Electronic Supplementary Information (ESI)

The attenuating ability of deep eutectic solvents towards carboxylated multiwalled carbon nanotubes induced-denatured β-lactoglobulin structure

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This supporting information contains 2 tables and 12 figures in 11 pages

Materials

β-lactoglobulin (Blg) from bovine milk and glycerol were purchased from Sigma-Aldrich, USA. Choline chloride (ChCl) (A15828) bought from Alfa Aesar, urea extrapure AR obtained from Sisco Research Laboratory Pvt. Ltd. (SRL), India. All chemicals were of high purity and analytical grade. The salts used for preparation of sodium phosphate buffer (sodium phosphate dibasic dehydrate and sodium phosphate monobasic anhydrous and carboxylated multiwalled CNTs (CA-MWCNTs) were synthesized by performing functionalization of crude MWCNTs obtained were purchased from SRL. The characterization and functionalization method has already been provided by our research group.[1] The two DESs, ChCl-urea and ChCl-glycerol were synthesized by following our previous work.[2,3]

Spectroscopic measurements

UV-visible absorption spectra of Blg in presence of DESs, CA-MWCNTs and their mixtures

The absorption spectra of Blg in buffer, DESs, CA-MWCNTs and their mixtures were recorded using Shimadzu UV-visible spectrophotometer (UV-1800) (Japan) with the highest resolution (1 nm) using 1 cm path length quartz cuvette. The spectra were recorded at room temperature in the range of 200-600 nm. All spectra were averaged over three scans. All the spectra presented here were obtained after subtracting respective blank solutions.

Steady state fluorescence spectra of Blg in presence of DESs, CA-MWCNTs and their mixtures

The steady-state fluorescence experiments were performed on a Cary Eclipse spectrophotometer from Varian optical spectroscopy instruments, Mulgrave, Australia. The temperature was maintained at 25 ˚C using a Peltier device attached to the thermostatic cell holder. To record the role of Tryptophan (Trp) residue, the excitation wavelength was 295 nm with slit width of 5 nm was set for both excitation and emission. All the spectra presented here were obtained after subtracting respective blank solutions.

Circular dichroism (CD) studies for Blg in presence of DESs, CA-MWCNTs and their mixtures

Circular dichroism (CD) spectroscopic studies were performed by employing Jasco-185 spectrophotometer (USA) for studying the structural alterations in protein in presence of DESs, CA-MWCNTs and their mixtures. The temperature was controlled with a Peltier system having an accuracy of ±0.1 °C. Far- and near-UV CD spectra were observed in the range of 190-250 and 250-350 nm, at 25 ˚C respectively. The parameter values were as follows: response time, 1 s; bandwidth, 1 nm; and scan rate, 50 nm min-1. The composition of secondary structures for Blg under different conditions was calculated by using an online CD analysis program, DICHROWEB, with the help of CONTIN/LL algorithm. All the spectra were a result of averaging three accumulations. All the spectra presented here were obtained after subtracting respective blank solutions.

Dynamic light scattering measurement for Blg in presence of DESs, CA-MWCNTs and their mixtures

For analyzing the effect of CA-MWCNTs, DESs and their mixture on hydrodynamic diameter (d_H) and zeta potential of Blg, dynamic light scattering (DLS) measurements were carried out by employing a Zetasizer Nano instrument (UK). The scattering angle was 90˚ having operating wavelength 633 nm. The instrument was fitted with a 4 mW He-Ne laser.

Thermal stability analysis of Blg in presence of DESs, CA-MWCNTs and their mixtures

The thermal stability of Blg in mixtures of DESs, CA-MWCNTs and their mixtures was analysed with the help of same Cary Eclipse fluorescence spectrofluorimeter by employing temperature range between 25 to 98 °C. The heating rate was set at 2 °C min^{-1} and the excitation wavelength at 295 nm. The thermal unfolding transition was determined by using the two-state unfolding mechanism.

