Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting Information

Unveiling the potential of deep eutectic solvents to improve the conformational and colloidal stability of immunoglobulin G antibodies

Diksha Dhiman[a], Ana S. C. Marques[b], Meena Bisht[a,b], Ana P. M. Tavares[b], Mara G. Freire[b]* and Pannuru Venkatesu[a]*

[a]Department of Chemistry, University of Delhi, Delhi-110 007, India

[b]CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Results

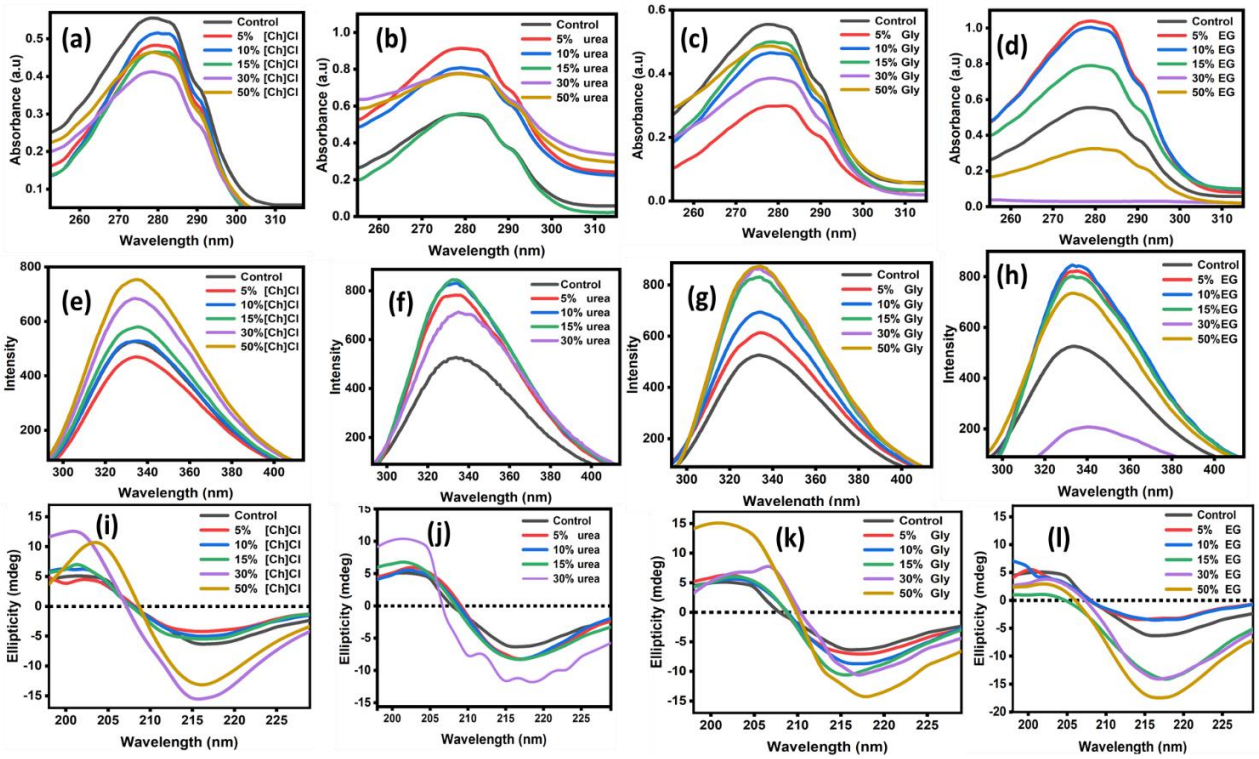

Fig. S1. Spectroscopic analysis of IgG. (a) (b) and (c) are UV visible absorption spectra; (d), (e) and (f) are fluorescence emission spectra; (g), (h) and (i) are far-UV CD spectra in the presence of sodium phosphate buffer pH 7.0, 10 mM (control) and at various concentrations of [Ch]Cl, Urea, Gly and EG at 25 °C.

