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ARTICLE TYPE

Dual hydrophilic and salt responsive schizophrenic block copolymers – synthesis and study of self-assembly behavior

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A new class of dual hydrophilic diblock copolymers (BCPs) possessing poly(ethylene glycol) (PEG) and zwitterionic polysulfabetaine (PSB) was synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization. These BCPs formed schizophrenic micelles undergoing core-shell transitions upon changing the medium from deionized water to electrolyte solution. “Conventional” micelle, i.e. PSB at the core and PEG at the periphery were formed in deionized water. The micelles “inverted” to form PSB at the shell and PEG at the core in electrolyte solutions. The reversal of core-shell structures were thoroughly studied by ^{1}H-NMR spectroscopic analysis, dynamic light scattering (DLS) and transmission electron microscopic (TEM) techniques. Antifouling evaluation in the sea indicated that BCPs displayed antifouling behaviour to some extent. The dual hydrophilic BCPs reported here are potentially useful as stimuli responsive materials.

Introduction

Stimuli responsive polymers are considered as smart materials and are proposed to be useful in applications spanning from biomedicine to release of active ingredients in various domains. Quite a large variety of stimulants have been reported such as pH, temperature, changing the nature of aqueous medium, light, magnetic field, electric field, CO_2, etc.[1-5] Stimuli responsive block copolymers that convert from “conventional” to “inverse” micelles are called “schizophrenic” block copolymers.[6-11] Some of the early examples of schizophrenic block copolymers responded to changes in pH and electrolyte concentration.[12] The latter generation of such block copolymers were stimulated by changes in pH, temperature and ionic strength of the medium.[11] In subsequent developments block copolymers which responded to highly desirable single stimulant have been reported.[13-16]

Schizophrenic copolymers are typically made up of segments which possess similar characteristics, for example, hydrophilicity, however, with minor differences, i.e. one of the segments may exhibit preference for water and the other segment may show preference for weak or strong aqueous electrolyte solutions. Polyethylene glycol (PEG) and ionic or zwitterionic polymers[17-23] are good examples for polymers showing such behavior whereby PEG is miscible in water and zwitterionic polymers are miscible with aqueous electrolyte solutions. Among zwitterionic polymers, zwitterionic sulfonates commonly known as sulfobetaines are the predominantly reported class of zwitterionic polymers. As a variance to this, we recently introduced “halophilic” polysulfabetaines (so called because of their solubility in brine solution alone. The nomenclature is derived from “halophilic bacteria” which live in aqueous solutions of concentrated sodium chloride) which are derived from zwitterionic sulfates.[24] In order to generate novel stimuli responsive polymers, we combined hydrophilic and electrolyte responsive polymeric systems to make interesting block copolymers and studied its responsive behavior.

Developments in radical polymerization like atom transfer radical polymerization (ATRP)[25-28] and reversible addition-fragmentation chain transfer (RAFT) polymerization[29-32] allow the facile formation of block copolymers. In the recent past, we have reported PEG derived macroinitiators for synthesizing block copolymers under ATRP conditions.[33, 34] Since RAFT polymerization process is relatively better suited for changes in polymerization medium, reaction conditions as well as monomer diversity we made use of PEG derived macroRAFT agents to introduce PEG segment into the block copolymer. Zwitterionic vinylbenzylammonio sulfates were polymerized using the macroRAFT agent to yield block copolymers bearing two hydrophilic segments. Since the segment composed of PEG is miscible with water and zwitterionic polysulfate block is miscible with electrolyte solution preferentially, the term dual hydrophilic was used instead of double hydrophilic in order to emphasize the difference in interaction with different aqueous media. Extensive studies by proton nuclear magnetic resonance spectroscopy (^{1}H - NMR), dynamic light scattering (DLS) and transmission electron microscopic (TEM) techniques have shown that these dual hydrophilic block copolymers undergo changes in micellar structure upon changing an aqueous medium from water to electrolyte solution.

These polymers were originally designed and synthesized to study their suitability as antifouling materials under Innovative Marine Antifouling Solutions (IMAS) for high value applications programme. Hence these block copolymers were evaluated for their antifouling behavior in the sea. We would like to highlight here that antifouling results are predominantly reported based on the settlement studies involving larvae of fouling organisms in lab based assays. This should be considered more as a study of antisettlement than antifouling behavior. As more and more reserachers find it difficult to translate the results developed from the lab based assays into antifouling behavior in the actual marine environment many recent publications have recommended to conduct the antifouling evaluation in the sea.[35-37]