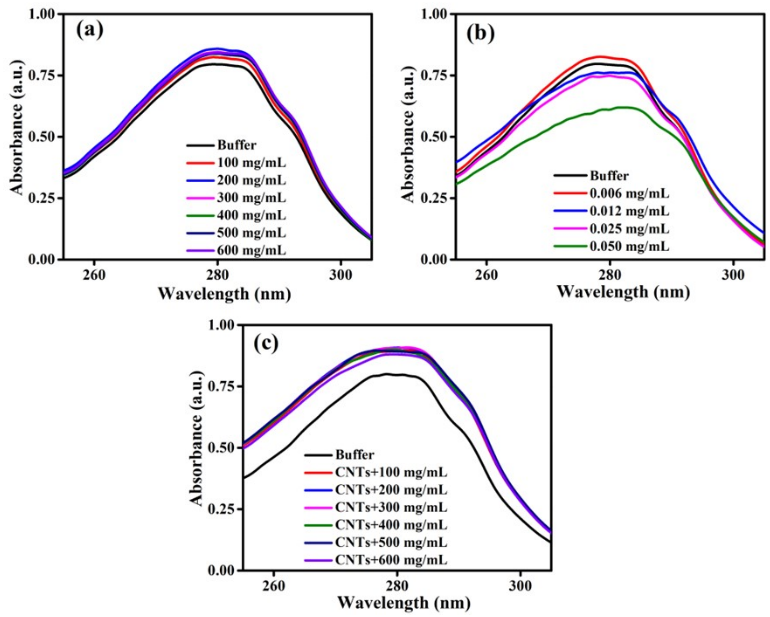

Fig. S1 UV-visible absorption spectra of Blg in presence of various concentrations of (a) DES-1(100-600 mg/mL), (b) CA-MWCNTs (0.006-0.050 mg/mL) and (c) mixture of DES-1(100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

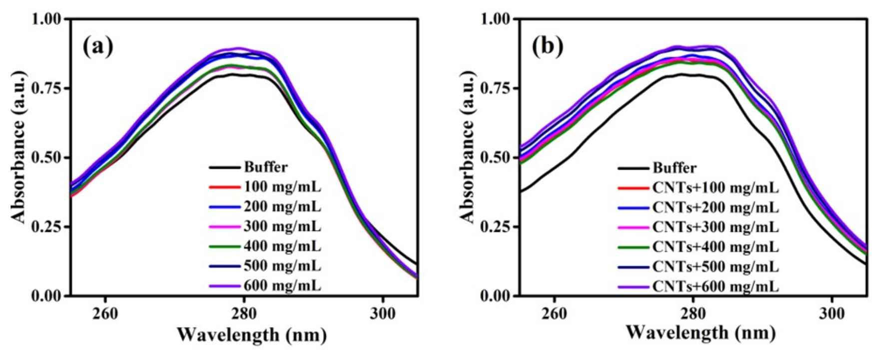

Fig. S2 UV-visible absorption spectra of Blg in presence of various concentrations of (a) DES-2 (100-600 mg/mL) and (b) mixture of DES-2 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

