Mixed-charge copolymer as environmentally friendly low fouling marine coatings

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A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Master of Science in Chemical Engineering

University of Washington

2016

Reading Committee:

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Program Authorized to Offer Degree:

Chemical Engineering

University of Washington

Abstract

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Self-polishing marine coatings made of mixed charged and other hydrophobic methacrylates are developed via free radical polymerization. By introducing the mixed-charge structure into the copolymer system, the marine coatings are able to resist bio-foulants. We observe that this mixed-charge structure on the copolymer is able to reduce non-specific protein adsorption from ELISA experiments. By varying the content of the copolymer, the coatings have different non-fouling properties and self-polishing rates in seawater.

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ACKNOWLEDGEMENTS

The research was supported by Office of Naval Research (ONR). I appreciate the help of Prof. Shaoyi Jiang and Harihara Subramanian Sundaram for guidance and discussions, Kan Wu for NMR and organic synthesis support, Caroline Tsao for ELISA support, Timothy Fujuhara and Jiawei Xue for general support and Prof. John Berg’s group for contact angle measurements. The members of Prof. Jiang’s group support me to review my thesis.

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INTRODUCTION

Bio Foulants

Bio-accumulation, such as barnacles, algae and fungi on ship hull is generally undesirable. They easily aggregate on hull once the extracellular polymeric substances (EPS) form. These extracellular polymeric substances in barnacle, for example, consist of different kinds of cement proteins (CP), which provide curing, binding and anchor mechanisms to help barnacle adhesive on substrate. Ulva uses adhesive vesicles to discharge adhesive once it finds an appropriate location. Biological adhesives efficiency is remarkable and performs in a way different than artificial adhesives. Unlike artificial adhesive chemical bond, biological adhesive is made up of proteins that secret by glands. Chemical bond could be weaken under water. However, these biological adhesives are extremely effective under water. For centuries, when people started sailing in ocean, bio-accumulation raises the problems. Once bio-accumulation forms and becomes cluster, it greatly increases drag and fuel consumption. Also, it require a lot of labor to remove bio-foulants.

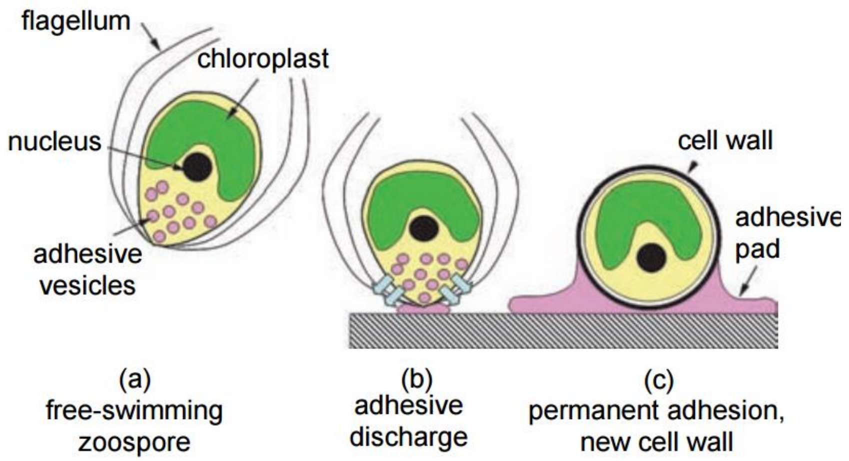

Fig. 1 The Ulva spore forming adhesive pad on substrate [19].

