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Electronic Supplementary information

A green and sustainable approach to utilize bio-ionic liquids for the selective precipitation of high purity agarose from an agarophyte extract

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General Experimental Section

Gelling Properties: Agarose gels (0.75 % *w/v*) were prepared by dissolving in demineralised water in an autoclave at 120 ºC. Gel strength measurements were done on a Gel Tester (Kiya Seisakusho, Ltd., Tokyo, Japan). Gelling and melting temperatures were also measured in the 0.75 % *w/v* gel samples following the method described in our previous work.[1]

Gel electrophoresis: DNA gel electrophoresis was carried out under identical experimental conditions in the gel sample of agarose prepared in the present study from *G. dura* extract through choline laurate precipitation and compared with commercially available Sigma agarose (Cat. No.: A0576) [using gel concentrations of 0.7 % for *G. dura* agarose of this study (Figure 6b) and 1.0 % for Sigma agarose (Figure 6a)]. Appropriate amount of agarose samples were dissolved in 50 mL 1x TBE buffer using microwave oven. The solutions were cooled down to 60 °C and 2 µL of ethidium bromide (10 mg per mL) solution was added and gels were casted in the gel tray.[1] DNA ladder and four different DNA samples were loaded on both the gels and subjected to electrophoresis using 1x TBE buffer at 50 V for 90 min. The gels were visualized under UV light (UV tube 8W, 4 nm).[1]

Electroendoosmosis (EEO) measurement: For this 1 % (*w/v*) agarose (Table 3) gel in 0.05 M barbital buffer was prepared.[1] 3 mL of the solution was poured on a clean slide and allowed to formed gel, and placed in a plastic container with a moistened piece of filter paper to prevent evaporation and stored in the refrigerator (4 °C) for 1 h. Two wells were makes in parallel of distance 2.54 cm from each other by a squared off No. 13 needle and gel plugs were removed. 10 mg/mL Dextran 500 (Pharmacia) and 2 mg/mL crystalline (4x) human albumin in 0.05 M barbital buffer were used as standard test solution. 2 µL of standard solution and Bromophenol blue as an indicator were added to the aspirated wells, and slide was placed in electrophoresis chamber. A potential of 10 V/cm (75 V) was applied at constant voltage for 90 min until indicator dye shows accelerated albumin travel. After completion, slide was placed in denatured ethanol for 15 min after which the position of the dextran was measured with respect to the origin (OD = distance from origin to dextran), and was transferred to protein staining solution (0.5 g amido black in 50 mL glacial acetic acid) then make up to 500 mL with ethanol. After 15 minutes slide was washed with 1:1 acetic acid

(5 % *w/v*): ethanol solution to remove excess stain. EEO value was calculated as mentioned in the literature.[1]

Reference:

1. R. Meena, J. P. Chaudhary, P. K. Agarwal, P. Maiti, S. Chatterjee, H. D. Raval, P. Agarwal, A. K. Siddhanta, K. Prasad and P. K. Ghosh, *RSC Adv.*, 2014, **4**, 28093-28098.