Experimental

Materials

All reactions and polymerizations were performed with Schlenk technique under argon atmosphere. 1, 2-ethylene sulfate, 1, 3-propylene sulfate, and 4, 4′-azobis(4-cyanovaleric acid) (ACVA) were purchased from Aldrich and used as received. N-(4-Vinylbenzyl)-N, N-dimethylamine was purchased from ACROS. The macro-RAFT agents such as poly(ethylene glycol) methyl ether (4-cyano-4-pentanoate dodecyl trithiocarbonate) with average M_n = 5,400 (PEG-RAFT 1) and poly(ethylene glycol) methyl ether (4-cyano-4-pentanoate dodecyl trithiocarbonate) with average M_n =1,400 (PEG-RAFT 2) were purchased from Sigma-Aldrich and used as received. 1,1,1,3,3,3-hexafluoro-2-propanol (HFIPA) and 2,2,2-trifluoroethanol (TFE) were purchased from Sigma-Aldrich and used as received. Acetonitrile (ACN), N, N-dimethylformamide (DMF), toluene and tetrahydrafuran (THF) were freshly dispensed from Glass Contour - Solvent Purification System. All other solvents and reagents used were of analytical grade. Deionized water was used for micellization and other aqueous solution studies. Artificial sea water (ASW) was prepared from sea salt obtained from Sigma Aldrich and was used at the concentration of 40 g/ L.

Methods

General procedure for RAFT polymerization

Synthesis of poly(ethylene oxide)-b-poly-[2-(dimethyl(4-vinylbenzyl)ammonio)ethyl sulfate] [$(EG)_{19}$-b-$(ZSB)_{10}$] ***(BCP 3).*** The zwitterionic sulfabetaine monomers, 2-(dimethyl(4-vinylbenzyl)ammonio)ethyl sulfate (ZSBM **1**) and 3-(dimethyl(4-vinylbenzyl)ammonio)propyl sulfate (ZSBM **2**) were synthesized as reported by us before.[24] In a 100 ml dried Schlenk flask, PEG-RAFT 2 (1.96g, 1.43mmol), ZSBM **1** (4.0g; 14.3mmol), ACVA (0.10 g; 1.42 mmol) and HFIPA (15 mL) were added, and the flask was purged with argon gas for 45 min. A dark yellow brown solution was obtained. The flask was degassed under vacuum and backfilled with argon gas three times. Then flask was sealed and immersed in an oil bath maintained at 60 °C and polymerization for 23 h. The highly viscous solution was diluted with HFIPA and transferred to a dialysis bag with MWCO 3500. It was dialyzed against water for 3 day with repeatedly changing in water. The collected polymer was lyophilized and dried in vacuo at 50 °C to yield 4.95 g (83.05 %) of BCP 3.

FTIR (ν cm^{-1}, film): 3600-3200 (moisture absorbed by the polymers), 3060-3010 (aromatic -CH), 2910, 2890 (aliphatic-CH), 1635 (C=C aromatic), 1480 (aliphatic –CH bending), 1255-1230 (S-O stretching), 1033 (S=O) and 1113 (C-O, PEG) (Fig. S1†).

The molecular weights of PEG-RAFT macrointitator and BCPs were determined by ^1H NMR (Fig. S2†) using the equation 1 and 2. $DP = (I_{b,c}/4)/(I_h/3)$ and $M_{n,NMR}$= DP x44.05 + 428.63 (Equ. 1) and $DP_{PZSB} = (I_k/I_{PEG})$x 4/4 x DP_{PEG}; $I_{PEG} = I_{b,c} - (2/4)$x I_k (Equ. 2). The molecular weight of PEG-RAFT was found to be 5100 and 1300.

For BCP 1, $(EG)_{105}$-*b*-(ZSB **1**)$_{23}$, $M_{n,\ NMR}$ = 11700, $M_{n,\ GPC}$ = 7100; PDI = 1.11.

For BCP 2, $(EG)_{105}$-*b*-(ZSB **2**)$_8$, $M_{n,\ NMR}$ =7600, $M_{n,\ GPC}$ = 6000; PDI = 1.22.

For BCP 3, $(EG)_{19}$-*b*-(ZSB **1**)$_{10}$, $M_{n,\ NMR}$ = 4100, $M_{n,\ GPC}$ = 1200; PDI = 1.44.

For BCP 4, $(EG)_{19}$-*b*-(ZSB **1**)$_{24}$, $M_{n,\ NMR}$ = 8200, $M_{n,\ GPC}$ = 1400; PDI = 1.19.