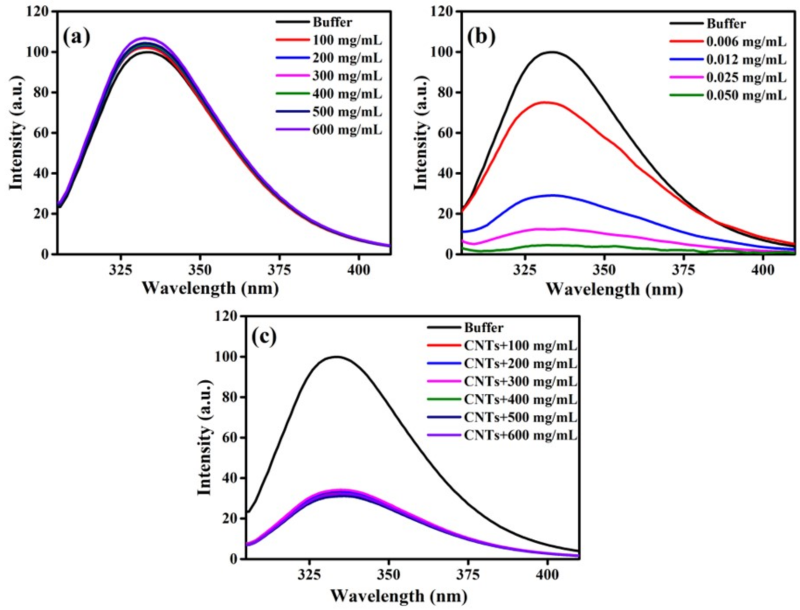

Fig. S3 Steady state fluorescence spectra of Blg in presence of various concentrations of (a) DES-1(100-600 mg/mL), (b) CA-MWCNTs (0.006-0.050 mg/mL) and (c) mixture of DES-1 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

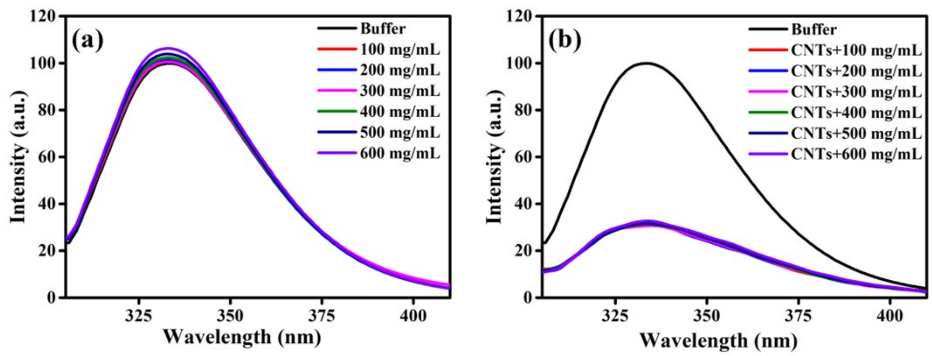

Fig. S4 Steady state fluorescence spectra of Blg in presence of various concentrations of (a) DES-2 (100-600 mg/mL) and (b) mixture of DES-2 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

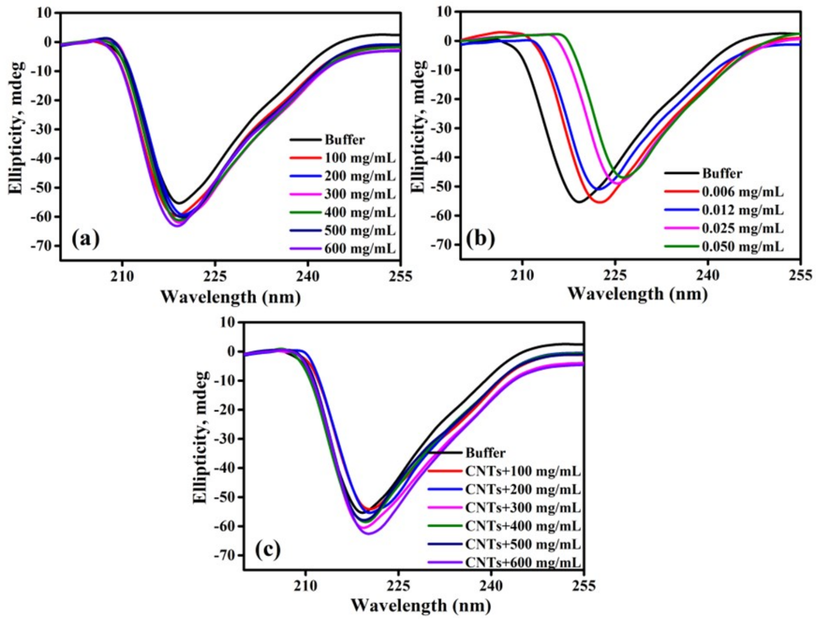

Fig. S5 Far UV-CD spectra of Blg in presence of various concentrations of (a) DES-1(100-600 mg/mL), (b) CA-MWCNTs (0.006-0.050 mg/mL) and (c) mixture of DES-1 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