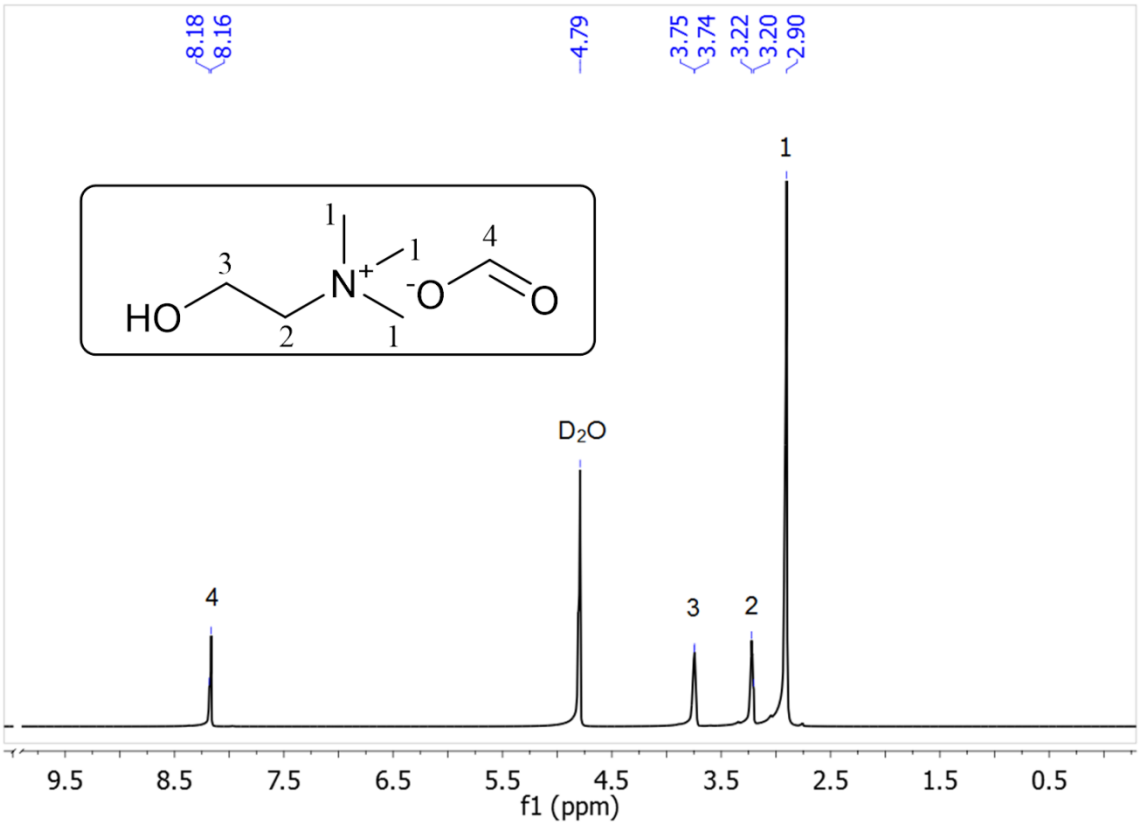

Figure S1: ^{1}H NMR spectra of choline formate. ^{1}H NMR (D_2O, 200 MHz, δ/ppm relative to TMS): 2.9 (*s*, 9H, -N-CH_3), 3.20 (*d*, 2H, -CH_2-N-), 3.74 (*d*, 2H, -O-CH_2-), 8.17 (*d*, 1H, H-COO^-).

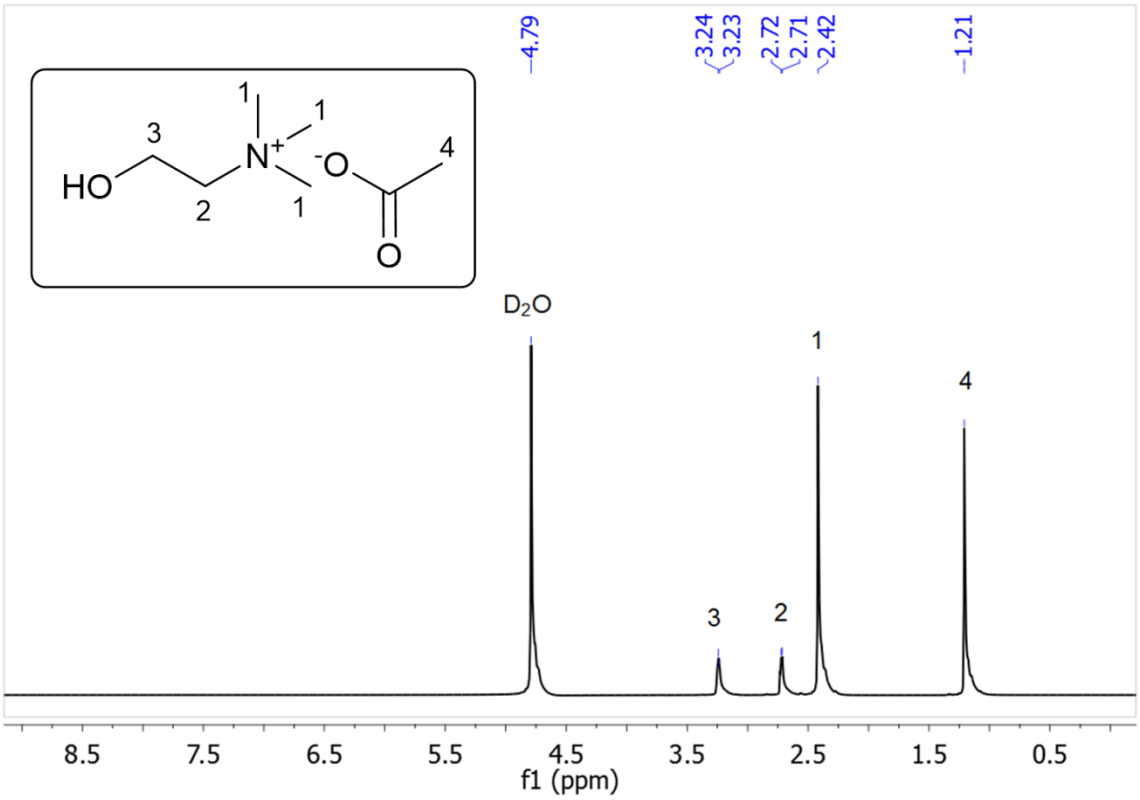

Figure S2: ^{1}H NMR spectra of choline acetate.

^{1}H NMR (D_2O, 200 MHz, δ/ppm relative to TMS): 1.21 (s, 3H, -CO-CH_3), 2.42 (s, 9H, -N-CH_3), 2.71 (d, 2H, -CH_2-N-), 3.23 (d, 2H, -O-CH_2-).