Preparation of micelles of PEG-b-PZSBs

The dual hydrophilic block copolymers PEG-b-PZSBs (BCPs) were dissolved in HFIPA at a concentration of 1 mg/mL. To obtain PZSB core micelles, DI water was added to the HFIPA solution drop wise under vigorous stirring for 15 min. The aqueous solution was then stirred for 24 h followed by dialysis using MWCO 1000 against DI water to remove HFIPA. In order to get inverse micelle, the ASW was added to HFIPA solution and dialyzed against ASW to remove solvent.

Preparation of coated glass slides.

To evaluate the antifouling properties, the BCPs were dispersed in a commercial primer (Primocon available from International Paints) commonly employed in marine coatings. This of our decision was to make the coated surfaces stable under sea thereby making it suitable for prolonged periods of evaluation. Since hydrophilic polymer films of BCPs upon prolonged immersion in water, most often can be dislodged from coated surfaces, primer was employed as a means of film forming material and the BCP as a functional surface modifying additive. Thus, frosted glass slides (7cm x 2.5 cm), were coated by primer dispersed with BCPs (about 1g of fine, powdered polymer in 10 mL of primer to give an effective concentration of 5 wt% in the dried film) using doctor's blade set at a gate height of 150-200 µm. The coated glass slides were dried under ambient conditions for one week.

Characterizations

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 soluble in 0.9MNaCl/D_2O solution and deuterated hexafluoroisopropanol (HFIPA-D_2). The micellization behaviour was studied using different deuterated solvents like D_2O, $CDCl_3$, 0.9M NaCl/D_2O and 4.3M NaCl/D_2O. Residual solvent peaks were used as internal standard. The DMF run SEC system was equipped with a Viscotek GPCmax Pump module, a Viscotek TDA 302 refractive index detector unit, fitted with TOSOH HHR Guard Column and one TOSOH GMHHR-M mixed bed column (5 µm, ID 7.8 mm x 300 mm). The eluent flow rate was 1.0 mL/min, and the columns were maintained at 60 °C. The results were obtained using poly(methyl methacrylate) (PMMA) calibrations. FT-IR spectra were recorded by Digilab Excalibur FTIR Spectrometer. A thin film was casted on the KBr pellet using HFIP as a solvent. 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).

Thermogravimetric Analysis (TGA)

TGA was performed under nitrogen atmosphere at a heating rate of 10 °C/min from 100 to 600 °C using TA instruments (SDT-TGA). For each measurement, the sample cell was maintained at 100 °C for 30 min to remove the absorbed moisture in the sample before measurement. Differential scanning calorimetry (DSC) was performed with a Perkin Elmer Pyris Diamond Hyper DSC. The scans were recorded in N_2 atmosphere with a heating rate of 10 °C/min. The melting temperature (T_m) was determined from the endothermic peak maximum of the first heating cycle.

Dynamic light scattering (DLS)

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 to intensity averaged 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 at 25°C. The micellar solution was filtered using a 0.45 µm disposable membrane filter to remove any dust in the solution.

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. Filtered 10 µL of the 1 mg/mL micellar solution from the DI water and ASW were placed onto the copper grid coated with carbon. The excess solution was removed by a filter paper.

Water contact angle (WCA)

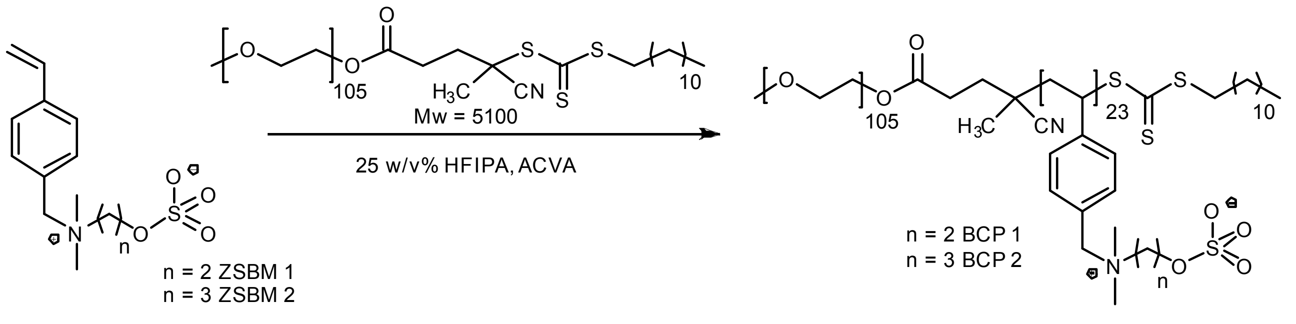
Static contact angle measurements were performed on a KWX 100 stable contact angle analyzer at room temperature with DI water and ASW as the test liquid by the sessile drop method. The block copolymer (1 % wt) in HFIPA was spin coated on a glass slide and dried in vacuum at 50 °C for 45 min. The contact angle of the blank glass slide was also estimated for comparison. The contact angles were measured after allowing the droplets to stabilize for 2 min. With each specimen, the θ values were determined three times in three different locations to obtain an average value.