The picture is adapted from p 64, *Biological Adhesives*, J. A. Callow, A. M. Smith (Eds) (Springer, Berlin, 2009)

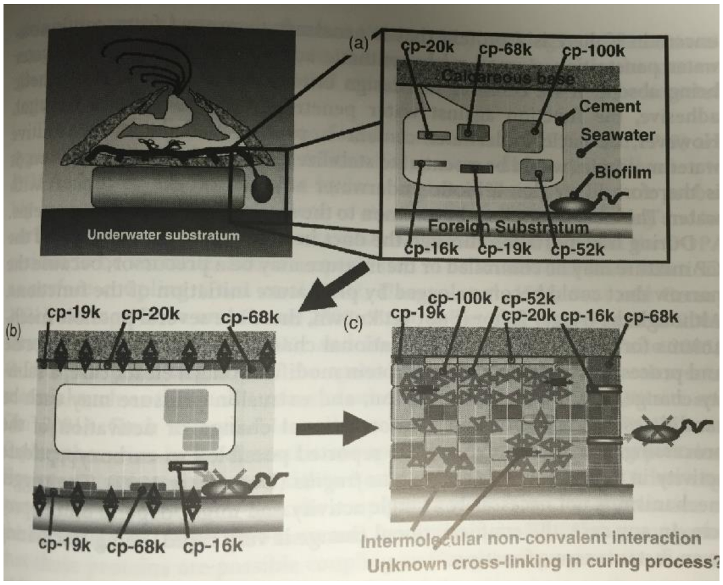

Fig. 2 Barnacle adhesion mechanism hypothesis [19].

The picture is adapted from p 159, *Biological Adhesives*, J. A. Callow, A. M. Smith (Eds) (Springer, Berlin, 2009)

Marine Coatings

For decades, people tried to solve bio-accumulation by marine coatings and nowadays the product development still on going. Conventional self-polishing marine coating (SPC) contain tributyltin (TBT) monomer which can be hydrolyzed and release tin metal ions. By hydrolyzing the functional groups on surface, coating surface slowly release metal ions at certain level concertation which is able to kill and preventing microorganism adhesive on the surface. Once the surface hydrolyzed, the polymer becomes soluble and finally dissolve in the ocean renew the surface. Thus, tin based self-polishing coating has extremely effective non-fouling property for long term. However, TBT has been banned by International Maritime Organization due to tremendous toxicity of tin metal ions. Later, people use copper and zinc pigments as substitution to kill microorganism. These substitute is less toxic than TBT but still create tremendous pollination in ocean. Those heavy metal ions can remain in organism and through food chain finally go into human body.

There is another group of marine coating called fouling-release marine coating (FRC). They comprise by PDMS which has low surface energy. Although fouling-release marine coating gets fouling easily, high speed of shear pressure can easily remove the fouling on hull once the ship is sailing. It is suitable for speedboat, but not effective for low-speed ships. Thus, it is important for searching a new approach which is clean to ocean and effective as well.

In the future, we foresee that restriction for marine coatings will become stricter with usage of biocides. Thus, the objective of this work is to develop eco-friendly marine coatings based on mixed-charge materials.

Mixed-Charge Structure

Mixed-charged materials have relatively strong hydration via electrostatically induced hydration and have positive and negative charges. Due to their strong hydration, the surfaces with mixed charged materials have a layer of water to prevent protein adsorption. This novel approach prevents proteins and microorganisms from ship hulls. As compared to traditional marine coatings, it is more eco-friendly and less costly. However, to achieve this goal, we have to create a renewable surface for longer-term applications. If the negative group in the mixed-charged material is replaced with a hydrolysable group, these coatings will become self-polishing to increase the life span of these coatings. After hydrolyzing, the

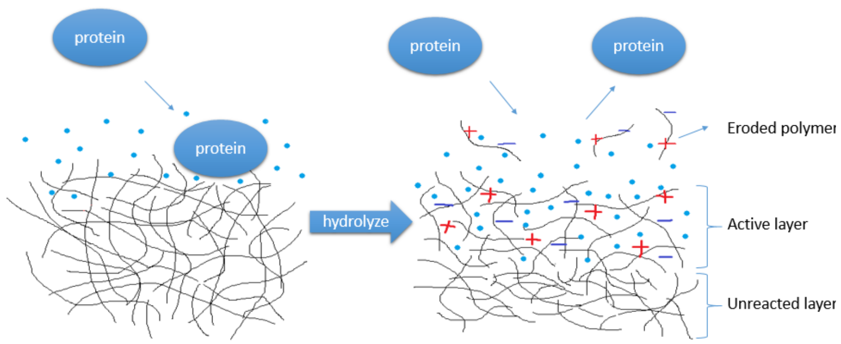

Fig. 3. Upon hydrolysis, the surface becomes hydrophilic and non-fouling due to the mixed-charge structure.