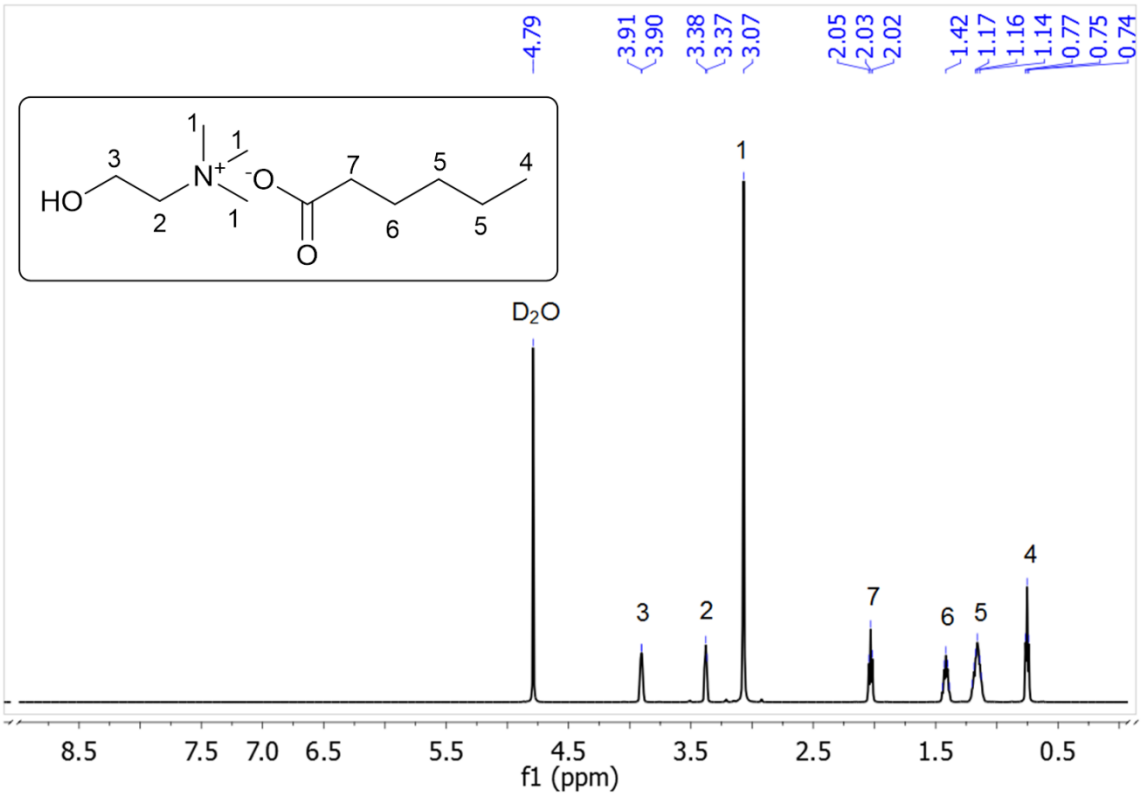

Figure S3: ^{1}H NMR spectra of choline caproate.

^{1}H NMR (D_2O, 200 MHz, δ/ppm relative to TMS): 0.75 (t, 3H, -C-CH_3), 1.16 (m, 4H, -CH_2-), 1.42 (m, 2H, -CH_2-), 2.03 (t, 2H, -CH_2-), 3.07 (s, 9H, -N-CH_3), 3.37 (d, 2H, -CH_2-N-), 3.90 (d, 2H, -O-CH_2-).

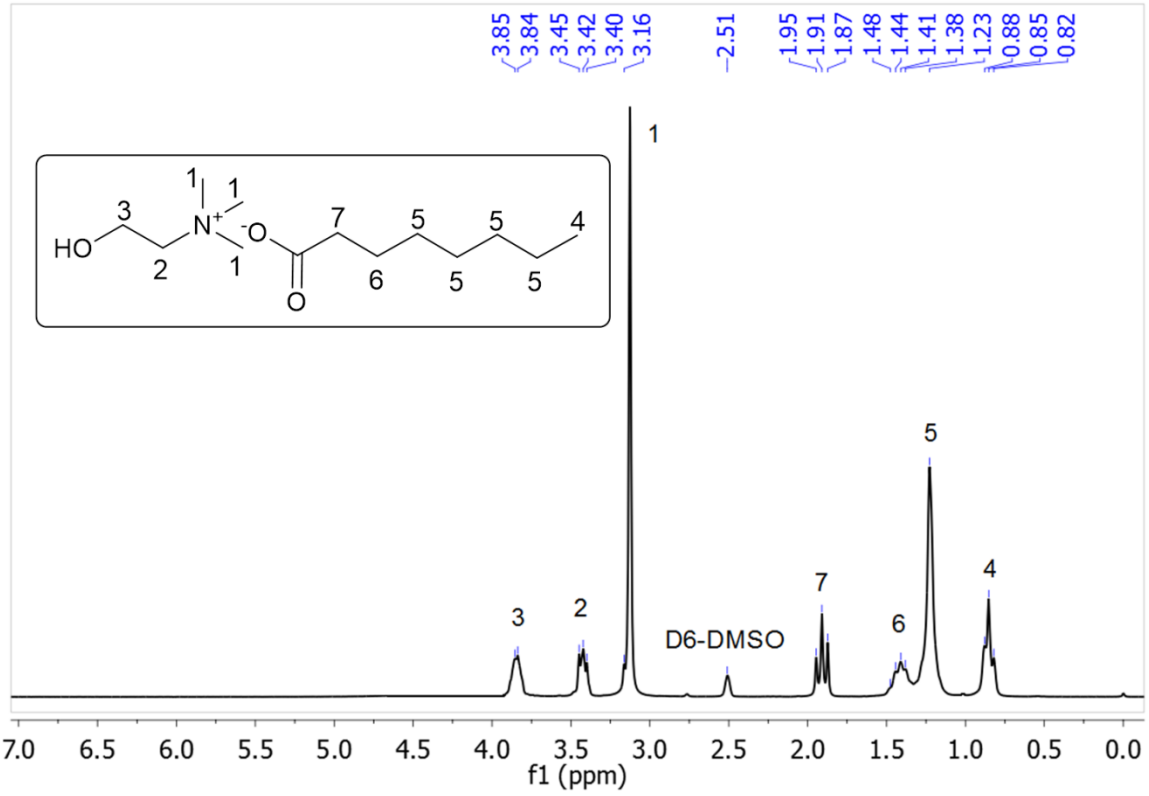

Figure S4: ^{1}H NMR spectra of choline caprylate

^{1}H NMR (D_6-DMSO, δ/ppm relative to TMS): 0.85 (t, 3H, -C-CH_3), 1.23 (s, 8H, -CH_2-), 1.41 (m, 2H, -CH_2-), 1.91 (t, 2H, -CO-CH_2-), 3.16 (s, 9H, -N-CH_3), 3.42 (t, 2H, -CH_2-N-), 3.84 (d, 2H, -O-CH_2-).

