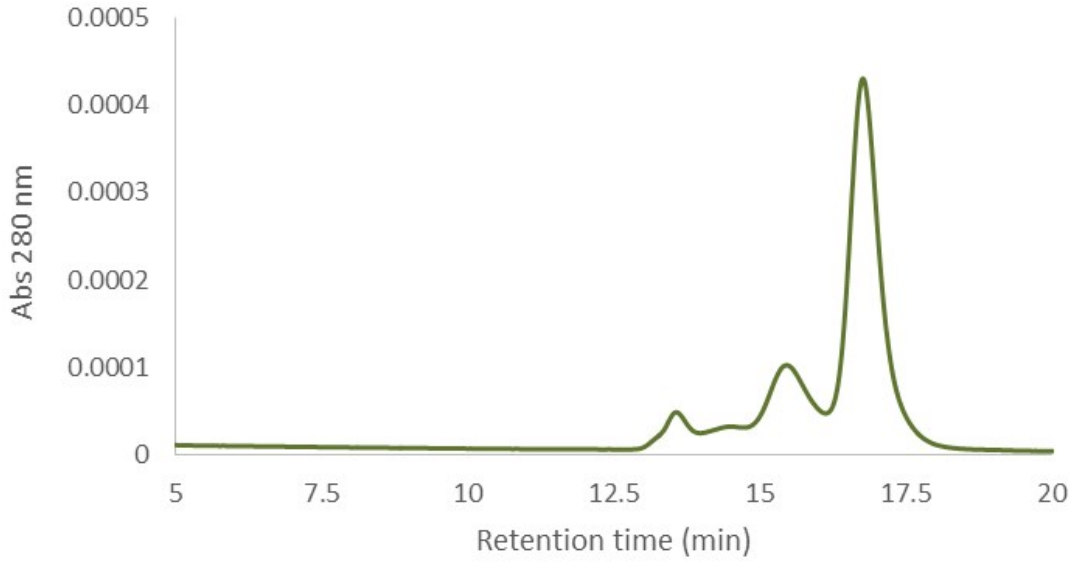


**Figure S10:** Size-exclusion chromatograms of commercial rabbit serum. The IgG peak is characterized by a retention time around 15.6 min. Peaks corresponding to lower retention times correspond to protein aggregates, also present in the commercial pure IgG, while the peak corresponding to the higher retention time corresponds to serum albumin.

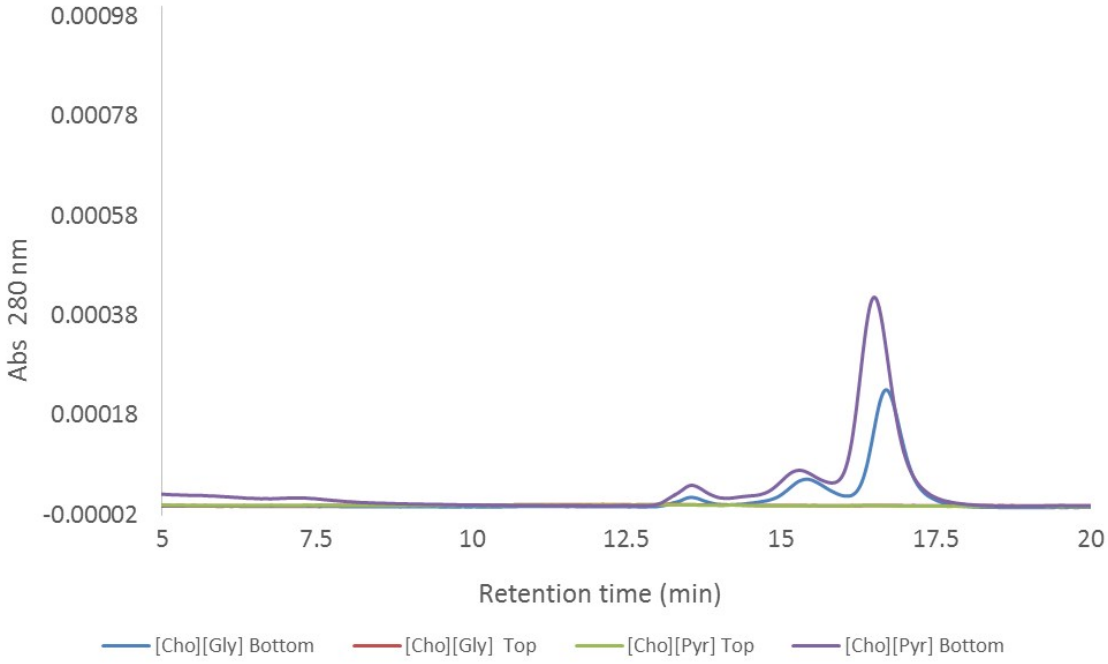


**Figure S11 :** Size-exclusion chromatograms of commercial rabbit serum (top and bottom phases) after the extraction with ionic-liquid-based ABS composed of [Cho][Gly] and [Cho][Pyr].

