Electronic Supplementary Information (ESI)

Surfactant-induced coagulation of agarose from aqueous extract of *Gracilaria dura* seaweed as an energy-efficient alternative to the conventional freeze-thaw process

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General Experimental Section

Gelling Properties: Agarose gels (1.0 % *w/v*) were prepared by dissolving in demineralised water in an autoclave at 120 ^{o}C. Gel strength measurements were done on a Gel Tester (Kiya Seisakusho, Ltd., Tokyo, Japan). Gelling and melting temperatures were also measured in the 1 % *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 from *G. dura* and compared with commercially available Sigma agarose (A05066), using gel concentrations of 0.7% for *G. dura* and 1.0% for Sigma agarose (Figures 4a). The appropriate amounts of these two agarose samples were dissolved in 50 ml 1x TBE (10.8 g Tris base, 5.5 g Boric acid, 0.744 g Na_2EDTA) buffer by heating in microwave oven. The agarose solutions were cooled down to 60^{o}C and 2 µl of ethidium bromide (10 mg per ml) solution was added and gels were casted in the gel tray.

DNA ladder and two different DNA samples were loaded on both the gels and subjected to electrophoresis using 1x TBE buffer at 50 V for 90 min. After running, gel piece containing the DNA was cut from the gel and DNA was extracted using QIA-quick Gel Extraction Kit (Qiagen, USA) in 50 µl sterilized mili Q water. After elution, 5 µl DNA was loaded in the freshly cast gel to see the concentration and the quality of the eluted DNA. The eluted DNA was again amplified by using gene specific primers in the standard PCR conditions (25 ng DNA template, 1 x Taq Polymerase buffer, 150 ng PCR primers, 200 µM dNTPs and 2.5 U of Taq Polymerase enzymes in 50 µl reaction volume). The PCR conditions were 94^{o}C -5 min, 1 cycle; 94 ^{o}C -1 min, 55^{o}C -1 min, 72^{o}C -2 min, 35 cycles; 72^{o}C -7 min. The PCR product was checked by electrophoresis using 0.7 % gels. The gels were visualized under UV light (UV tube 8W, 312

nm; Bangalore Genei, India). Similarly comparison of the *G. dura* agarose prepared in the present study was also compared with the agarose prepared by conventional method "freeze-thaw process" (Figure S2) and Genei agarose (Figure 4b). For testing the electrophoresis of plant RNA samples, 10 µg RNA sample were run in the 0.7% *G. dura* agarose and 1% Sigma agarose. All precautions were taken for RNA run to avoid the RNA degradation.

Electroendoosmosis (EEO) measurement: EEO was measured in 1% (*w/v*) agarose (Table 3) gel in 0.05 M barbital buffer having pH 8.6. Three milliliters of the solution was poured on a clean microscope slide and allowed to gel at room temperature. Further gel was placed in plastic container with a moistened piece of filter paper to prevent evaporation and stored in the refrigerator (4°C) for an hour. Using a squared off No. 13 needle, two wells were cut in parallel of distance 2.54 cm from each other and gel plugs were removed. A standard test solution was prepared which consists of 10 mg/ml Dextran 500 (Pharmacia) and 2 mg/ml crystalline (4x) human albumin in 0.05 M, pH 8.6, barbital buffer. Using a small bore dropper, 2 µl of standard solution and Bromophenol blue as an indicator of electrophoretic migration were added to the aspirated holes. Then slide was placed in position for electrophoresis using paper wicks and chamber was filled with fresh Barbital buffer. A potential of 10 V/cm (75 V) was applied using constant voltage settings. Electrophoresis was continued for 90 min at this voltage until indicator dye shows accelerated albumin travel, then the slide was removed. Further, visualization was accomplished in two stages. The slide was first placed in denatured ethanol for 15 min after which the position of the dextran was measured with respect to the origin (OD). After measuring, slide was transferred to protein staining solution prepared from 0.5 g amido black in 50 ml glacial acetic acid, then made up to 500 ml with ethanol. After 15 minutes slide was washed in a

1:1 acetic acid (5%): ethanol solution to remove excess stain. The distance from the center of the spot to the center of the origin was measured (OA = distance from origin to albumin).

Recovery of Surfactant: Surfactant (Triton X-100) was recovered by RO membrane process using Hydranautics make SWC5–LD-4040 (Low fouling technology) at pressure: 150 psig in recirculation mode.