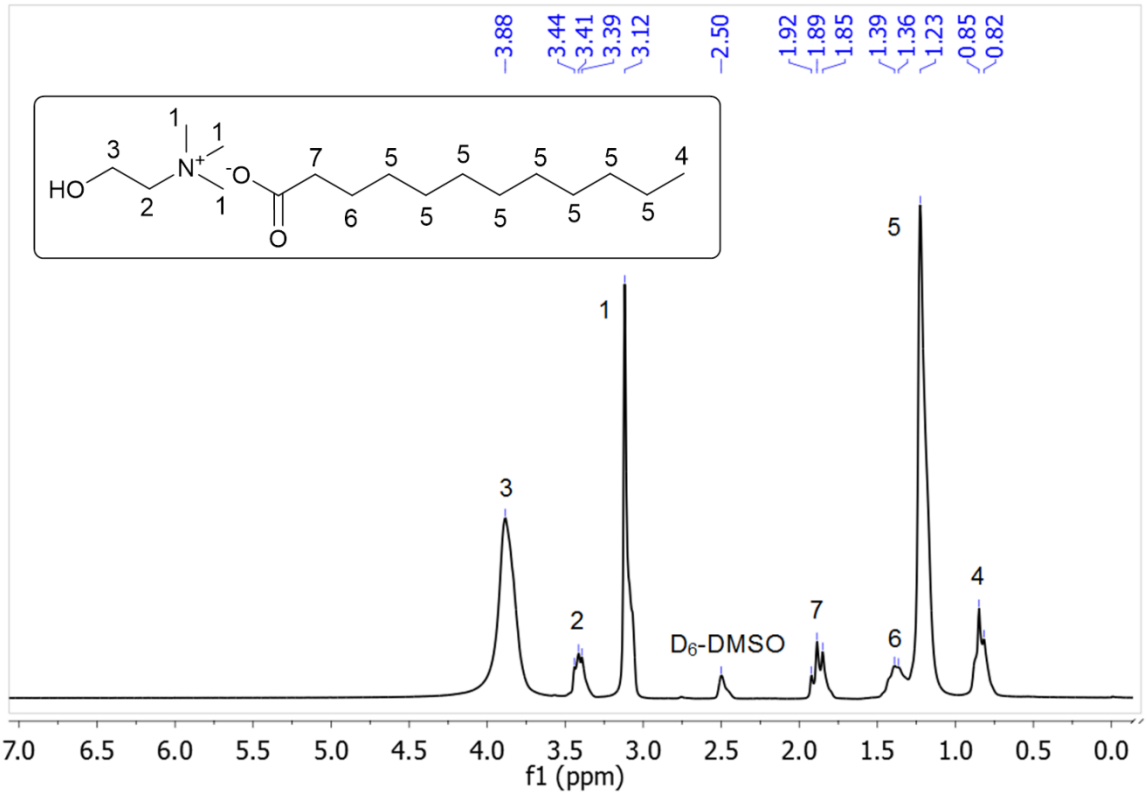

Figure S5: ^{1}H NMR spectra of choline laurate.

^{1}H NMR (D_6-DMSO, δ/ppm relative to TMS): 0.84 (t, 3H, -CH_3), 1.23 (s, 16H, -CH_2-), 1.37 (m, 2H, -CH_2-), 1.89 (t, 2H, -CO-CH_2-), 3.12 (s, 9H, -N-CH_3), 3.42 (d, 2H, -CH_2-N-), 3.88 (d, 2H, -O-CH_2-).