Evaluation of antifouling properties

Prior to field test, a simple assay was conducted to determine if any toxicity was present in the coatings. All the coated slides were individually soaked in 50ml of 2.7% salinity, 0.2 µm filtered seawater (FSW) at 24°C, without agitation. There were five replicates for each treatment. After 24 hours, the slides were removed and the leachates collected tested against Stage II barnacle nauplii. For the toxicity assay, 20-30 freshly spawned, stage II barnacle nauplii of the barnacle, *Amphibalanus amphitrite*, in 500µl of 2.7% FSW was added to 500µl of leachate resulting in 2-fold dilution of the leachate. The assay was incubated at 24°C for 24 hours. The number of dead and living nauplii was enumerated after 24 hours. Controls consisted of leachates from Primer and uncoated glass slides.

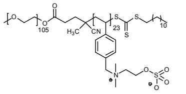
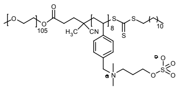
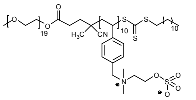
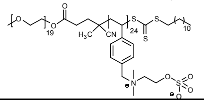
After the leaching, the slides were immediately transferred to the field. Coated glass slides were randomized and slotted into a frame constructed from a slide box such that they were at least 5mm apart, with the coated side facing down when the box was secured upright on a PVC frame. Frame boxes containing coated glass slides were immersed at sea on a floating test platform located in a marina in the south-western part of Singapore Island (1°17’40’’N, 103°45’35’’E), suspended at 0.5 m depth. The coated glass slides were inspected once a week.

After 2 weeks immersion, the slides were removed from the sea and gently washed to remove any detritus or debris. The coated glass slides were removed and photographed with a high resolution 10 megapixel digital camera (Canon Powershot G11) mounted on a camera stand. Enumeration of fouling organisms present on the coated glass slides was performed on the digital images of the slides to quantify the fouling present. To reduce edge effects, a border of 4mm and 2.5 mm from the width and length respectively of the image was digitally cropped using PhotoShop CS. Organisms were classified using the categories defined in the ASTM D6990-03. Only organisms that had settled directly on the surface of the test coupon were considered. Total hard fouling was quantified and presented as total counts (Figure 6) as coverage was < 20%.



Scheme 1 Polymerization of zwitterionic sulfabetaine monomers using PEG macro-RAFT agent.

Table 1 Summary of RAFT polymerization of zwitterionic sulfobetaines.

Sample Code	Composition*	Chemical structures	PEG-RAFT (Mn, NMR)	aMolar ratio	cYield (%)	dMn, theor.	eMn, GPC	PDI	fMn, NMR
BCP 1	$(EG)_{105}$-*b*-$(ZSB\ 1)_{23}$		5100	25: 1: 0.5[b]	98	12400	7100	1.11	11700
BCP 2	$(EG)_{105}$-*b*-$(ZSB\ 2)_{8}$		5100	10: 1: 0.5	69	7500	6000	1.22	7600
BCP 3	$(EG)_{19}$-*b*-$(ZSB\ 1)_{10}$		1300	10: 1: 1	83	3800	1200	1.44	4100
BCP 4	$(EG)_{19}$-*b*-$(ZSB\ 1)_{24}$		1300	25: 1: 1	91	7900	1400	1.19	8200

* composition of block copolymers as determined by ^{1}H NMR; aMonomer: PEG-RAFT: ACVA using 15mL HFIPA as a solvent and busing 15mL of 10 wt% KBr solution; cdetermined by gravimetry after drying the polymer at 50 °C *in vacuo*. d$M_{n,\ theor.} = M_{nPEG} + (DP_{PZSB} \times M_{wPZSB} \times \text{conversion})$; emeasured by DMF GPC; f$M_{n,\ NMR} = M_{nPEG} + (DP_{PSB} \times M_{wPSB})$.

