Halophilic polysulfabetaines – synthesis and study of gelation and thermoresponsive behavior

Vivek Arjunan Vasantha,[*a] **Satyasankar Jana,**[a] **Anbanandam Parthiban**[*a] **and Julius G. Vancso**[a,b]
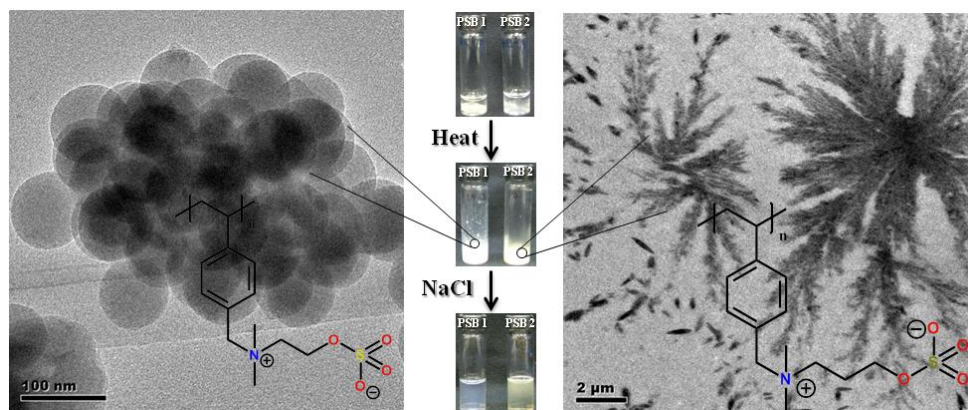
[a]*Institute of Chemical and Engineering Sciences (ICES), Agency for Science, Technology and Research (A*STAR), 1 Pesek Road, Jurong Island, Singapore 627833.*

[b]*MESA+ Research Institute for Nanotechnology, Faculty of Science and Technology, University of Twente, P. O. Box 217, 7500 AE Enschede, The Netherlands.*

E-mail: *vivek_vasantha@ices.a-star.edu.sg; aparthiban@ices.a-star.edu.sg*

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Graphical Abstract

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Vivek Arjunan Vasantha,*[a] Satyasankar Jana,[a] Anbanandam Parthiban*[a] and Julius G. Vancso[a,b]

[a]*Institute of Chemical and Engineering Sciences (ICES), Agency for Science, Technology and Research (A*STAR), 1 Pesek Road, Jurong Island, Singapore 627833.*

[b]*MESA+ Research Institute for Nanotechnology, Faculty of Science and Technology, University of Twente, P. O. Box 217, 7500 AE Enschede, The Netherlands.*

E-mail: vivek_vasantha@ices.a-star.edu.sg; aparthiban@ices.a-star.edu.sg

Materials

All reactions and polymerizations were performed with Schlenk techniques under Argon atmosphere. 1, 2-ethylene sulfate, 1, 3-propylene sulfate, and 4, 4′-azo bis(4-cyanovaleric acid) (ACVA), 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIPA) were purchased from Aldrich and used as received. N-(4-Vinylbenzyl)-N, N-dimethylamine is purchased from ACROS. Solvents like acetonitrile (ACN), N, N-dimethylformamide (DMF), toluene, tetrahydrofuran were freshly dispensed from Glass Contour - Solvent Purification System. All other solvents used were of analytical grade. Deionized water is used for swelling and other aqueous studied. Other chemicals for this study were analytic reagents obtained from Aldrich.

Instrumentation

[1]H nuclear magnetic resonance (NMR) spectra were recorded at room temperature on 400 MHz Bruker UltraShield AVANCE 400SB spectrometer. The zwitterionic polymers showed limited solubility in most common dueterated solvents, but were partially soluble in 2% $NaCl/D_2O$ solution and dueterated hexafluoroisopropanol (HFIPA-D_2). Residual solvent peaks were used as internal standard. The aqueous GPC system was equipped with a Delta 600 HPLC pump, a 600 controller, a 717 plus autosampler, a 2487 dual absorbance detector and a 2414 refractive-index detector, all from Waters. The following GPC columns were arranged in series: Ultrahydragel guard, and Ultrahydragel 120 (7.8 mm ID × 300 mm) and an Ultrahydragel Linear (7.8 mm ID × 300 mm). The eluant (0.1 M $NaNO_3$ in deionized water) flow rate was 0.7 mL/min and the columns were maintained at 30 °C. The results were obtained using poly(ethylene oxide) (PEO) calibrations. The molecular weights of polymers were determined using Mark-Houwink-Sakurada method using $D = KM^a$ equation, where K and a are constants. The value of K and a are found empirically for polymer/solvent pair. The empirical constants from the literature were used to calculate the molecular weight from the diffusion coefficient (D) which was determined from the autocorrelation function of the scattered light from Zetasizer NanoZS (Malvern Instruments, DLS). Infrared spectra were run as KBr pellets for solids using Digilab Excalibur FTIR. High resolution mass spectra were recorded using electrospray ionisation (ESI) techniques in positive and negative ion modes by Thermo Finnigan MAT 95 XP. Mass spectral data is reported as the mass-to-charge ratio (*m/z*). UV absorption spectra were measured on a Shimadzu UV-VIS UV 2550 spectrometer. The solution samples were measured in 1 cm length cell and measured in the 200-600 nm

regions. The transmittance studies for critical salt concentration or cloud point measurements were recorded at 600 nm.