hydrolysable group will reveal negative charge and the coatings become mixed-charge. These mixed-charge structures are able to resist non-specific protein adsorption Furthermore, different counter ions are added to stabilize the structure. . These marine coatings are non-fouling, self-polishing and eco-friendly without biocides.

EXPERIMENT&MATERIALS

Chemicals

1,4-Butanediol diglycidyl ether (BDDE, ≥95%) and 2,2′-Azobis(2-methylpropionitrile) (AIBN, 98%) were purchased from Sigma-Aldrich (Milwaukee, WI).

Protein

Fibrinogen from human plasma (≥90%) was purchased from Sigam-Aldrich. Anti-human plasma fibrinogen IgG, HRP conjugate was purchased from Alpha Diagnostic International.

General supplies

InterProtect 2000E primer was purchased from International Paint Ltd. 5052-H32 aluminum sheet was purchased from OnlineMetals.com.

Polymer Synthesis

Dissolve 10g of monomers in an organic solvent. Add in 1% mole initiator (AIBN). Use nitrogen bubbling to remove oxygen for 20min. React under 70℃ close system for one day.

Additive applications

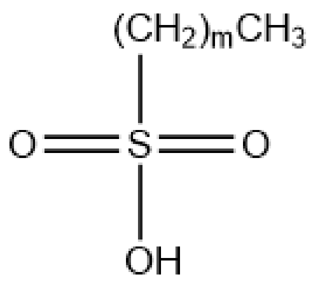

Fig 4. The structure of a counter ion added into coating

For the counter ions, we choose sulfonic acid with long carbon chain. We add these counter ions into a polymer solution until it well dissolve.

Coating Process

Primer coating

All the aluminum panels are sandblasted first. Then, use water, acetone and hexane to wash panels in order to remove aluminum particles on surfaces. Use spray gun to apply InterProtect 2000E primer on panels.

Polymer coating

Take 14g polymer solution, and drop on panels covered with primer. Let coating completely dry for 3 days. Then, put polymer the coating into ASW for long-term stability tests.

ELISA

In order to test the non-fouling property of copolymer formulations, we use direct ELISA to test the amount of protein adhesive on coating samples.

1. Take fibrinogen out of the -20ºC fridge and let it sit to room temp.
2. Let all the sample chips soak in PBS for 5min. Ensure each sample is not floating.
3. Dissolve fibrinogen into PBS with the concentration of 1 mg/mL. Let fibrinogen/PBS solution spin for 5 min (for protein to fully dissolve).

   NOTE: Each well of sample requires 1 mL of fibrinogen/PBS. Weight fibrinogen according to the amount of samples one has.
4. Soak the samples in 1 mL fibrinogen/PBS solution, and make sure that samples are not floating and let it sit for 1 hr.
5. Take the anti-fibrinogen-HRP concentrated solution out from the -20ºC fridge and let it sit to room temp.
6. Discard the fibrinogen/PBS solution. Place 1 mL PBS into the wells with samples, let them sit 5 min for each interval then discard solution. Repeat this procedure for 5 times in the original well.
7. Dilute 10,000 times anti-fibrinogen-HRP into PBS. Spin for 5 min.
8. Soak the samples in 1 mL anti-Fibrinogen-HRP/PBS solution in the new wells and make sure that samples are not floating and let it SIT for 30 min. Do NOT shake.
9. Discard the anti-fibrinogen-HRP/PBS solution. Place 1 mL PBS into the wells with samples, let them sit 5 min for each interval then discard solution. Repeat for 5 times in the original well.
10. Take 15 mL tube, add in 9 mL ddH_2O and 1 mL of 1 M citrate buffer (pH 5.0, 0.02% NaN_3). Protect the tube from light.
11. Weight OPD (in -20ºC fridge in R335) 10 mg and dissolve in 10 mL working citrate buffer prepared previously.