Results and discussion

Earlier we reported sulfate based zwitterions obtained by the ring opening of cyclic sulfates with vinyl monomers bearing tertiary amines.[24] In the present study, zwitterionic vinylbenzylammonio sulfate monomers were polymerized by RAFT agents derived from polyethylene glycol (PEG). Two types of PEG-macroRAFT agents i.e. PEG terminated with RAFT functionality of the type trithiocarbonate (Scheme 1) were used. The block copolymers thus obtained are unique in that they are composed of hydrophilic segments of contrasting nature and hence the classification dual hydrophilic. The PEG segment interacts with water as donor for hydrogen bond through its oxygen atoms whereas zwitterionic polysulfabetaines interact with water through dipoles. In addition, zwitterionic polysulfabetaines also exhibited salt responsive behavior.[24] The number average molecular weights (av. M_n) of RAFT agents were 5400 (PEG-RAFT 1) and 1400 (PEG-RAFT 2). Each of these RAFT agents were used at monomer: RAFT agent ratio of 25:1 and 10:1 to make block copolymers, BCP 1 to 4. The preparation of block copolymers is shown in Scheme 1.

Table 1 shows the chemical structure of BCPs and the molecular weight characteristics of BCPs. BCP 1 was prepared in 10 wt% potassium bromide solution and the remaining BCPs were prepared using hexafluoroisoprapanol as solvent. ACVA was used as radical initiator in all cases. The polymerization proceeded smoothly at 60°C to give moderately high to very high yields of BCPs. The theoretically estimated molecular weights were closer to those determined by NMR analysis. In general, the molecular weights determined by gel permeation chromatography (GPC) (Fig. S3†)using N,N-dimethyl formamide as eluent were lower than that determined by ^{1}H-NMR spectroscopic analysis. This difference is most likely due to the solution conformation of BCPs. It appears that solution conformation is predominantly dependent on the composition of BCPs. The low zwitterionic content of BCP 2 did not influence the solution behavior as evident from the comparatively lower difference between the molecular weights obtained in GPC and NMR analysis. The polydispersity of block copolymers were in general low and comparable to the block copolymers reported by Armes et al.[38] However, unlike the previous block copolymers where zwitterions were introduced through a post polymerization modification, we used preformed zwitterionic monomers. It should also be emphasized that Armes et al.[38] quaternized a block copolymer which possessed two different types of 3° nitrogen atoms which could potentially compete in the quaternization reaction. The polydispersity of BCPs 1, 2 and 4 are well below than the block copolymers reported by Laschewsky et al.[14] As compared with the previously reported schizophrenic block copolymers, these block copolymers have been prepared in multigram scale in high yields from readily available starting materials. The ^{1}H-NMR spectra of macro-RAFT agent and BCPs 1 and 2 are shown in Fig. S2†. As expected the signal intensities varied with the degree of polymerization.

Dual hydrophilic BCPs showed stepwise degradation in the thermogravimetric analysis (TGA). Stepwise degradation is normally observed for block copolymers which are constituted of segments of contrasting thermal stability[39-41] as well as in those polymers where the loss of side chain precedes the backbone degradation.[33] The TGA behavior of BCPs studied here are due to a combination of these two phenomenons. The difference in thermal stability between PEG and zwitterionic polysulfate segments as shown in Fig. S4† is large and also the loss of zwitterionic moiety occurs before the degradation of the corresponding backbone.

Table S1† compares the melting transition of PEG segment in the macroRAFT agent with that of BCPs. Interestingly moderate increase in melting temperature was observed with lower incorporation of zwitterionic segment (BCP 2) which may be due to chain stiffening caused by the polysulfate block. This trend was reversed in the case of lower molecular weight macroRAFT agent. The zwitterionic polysulfate block also induced chain stiffening in BCPs 3 and 4 as a result of which melting transition of PEG was observed in these block copolymers which was absent in macroRAFT agent.

Schizophrenic micellization behavior of dual hydrophilic BCPs

As described previously the BCPs possess water miscible PEG blocks and salt responsive zwitterionic sulfate blocks. Due to this dual nature these BCPs can be expected to form "conventional" and "inverse" micelles in aqueous and electrolyte solutions respectively as schematically represented in Fig. 1. The schizophrenic micellization and self-assembly behavior were studied by ^{1}H-NMR spectroscopy, dynamic light scattering and transmission electron microscopic techniques as described below.

^{1}H-NMR studies

In order to follow the structural changes occurring in the polymer chain, BCPs in various solvents viz., HFIPA-D_2, D_2O, 0.9M NaCl and 4.3M NaCl in D_2O were analyzed by ^{1}H-NMR spectroscopic analysis (Fig. 2). In HFIPA-D_2, BCP 1 dissolved completely. Due to the prevalence of well solvated stretched polymer chains the signal observed was as expected and the interpretation was fairly straightforward. However, in D_2O, lessened intensity of signals corresponding to aromatic protons as well as the signals corresponding to the $–CH_3$ groups attached to the quaternized nitrogen atom were noticed. This is due to the prevalence of core-shell structures with PEG block forming the shell and the zwitterionic sulfate constituting the core ("conventional" micelle). This core-shell composition was inversed in aqueous salt solutions. It has been reported that aqueous solutions of PEG form two phase systems upon the addition of electrolyte solution.[42, 43]