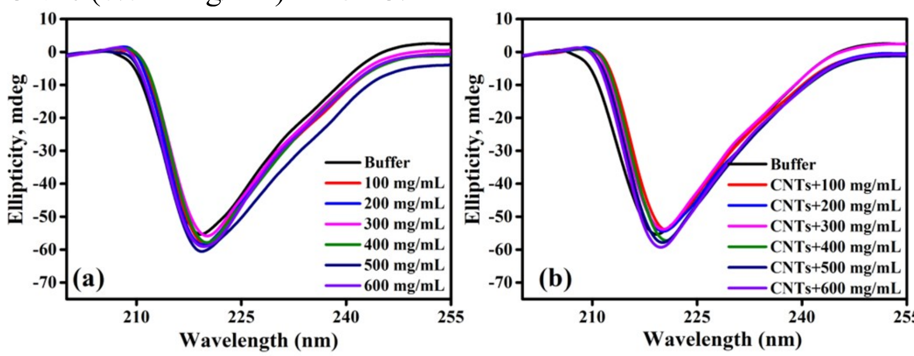

Fig. S6 Far UV-CD spectra of Blg in presence of various concentrations of (a) DES-2 (100-600 mg/mL) and (b) mixture of DES-2 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

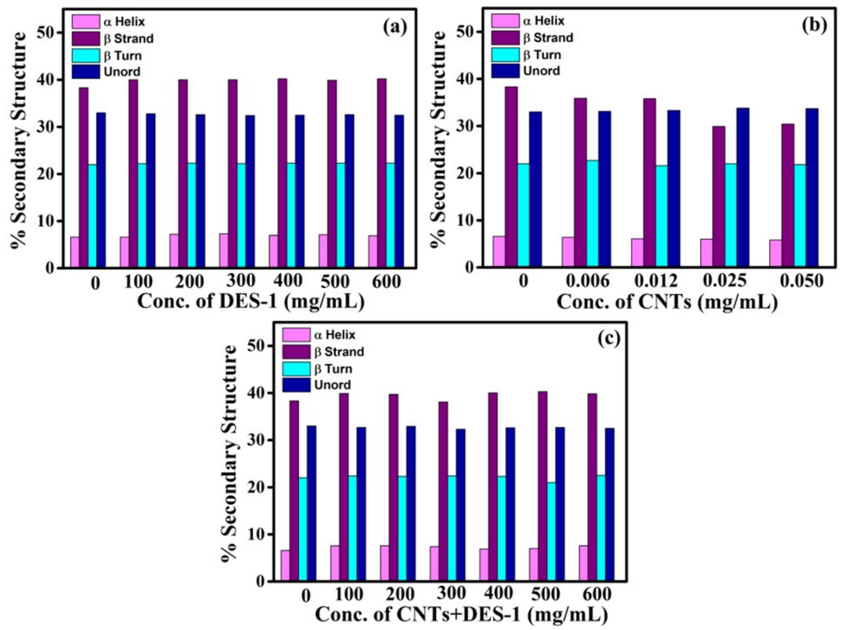

Fig. S7 % Secondary spectra of Blg in presence of various concentrations of (a) DES-1(100-600 mg/mL), (b) CA-MWCNTs (0.006-0.050 mg/mL) and (c) mixture of DES-1 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