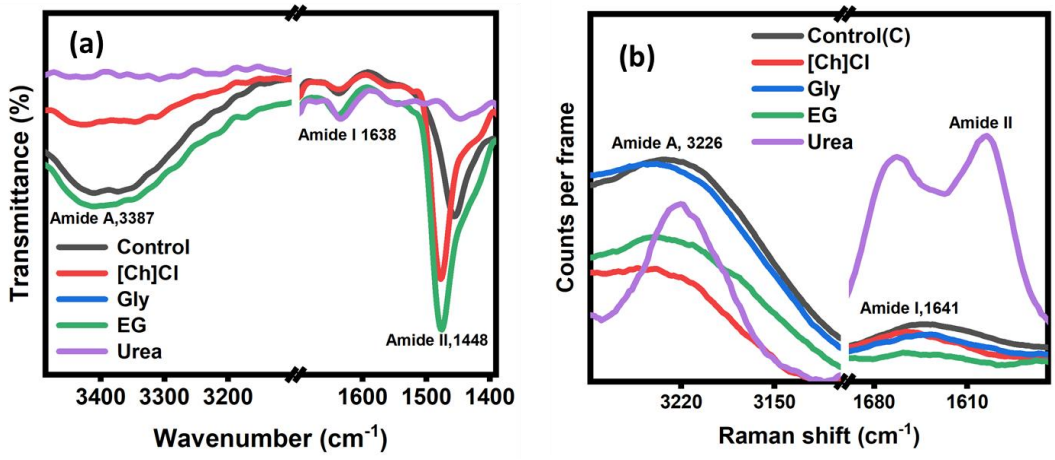

Fig. S2. (a) FT-IR spectra of IgG at 25°C in the presence of control and at 30% of DESs. (b) Raman spectra of IgG at 25°C in the presence of control and at 30% of DESs.

Thermodynamic stability studies of IgG

Sigmoidal fluorescence intensity curves were obtained for IgG in the presence of various concentrations of DESs. As indicated by Eqs. (1-3) below, the fractions of folded protein (f_f) and unfolded protein are (f_u) are determined by combining the intensity, Y, of protein found at a temperature, T, with the experimentally observed intensities of the pre-and post-transition values, and, respectively, of the native and denatured Y_f and Y_u protein. Using a linear fitting method, the latter values are obtained by extrapolating the pre-and post-transition baseline values. Upon each change in temperature, the system is assumed to be at thermal equilibrium. It is then possible to use Eq. 4 to define an effective equilibrium constant, K. This equilibrium constant is related to the standard free energy of formation, according to Eq. 5. The condition can define the melting temperature, Y=1/2 ($Y_f + Y_u$), for which K=1 and $\Delta_{fu} G = 0$.

$$N \leftrightharpoons D \quad (1)$$

$$f_u = (Y_f - Y) / (Y_f - Y_u) \quad (2)$$

$$f_u + f_f = 1 \quad (3)$$

$$K = f_u / f_f = (Y_f - Y) / (Y - Y_u) \quad (4)$$

$$\Delta_{fu} G = -RT \ln K \quad (5)$$

$$\Delta_{fu} H = T_m \Delta_{fu} S \quad (6)$$

If ΔC_p represent the difference in the isobaric heat capacities of the two forms of the protein, the integral of the Gibbs – Helmholtz equation leads to Eq. (7).[1,2]

$$\Delta_{fu} G(T) = \Delta_{fu} H(T_m) [1 - (T/T_m)] + \Delta C_p [(T - T_m) - T \ln (T/T_m)] \quad (7)$$

References:

(1) P. Venkatesu, M. J. Lee and H. M. Lin, *Journal of Physical Chemistry B*, 2009, **113**, 5327–5338.
(2) M. M. Santoro and D. W. Bolen, *Biochemistry*, 2002, **31**, 4901–4907.