1. Experimental

Synthesis of sulfabetaine monomers

2-(dimethyl(4-vinylbenzyl)ammonio)ethyl sulfate (SBM 1)

In a 250 mL flask, 6.66 g (0.054 mol) of 1, 2 ethylene sulfate was dissolved in dry acetonitrile. 8.65 g (0.054 mol) of N, N-dimethylvinylbenzylamine in acetonitrile was added through the additional funnel dropwise under argon atmosphere at room temperature. The reaction mixture was heated at 50 °C for 48h. A white precipitate was obtained by adding acetone which was filtered, washed thoroughly with acetone and dried in a vacuum. The solid was recrystallized from hot water and dried.   Yield = 12.68 g (82.8 %). mp 237.50 °C (DSC). ^{1}H NMR (D_2O): 7.5-7.6 (m, 4H) $CH_{aromatic}$; 6.7-6.86 (dd, 1H) $CH_{olefine}$; 5.96 and 5.4 (d, 2H) $CH_{cis \ and \ trans}$; 4.56 (s, 2H) $CH_{2 \ benzyl}$; 4.5 (t, 2 H) $O-CH_2$; 3.72 (t, 2H) $-N-CH2$; 3.09 (s, 6 H) $N(CH_3)_2$.
MS signal at m/z =286.11 $[M-H]^+$

3-(dimethyl(4-vinylbenzyl)ammonio)propyl sulfate (SBM 2)

In a 250 mL flask, 10 g (0.072 mol) of 1, 3-propylene sulfate was dissolved in dry acetonitrile. 11.67 g (0.072 mol) of N, N-dimethylvinylbenzylamine in acetonitrile was added through the additional funnel dropwise under argon atmosphere at room temperature. The reaction mixture was heated at 50 °C for 48h. A white precipitate was obtained upon adding acetone. It was filtered, washed thoroughly with acetone and recrystallized from hot water followed by drying in the oven. Yield = 18.89 g (87.19 %). mp 245.02 °C (DSC). ^{1}H NMR (D_2O): 7.4-7.6 (m, 4H) $CH_{aromatic}$; 6.7-6.8 (dd, 1H) $CH_{olefine}$; 5.95 and 5.38 (d, 2H) $CH_{cis \ and \ trans}$; 4.47 (s, 2H) $CH_{2 \ benzyl}$; 4.15 (t, 2H) $-O-CH2$; 3.42 (t, 2 H) $N-CH_2$; 2.99 (s, 6 H) $N(CH_3)_2$; 2.29 (m, 2H) CH_2.
MS signal at m/z =300.12 $[M-H]^+$

General procedure for the preparation of polyzwitterions (PSB 1 and 2)

In a dried Schlenk tube (100 mL), SBM (21 mmol) was introduced and added in 0.5 M NaBr solution (20% w/v, 30 mL). The solution was purged with Argon gas for 45 min. The initiator ACVA (0.05 mmol) was added all at once. The system was degassed by three consecutive vacuum-argon sweeping cycles and sealed-off under Ar atmosphere at room temperature. The reaction flask was then placed in a constant temperature bath at 90 °C for 24 h. The resulting viscous polymer solution was cooled and diluted with 10mL of sat. NaCl solution. The solution was then dialyzed against deionized water for 3 days (MWCO = 1500) at 50 °C. The resulting water-insoluble polymer was lyophilized and dried *in vacuo* at 50 °C for 48 h.

Poly-[2-(dimethyl(4-vinylbenzyl)ammonio)ethyl sulfate] (PSB1)

PSB **1** was synthesized from SBM **1** (6.0 g, 21 mmol) and ACVA (14.7 mg, 0.053 mmol) in 0.5 M NaBr solutions (30mL) for 20 h at 90 °C. ^{1}H NMR (HFIPA-D_2): 6.59-7.1 (4H) $CH_{aromatic}$; 4.38 (2H) $CH_{2 \ benzyl}$; 4.38 (2 H) $O-CH_2$; 3.54 (2H) $-N-CH_2$; 2.99 (6 H) $N(CH_3)_2$; 2.3 (1H) $CH_{backone}$; 1.65 (2H) $CH_{2 \ backbone}$. GPC analysis: M_n = Not determined due to insolubility in eluent. UV-Vis (22.6 wt % NaCl): 261 nm.

Poly-[3-(dimethyl(4-vinylbenzyl)ammonio)propyl sulfate] (PSB 2)

PSB **2** was synthesized from SBM **2** (6.0 g, 20 mmol) and ACVA (14mg, 0.05 mmol) in 0.5 M NaBr solutions (30 mL) for 24 h at 90 °C. ^{1}H NMR (HFIPA-D$_2$): 6.56-7.2 (4H) CH$_{aromatic}$; 4.4 (2H) CH$_{2\ benzyl}$; 4.2 (2 H) O-CH$_2$; 3.41 (2H) –N-CH$_2$; 2.9 (6 H) N(CH$_3$)$_2$; 2.17-2.28 (2 H) C-CH$_2$-C; 1.85 (1H) CH$_{backone}$; 1.3 (2H) CH$_{2\ backbone}$. GPC analysis: M$_n$ = 3500 (PDI 1.13). UV-Vis (22.6 wt % NaCl): 262 nm.

2 Characterizations

Solubility

Solubility of PSB **1** and **2** in organic solvents at room temperature was determined by dispersing 1 wt % of polymer in various solvent and heating the mixture at 70 °C for 1h.

Critical salt concentration or cloud point measurement

The cloud point titration was carried out using 1.11 wt % of PSB**1** and 0.87 wt % of PSB **2** in 22.6 wt % NaCl at 25 °C. The CSC values obtained by visual determination of the cloud point (clear solution became turbid) with error value ± 0.2 %. The CSC values are reported in Table 2.

Phase behaviour measurements

UCST of diluted PSB solutions was determined with respect to temperature with varying NaCl concentration. The polymer solution (2 ml) was poured into a glass vial, and placed directly into temperature controlled bath (Lauda Alpha RA 8) (temperature variation ±0.1 °C). The solutions, which were turbid at room temperature, turned clear (transparent) upon heating. The transition point (UCST) was taken as the temperature at which the solution turned from cloudy to transparent.