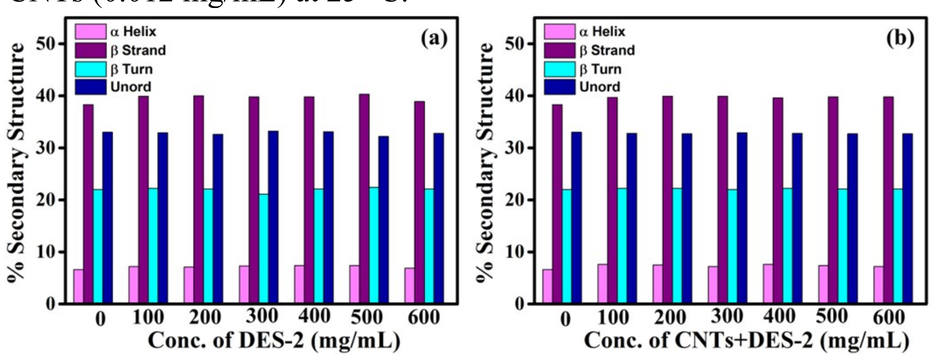

Fig. S8 % Secondary spectra of Blg in presence of various concentrations of (a) DES-2 (100-600 mg/mL) and (b) mixture of DES-2 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

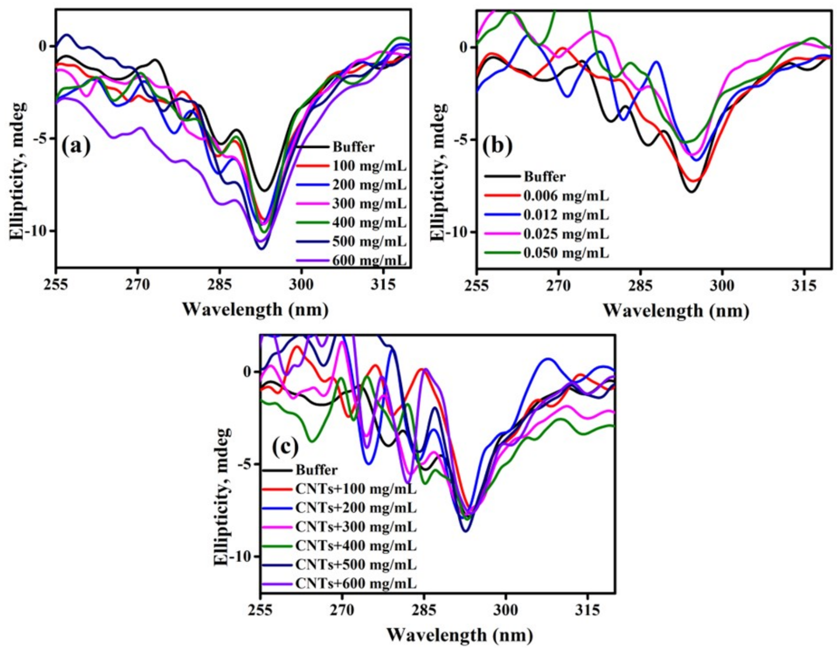

Fig. S9 Near UV-CD spectra of Blg in presence of various concentrations of (a) DES-1(100-600 mg/mL), (b) CA-MWCNTs (0.006-0.050 mg/mL) and (c) mixture of DES-1 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

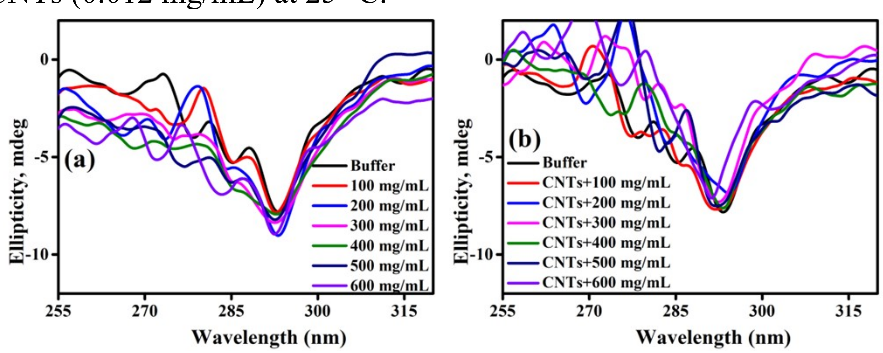

Fig. S10 Near UV-CD spectra of Blg in presence of various concentrations of (a) DES-2 and (b) mixture of DES-2 and CA-MWCNTs (0.012 mg/mL) at 25 °C.