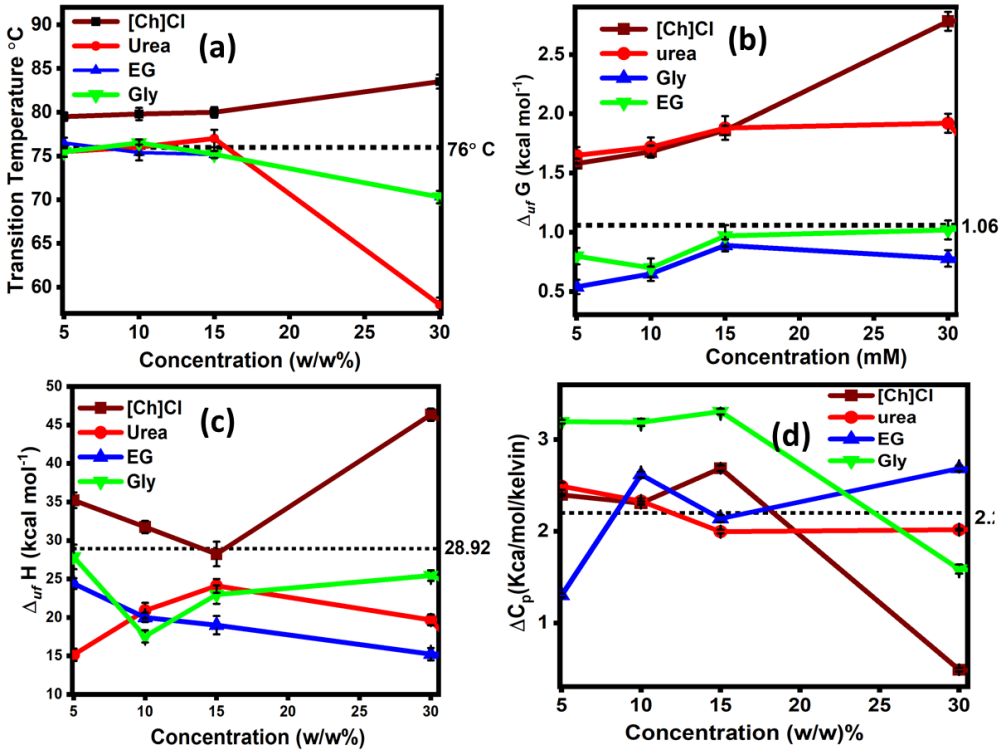

Fig. S3. Thermodynamic studies of IgG at 25ºC in the presence of sodium phosphate buffer pH 7.0, 10 mM (control) and as a function of concentration of DESs. (a) transition temperature (T_m); (b) Gibbs free energy changes ($\Delta_{fu}G$); (c) enthalpy change ($\Delta_{fu}H$) of unfolding; and (d) heat capacity change of unfolding (ΔC_p).