**Table S1:** Summary of the bio-based ILs synthesized.

| Cation | Anion | Sources of anion | Observation |
|---|---|---|---|
| Cholinium | Indole-3-acetate | Naturally occurring plant growth hormone found in all type of plants | Liquid at r.t. |
| | Indole-3-butyrate | Naturally occurring plant growth hormone found in all type of plants | Liquid at r.t. |
| | Glycolate | Sugarcane, sugar beets, pineapple and unripe grapes | Liquid at r.t. |
| | Pyruvate | Apple and wine | Liquid at r.t. |
| | Abeitate | Trees, pine wood & resins | Liquid at r.t. |
| | L-ascorbate | Orange, green pepper, papaya, kiwi, cauliflower, broccoli, citrus juice | Liquid at r.t. |
| | Coumarine-3-carboxylate | Tonka beans, vanilla grass, sweet grass and cassia cinnamon, Cherries, Apricot | Highly viscous at r.t. |
| | Genistate | African tree *Alchorneacordifolia* and wine | Semi-solid at r.t. |
| | D-(+)-Galactouronate | Fresh apple and trees | Liquid at r.t. |
| | D-(–)-Quinate | Cinchona bark & coffee beans | Liquid at r.t. |

**Note**: r.t. = room temperature (25 ºC)

**Table S2:** Experimental weight fraction data for the systems composed of IL (1) + PPG400 (2) + $H_2O$ (3) at 25 ºC.

| [Cho][IA] | | [Cho][Gal] | | [Cho][Asc] | | [Cho][Qui] | |
|---|---|---|---|---|---|---|---|
| W1 100 | W2 100 | W1 100 | W2 100 | W1 100 | W2 100 | W1 100 | W2 100 |
| 82.4468 | 2.6596 | 97.5816 | 1.2023 | 70.8522 | 1.9640 | 9.1611 | 9.1611 |
| 73.1132 | 6.1557 | 66.2352 | 1.8445 | 64.5122 | 3.0497 | 9.7281 | 9.7281 |
| 63.4076 | 11.8225 | 58.7615 | 2.7960 | 51.7866 | 5.7530 | 11.4003 | 11.4003 |
| 56.7454 | 17.1884 | 54.8822 | 3.6450 | 47.2744 | 6.8060 | 12.5340 | 12.5340 |
| 49.1595 | 24.4370 | 50.8973 | 4.2828 | 41.3080 | 7.9175 | 14.3345 | 14.3345 |
| 41.2015 | 33.1606 | 47.2442 | 5.0449 | 37.4845 | 8.8197 | 16.8416 | 16.8416 |
| 33.6189 | 42.5659 | 44.4952 | 5.1748 | 27.8751 | 12.6084 | 23.1121 | 23.1121 |
| 26.8445 | 51.9657 | 43.2505 | 5.4850 | 24.5975 | 16.0413 | 25.2966 | 25.2966 |
| 21.6163 | 59.4868 | 42.0451 | 5.7154 | 20.8751 | 19.9518 | 26.5002 | 26.5002 |
| 17.8716 | 64.9660 | 40.7003 | 6.1401 | 19.4996 | 20.2168 | 27.6180 | 27.6180 |
| 14.6600 | 69.6775 | 39.5427 | 6.4685 | 18.5338 | 21.1151 | 29.8614 | 29.8614 |
| 12.6603 | 72.5843 | 37.7845 | 6.8849 | 17.6260 | 21.8421 | 48.7142 | 48.7142 |
| 10.5809 | 75.5956 | 35.8236 | 7.0084 | 16.6897 | 22.7295 | 51.7196 | 51.7196 |
| 9.3627 | 77.4419 | 35.0000 | 7.3066 | 22.8388 | 15.2372 | 54.2745 | 54.2745 |
| 8.4887 | 78.7836 | 34.3175 | 7.9032 | 13.5938 | 26.4215 | 57.4239 | 57.4239 |
| 7.6642 | 80.1226 | 31.4114 | 8.0930 | 14.3913 | 25.2039 | 60.9038 | 60.9038 |
| 6.9984 | 81.0773 | 30.0260 | 8.4730 | 14.4669 | 23.9895 | 68.1847 | 68.1847 |
| 6.3774 | 82.0918 | 28.5624 | 9.1186 | 15.2372 | 22.8388 | | |
| 6.0837 | 82.4967 | 26.9978 | 9.6413 | 4.2536 | 71.1192 | | |
| 5.7082 | 83.1019 | 25.4148 | 10.1962 | 7.3126 | 51.4469 | | |
| 5.2862 | 83.7918 | 24.1379 | 10.6632 | 8.6776 | 33.5863 | | |
| 4.9432 | 84.2981 | 23.1365 | 11.2846 | 10.9115 | 27.6118 | | |
| 4.6220 | 84.8710 | 21.9884 | 11.7819 | 12.1787 | 25.4082 | | |
| 4.3539 | 85.2741 | 20.7284 | 12.5478 | | | | |
| 4.1528 | 85.5669 | 19.6796 | 13.0721 | | | | |
| 3.9487 | 85.8788 | 18.4484 | 13.7438 | | | | |
| | | 17.2470 | 14.8339 | | | | |

