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SEAFRONT

Synergistic Fouling Control Technologies

**Deliverable D1.16: Delivery of coatings possessing LCST/UCST for
evaluation of anti-biofouling activity**

Delivery date: M18 (June 2015)



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1 Introduction

Deliverable 1.3 has been completed, providing polymer coatings possessing thermoresponsive behaviours for evaluation of anti-biofouling activity within WP4. The following report details progress made in the development of polymer coatings based on the thermoresponsive polymers poly(oligoethylene glycol methyl ether methacrylate) (pOEGMA) and poly(*N*-isopropylacrylamide) (pNIPAM) (Figure 1).¹

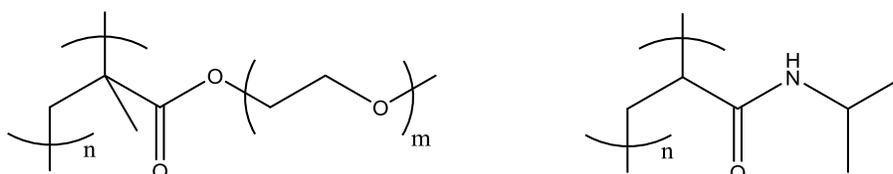


Figure 1: Poly(oligoethylene glycol methyl ether methacrylate) (pOEGMA), left, and poly(*N*-isopropylacrylamide) (pNIPAM), right.

At the critical solution temperature (CST) in aqueous media these thermoresponsive polymers switch from a solvated hydrophilic form to a desolvated hydrophobic form (Figure 2).

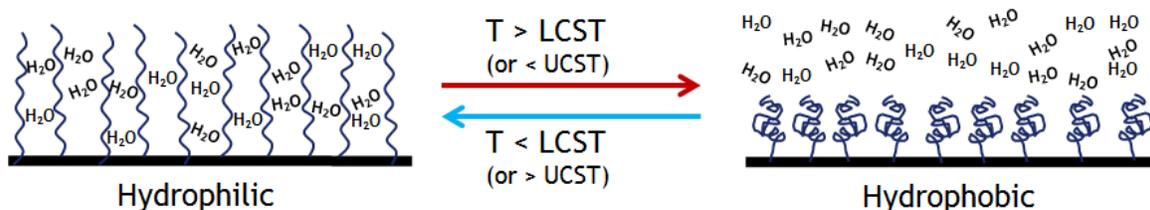


Figure 2: Representation of thermoresponsive behaviour in polymer coatings.²

The stimuli-responsive properties of p(NIPAM) have been shown to provide excellent foul-release character against *Halomonas marina*³⁻⁵ on cycling through the CST,⁶ indicating the concept is worthwhile evaluating further. Analogous research with p(OEGMA), a less-studied thermoresponsive coating, has also highlighted anti-biofouling behaviour,⁷ although this desirable feature has not yet been investigated in a marine environment.

It is anticipated that the dynamic nature of these surfaces, encompassing both hydrophilic to hydrophobic switching and rapid desolvation, will greatly deter the settlement of marine organisms. If successful, this 'proof-of-principal' investigation will allow the development of more advanced, synergistic coatings possessing other useful properties for robust and effective marine anti-fouling coatings.

1.1 Deliverable objective(s)

D1.3: Delivery of films possessing LCST/UCST for evaluation of anti-biofouling activity.

2 Partners involved

The University of Newcastle – School of Chemistry – (UNEW-SCL) performed the synthesis of LCST/UCST thermoresponsive coatings, which will be for anti-biofouling and foul-release

properties at University of Newcastle – School of Marine Science and Technology – (UNEW-MST). When excellent performance at lab scale testing is achieved, the thermoresponsive coatings will (probably) be down selected for upscaling and field test depending on the lab scale results of competitive anti-fouling technologies with comparable biofouling performance.

3 Description of technology delivered

3.1 Discussion of work for deliverable 1.3 and Results

Several thermoresponsive coatings have been produced including a number of p(OEGMA) samples, with variation of the oligoethylene glycol side-chain length, and samples of p(NIPAM) for comparison. A number of other achievements have also been made, including coating formulation, development of substrate functionalization techniques to provide coating adhesion and production of equipment for programmable cycling of the coatings through their CSTs, enabling the reproducible production of suitable thermoresponsive coatings and assessment for anti-biofouling / foul-release behavior.