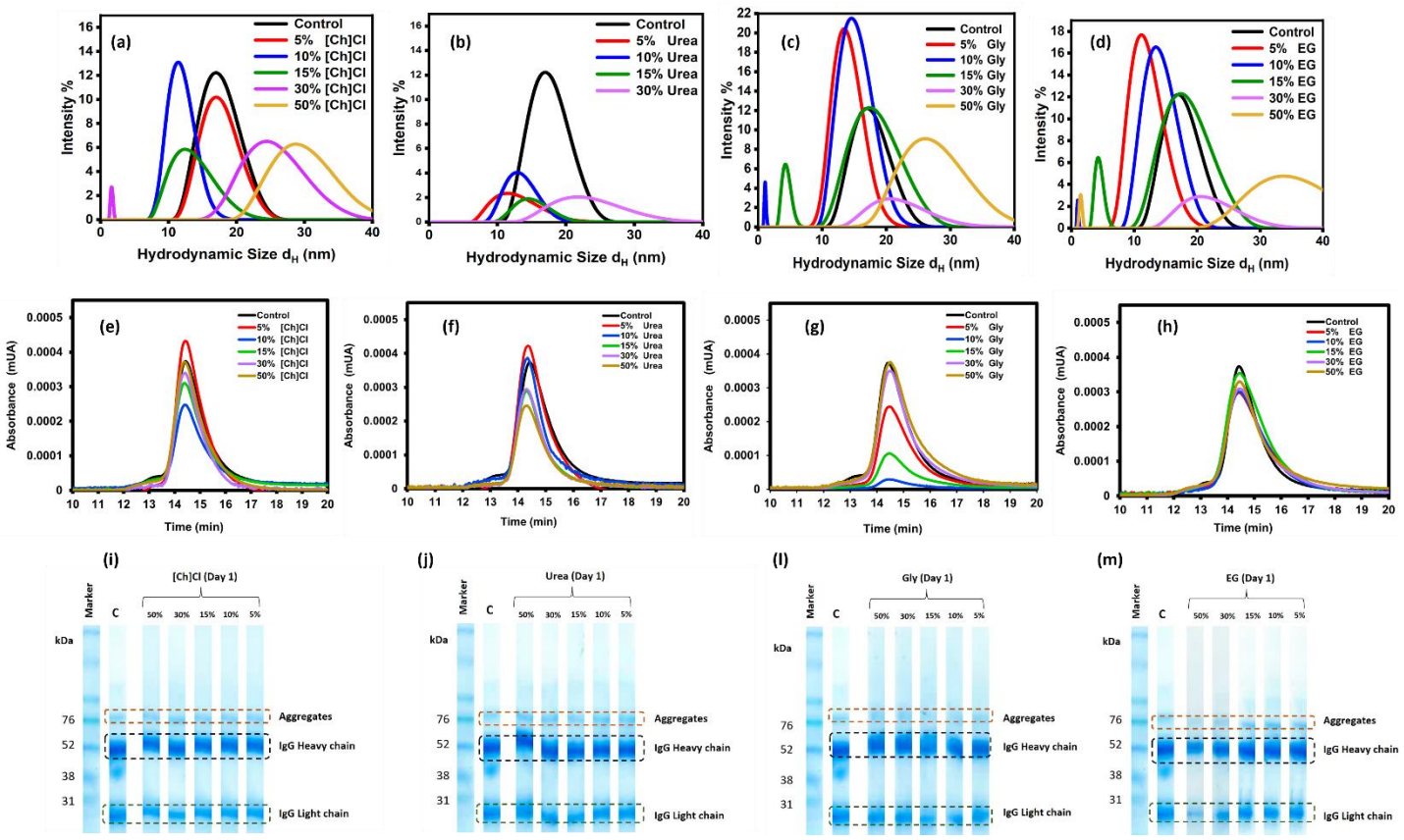

Fig. S4. Analysis of aggregation of IgG at 25 ºC in the presence of sodium phosphate buffer pH 7.0, 10 mM (control) and as a function of the concentration of DESs. (a), (b), (c) and (d) size-distribution plot. (e), (f), (g) and (h) SE-HPLC spectra. (i), (j), (l) and (m) SDS-PAGE.

Time-dependent studies of IgG stability in the presence of cholinium-based DESs

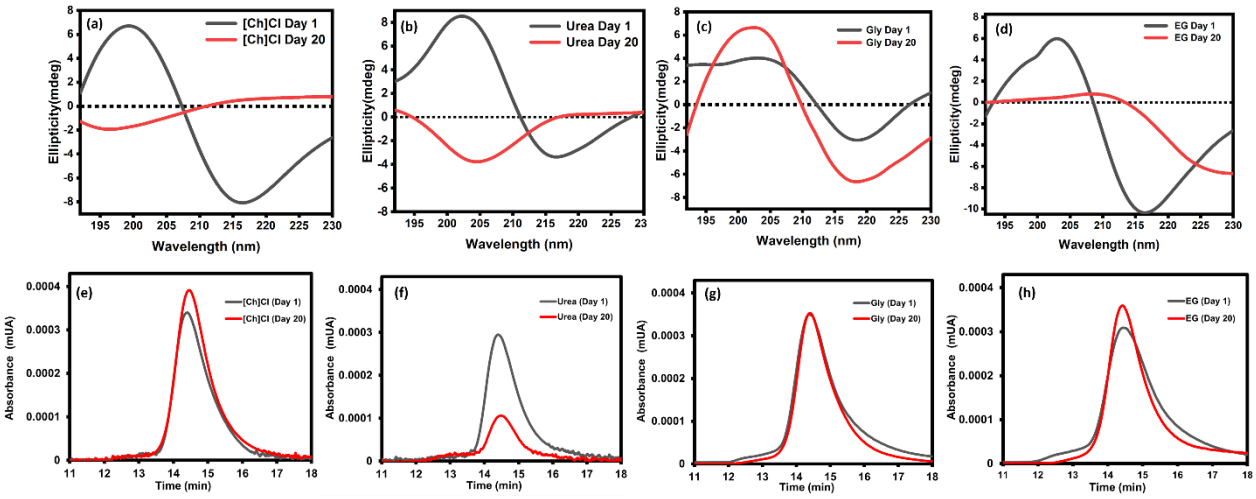

Fig. S5. Ellipticity versus wavelength as a function of time at 30% (a) [Ch]Cl; (b) urea; (c) Gly; and (d) EG. SE-HPLC chromatogram of IgG as a function of time at 30 % (e) [Ch]Cl; (f) urea; (g) Gly; and (h) EG.