12. Add in 10 uL H_2O_2 (in -20°C fridge in R335) to the OPD/citrate buffer, make sure it is mixed well (the solution should be light yellowish). Must protect from light.
13. Do it when the room is dark and wrap the plate with tin foil. Soak the samples in 1 mL OPD/citrate buffer/H_2O_2 solution and make sure that samples are not floating and let it SIT for 30 min.
14. Add 500 uL of 1 N HCl in a new well. Mix well with pipette and take 500 uL of the OPD/citrate buffer/H_2O_2 from the previous well and mix in with 1 N HCl. Make a blank-original OPD/citrate buffer/H_2O_2 with 1 N HCl.
15. Transfer 100 uL of HCl/(OPD/citrate buffer/H_2O_2) mixed solution into another 96 well plate and read the absorbance at 492 nm. Use the program DH_Full ELISA_492nm.

Weight Lost Study

In order to test self-polishing property, we use a gravity way to measure the weight loss of coating when it soaks into ASW.

1. Prepare 1x3 inch square aluminum sheets. Sandblast and wash sheets as panels pervious.
2. Apply primer on aluminum sheets. Let sheets dry for 1 day.
3. Weight sheets for empty weight.
4. Apply copolymer formulations on sheets. Let sheets dry for 3 day.
5. Weight sheets for initiate weight.
6. Soak samples at same time and at certain time pick samples out to stop soaking.
7. Weight sheets again and get the weight loss.

Gel Permeation Chromatography

Since we are using free radical method, we need to know molecular weight batch by batch to ensure the performance of copolymer each batch is the same.

Dynamic Scanning Calorimetry

The DSC is helping us to find the failure of roots in the formulations. Some monomer pendent group cause the main chain too rigid to move. Thus, cause glass transition arise above room temperature and make coating cracking. By using DSC, it can help us to find the window of applicable formulations.

RESULT AND DISCUSSION

Thermal analysis

In order to find formulations with good mechanical properties, we need to study the transition glass temperature of copolymer. Reference [23] indicates several homopolymer glass transition temperature.

The component such as methyl methacrylate has relatively high Tg. However, the pendent group on the hard component has ability to resist water from swelling coating. This bulky and rigid pendent group can work as anchor and cause high Tg for the hard component. When two pendent groups meet each other in the opposite parallel direction, these pendent groups block each other and cause chains hard to move, thus increasing Tg drastically. By choosing this hard