3.1.1 Development of thermoresponsive coatings

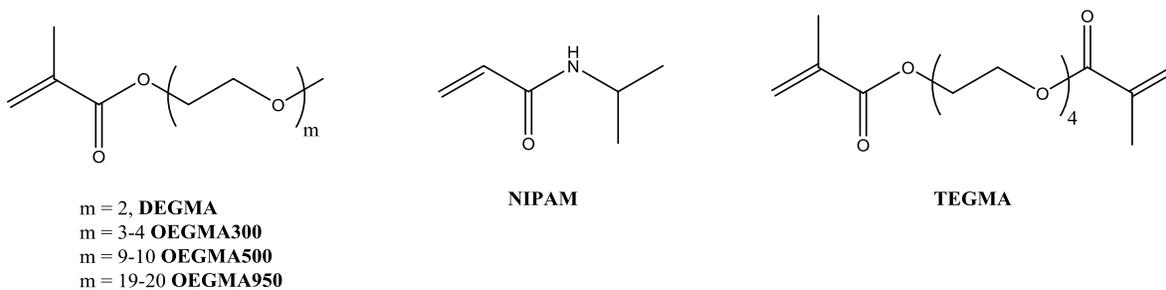
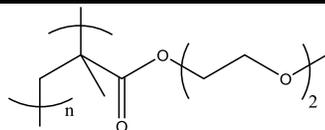
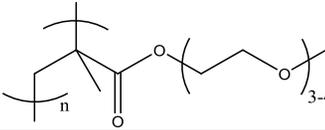
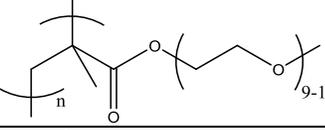
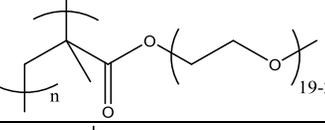
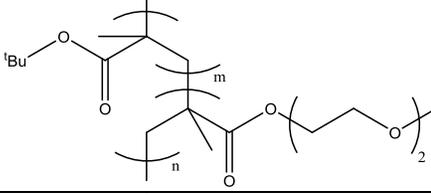
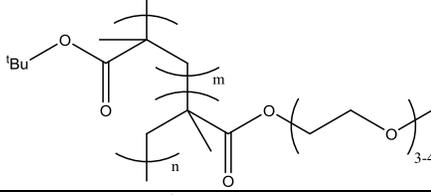
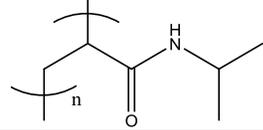


Figure 3: Structures of monomers and cross-linkers used to produce thermoresponsive polymer coatings.

A summary of the polymer coatings produced and some of their physical characteristics may be found in Table 1.

Table 1: Summary of thermoresponsive coatings produced.

Entry	Polymer	General name ^a	Replicates produced	CST ^{b,c}
1		p(DEGMA)	7 watch glasses, 7 slides	23 °C
2		p(OEGMA300)	7 watch glasses, 7 slides	61 °C
3		p(OEGMA500)	7 watch glasses, 4 slides	85 °C
4		p(OEGMA950)	7 watch glasses, 4 slides	N/A
5		p(DEGMAco ^t BuMA)	2 watch glasses, 2 slides	20 °C
6		p(OEGMA300co ^t BuMA)	2 watch glasses, 2 slides	57 °C
7		p(NIPAM)	8 watch glasses, 4 slides	21 °C

^a Mixed polymer systems are statistical co-polymers; all polymer coatings also contain 1 mol% TEGMA cross-linker (see experimental for full formulation); ^b CST measurements were performed using samples on glass microscope slides immersed in 33 PSU (approx. 34.1 g/L) 'Tropic Marin' synthetic sea water

Polymer coatings produced were all tested for anti-biofouling activity with the barnacle cyprid settlement assay (data not shown). Initial results suggest promising anti-fouling behavior even without repeated transition through the CST of the coatings and thus these coatings were re-produced with multiple replicates of each to enable a full barnacle cyprid settlement assay to be performed in the future, as well as algae and diatom assays (all within WP4).