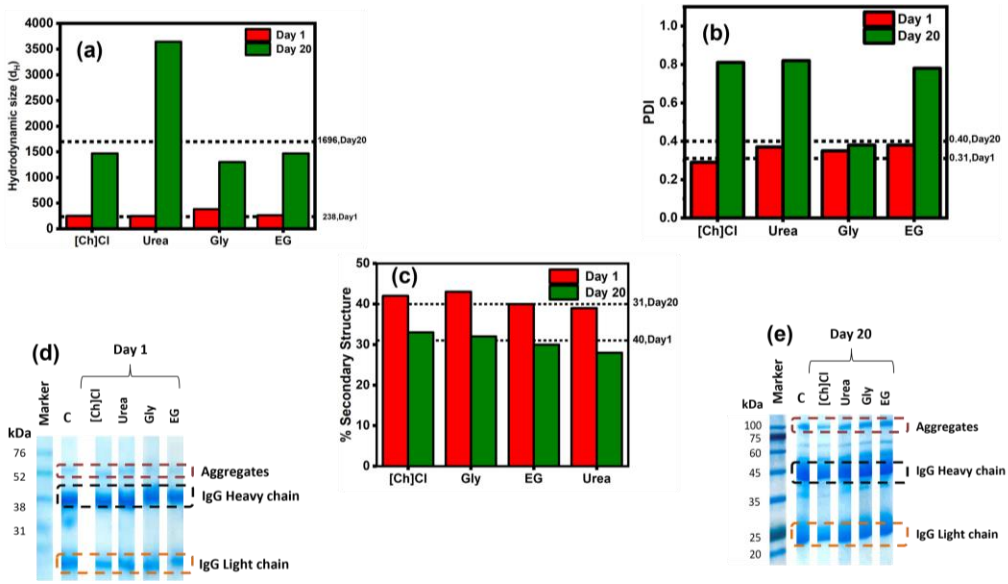

Fig. S6. (a) and (b) Aggregation studies of IgG as a function of time by calculating hydrodynamic size and PDI of IgG in the presence of 30% [Ch]Cl, urea, Gly and EG. (c) percentage secondary structure of IgG as a function of time. (d) and (e) SDS-PAGE spectra of IgG as a function of time, a1- day 1, a2- day 20. Lane 1 – molecular weight marker; lane 2 – IgG in sodium phosphate buffer pH 7.0, 10 mM (Control (C)) and DESs components at 30%.

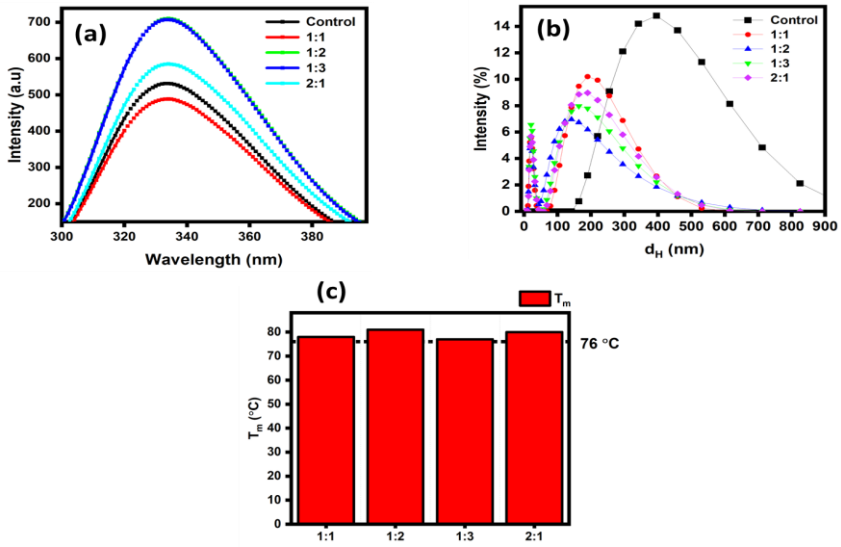

Fig. S7. (a) Fluorescence spectroscopy analysis; (b) hydrodynamic size as function of intensity of IgG in the presence of control and different DESs composition; and (c) bar graph of transition temperature (T_m) versus different composition of DESs; black dotted line reflects T_m of IgG in the presence of buffer.