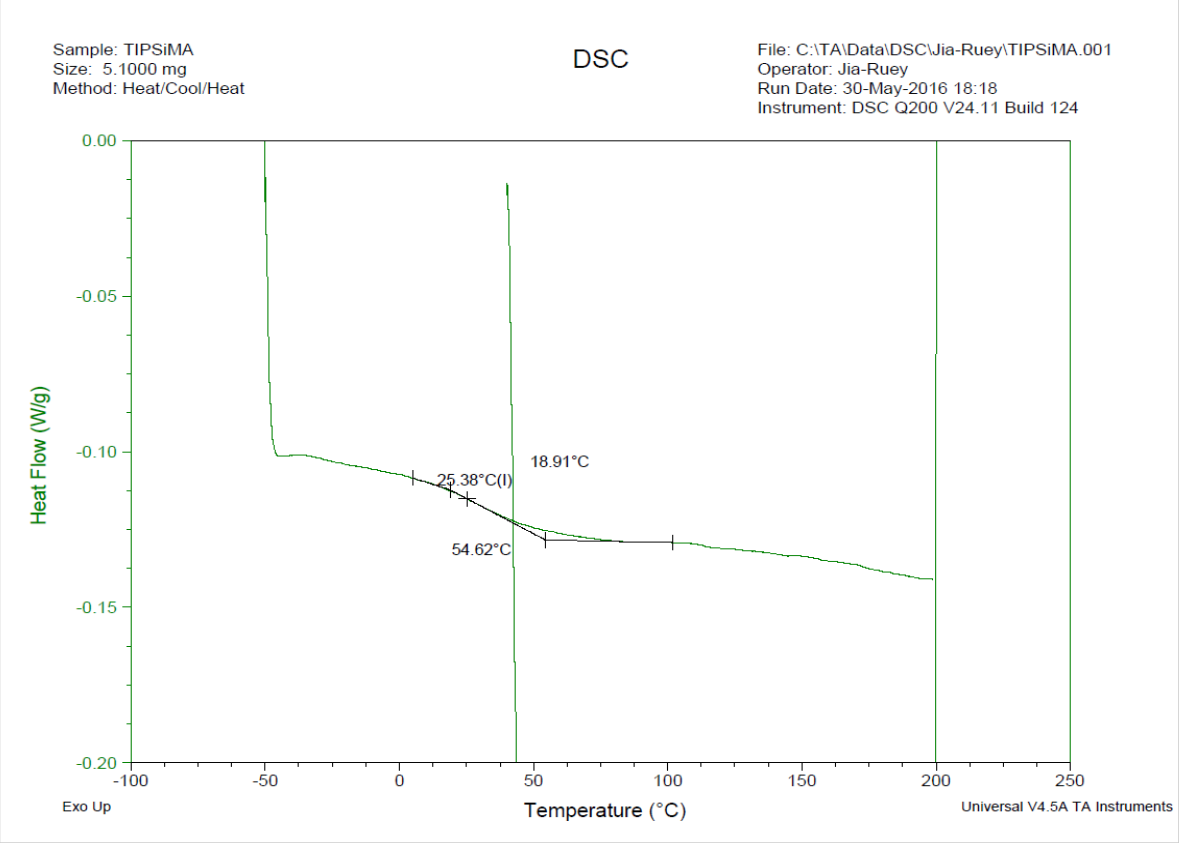

Fig. 5 DSC analysis for TIPSiMA homopolymer

component, we can increase the hydrophilic portion of the coating. On the other hand, the soft component was used to reduce Tg for copolymer to prevent cracking. The soft component has long and flexible pendent groups with space for chains, thus, decreases Tg.

Hard	Soft	hydrophilic
15	45	40
10	40	50
20	30	50
30	20	50
40	10	50
10	30	60
20	20	60
30	10	60
10	20	70
20	10	70