| | | | |
|---|---|---|---|
| 16.0030 | 15.4070 | | |
| 15.1593 | 16.1437 | | |
| 14.0615 | 17.1058 | | |
| 13.1140 | 17.7976 | | |

**Table S3:** Experimental weight fraction data for the systems composed of IL (1) + PPG400 (2) + $H_2O$ (3) at 25 ºC.

| [Cho][Pyr] | | [Cho][Gly] | |
|---|---|---|---|
| 100 w1 | 100 w2 | 100 w1 | 100 w2 |
| 51.6194 | 5.7660 | 29.9833 | 7.2523 |
| 40.8260 | 7.4312 | 29.5173 | 7.4783 |
| 35.6452 | 8.1962 | 22.8492 | 9.2222 |
| 31.4733 | 9.0681 | 21.0939 | 9.7013 |
| 29.0617 | 9.7930 | 19.9952 | 10.5799 |
| 27.1250 | 11.1490 | 17.6795 | 11.8493 |
| 25.1284 | 11.7514 | 15.3324 | 13.6604 |
| 23.6811 | 12.3272 | 14.3374 | 14.4192 |
| 20.9760 | 13.9474 | 13.5719 | 15.6594 |
| 19.8795 | 14.5234 | 12.6340 | 16.7270 |
| 19.2300 | 15.1080 | 17.6795 | 11.8493 |
| 17.7279 | 18.9364 | 4.2337 | 52.0548 |
| 13.1800 | 26.0942 | 3.8981 | 37.1061 |
| 12.6106 | 27.0895 | 5.7902 | 33.9906 |
| 12.1660 | 27.3838 | 5.8292 | 29.1543 |
| 11.6156 | 28.0929 | 6.4451 | 27.6432 |
| 11.0976 | 28.8153 | 7.8759 | 24.5846 |
| 12.9534 | 22.3279 | 7.7262 | 21.9783 |
| 16.5772 | 19.8381 | 8.7543 | 20.9681 |
| 16.4111 | 18.2344 | 11.3500 | 19.1677 |
| 20.2368 | 15.6078 | 12.0512 | 18.1374 |
| 3.8457 | 67.8161 | 13.6250 | 16.6966 |
| 5.5194 | 42.7979 | 13.8319 | 16.1968 |
| 5.3602 | 36.8585 | 14.0416 | 15.1628 |
| 7.9996 | 32.6353 | 14.6613 | 14.4244 |
| 10.8798 | 26.6710 | 17.8427 | 12.9492 |
| 10.6499 | 23.2414 | 23.1422 | 10.6558 |
| 17.6876 | 19.3579 | 33.8080 | 6.7471 |

| | | |
|---|---|---|
| 18.2525 | 17.0274 | |
| 19.7534 | 14.5141 | |
| 23.1474 | 12.9386 | |