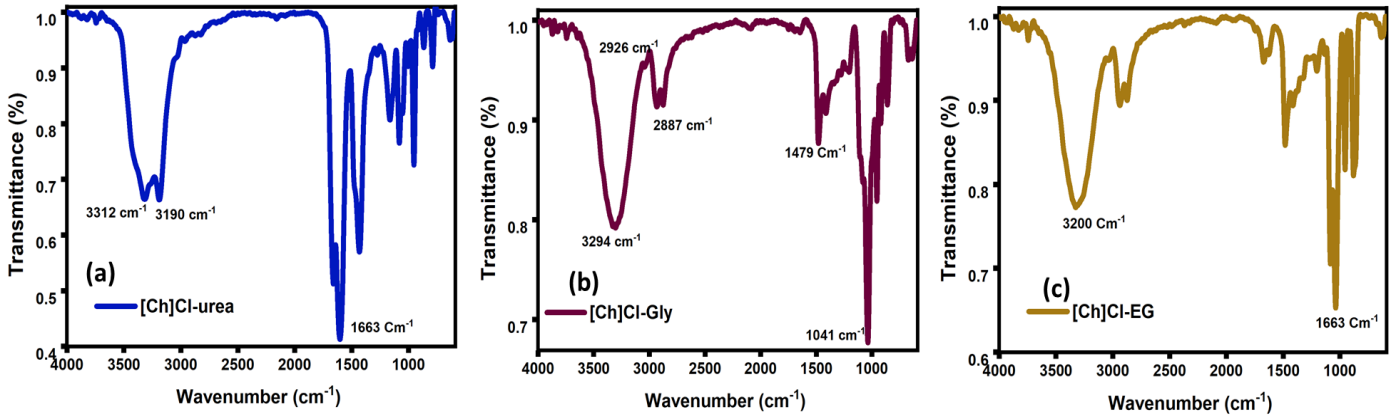

Fig. S8. FTIR spectra of DESs: (a) ChCl-urea (b) ChCl-Gly and (c) ChCl-EG.

Table S1. Wavenumber shift of IgG as a function of transmittance in various concentrations of [Ch]Cl-EG, [Ch]Cl, urea, Gly and EG.

Solvent	Wavenumber (nm) Amide A	Wavenumber (nm) Amide I	Wavenumber (nm) Amide II
Control	3390	1641	1455
Ch]Cl-urea	3390	1641	1448
[Ch]Cl-Gly	3390	1641	1448
[Ch]Cl-EG	3390	1641	1449
[Ch]Cl	3430	1638	1476
Gly	3407	1638	1477
EG	3409	1638	1476
Urea	-	1639	1449

Table S2. Raman shift of IgG as a function of transmittance in various concentrations of [Ch]Cl-urea, [Ch]Cl-Gly, [Ch]Cl-EG, [Ch]Cl, urea, Gly and EG.

Solvent	Raman Shift (nm) Amide A	Raman Shift (nm) Amide I
Control	3233	1643
[Ch]Cl-urea	3252	1652
[Ch]Cl-Gly	3252	1652
[Ch]Cl-EG	3234	1600
[Ch]Cl	3237	1652
Gly	3237	1643
EG	3247	1652
Urea	3220	1593

Table S3. Transition temperature (T_m), Gibbs free energy change of unfolding ($\Delta_{fu}G$), Enthalpy change ($\Delta_{fu}H$), Entropy change ($\Delta_{fu}S$) and heat capacity change (C_p) of unfolding at 25 ˚C, determined by thermal fluorescence analysis of thermal denaturation of IgG in absence and presence of cholinium-based DESs at various concentrations.