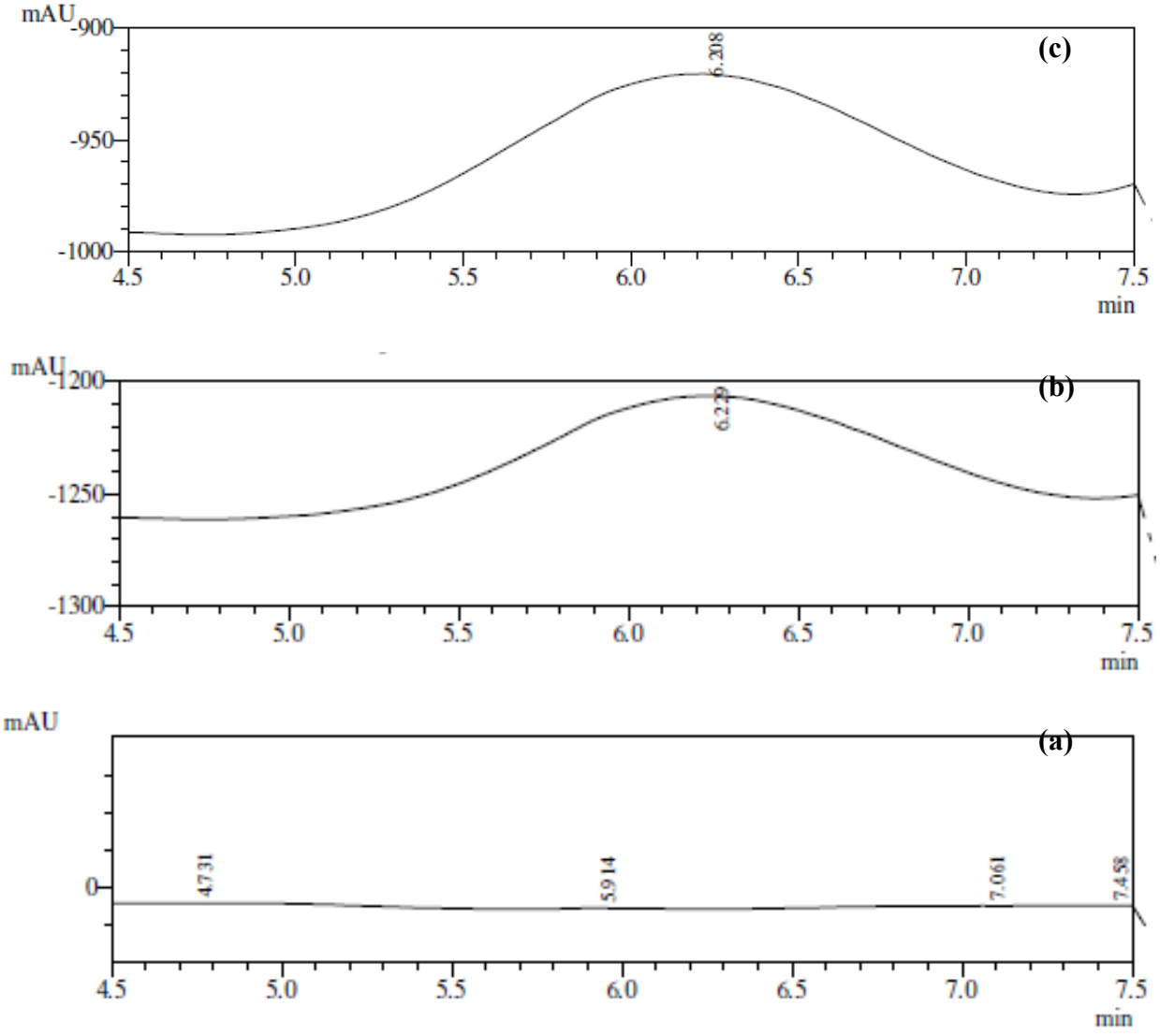

Figure S1 HPLC spectra of (a) permeate after 90 min, (b) native Triton X-100, and (c) retentate after 90 min (No peak at ca. 6.2 in HPLC spectrum of permeate confirmed the absence of Triton X-100 in the permeate).

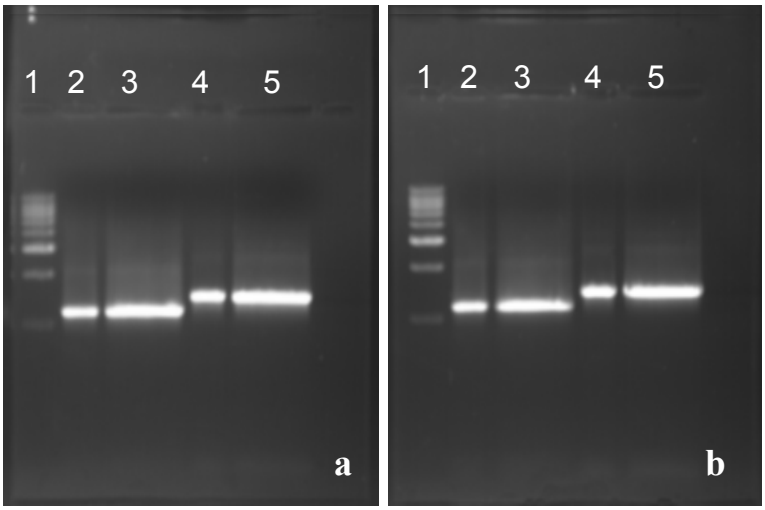

Figure S2 Gel electrophoresis: gel was prepared in 1x TBE buffer and DNA was electrophoresed at 50 V (a) 0.7% CSMCRI agarose gel (prepared through conventional method "Freeze-Thaw process") and (b) 0.7% CSMCRI agarose gel (prepared in the present study through "surfactant induced coagulation process"). Samples: Lane 1: 1 Kb ladder; Lanes 2-5: different DNA samples.