
FP7-OCEAN-2013.3

Grant Agreement Number 614034

SEAFRONT

Synergistic Fouling Control Technologies

Deliverable 2.23: Synthesis of at least 6 prototype zwitterionic surfaces for characterization and antifouling performance evaluation.

Delivery date: M18 (June 2015)



Table of contents

1	Introduction.....	3
1.1	Deliverable objective(s)	3
2	Partners involved.....	3
3	Description of technology delivered	3
3.1	Results and discussion.....	3
3.2	Experimental part	6
4	Conclusions.....	7
5	References.....	7

1 Introduction

Zwitterionic polymers have extensively demonstrated antifouling properties in the field of biomaterials¹⁻³ but they have not been extensively investigated in the context of marine fouling organisms. The aim of this deliverable is to prepare zwitterionic coatings for testing.

1.1 Deliverable objective(s)

Synthesis of at least 6 prototype zwitterionic surfaces for characterisation and antifouling performance evaluation.

2 Partners involved

Newcastle University – School of Chemistry performed the synthesis of the prototype coatings containing zwitterionic moieties. These coatings have been submitted to the Newcastle University – School of Marine Science and Technology for bio-assay testing in order to determine the antifouling performance on lab scale.

3 Description of technology delivered

3.1 Results and discussion.

Twenty three different zwitterionic polymer coatings have been synthesized on glass substrates, using either a sulfobetaine-containing monomer **1** or a mixture of positively and negatively charged monomers **2** and **3** (**Figure 1**).

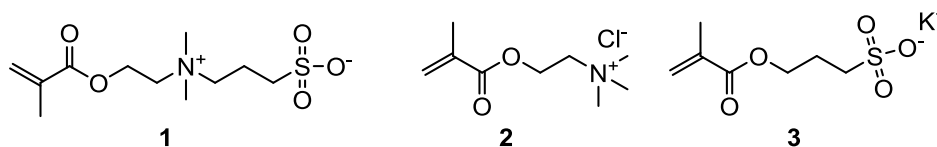


Figure 1 Structures of the three monomers selected for the preparation of zwitterionic surfaces

Neutral hydrophilic or hydrophobic co-monomers have been included to the polymer coatings in order to dilute the content of the zwitterionic units. The chosen monomers are shown in **Figure 2** and encompassed the hydrophilic oligo(ethyleneglycol) methyl ether methacrylate (OEGMEMA), ethylene glycol methyl ether methacrylate (EGMEMA) and 2-hydroxyethyl methacrylate (HEMA), and the hydrophobic *tert*-Butyl methacrylate (*t*-Bu MA), methyl methacrylate (MMA) and lauryl methacrylate (LMA). The different formulations prepared are gathered in **Table 1**.