Table S1 Tm values of Blg in buffer, CA-MWCNTs, DES-1, DES-2, mixture of DES-1+CA-MWCNTs and mixture of DES-2+CA-MWCNTs

Sample	**Tm (˚C)**
Pure Blg	83.96±0.21
0.012 mg/mL CA-MWCNTs	82.86±2.10
100 mg/mL DES-1	88.96±1.11
200 mg/mL DES-1	89.98±1.40
300 mg/mL DES-1	90.97±0.88
400 mg/mL DES-1	91.01±0.96
500 mg/mL DES-1	93.98±1.24
600 mg/mL DES-1	94.97±0.91

CA-MWCNTs+100 mg/mL DES-1	87.96±1.36
CA-MWCNTs+200 mg/mL DES-1	88.97±1.25
CA-MWCNTs+300 mg/mL DES-1	89.98±1.98
CA-MWCNTs+400 mg/mL DES-1	89.99±1.50
CA-MWCNTs+500 mg/mL DES-1	90.97±1.70
CA-MWCNTs+600 mg/mL DES-1	91.99±1.20
100 mg/mL DES-2	86.96±1.93
200 mg/mL DES-2	85.99±1.50
300 mg/mL DES-2	89.99±0.96
400 mg/mL DES-2	88.99±1.20
500 mg/mL DES-2	90.99±1.74
600 mg/mL DES-2	89.96±2.20
CA-MWCNTs+100 mg/mL DES-2	83.97±1.50
CA-MWCNTs+200 mg/mL DES-2	84.97±1.98
CA-MWCNTs+300 mg/mL DES-2	85.99±1.33
CA-MWCNTs+400 mg/mL DES-2	86.99±1.87
CA-MWCNTs+500 mg/mL DES-2	87.97±1.82
CA-MWCNTs+600 mg/mL DES-2	88.98±1.85

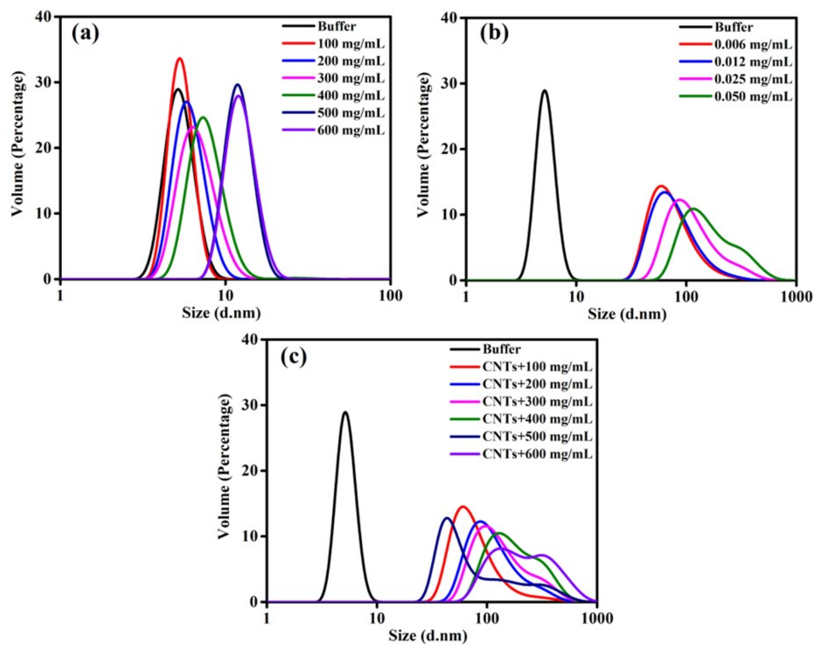

Fig. S11 Plot of volume distribution ofBlgin presence of various concentrations of (a) DES-1(100-600 mg/mL), (b) CA-MWCNTs (0.006-0.050 mg/mL) and (c) mixture of DES-1 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