In 0.9M sodium chloride solution as well as saturated sodium chloride solution in D_2O, the formation of "inverse" micelles whereby zwitterionic sulfate occupied the shell and the PEG block constituted the core was evident from the far reduced intensity of signals corresponding to PEG and also the reappearance of signals corresponding to aromatic protons along with the signals related to $–CH_3$ group attached to the quaternized nitrogen atom. The broadening of latter signals is more likely due to the poor relaxation caused by the increased viscosity of the medium as compared to HFIPA-D_2. Thus the ^{1}H-NMR spectroscopic analysis confirmed the schizophrenic nature of the self-assembled micelles.

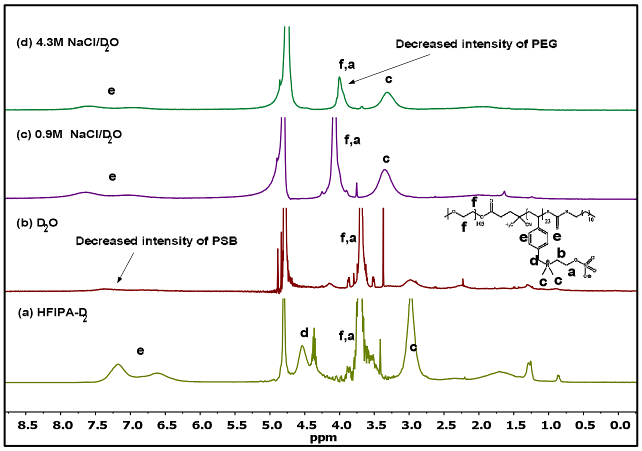


Fig. 2 ^{1}H NMR spectra of BCP 1 in various deuterated solvents (a) HIFPA-D_2; (b) D_2O; (c) 0.9M NaCl/D_2O and (d) 4.3M NaCl/D_2O.

Dynamic light scattering (DLS) studies

In order to investigate the size and distribution of schizophrenic micelles, DLS experiments were conducted in different media. BCP micelles were formed in deionized (DI) water, artificial sea water (ASW), and concentrated brine solution at 25°C for BCP 1 and 2. The resulting Z-average size of BCP micelles is given in Table 2. As mentioned before, BCP 1 formed completely solvated stretched polymer chains whose intensity average hydrodynamic diameter was about 10nm in HFIPA. The size of micelles increased to about 45nm in DI water (Fig. 3), indicating self assembly. The increase in size was higher for BCP 2 (Fig. S5†). We observed similar behaviour in zwitterionic polysulfates previously where the conformation changed from spherical to rod like upon moving from zwitterions where the counter charges were separated by 2 and 3 carbon atoms respectively.[24] This behavior is unique to zwitterions derived from sulfates. This is most likely due to the change in nature of ionic interactions from predominantly intramolecular to substantially intermolecular. This in turn is caused by the presence of additional oxygen atom which separates counter charges of the zwitterion. It is interesting

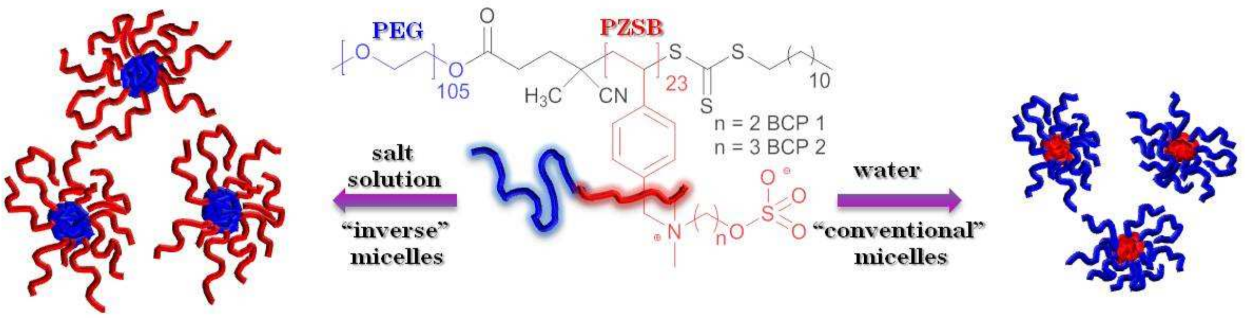


Fig. 1 Schematic representation of "schizophrenic" micellization behavior of dual hydrophilic block copolymers.

to note that higher diameter was observed in spite of the low degree of polymerization and thus shorter block length of sulfate derived zwitterions. It is also useful to note here that increased melting of PEG block was observed in BCP 2 (Table 1S†).