	T_m (ºC)	**T_m (K)**	**$\Delta_{uf}G$ (kcal/mol)**	**$\Delta_{uf}S$ (kcal/mol)**	**$\Delta_{uf}H$ (kcal mol^{-1})**	**ΔC_p (kcal mol^{-1})**
0	76.0 ± 0.9	349.0 ± 0.9	1.06 ± 0.04	0.08	28.9 ± 1.2	0.29 ±1.4
[Ch]Cl-urea						
5%	76.9 ± 0.3	351.0 ± 0.3	1.59 ± 0.05	0.25	87.8 ± 0.9	1.75 ± 0.07
10%	77.5 ± 0.2	349.8 ± 0.2	1.91 ± 0.09	0.31	80.4 ± 1.1	2.15 ± 0.05
15%	78.0 ± 0.2	350.4 ± 0.2	2.23 ± 0.1	0.30	85.1 ± 1.0	2.21± 0.04
30%	78.5± 0.4	350.5 ± 0.2	2.62 ± 0.2	0.11	112.0 ± 1.2	1.74 ± 0.02
50%	76.9 ± 0.4	335.0 ± 0.4	1.17 ± 0.01	0.04	12.4 ± 1.0	2.95 ± 0.03
[Ch]Cl-Gly						
5%	76.7 ± 0.3	344.7 ± 0.3	1.59 ± 0.05	0.15	51.7 ± 0.5	1.68 ± 0.02
10%	77.9 ± 0.2	347.9 ± 0.2	1.84 ± 0.08	0.36	86.8 ± 1.2	1.96 ± 0.02
15%	78.8 ± 0.2	348.8 ± 0.2	2.12 ± 0.05	0.33	95.1 ± 0.7	2.1 ± 0.1
30%	80.0 ± 0.4	353.0 ± 0.4	2.98 ± 0.04	0.06	125.2 ± 0.4	0.87 ± 0.06
50%	76.0 ± 0.4	349.0 ± 0.4	1.93 ± 0.07	0.11	38.4 ± 2.0	3.92 ± 0.08

[Ch]Cl-EG						
5%	76.0 ± 0.2	349.0 ± 0.2	1.41 ± 0.06	0.20	46.76 ± 1.2	2.71 ± 0.08
10%	76.1 ± 0.2	349.0 ± 0.2	1.80 ± 0.07	0.32	68.7 ± 0.8	2.3 ± 1.0
15%	76.5 ± 0.2	349.5 ± 0.2	1.89 ± 0.08	0.28	72.9 ± 1.5	2.5 ± 0.9
30%	76.5 ± 0.4	349.5 ± 0.4	1.95 ± 0.08	0.15	98.0 ± 1.1	1.3 ± 1.2
50%	74.0 ± 1.2	347.0 ± 0.4	1.8 ± 0.1	0.04	13.9 ± 0.7	3.4 ± 0.9
[Ch]Cl						
5%	79.5 ± 0.7	352.5 ± 0.7	1.58 ± 0.04	0.10	35.2 ± 1.0	2.4 ± 0.6
10%	79.8 ± 0.9	352.8 ± 0.9	1.68 ± 0.04	0.09	31.8 ± 1.2	2.3 ± 0.5
15%	80.0 ± 0.7	353.0 ± 0.7	1.86 ± 0.04	0.08	28.2 ± 1.5	2.68 ± 0.06
30%	83.5 ± 0.8	356.5 ± 0.4	2.78 ± 0.08	0.13	46.3 ± 1.3	0.49 ± 0.05
50%	54.0 ± 1.4	327.0 ± 0.7	2.93 ± 0.07	0.08	26.2 ± 0.9	0.19 ± 0.05
Urea						
5%	75.5 ± 0.8	348.5 ± 0.5	0.80 ± 0.04	0.23	15.2 ± 0.8	2.49 ± 0.08
10%	76.0 ± 0.9	349.0 ± 0.6	0.70 ± 0.07	0.22	20.9 ± 1.0	2.33 ± 0.04
15%	77.0 ± 0.9	350.0 ± 0.9	0.97 ± 0.04	0.21	24.1± 0.9	1.99 ± 0.07
30%	58.0 ± 0.7	331.0 ± 0.7	1.0 ± 0.7	0.06	19.7 ± 0.7	2.01 ± 0.05
50%	43.6 ± 1.2	316.6 ± 1.2	1.3 ± 0.7	-0.07	22.2 ± 0.9	2.10 ± 0.03