Aggregation Studies

Dynamic light scattering (DLS) was performed by using a Zetasizer NanoZS Instrument (Malvern Instruments, UK) equipped with a He–Ne laser (633 nm) and with non invasive backscattering (NIBS) detection at a scattering angle of 173°. The autocorrelation function was converted in a volume-weighted particle size distribution with Dispersion Technology Software from Malvern Instruments. Each measurement was repeated at least three times, and the average result was reported as the final Z average diameter (nm). The measurements were performed in the temperature range of 20–60°C with a temperature interval of 5 °C and an equilibration time of 10 min. Polymer solutions with various concentrations were prepared in NaCl solution at 50 °C and filtered using a 0.45 μm disposable membrane filter to remove any dust in solution.

Viscosity measurements

Viscosity of the zwitterionic polymers was measured on an Anton Paar MCR 501 rheometer. The viscosity was determined by a rotary viscometer at UCST concentration. The polymers were dissolved in salt solution at 40 °C. The measure geometry used was a spindle with cyclinder, equipped with a size 19 cylindrical spindle set for constant shear rate at 1000 s^{-1}. The temperature dependance of the viscosity was fitted by Stettin analysis method. For temperature dependence studies, the samples were heated at 40 °C and cooled to 15 °C at the rate of 1 °C /min until the phase transition. The effect of shear rate with respect to viscosity was studied by placing the samples into the sample cup, equilibrating for 10 min at the required temperature and then subjecting to linearly increasing shear rate from 1 to 2000 s^{-1} as a function of viscosity at constant temperature, 40 °C using 5 ml of PSB solutions. The flow curve of the material with shear thickening behaviour was fitted by Herschel-Bulkley analysis method.

Hydration Studies

The samples in triplicate of known weight of PSBs were immersed in excess of DI water. The polymer kept in sealed containers were placed in water bath at 23 °C and equilibrated for 48 h. The samples were removed and blotted dry. The swelling ratio of the polymer gel was determined gravimetrically. The swelling ratios were calculated from the ratio of the weight of the equilibrated gel to the dry weight. For each polymer, the average value was calculated. The swelling of PSB **1** was studied in the form of disc and PSB **2** was carried out in a polyester tea bag.

Hydrolytic stability

The hydrolytic stability test was performed in aqueous solution. The polymer is heated with DI water (10 wt %) for 24 h at 95 °C. The reaction mixture allowed to cool an then dialyzed with DI water using MWCO 3500, lyophilized and dried in vacuum oven at 50 °C for 24 h. The dried polymer samples were analyzed for elemental composition.

Rheological measurement

The mechanical properties of PSB hydrogels were studied using MCR 501 Anton Paar parallel plate with configuration of PP25 at controlled temperature of 25 ± 0.1 °C. The polymer swollen to its maximum. The hydrogels were directly placed between the parallel plates of the rheometer with 1mm gap. A frequency of 1 Hz and a strain of 1% were used throughout the experiments. The storage (G') and loss (G") moduli of the hydrogel were investigated using time, amplitude, frequency sweep at constant temperature (25 °C). Frequency sweep (with constant 1 % strain) were conducted in the linear viscoelastic regime of the hydrogel from amplitude sweep measurement (with constant 1Hz frequency). The phase transition of the hydrogel was assessed by ramping the temperature from 25 to 75 °C with a heating and cooling rate of 1 °C/min and measured their storage and loss moduli at constant frequency (1 Hz) and strain (1 %).

Transmission Electron Microscopy (TEM)

TEM analyses were conducted on a Tecnai TF 20 S-twin transmission electron microscope at an acceleration voltage of 200 kV. 1 wt% in DI water of polymer sample (PSB **1** and **2**) was

heated at 70 °C for 15 min. Aliquots of top aggregated layer (turbid phase) were placed on copper grids coated with thin films of Formvar and carbon.
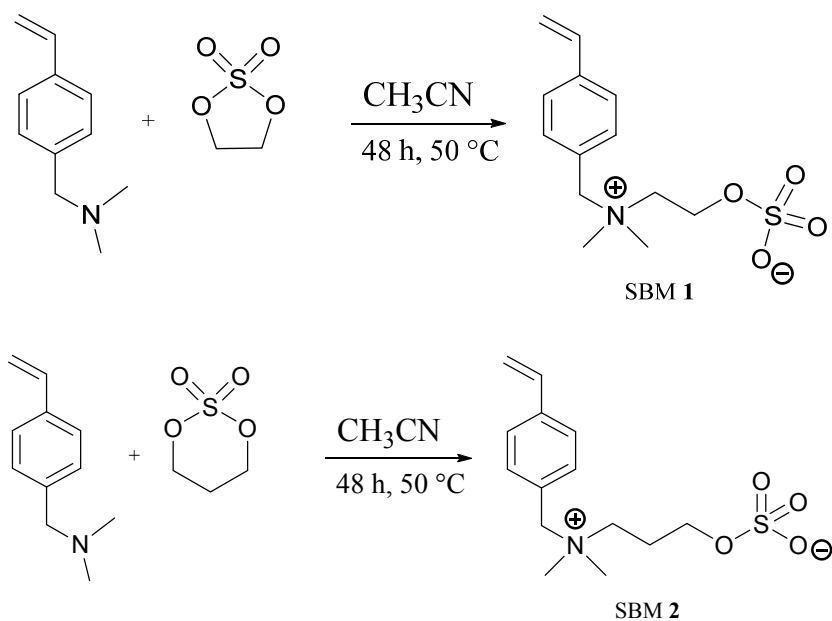
Scheme S1 Synthesis of zwitterionic sulfabetaine monomers SBM **1** and **2**

Table S1 Characterization of sulfabetaine zwitterionic monomers

Zwitterionic Monomers	Cation	Anion	Yield (%)	ESI-MS [M-H]$^+$	Mol. wt g/mol	m.p (°C, DSC)
SBM **1**	-CH$_2$N$^+$(CH$_3$)$_2$CH$_2$-	-CH$_2$OSO$^-_3$	82.80 %	286.11	285.36	237.50 °C
SBM **2**	-CH$_2$N$^+$(CH$_3$)$_2$CH$_2$-	- CH$_2$CH$_2$OSO$^-_3$	87.19 %	300.12	299.12	245.02 °C

Table S2 Summary of free-radical polymerization of sulfabetaine monomers SBM **1** and **2**