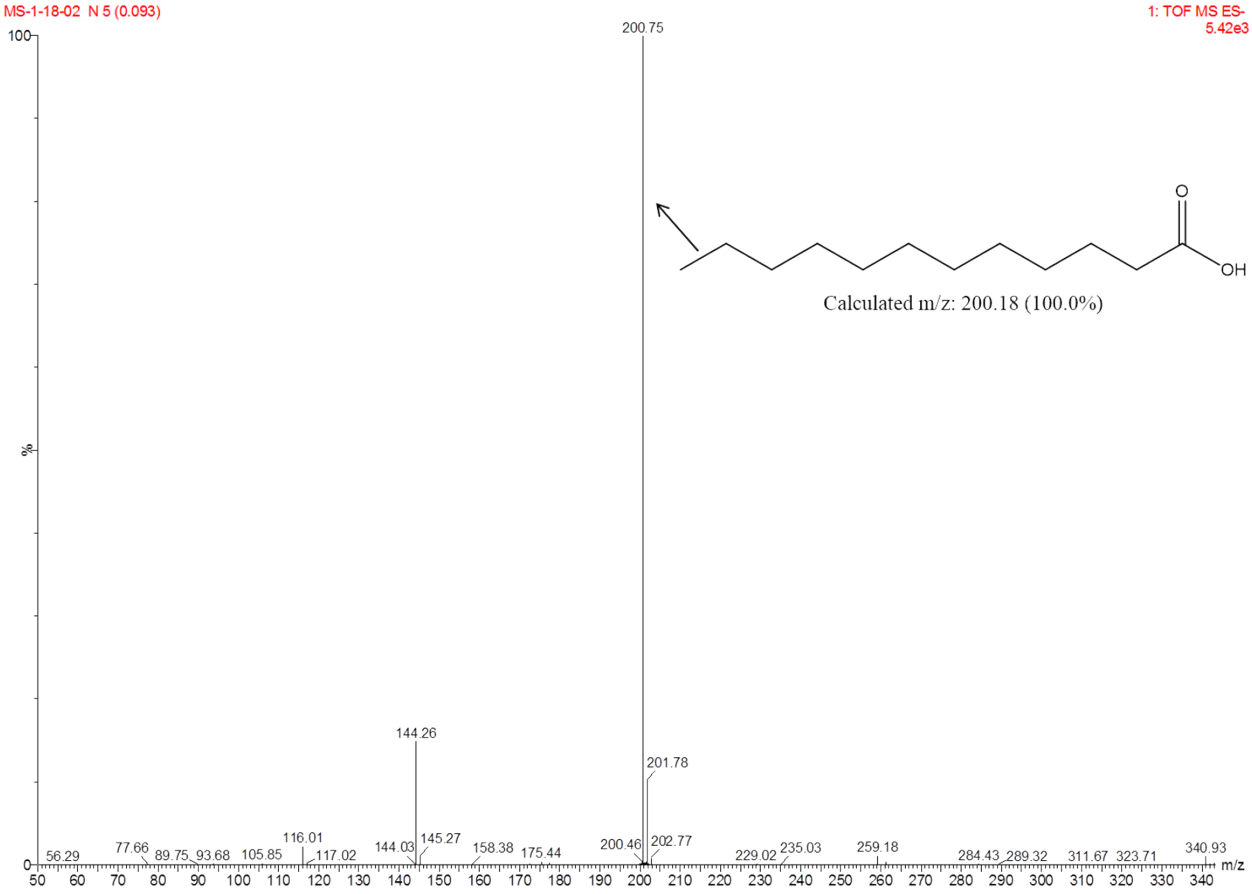

Figure S6 (a): ESI-MS^{-} spectra of choline laurate

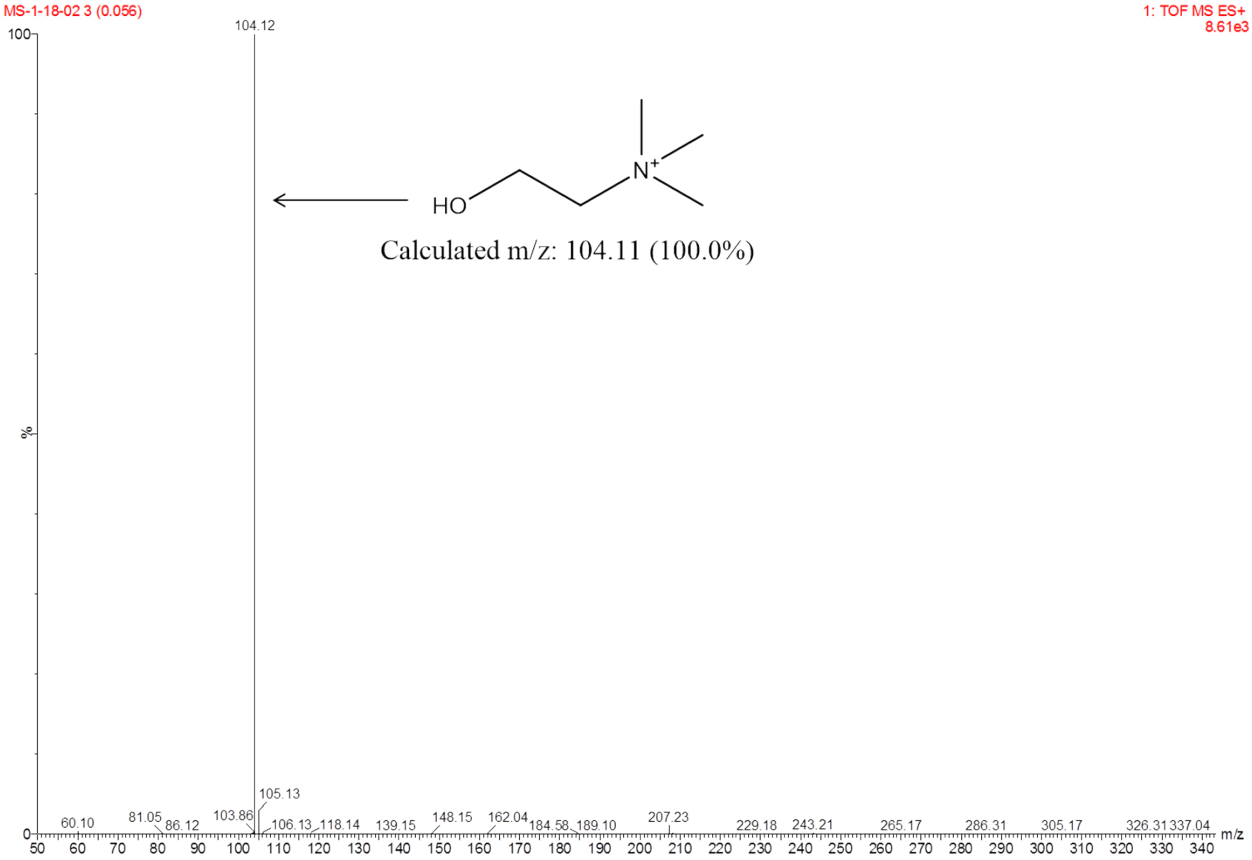

Figure S6 (b): ESI-MS^{+} spectra of choline laurate