Interestingly, the CSTs of the polymer coatings were reduced on account of the salinity of the testing medium; this behavior has been well documented previously.⁸ The salt induced reduction in CST from 26°C to 23°C in pDEGMA and 32°C to 21°C in pNIPAM, inadvertently produced CST transitions of these two materials during the barnacle cyprid settlement assay, which was performed at 28°C. During this assay the coatings are removed from the incubator for analysis every 24 h at which point the temperature of the coating returns below the CST temporarily before being returned to the incubator.

As a consequence of the inherent hydrophilicity of these coatings, the usual methods used in the barnacle cyprid settlement assay were not suitable since the test requires the surface to hold 1 mL of water. In contrast, the surface tension of hydrophobic surfaces is sufficient to hold this volume of liquid on the sample surface of a flat substrate. To accommodate this requirement, hydrophilic samples were produced on 'watch-glasses' (in addition to the microscope slides used for the algae and diatom assays), which were readily available and could be functionalized in the same manner as the microscope slides. Microscope slides and 'watch-glasses' could be spaced appropriately using disposable needles (21 – 30 gauge) (Figure 4).

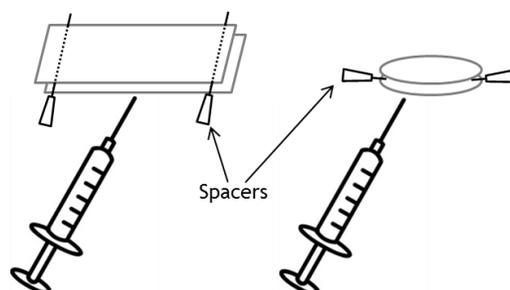


Figure 4: Schematic representation of polymer coating methodology.

To establish a suitable formulation for the production of p(OEGMA) and p(NIPAM) coatings, a number of parameters were varied including monomer and cross-linker concentrations, coating thickness and solvent composition. From these tests, appropriate working ranges were identified for the most important parameters (Table 2).

Table 2: Summary of variables screened in reagent formulation for polymer coating synthesis.

Entry	Variable	Suitable Conditions
Initiator	Ammonium persulfate (APS) Sodium metabisulfide (SMS)	APS
Monomer concentration	10, 25, 50%vol	25 – 50%vol ^a
Molar equivalents cross-linker (<i>cf.</i> monomer)	0, 0.02, 0.04, 0.07, 0.09	0.02
Coating Thickness	0.31, 0.41, 0.51, 0.82 mm	0.51 mm glass slides 0.31 mm watch glasses
Curing method	60 – 85 °C / RT + TMEDA	RT + TMEDA
Solvents	Ethylene glycol / Ethanol / H ₂ O (1:1:1, 1:1:2, 2:1:2, 1:2:2, 2:2:1)	1:1:1
Time	2 h (60 – 85 °C), 24 h (RT + TMEDA)	24 h (RT + TMEDA)

^a Conditions 'optimized' using OEGMA300 where 50%vol = 35 mmol + 1 mL solvent

3.1.2 Development of glass substrate functionalization procedures to improve polymer coating adhesion

Preliminary attempts to produce polymer coatings possessing thermoresponsive behavior revealed that the coatings produced required immobilization on a suitable substrate to facilitate biological testing.

Glass was chosen as a substrate on account of its ease of use and commercial availability, enabling facile functionalization with trimethoxysilyl-derivatised reagents to chemically bind the coatings to the glass. A number of trimethoxysilyl-containing compounds were screened to this effect and some simple contact angle measurements performed on the functionalized glass (Table 3).

Table 3: Contact angle measurements on functionalised glass surfaces.

Surface Functionalization	Deposition Method	Average Contact Angle ^a
Clean Glass	N/A	11°
Trimethoxy(oct-7-en-1-yl)silane	Solution phase deposition	83°
Trimethoxy(vinyl)silane	Solution phase deposition	77°
3-(trimethoxysilyl)propyl methacrylate	Solution phase deposition	80°

^aContact angle measurements were conducted using distilled water in air and averaged from 5 measurements

These contact angle measurements suggest that a sufficient degree of functionalization could be achieved by immersing the glass substrate in a 0.07 M solution of the desired trimethoxysilyl-reagent in toluene for 24 h, and no significant deviation from the measured contact angles was observed looking at different areas of the glass substrate, implying an even coverage. Thus, these conditions were adopted as the general procedure for functionalization of the glass substrates, and 3-(trimethoxysilyl)propyl methacrylate was selected as the functionalization agent to best match the reactivity of the monomers being used. All polymer coatings listed in Table 1 utilized this glass-functionalization chemistry.