EG						
5%	75.5 ± 0.6	348.5 ± 0.6	0.54 ± 0.03	0.07	24.4 ± 1.7	1.29 ± 0.06
10%	76.5 ± 0.5	349.5 ± 0.5	0.65 ± 0.07	0.24	20.9 ± 1.5	2.61 ± 0.09
15%	75.2 ± 0.8	348.2 ± 0.8	0.89 ± 0.04	0.27	19.0 ± 1.2	2.13 ± 0.02
30%	70.3 ± 0.9	343.3 ± 0.9	0.78 ± 0.03	0.19	15.2 ± 1.5	2.68 ± 0.08
50%	51.7 ± 0.8	324.7 ± 0.7	0.56 ± 0.07	0.05	16.2 ± 1.2	2.00 ± 0.06
Gly						
5%	76.5 ± 0.4	349.5 ±0 .4	1.65 ± 0.05	0.28	27.9 ± 1.6	3.19 ± 0.02
10%	75.4 ± 0.6	348.4 ± 0.6	1.72 ± 0.06	0.28	17.6 ± 1.3	3.18 ± 0.08
15%	75.2 ± 0.6	348.2 ± 0.6	1.88 ± 0.04	0.29	22.9 ± 1.8	3.30 ± 0.06
30%	86.0 ± 0.7	359.0 ± 0.7	1.92 ± 0.08	0.16	25.4 ± 1.3	1.58 ± 0.04
50%	81.0 ± 0.9	354.0 ± 0.9	-1.05 ± 0.07	0.13	10.0 ± 1.3	1.28 ± 0.07

Table S4. Hydrodynamic diameter (d_H) values in various concentrations of [Ch]Cl-urea, [Ch]Cl-Gly, [Ch]Cl-EG, [Ch]Cl, urea, Gly and EG.

Solvent (w/w%)	Hydrodynamic size (d_H) (nm)	PDI values
[Ch]Cl-urea		
0	15.07	0.51
5%	13.31	0.44
10%	15.98	0.37
15%	16.03	0.47
30%	18.83	0.59
50%	24.53	0.69
[Ch]Cl-Gly		
5%	10.90	0.33
10%	13.23	0.35
15%	14.37	0.33
30%	17.86	0.66
50%	31.34	0.94
[Ch]Cl-EG		
5%	14.96	0.24
10%	14.99	0.32
15%	18.04	0.59
30%	20.08	0.87
50%	30.73	0.94
[Ch]Cl		
5%	17.10	0.41
10%	11.53	0.42
15%	12.95	0.56
30%	24.72	0.78
50%	29.14	0.97

Urea

5%	12.23	0.83
10%	13.15	0.55
15%	14.66	0.33
30%	33.54	0.54

Gly

5%	13.61	0.30
10%	14.80	0.43
15%	17.54	0.42
30%	21.21	0.95
50%	26.57	0.62

EG

5%	12.61	0.25
10%	14.80	0.33
15%	16.54	0.32
30%	18.21	0.75
50%	21.57	0.42

Table S5. Values of the peak areas of the [Ch]Cl-Gly chromatograms shown in the Figure 4e.

Sample	Area
Control	1.395×10^{-3}
[Ch]Cl-Gly 5%	1.375×10^{-3}
[Ch]Cl-Gly 10%	1.182×10^{-3}
[Ch]Cl-Gly 15%	9.605×10^{-4}
[Ch]Cl-Gly 30%	6.242×10^{-4}
[Ch]Cl-Gly 50%	5.329×10^{-4}

Table S6. Hydrodynamic diameter (d_H) values in various concentrations of [Ch]Cl-urea, [Ch]Cl-Gly, [Ch]Cl-EG, [Ch]Cl, Gly, EG and urea on day 1 and day 20.

Day-1		
Solvent System	**Hydrodynamic size (dH)**	**PDI**
Control	238	0.30
[Ch]Cl-urea	166	0.21
[Ch]Cl-Gly	177	0.24
[Ch]Cl-EG	213	0.23
[Ch]Cl	252	0.29
EG	264	0.37
Gly	382	0.35
Urea	249	0.38
After day - 20		
Control	1696	0.49
[Ch]Cl-urea	989	0.45
[Ch]Cl-Gly	1584	0.48
[Ch]Cl-EG	1610	0.35
[Ch]Cl	1468	0.81
EG	1468	0.78
Gly	1300	0.38
Urea	4643	0.82

Table S7. Transition temperature (T_m) and hydrodynamic (d_H) values of IgG in the presence of various DESs composition.

[Ch]Cl:urea	Transition Temperature (^oC)	Hydrodynamic size (d_H) (nm)
Control	76	414
1:1	78	85
1:2	81	196
1:3	77	213
2:1	80	204