Tie-lines (TLs) and tie-line lengths (TLLs) were determined by the lever–arm rule through the relationship between the top phase composition and the overall system composition, according to the following equations (Eqs.S1-4):

$$[PPG]_{PPG} = A\ exp\left[\left(B[IL]_{PPG}^{0,5}\right) - \left(C[IL]_{PPG}^{3}\right)\right] \quad \text{(S1)}$$

$$[PPG]_{IL} = A\ exp\left[\left(B[IL]X_{IL}^{0,5}\right) - \left(C[IL]_{IL}^{3}\right)\right] \quad \text{(S2)}$$

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

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

where subscripts "PPG", "IL", and "M" correspond to the top (PPG-400-rich), bottom (IL-rich) and mixture phases, respectively. The parameter, α is the ratio between the mass of the PPG rich phase and the total mass of the two phases. The system solution results in the composition (wt%) of the PPG 400 and IL in the top and bottom phases. For the calculation of each tie-line length (TLL), EqS5 was used:

$$TLL = \sqrt{\left([IL]_{PPG} - [IL]_{IL}\right)^2 + \left([PPG]_{PPG} - [PPG]_{IL}\right)^2} \quad \text{(S5)}$$

**Table S4.** Correlation parameters obtained from the fitting of the experimental binodal data by Eq. 1, respective standard deviations (σ) and correlation coefficients ($R^2$).

| IL | $A \pm \sigma$ | $B \pm \sigma$ | $10^{-5}$ ($C \pm \sigma$) | $R^2$ |
|---|---|---|---|---|
| [Cho][Gly] | 313.3 ± 38.6 | -0.883 ± 0.046 | -8.9 ± 1.7 | 0.9872 |
| [Cho][D-Gal] | 131.5 ± 0.7 | -0.475 ± 0.003 | 7.1 ± 0.8 | 0.9963 |
| [Cho][Qui] | 975.0 ± 175.3 | -1.146 ± 0.072 | -7.8 ± 0.3 | 0.9862 |
| [Cho][Pyr] | 284.3 ± 23.6 | -0.722 ± 0.029 | -3.0 ± 0.4 | 0.9620 |
| [Cho][Asc] | 217.5 ± 19.7 | 0.567 ± 0.029 | -13.3 ± 0.1 | 0.9967 |
| [Cho][lA] | 162.0 ± 6.1 | -0.504 ± 0.020 | 0.5 ± 0.02 | 0.9986 |

**Table S5.** Tie-lines (TLs) and tie-line lengths (TLLs). Initial mixture compositions are represented as $[IL]_M$ and $[PPG]_M$ whereas $[IL]_{IL}$ and $[PPG]_{IL}$ are the compositions of IL and PPG at the IL-rich phase, respectively, and *vice-versa*.

| IL | Weight fraction composition / wt % | | | | | | |
|---|---|---|---|---|---|---|---|
| | $[PPG]_{PPG}$ | $[IL]_{PPG}$ | $[PPG]_M$ | $[IL]_M$ | $[PPG]_{IL}$ | $[IL]_{IL}$ | TLL |
| [Cho][IA] | 48.27 | 1.73 | 40.16 | 39.77 | 1.73 | 82.53 | 69.63 |
| [Cho][D-Gal] | 85.66 | 0.81 | 29.76 | 26.20 | 0.078 | 39.68 | 93.99 |
| [Cho][Pyr] | 93.02 | 1.38 | 29.99 | 25.01 | 7.32 | 33.50 | 91.52 |
| [Cho][Qui] | - | - | 29.99 | 25.84 | - | - | ND* |
| [Cho][Gly] | - | - | 29.99 | 24.97 | - | - | ND |
| [Cho][Asc] | 72.96 | 3.71 | 30.20 | 25.36 | 6.52 | 36.85 | 74.25 |

*ND: not determined

**Table S6**: Extraction efficiencies of IgG ($EE_{IgG}$%), IgG recovery yield ($Y_{IgG}$%) and IgG percentage purity (%) from rabbit serum in ABS composed of PPG 400 (30 wt%) + bio-based IL (25 wt%) + 45 wt% of rabbit serum (diluted at 1:10 (v/v); pH ≈ 7).

| Bio-based IL | $EE_{IgG}$% | $Y_{IgG}$% | % purity |
|---|---|---|---|
| [Cho][Gly] | 100 | 45.1 | 24.8 |
| [Cho][Pyr] | 100 | 58.9 | 23.3 |
| [Cho][Asc] | 100 | 85.4 | 30.4 |
| [Cho][Qui] | 100 | 51.5 | 27.1 |