3.1.3 Improvement of mechanical stability of polymer coatings

In general, the mechanical stability of all the coatings produced was sufficient for practical manipulations and 'settlement testing'. The mechanical stability of the pure OEGMA coatings (found in Table 1, entries 1–4) was also shown to be acceptable for foul-release testing in the 'flow cell' though too low for 'jet-washing', a key foul-release test utilized in WP1 and WP4, in which a programmable arm passes a jet of water over the samples at increasing pressures (Figure 5).

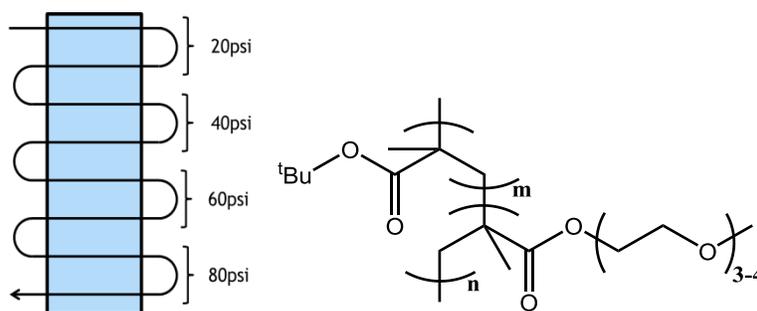


Figure 5: Schematic representation of 'jet removal' test (left) and *p*(OEGMA) compositions with increased mechanical stability (right)

It was found that addition of *t*-butylmethacrylate (*t*BuMA) to OEGMA300 greatly improved mechanical stability of the resultant coating. Coatings formulated using a molar ratio of 8:2, OEGMA300:*t*BuMA were shown to be stable up to the highest pressures used (80 psi) whereas the analogous pure OEGMA300 coating disintegrated at just 20 psi. In addition to the increased mechanical stability, the addition of the more hydrophobic *t*BuMA monomer also serves to lower the CST of the resultant co-polymer by around 4 °C, (Table 1, Entries 6 and 7). This modification allows the tuning of the CST to a useful range for use in marine environments. The addition of *t*BuMA to the polymer coatings was shown to have no deleterious effects on the anti-fouling properties of the coatings in the barnacle cyprid assay.

3.1.4 Development of custom made apparatus to enable temperature cycling during anti-biofouling / foul-release assessment of polymer coatings

To test the anti-biofouling / foul-release properties of the thermoresponsive polymer coatings a waterproof heating platform was designed incorporating a thermocouple controller with RTD input ('Tempatron IR32') (Figure 6).

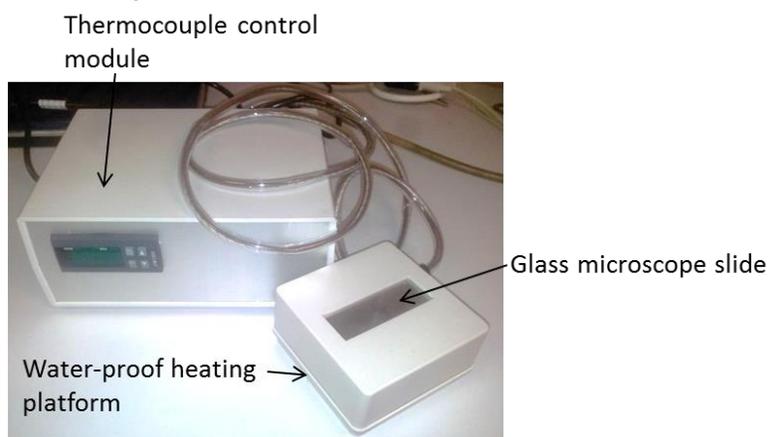


Figure 6: Bespoke heater-block apparatus for subversive temperature control in thermoresponsive coatings

The capabilities of this device will allow accurate cycling of the polymer through its CST and can be programmed to repeatedly raise and lower the surface temperature as required (Figure 7).