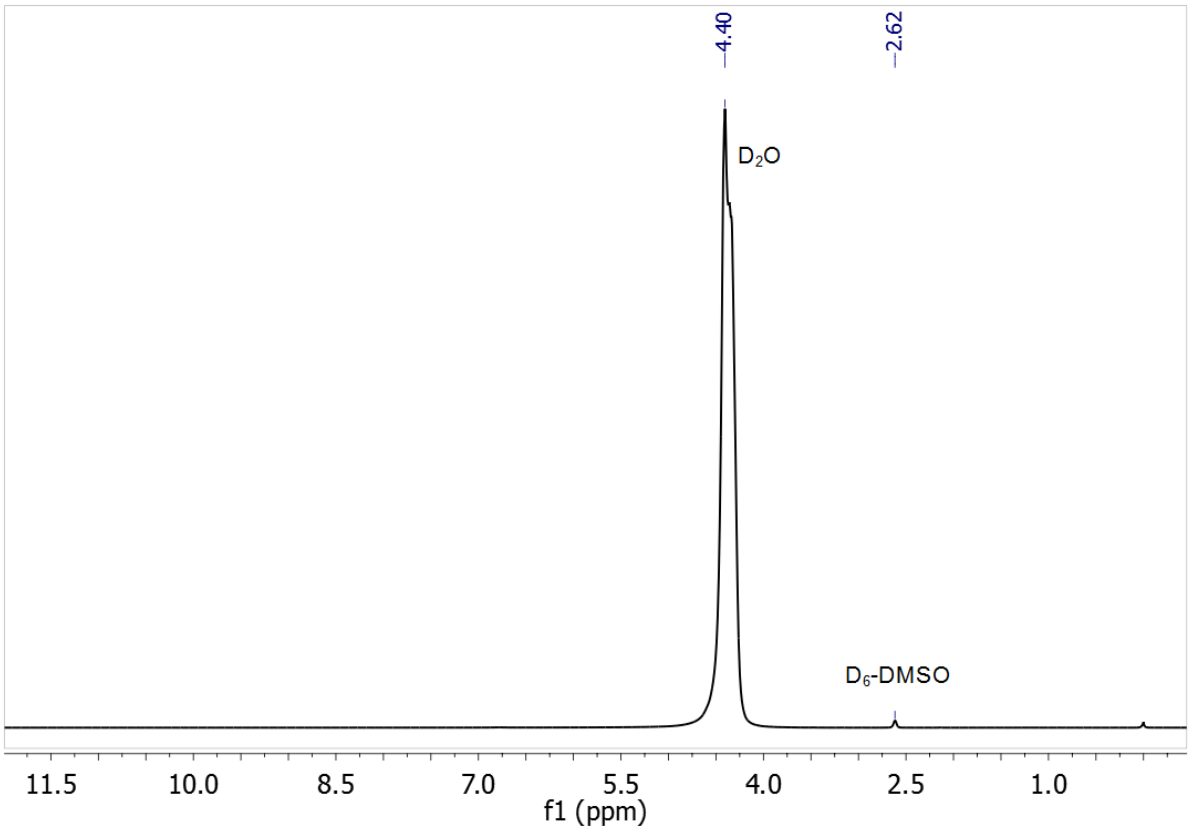

Figure S7: NMR spectra of permeate stream obtained after forward osmosis.

^{1}H NMR (D_6-DMSO & D_2O, δ/ppm relative to TMS): 2.62 (D_6-DMSO) and 4.40 (D_2O).