Monomer/ Initiator (mmol)	Time	Yield[a]	$M_{n, GPC}$ (g/mol)	PDI	Mol. Wt Est.[e] (kDa)
SBM **1** (21) / ACVA (0.053)	20 h	98 %	ND[b]	-	2110
SBM **2** (20) / ACVA (0.05)	24 h	>98 %	3500[c]	1.13[d]	4500

[a]Determined by gravimetry after drying the polymer at 50 °C *in vacuo;*[b]ND – Not determined; due to insolubility in eluting solvent; [c]Fraction soluble in the eluent (0.1M aqueous NaNO$_3$ solution); [d]The low PDI could be the result of imperfect SEC separation which led to an underestimation of molecular weight distribution. [e]molecular weight determined from DLS using Mark-Houwink-Sakurada method with known 'K' (4.4 x 10^{-5} cm^{2}s^{-1}) and 'a' (0.38) (parameter of polyvinylsulfonic acid at 20 °C) using aqueous NaCl. The estimated molecular weight is based on linear polysaccharide.
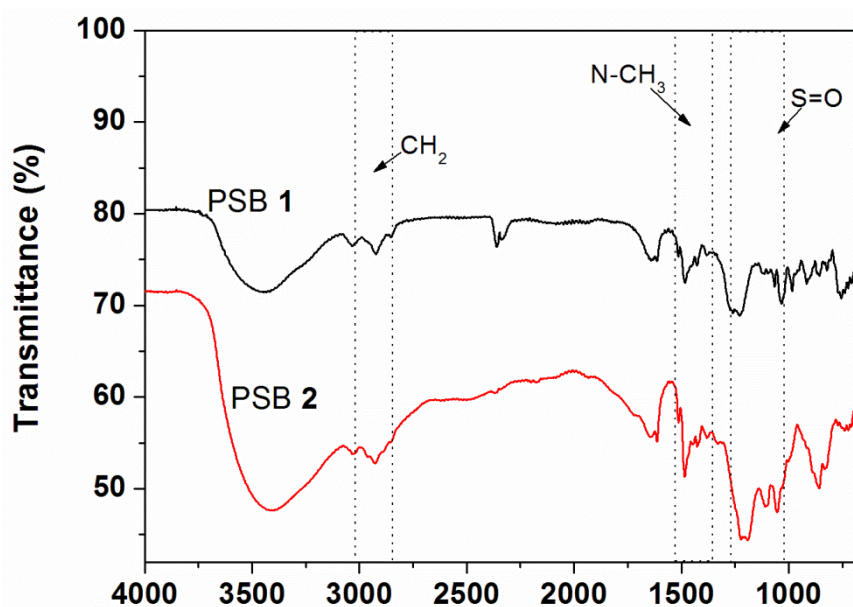
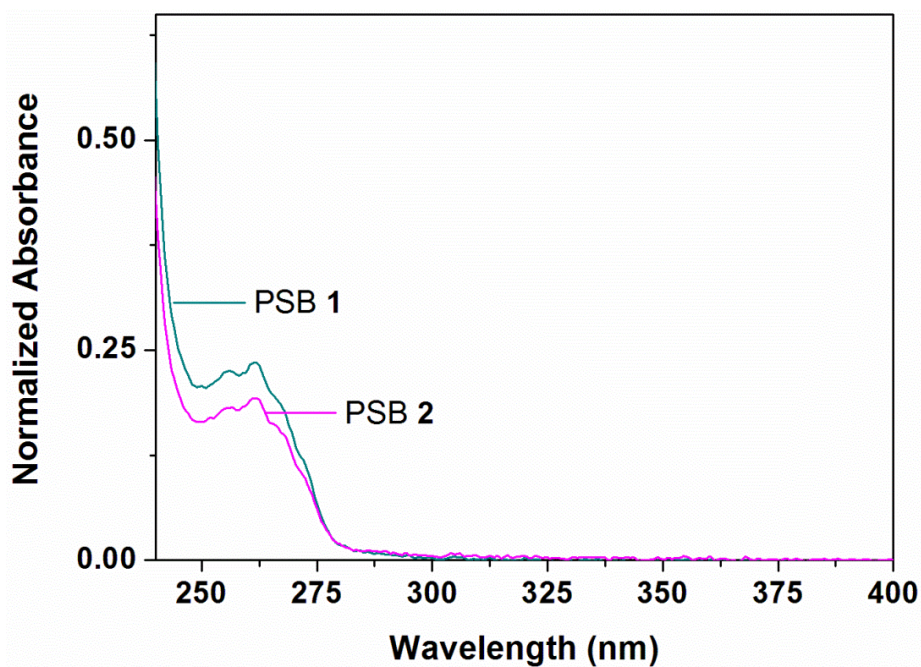**Fig. S1** FTIR of polysulfabetaine PSB **1** and **2**

Fig. S2 UV-Vis of polysulfabetaines PSB **1** and **2** in 22.6 wt % NaCl

Tabel S3 Solubility of polysulfabetaines PSB **1** and **2** in protic and aprotic solvents

Solvents	PSB 1	PSB 2
Water	-	-
Methanol	-	-
Chloroform	-	-
Acetic acid	-	-
DMF	-	-
DMSO	-	-
DCA	-	-
Formic acid	+	+
Trifluoroethanol	+	+
Hexafluoroisopropanol	+	+

Brine (Sat. NaCl)	+	+
Formamide	+	±
Hexamethyl phosphoramide	±	-
Glycerol	-	-
Trichloroacetic acid	+	+
Trifluoroacetic acid	±	±

1 w/w % of polymer solution was made after preheating at 70 °C for 1h.
b'+' indicates soluble; '-' indicates insoluble; '±' indicates partially soluble.