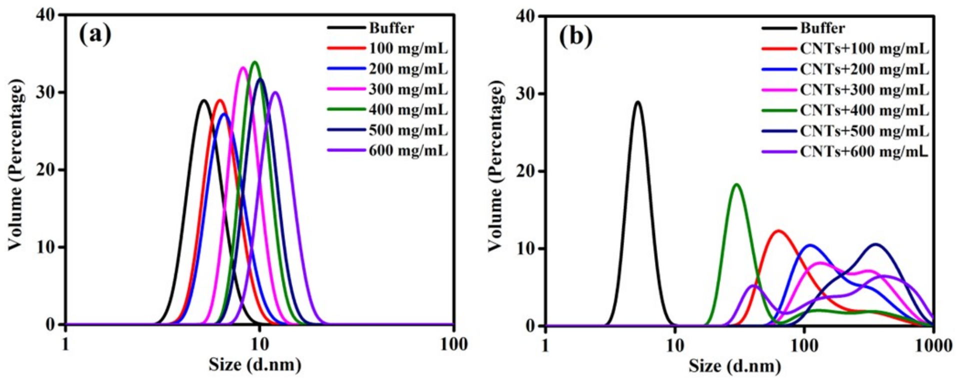

Fig. S12 Plot of volume distribution ofBlgin presence of various concentrations of (a) DES-2 (100-600 mg/mL) and (b) mixture of DES-2 (100-600 mg/mL) and CA-MWCNTs (0.012 mg/mL) at 25 °C.

Table S2 D_H values of Blg in buffer, CA-MWCNTs, DES-1, DES-2, mixture of DES-1+CA-MWCNTs and mixture of DES-2+CA-MWCNTs

Sample	**d_H (nm)**
Pure Blg	5.64±0.25
0.012 mg/mL CA-MWCNTs	136.60±3.20
100 mg/mL DES-1	5.55±0.78
200 mg/mL DES-1	5.76±0.16
300 mg/mL DES-1	6.81±0.49
400 mg/mL DES-1	7.75±0.58
500 mg/mL DES-1	12.20±0.29
600 mg/mL DES-1	12.50±0.11
CA-MWCNTs+100 mg/mL DES-1	152.80±1.86
CA-MWCNTs+200 mg/mL DES-1	179.60±2.32
CA-MWCNTs+300 mg/mL DES-1	201.90±2.78
CA-MWCNTs+400 mg/mL DES-1	225.00±3.10
CA-MWCNTs+500 mg/mL DES-1	242.50±4.20
CA-MWCNTs+600 mg/mL DES-1	267.60±3.96
100 mg/mL DES-2	6.82±0.16
200 mg/mL DES-2	7.36±0.22
300 mg/mL DES-2	8.63±0.26
400 mg/mL DES-2	9.90±0.18
500 mg/mL DES-2	10.68±0.12
600 mg/mL DES-2	12.91±0.33
CA-MWCNTs+100 mg/mL DES-2	205.20±2.78
CA-MWCNTs+200 mg/mL DES-2	233.80±3.20
CA-MWCNTs+300 mg/mL DES-2	267.60±3.80
CA-MWCNTs+400 mg/mL DES-2	285.50±4.11
CA-MWCNTs+500 mg/mL DES-2	321.00±3.60
CA-MWCNTs+600 mg/mL DES-2	364.90±2.94

0.006 mg/mL CA-MWCNTs	128.60±4.12
0.012 mg/mL CA-MWCNTs	136.60±3.20
0.025 mg/mL CA-MWCNTs	165.20±2.85
0.050 mg/mL CA-MWCNTs	191.41±3.64

References

1. R. Yadav and P. Venkatesu, *Polymer,* 2019, **178**, 121573.

2. N. Yadav, K. Bhakuni, M. Bisht, I. Bahadur and P. Venkatesu, *ACS Sustain. Chem. Eng.,* 2020, **8**, 10151–10160.

3. N. Yadav, M. Halanur, M. Bisht, S. K. Nataraj, P. Venkatesu and D. Mondal, *Chem. Commun.,* 2020, **56**, 9659–9662.