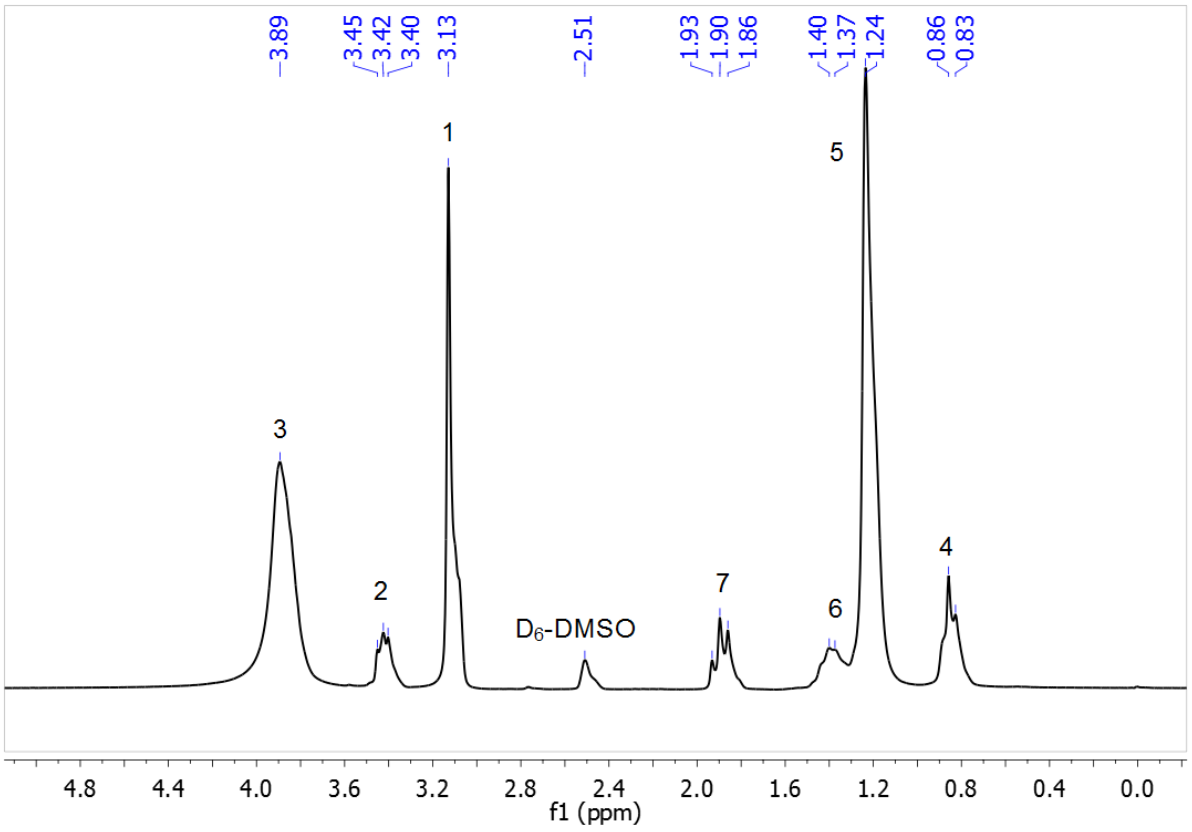

Figure S8: NMR spectra of regenerated choline laurate

^{1}H NMR ($CDCl_3$, δ/ppm relative to TMS): 0.77 (t, 3H, -CH_3), 1.15 (s, 16H, -CH_2-), 1.44 (s, 2H, -CH_2-), 2.03 (t, 2H, -CO-CH_2-), 3.15 (s, 9H, -N-CH_3), 3.46 (d, 2H, -CH_2-N-), 3.91 (s, 2H, -O-CH_2-).

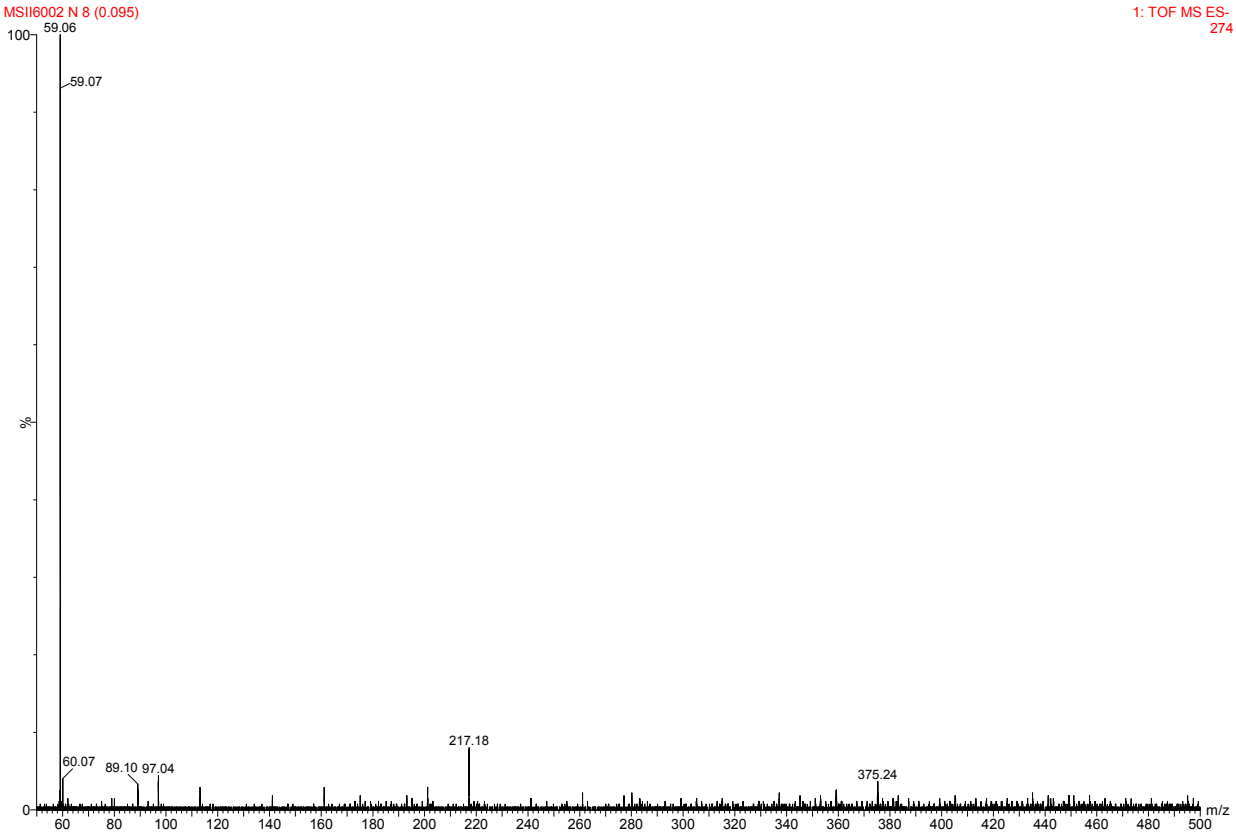

Figure S9 (a): ESI-MS$^-$ of isolated agarose obtained from *G. dura* seaweed using choline laurate. Absence of peak at m/z = 200.75 indicated the absence of residual laurate in the agarose.