Tabel S4 Hydration studies of polysulfabetaines

Physical Properties	PSB 1*	PSB 2[#]
Hydration / Water Content (%) @ 23 ^{0}C (Mean±SD)	48.56 ± 3.0 %	76.83 ± 5.05 %

Water Content (%) =100 x [(Wt of hydrogel-Wt of dry polymer)/Wt of hydrogel]; *Dried polymer made into disc. [#]Swelling was studied by tea bag method.
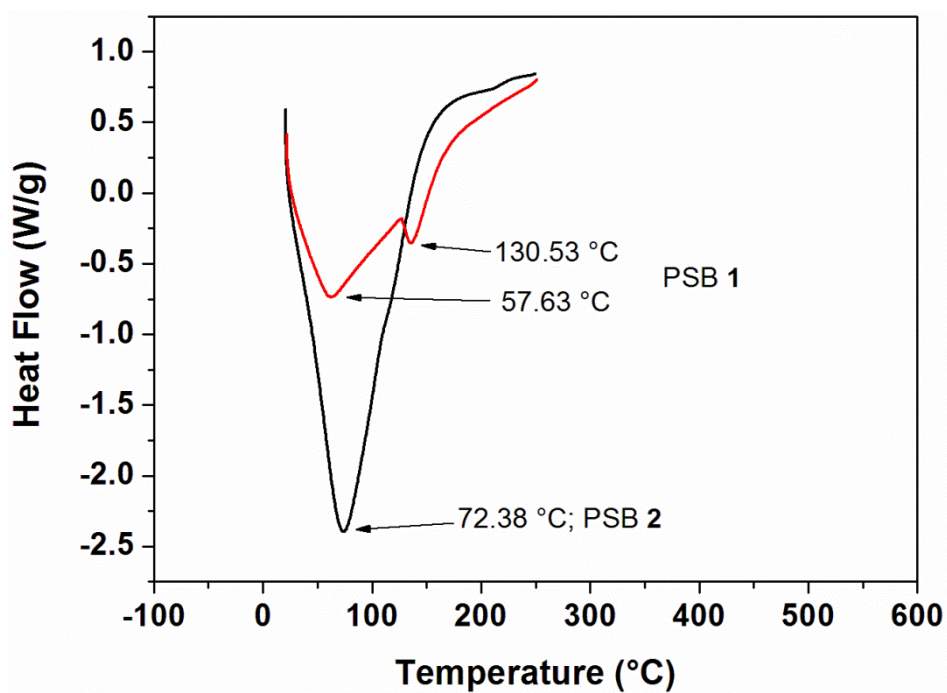
Fig. S3 DSC thermogram of physical hydrogel of PSB 1 and PSB 2
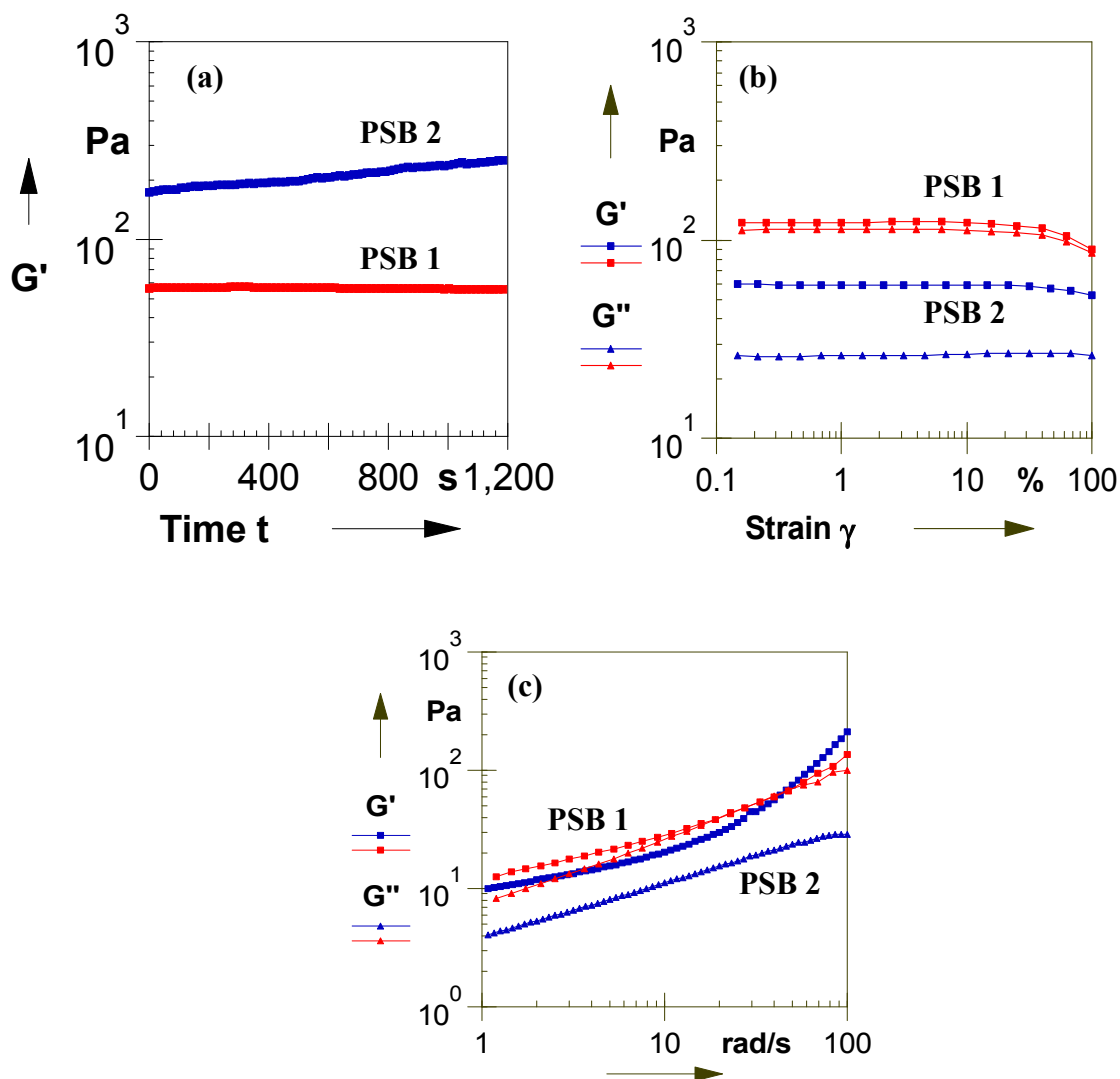
Fig. S4 Rheological characterization of polysulfabetaine hydrogel PSB**1** (51 wt %) and PSB **2** (17.69 wt %) at 25 °C respectively. (a) Time sweep rheology measurements for polysulfabetaine hydrogel PSB **1** and **2** with storage modulus (G'), reported as red (PSB **1**) and blue (PSB **2**) with constant strain of 1 % and constant frequency of 1Hz (b) Amplitude sweep rheology measurements for polysulfabetaine hydrogel PSB **1**(red) and PSB **2** (blue) with storage (G′) and loss (G″) moduli with constant frequency of 1Hz (c) Frequency sweep of polysulfabetaine hydrogel PSB **1**(red) and PSB **2** (blue) with storage (G′) and loss (G″) moduli with constant strain of 1 %.
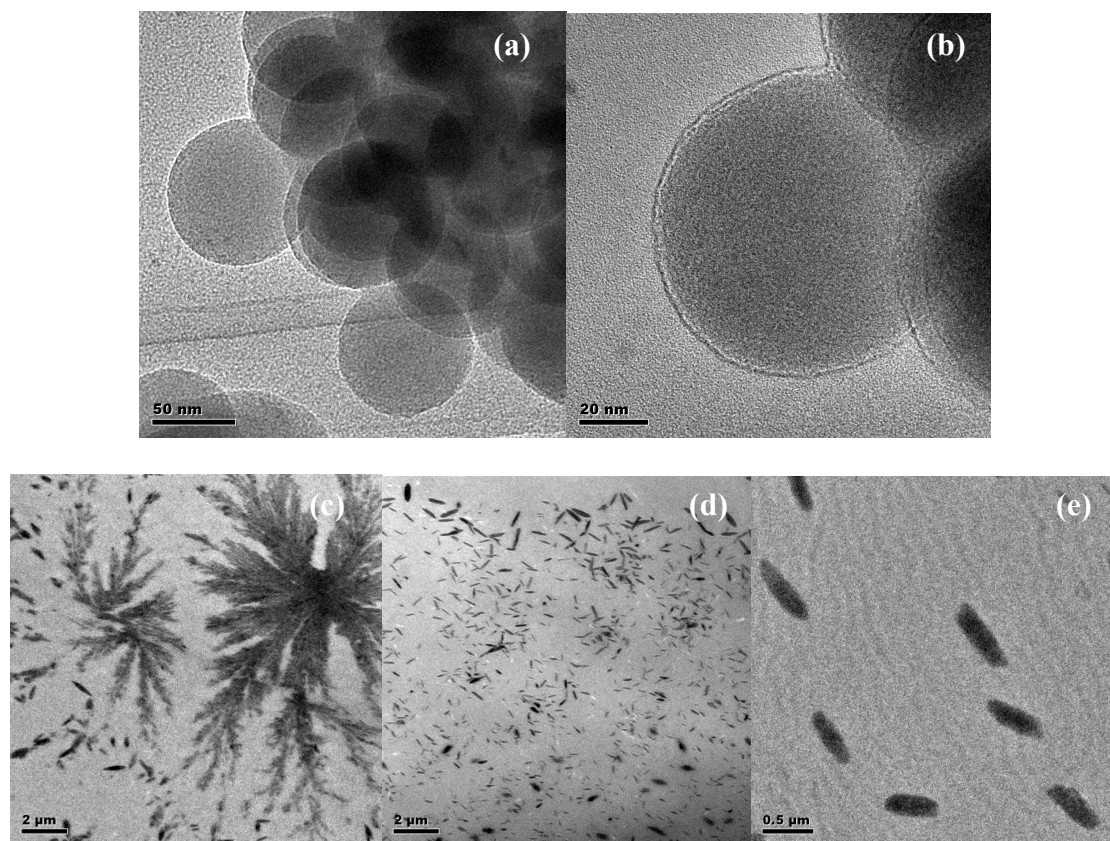
Fig. S5 TEM micrographs of 1 wt% PSB hydrogel in aqueous solution after heat treatment at 70 °C, PSB **1** (a) and (b); PSB **2** (c), (d) and (e).
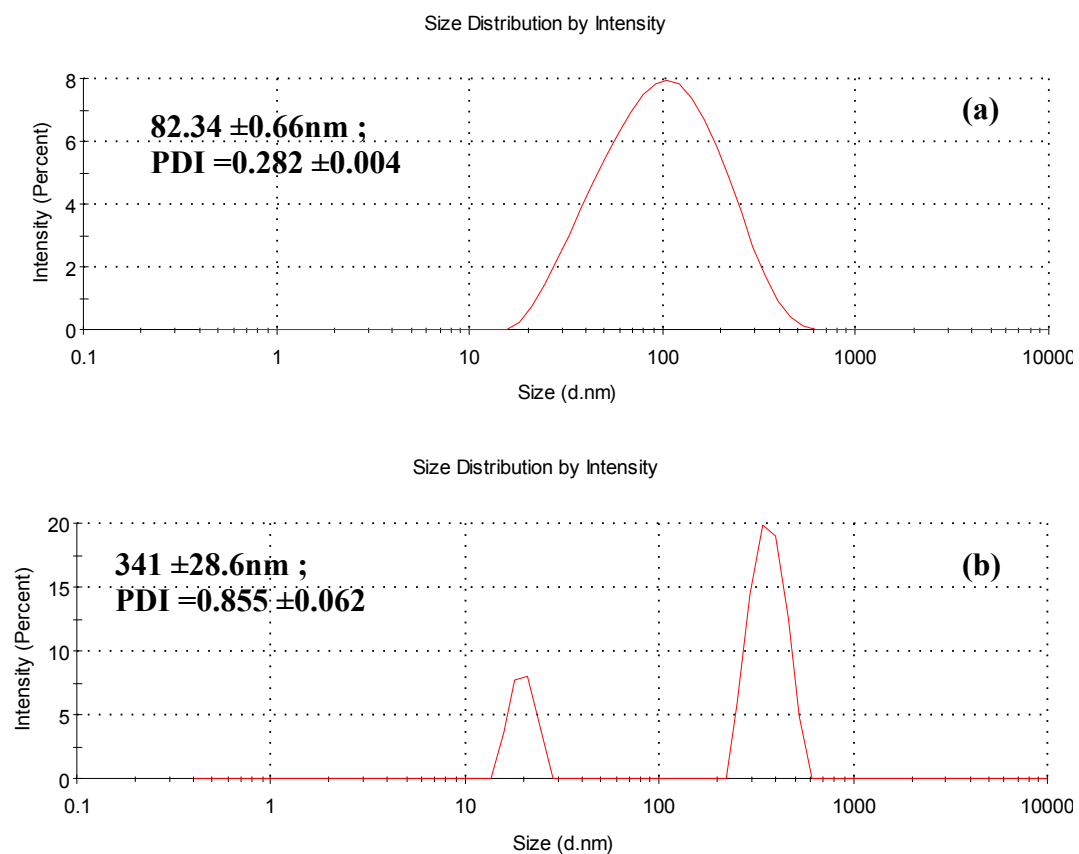
Size Distribution by Intensity