Table 2 Summary of particle sizes for BCPs by DLS and TEM in water and brine solution.

Polymer	Solvents	Method		
		^{b}DLS (D_z, nm)	PDI (DLS)	^{c}TEM (nm)
BCP 1	HFIPA	11	0.66	-
	DI water	45	0.09	50
	ASW	149	0.1	120
	4.3M NaCl^a	57	0.7	-
BCP 2	HFIPA	7	0.55	-
	DI water	144	0.13	110
	ASW	126	0.6	90
	4.3M NaCl	45	0.63	-

[a] 25 wt% NaCl solution; [b] the average size by intensity at 25 °C, (peak maximum); [b] size determination using TEM.

The addition of electrolyte in the form of 4.3M aqueous sodium chloride solution caused the shrinking of micelles more on BCP 2 which is again an indication of lessened interchain interaction induced by solvation. Once again the schizophrenic nature of these micelles were confirmed by measuring the zeta potential which was found to be -5.06 mV for BCP 1 and -4.74 mV for BCP 2 in ASW. The negative values are indicative of the anionic behavior caused by sulfate unit occupying the shell. Fig. 3 shows the size distribution of BCP 1 in DI water and ASW. The stability of each of BCP micellar solutions were examined after two months using DLS (Table S2). After two months of storage under ambient conditions minor variations due to aggregation was more prominent in DI water (Fig. S6†). Thus the low zeta potential values are more of an indication of the net ionic interaction prevailing in the electrolyte solution.

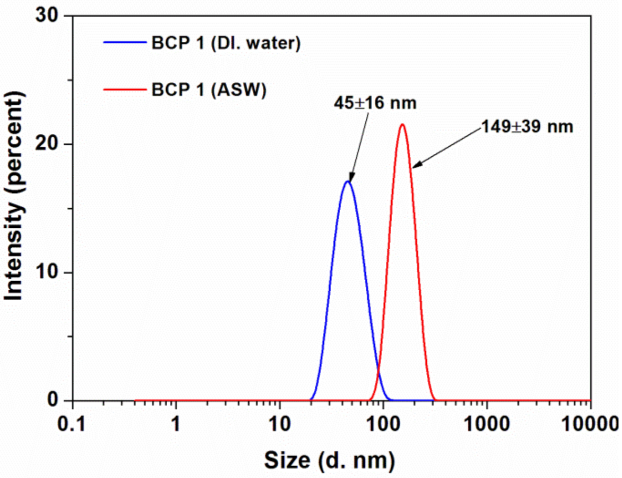


Fig 3 Size (Z-Average) distribution of micelles of BCP 1 in DI water and ASW.

Transmission electron microscopic (TEM) studies

TEM studies were conducted to further analyze the nature of micelles such as its size and shape. Fig. 4 shows transmission electron micrographs of BCPs 1 and 2 in DI water and ASW. As summarized in Table 2, significant differences in the size of micelles were observed. As revealed by TEM, BCP 1 and 2 remained as spheres (Fig. S7†). The bilayer structure of BCP 2 indicated the formation of vesicles with a radius of 150 nm. The difference in diameter observed between TEM and DLS is most likely due to the shrinkage of PEG shell. It may be noted that DLS measurements were performed in aqueous solution whereas TEM was conducted after drying the droplet. Drying can also be expected to induce structural changes in the micelles. However, the trend observed i.e. change in size upon moving from DI water to ASW was same in both DLS and TEM techniques.

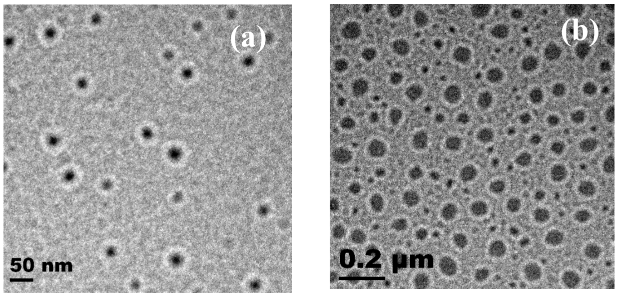


Fig 4 TEM of BCP 1 (a) and 2 (b) in DI water at the concentration of 1 mg/mL.

Contact angle measurements

Static contact angle measurements in DI water (Table 4) indicated that coated slides retained their hydrophilicity thereby indicating the predominance of BCPs on the surface of the coated glass slides. Further evaluation of surface structure is currently underway.

Table 4 Nature of coated surfaces as determined by contact angle measurements.