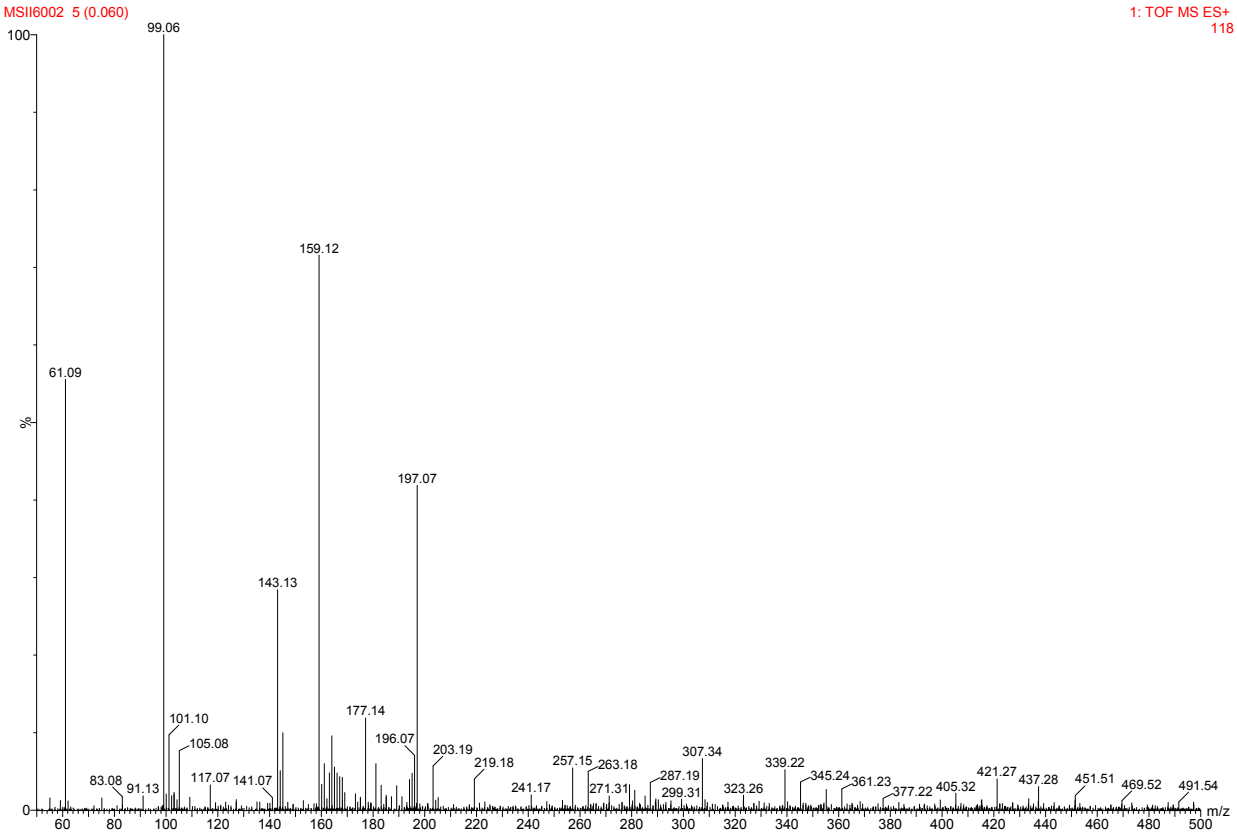

Figure S9 (b): ESI-MS^{+} of isolated agarose obtained from *G. dura* seaweed using choline laurate. Absence of peak at m/z = 104.12 indicated the absence of residual choline in the agarose.

Table S1: Critical micelle concentration (CMC) and viscosity of bio-ionic liquids

bio-ionic liquids	CMC (mM)	Viscosity (Pa.S)	T_{dec}[a] (°C)
Choline formate	ND	2.16	241
Choline acetate	ND	3.60	213
Choline caproate	ND	3.98	203
Choline caprylate	33.5	4.88	205
Choline laurate	21.7	572	206

Note: ND = Not detected; [a]T_{dec} = Decomposition temperature.

Table S2: Comparison of metal ion contents in alkali treated seaweeds and agarose of *G. dura* precipitating with choline laurate [a]

Entry	Metal Ions	Alkali treated seaweed (ATS) ($mg.kg^{-1}$)	Agarose of current study ($mg.kg^{-1}$)
1.	Na^{+}	≤ 4270	≤ 187
2.	K^{+}	≤ 1191	≤ 11.87
3.	Mg^{2+}	≤ 5526	≤ 221
4.	Ca^{2+}	≤ 7895	≤ 457
5.	Cr^{3+}	ND	ND
6.	Mn^{2+}	≤ 156	ND
7.	$Fe^{2+/3+}$	≤ 43.47	ND
8.	Co^{2+}	ND	ND
9.	Ni^{2+}	≤ 5.81	ND
10.	Cu^{2+}	≤ 4.31	ND
11.	Zn^{2+}	≤ 40.47	≤ 1.87
12.	Pb^{2+}	ND	ND
13.	Cd^{2+}	ND	ND
14.	B^{3+}	≤ 11.5	≤ 0.67
15.	Al^{3+}	≤ 113	≤ 0.56
16.	As^{3+}	ND	ND

Note: ND = Not detected.

Table S3: CHN data of Sigma agarose and isolated agarose obtained from *G. dura* using choline laurate.

Entry	Sample	% C	% H	% N
1.	*G. dura* agarose (Present study)	37.81	6.55	0.00
2.	Sigma agarose (Cat. No. A0567)	40.03	5.96	0.00