82.34 ±0.66nm ;
PDI =0.282 ±0.004

(a)

Intensity (Percent)

Size (d.nm)

Size Distribution by Intensity

341 ±28.6nm ;
PDI =0.855 ±0.062

(b)

Intensity (Percent)

Size (d.nm)

Fig. S6 Heat induced aggregation of PSBs through DLS measurement (a) PSB **1** and (b) PSB **2**.
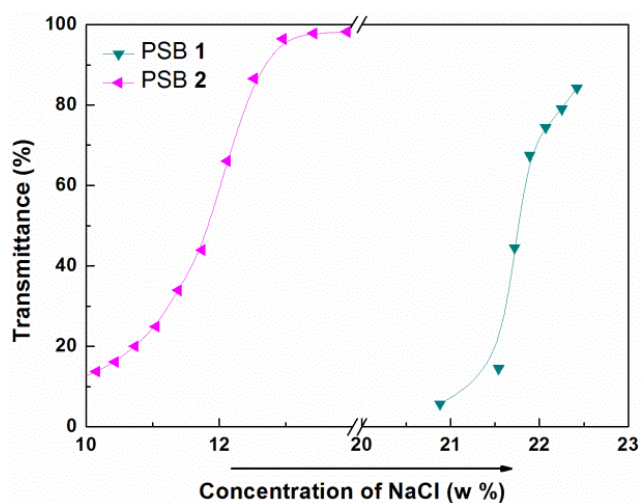
Fig. S7 Effect of salt concentration with respect to UV- transmittance using 1.11 wt % of PSB**1** and 0.87 wt % of PSB **2** in 22.6 wt % NaCl and titrated with deionised water at 25 °C
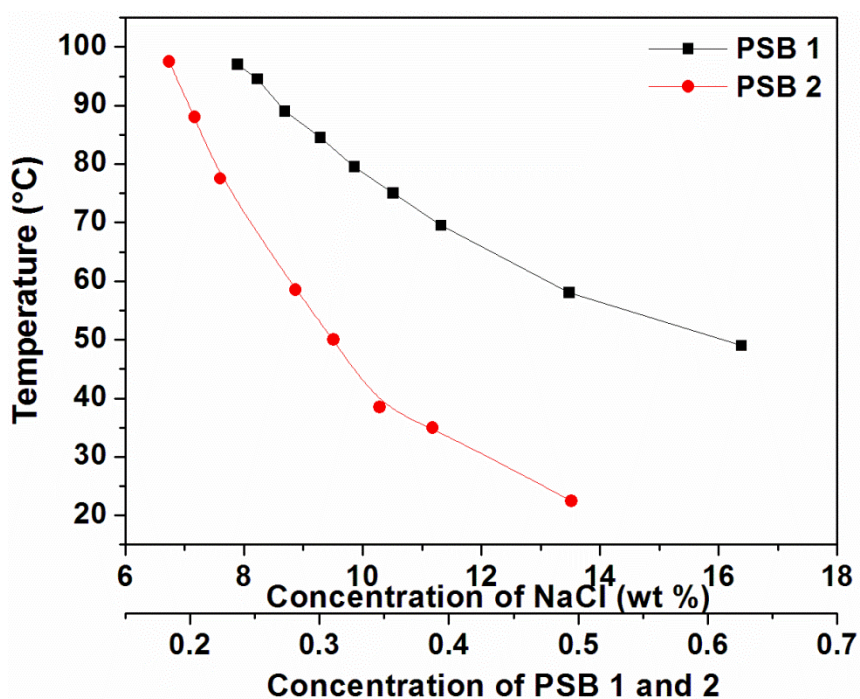
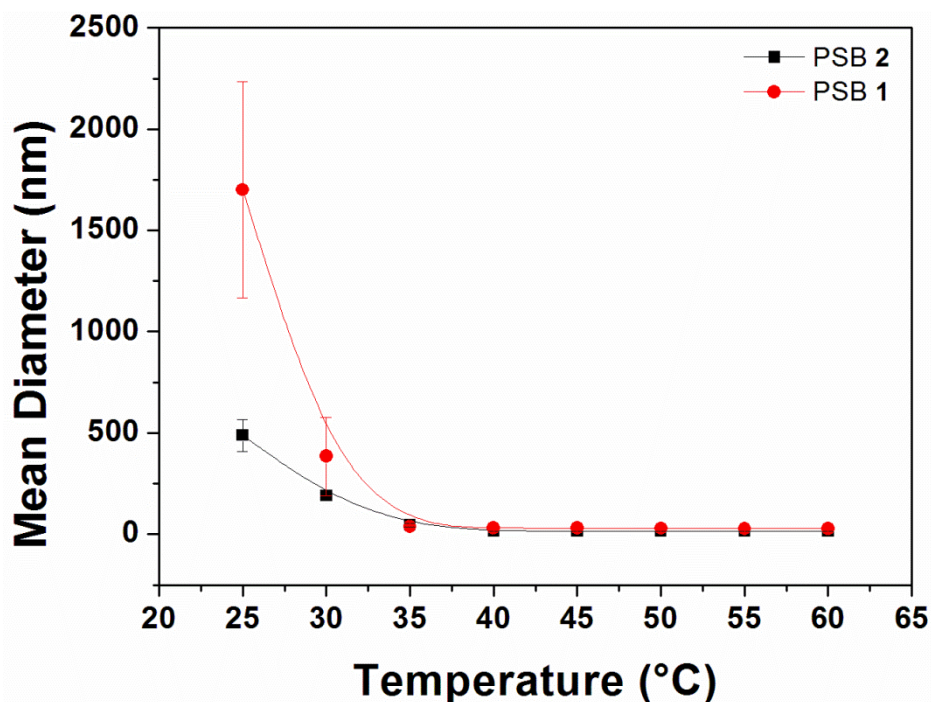**Fig. S8** UCST of PSB in NaCl solutions during heating and cooling process.