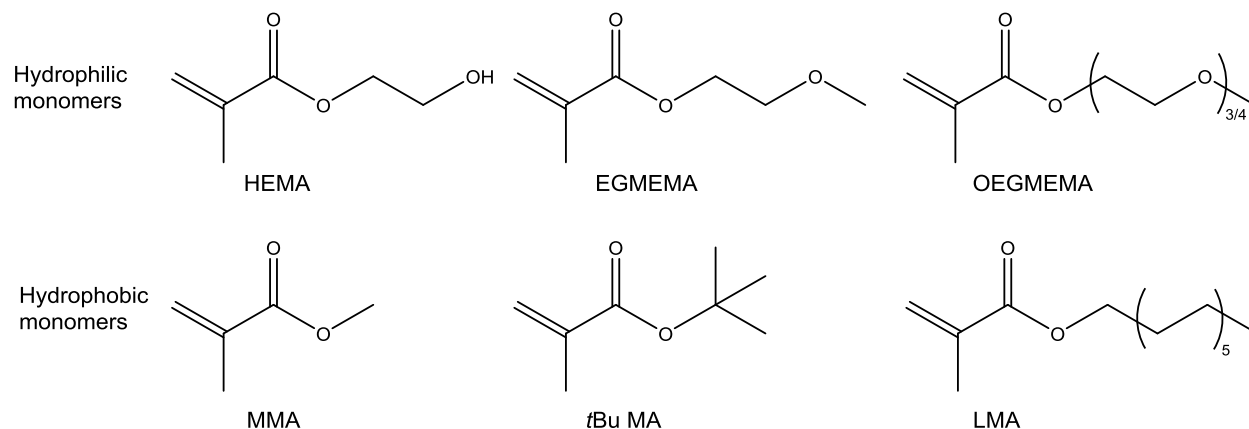


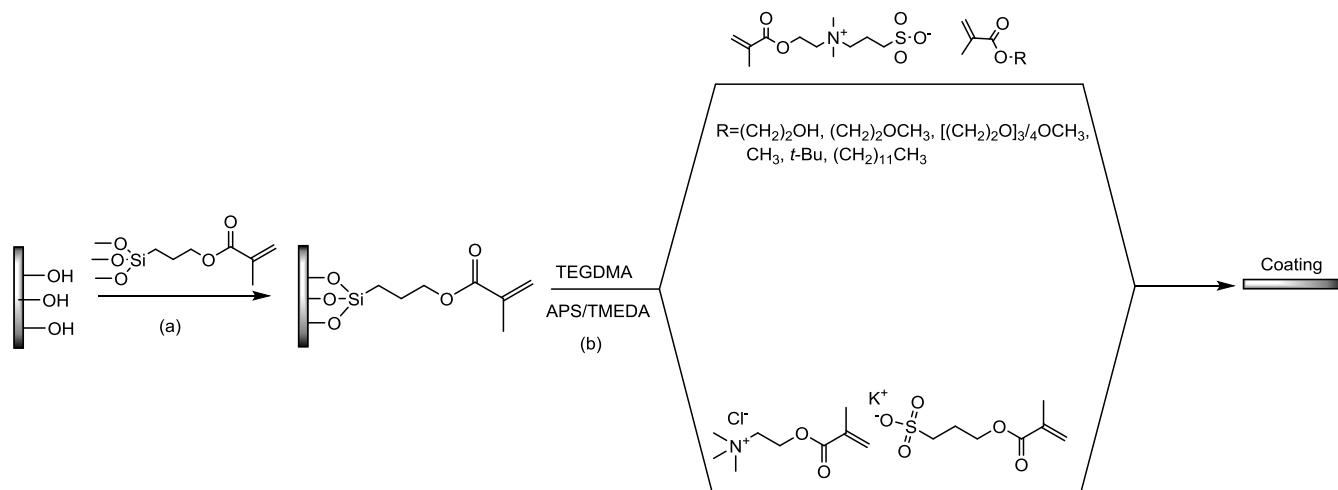
Figure 2 Structures of the neutral monomers added to the polymerization of **1**

Table 1 Summary of the zwitterionic polymer coatings prepared

Entry	Content of 1 (%) ^(a)	Content of 2 (%) ^(a)	Content of 3 (%) ^(a)	Neutral monomer	Content of Neutral monomer (%) ^(a)	Number of replicates
C1	100	0	0	-	-	4
C2	90	0	0	OEGMEMA	10	1
C3	80	0	0	OEGMEMA	20	1
C4	70	0	0	OEGMEMA	30	1
C5	60	0	0	OEGMEMA	40	1
C6	50	0	0	OEGMEMA	50	1
C7	40	0	0	OEGMEMA	60	1
C8	25	0	0	<i>t</i> -Bu MA	75	2
C9	50	0	0	<i>t</i> -Bu MA	50	2
C10	75	0	0	<i>t</i> -Bu MA	25	2
C11	25	0	0	EGMEMA	75	2
C12	50	0	0	EGMEMA	50	2
C13	75	0	0	EGMEMA	25	2
C14	25	0	0	HEMA	75	3
C15	50	0	0	HEMA	50	3
C16	75	0	0	HEMA	25	3
C17	50	0	0	MMA	50	1
C18	25	0	0	LMA	75	20
C19	50	0	0	LMA	50	20
C20	75	0	0	LMA	25	20
C21	0	25	75	-	-	2
C22	0	50	50	-	-	2
C23	0	75	25	-	-	2

(a) Percentage in composition

As shown in **Scheme 1**, a simple route for preparing zwitterionic coatings has been developed and optimized. Firstly, the glass substrate was derivatized with a methyl methacrylate functionality capable of polymerizing with the monomer formulation in order to covalently bond the coating to the glass substrate. Then, mixtures of the selected monomer, the cross-linker triethyleneglycol dimethacrylate (TEGDMA), and the redox initiator system ammonium persulfate/*N,N,N',N'*-tertamethylethylenediamine (APS/TMEDA) were injected in a sandwich made of two glass slides (or watch glasses) and the polymer coating was formed after 24-48 h (**Figure 3**). The coatings were prepared on two different glass substrates (glass slides and watch glasses) to allow their evaluation in biological assays.



Scheme 1 Preparation of zwitterionic polymer coatings on a glass substrates from zwitterionic-containing monomers (top route) and positively charged compounds and negatively charged compounds (bottom route). Reaction conditions: (a) EtOH, room temperature, 16 h; (b) Salt water/ethylene glycol/dimethyl formamide (DMF) if necessary, room temperature, 24-48h.