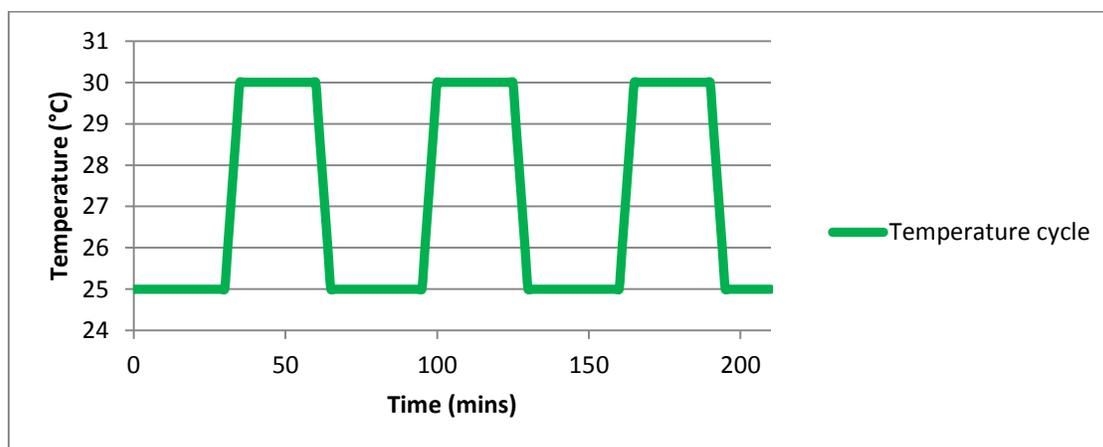


Figure 7: A proposed heating/cooling cycle for thermoresponsive coatings during marine anti-fouling assay

3.2 Experimental procedures – Polymer Coating Production

Materials and methods: Toluene, ethanol, ethylene glycol were purchased from Fisher Scientific and used as received. Tetraethylene glycol dimethacrylate (TEGMA), diethylene glycol methylether methacrylate (DEGMA), oligoethylene glycol methylether methacrylate (OEGMA300, av. M_w 300 gmol^{-1}), OEGMA500 (av. M_w 500 gmol^{-1}), OEGMA950 (av. M_w 950 gmol^{-1}), ammonium persulfate and *N,N,N,N*-tetramethylethylenediamine (TMEDA) were all purchased from Sigma-Aldrich and used as received.

General procedure for the production of p(OEGMA) coatings:

Two glass microscope slides or watch-glasses were cleaned using detergent then rinsed with distilled water followed by ethanol before being placed in ethanol and sonicated for 15 min. The substrates were then dried under a stream of nitrogen before being placed in a plasma asher and subjected to oxygen plasma at 70 W for 15 min. One substrate was then immediately placed into a solution of 3-(trimethoxysilyl)propyl methacrylate in toluene (0.07 M) and covered. After 24 h the substrate was removed from the solution and dried under a stream of nitrogen then placed in an oven at 115 °C for 20 min. Once cool, the substrate was rinsed with toluene then dried with nitrogen.

The clean substrate was then placed on top of the 3-(trimethoxysilyl)propyl methacrylate functionalized substrate, spacing with 0.51 mm (for glass slides) or 0.31 mm (for watch-glasses) needles, and the 'sandwich' clamped together with 2 clips.

The appropriate monomer (35 mmol) was dissolved in a mixture of ethanol, H₂O and ethylene glycol (1:1:1, 10 mL) before the addition of TEGMA (100 μL) and ammonium persulfate (40% w/v in H₂O, 1.75 M, 230 μL) then mixed thoroughly. Immediately before use, TMEDA (30 μL) was then added and mixed thoroughly before the mixture was injected into the 'sandwich', filling to the edge of the glass and ensuring no air bubbles remained.

The sample was then covered and left at room temperature for 24 h before the top glass substrate was removed and the resultant polymer coating placed in 100 mL of distilled H₂O to leach residual impurities for 24 h.

Samples were then placed in a distilled water bath with continuous water purification (Amberlyst Na⁺, activated carbon) for 3 days prior to testing.

4 Conclusions

In conclusion, the production of several thermoresponsive coatings has been achieved. These include a number of p(OEGMA) samples, with variation of the oligoethylene glycol side-chain length, and samples of p(NIPAM) for comparison. Factors affecting the quality of the coatings have been identified and the formulations adjusted to reproducibly provide uniform coatings of sufficient quality and mechanical strength for screening of anti-biofouling properties as part of WP4. These coatings can be modified to provide CSTs in the desired range (10 – 30 °C). In addition to these achievements, equipment has been developed to facilitate cycling of the temperature to induce repeated CST transitions during anti-biofouling assessment.

5 References

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