	Contact Angle, (θ) °					
Solvent	Blank glass	BCP 1	BCP 2	BCP 3	BCP 4	ZSB
DI water	59.6±0.4	8.6±2.9	14.2±0.8	15.1±0.9	19.2±1.7	17.4±1.4
ASW	43.0±4.4	8.6±1.3	11.1±1.1	15.6±3.4	22.2±0.2	-

Antifouling properties

The coated glass slides were then immersed in sea water for 24h to collect leachates for nauplii toxicity test. The toxicity assay revealed low levels of toxicity associated with BCPs 2, 3 and 4 which may be the result of chemical residues left over in the formulation such as traces of solvents (Fig. 5). However, these differences were not strongly reflected in subsequent antifouling activity in the field test possibly due to dilution factors in open sea. Barnacles and tubeworms were the dominant macrofoulers present during the field test. After two weeks of immersion in the sea, the glass slides were scored for settlement of macroorganisms (Fig. 6). Coated surfaces remained intact after immersion in sea and no anomaly was noticed thereby validating our approach.

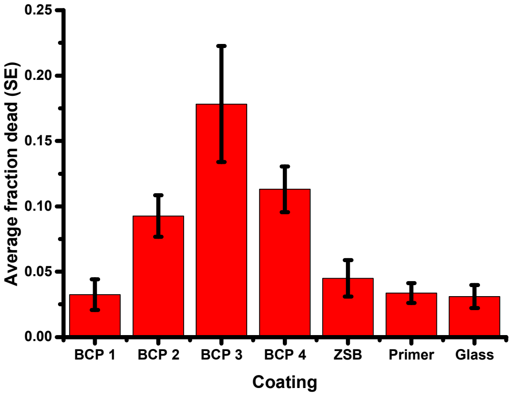


Fig. 5 Nauplii toxicity of BCPs.

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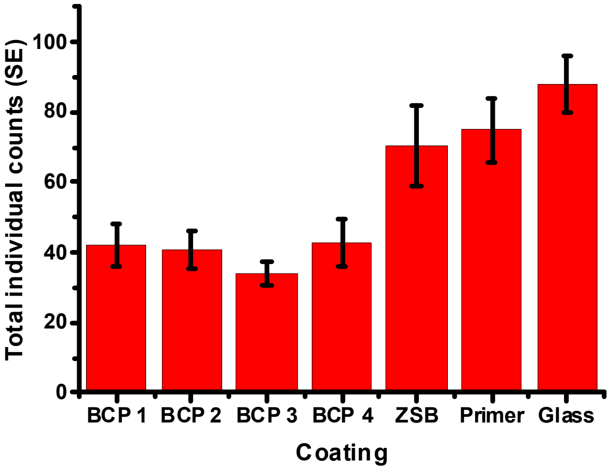

Fig. 6 Antifouling behaviour of surfaces coated with BCPs.

The combination of PEG and zwitterion embedded in a coating exhibited anti-fouling properties in the field test (Fig. S8†). The effectiveness of PEG is highly apparent when one takes into consideration that the homopolymer of zwitterionic sulfate, ZSB was not mixed with primer and coated on frosted glass slide by solution casting technique. ZSB upon mixing with the primer also did not show any antifouling property. It is worth noting that these antifouling results have been achieved after mixing BCPs with a primer commonly employed in marine antifouling paints. The observed antifouling property is most likely due to the formation of microstructures induced by BCP. This conclusion is based on the fact that PEG coated surfaces did not show any antifouling behaviour in the same location of the sea. The nature of microstructure formed and the analysis of surface chemical composition will be the subject of future publications. We would like to stress the fact that for the first time we've demonstrated the effectiveness of combination of PEG and zwitterions in the antifouling evaluation in conditions resembling actual application. By considering the fact that these results were obtained by mixing the BCPs with the primer employed in marine coating, this result possesses enormous potential.

Conclusions

Dual hydrophilic block copolymers capable of forming schizophrenic micelles were synthesized and characterized. The schizophrenic nature was confirmed by ^{1}H-NMR spectroscopic analysis of BCPs. DLS and TEM studies further confirmed the changes occurring in micelles upon changing the nature of medium. The ability of BCPs to prevent the settlement of marine organisms was also studied in the sea. BCPs composed of hydrophlic PEG and salt responsive zwitterionic sulfate segments exhibited better antifouling behavior than the homopolymer of salt responsive zwitterionic sulfate or PEG alone in the sea. We believe that these BCPs are highly useful as stimuli responsive materials.

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† Electronic Supplementary Information (ESI) available: FTIR, GPC, TGA, TEM and images of field evaluation. See DOI: 10.1039/b000000x/

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