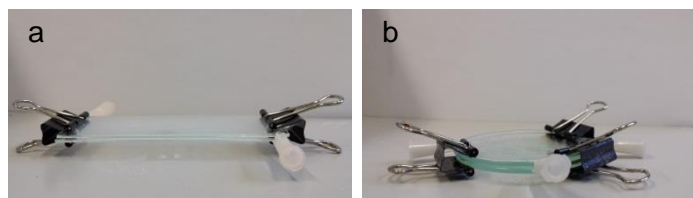


Figure 3 Pictures of the sandwich systems made of glass slides (a) and watch glasses (b)

The polymerization has been extensively studied, varying several key conditions, including the type of initiator, the ratio between monomer and cross-linker, the solvent, the curing method and the thickness of the films (**Table 2**). All the coatings were formed within 48 h. Afterwards, the sandwich system was soaked in salt water (approximately the same salinity as seawater) and left for 2-4 days in order to remove the top glass. After removing the top glass, the coatings were purified through immersion in fresh salt water daily for one week. The coatings endurance was tested leaving the samples soaking in salt water for 2-3 weeks. C12, C14, C17, C19, C22 were submitted to marine biology for preliminary studies on barnacle cyprid settlement. All the coatings exhibited very good antifouling properties, in particular no barnacles settled on C19 even after 72 h. Therefore, the coatings C18, C19 and C20 were 'scaled-up' and the required

number of replicates submitted to the marine biology department for systematic studies on their antifouling properties.

Table 2 Summary of the polymerization reaction conditions.

Entry	Tests	Best Conditions
Initiator	Ammonium Persulfate (APS) Sodium metabisulfite (SMS)	APS
Monomer/Cross-linker ratio	From 52/1 to 2.6/1	6.5/1
Thickness	From 0.31 to 0.82 mm	0.31 and 0.41mm
Curing methods	T = 60-85°C /TMEDA Room T	TMEDA Room T
Solvents	DI Water/Salt Water EtOH/Ethylene Glycol	Salt Water/Ethylene glycol =1/1
Time	From 2h to 48h	48h

3.2 Experimental part

Materials

Absolute Ethanol was purchased from Fisher Scientific, all the chemicals were obtained from Sigma Aldrich and used as received. Glass slides were purchased from VWR and watch glasses were obtained from Scientific Glass Laboratory.

Surface Preparation

Prior to use, the glass substrates were washed with detergent, rinsed with DI water, sonicated in DI water for 15 min, sonicated in EtOH for 15 min, dried with a stream of N₂ gas and placed in a plasma asher subjected to oxygen plasma at 70 W for 15 min.

Methacryloxypropyltrimethoxysilane-modified glass substrates were prepared by submersion in a solution of γ -methacryloxypropyltrimethoxysilane (MPS) in EtOH (0.07M) and covered. After 16 h, the substrates were removed from the bath and dried with a stream of N₂ gas. The silane layer was then cured by placing the substrates in an oven at 115° for 30 min. Once cooled, the substrates were sonicated in EtOH to remove any silane multilayers and then dried with nitrogen.

The coatings were prepared by polymerizing them directly onto the functionalized substrates. A sandwich system of a clean glass substrate (top) and a functionalized glass substrate (bottom) was assembled, using 0.31 mm (for glass slides) or 0.41 mm (for watch glasses) as spacers and clips as holders (**Figure 3**).

Coating preparation

4 mmol of the selected mixture of monomers in the appropriate ratio were dissolved in 2 ml mixed solvent of ethylene glycol/salt water (1:1) and stirred for 20 min. Then, 160 μ L (0.61

mmol) of TEGDMA were mixed with the solution and stirred vigorously for 10 minutes. At last, 80 μL of APS (0.22M in salt water) and 2.64 μL of TMEDA were added to the mixture and stirred for further 2 min. The mixture was quickly injected in the sandwich system previously described and left at room temperature for 24-48 h. Then, the sandwich was soaked in a salt water bath for 2-4 days to easily remove the non-functionalized top glass substrate. The samples were placed in a salt water bath to leach residual impurities using fresh salt water daily for one week.

Note: Mixed solvent ethylene glycol/salt water/DMF (2:2:1) was used for Coatings C8-C13 and C17-C20.

4 Conclusions

In this work, we developed and optimized a simple route for the synthesis of zwitterionic coatings on both glass slides and watch glasses, varying either the zwitterionic content or the net charge of the final materials. C12, C14, C17, C19, C22 (**Table 1**) underwent to preliminary tests on barnacle cyprid settlement and showed very good antifouling properties. Therefore, C18, C19 and C20 were produced in a large scale and are currently submitted to the marine biology department for antifouling performance evaluation.

5 References

1. S. Chen and S. Jiang, *Adv. Materials* 2008, **20**, 335-338.
2. Z. Zhang, T. Cao, S. Chen and S. Jiang *Langmuir* 2006, **22**, 10072-10077.
3. J. Yuan, X. Huang, P. Li, L. Li and J. Shen *Polym. Chem.* 2013, **4**, 5074.