Fig. S9 Temperature dependence size variation of PSBs in NaCl solution. ● (red): 1.03 wt % of PSB **1** in 21.04 wt % NaCl; ■ (black): 0.49 wt % of PSB **2** in 12.93 wt % NaCl
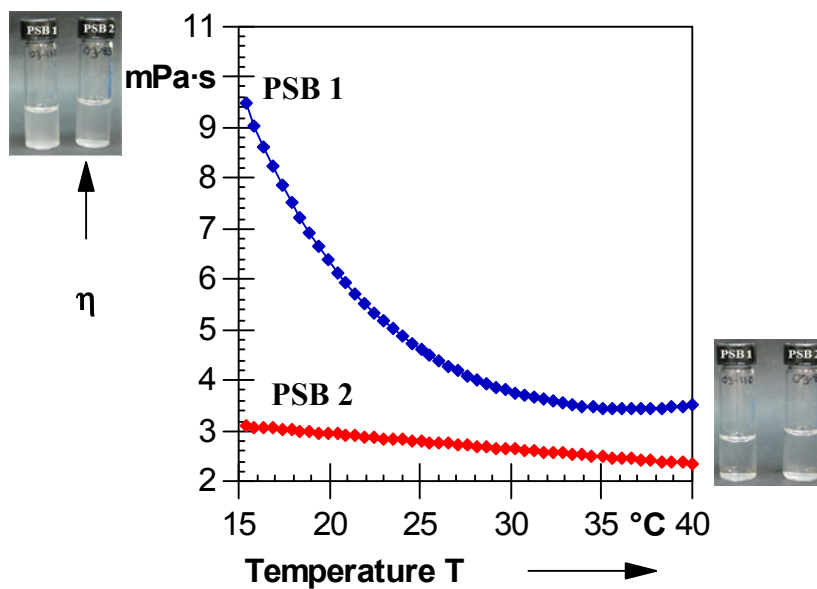

Fig. S10 Change of viscosity of zwitterionic polymers PSB **1** and **2** with temperature. ed): ■ (blue): 1.03 wt % of PSB **1** in 21.04 wt % NaCl; ■ (red): 0.49 wt % of PSB **2** in 12.93 wt % NaCl.
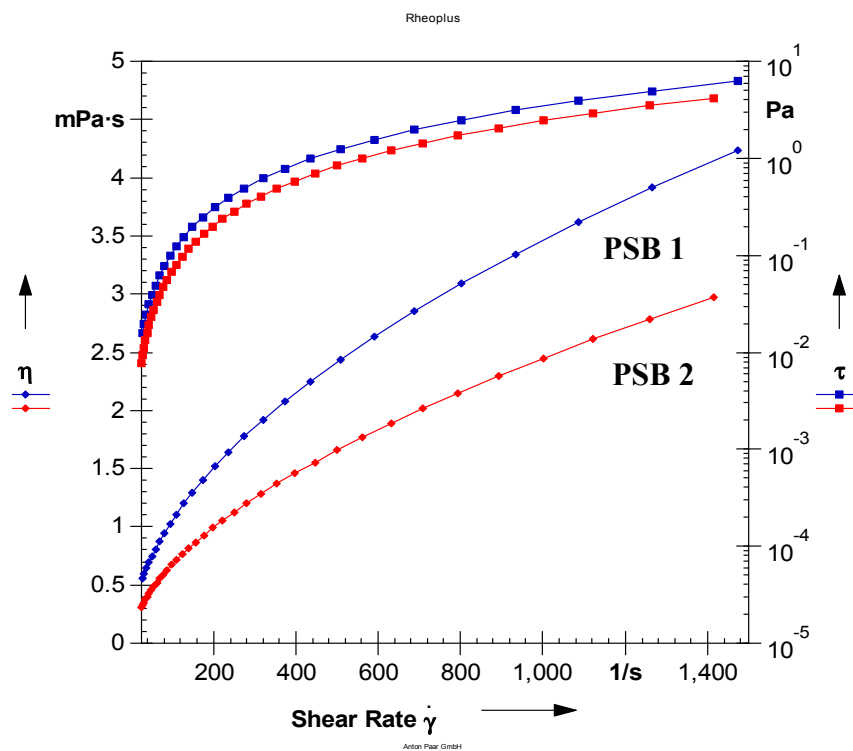
Fig. S11 Viscosity and shear stress of PSB **1** and **2** salt solutions as a function of shear rate. ■ (blue):1.03 wt % of PSB **1** in 21.04 wt % NaCl; ■ (red): 0.49 wt % of PSB **2** in 12.93 wt % NaCl.