Table 1. The formulations for mixed-charge copolymer

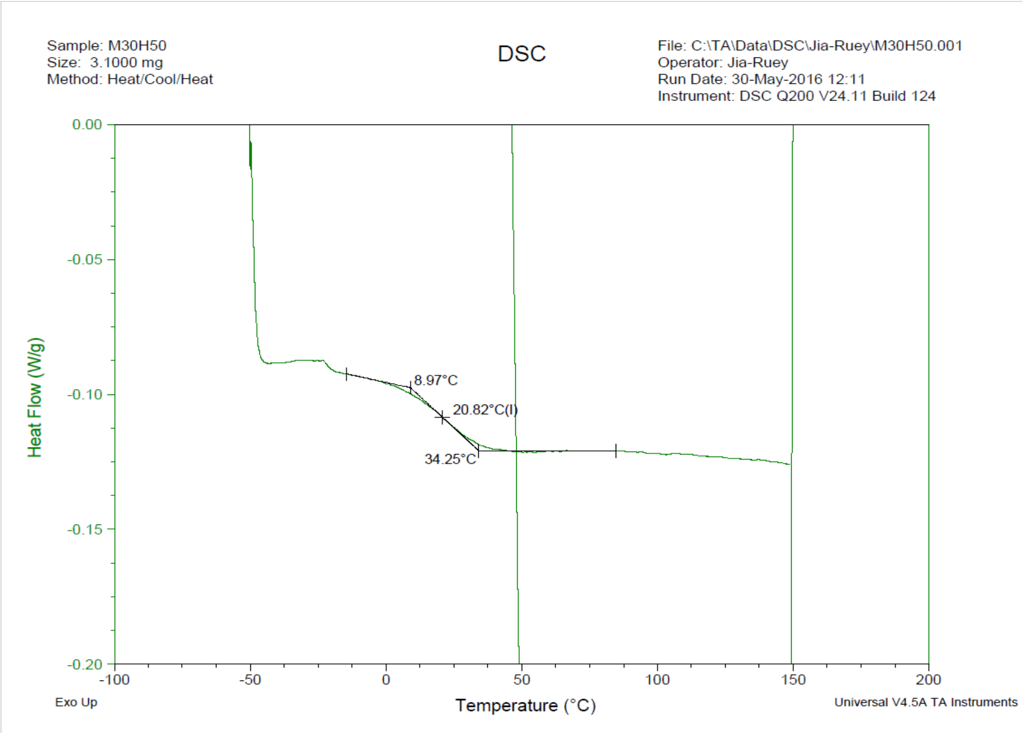

Fig 6. DSC analysis for ML30H50 formulation

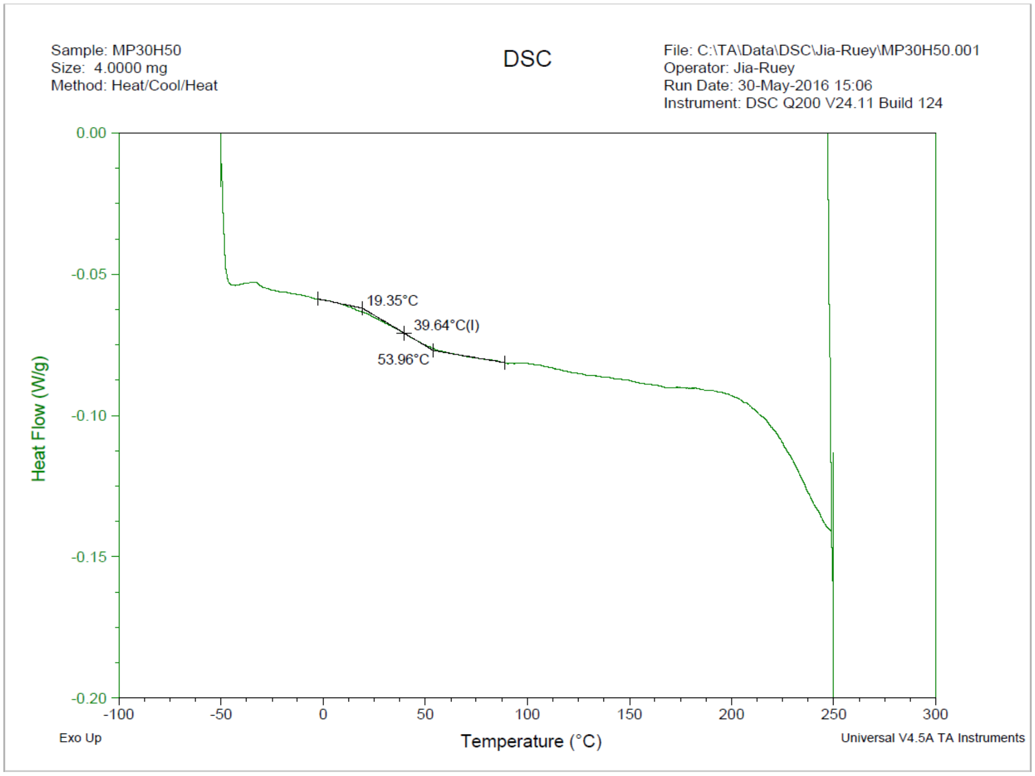

Fig 7. DSC analysis for MS30H50 formulation

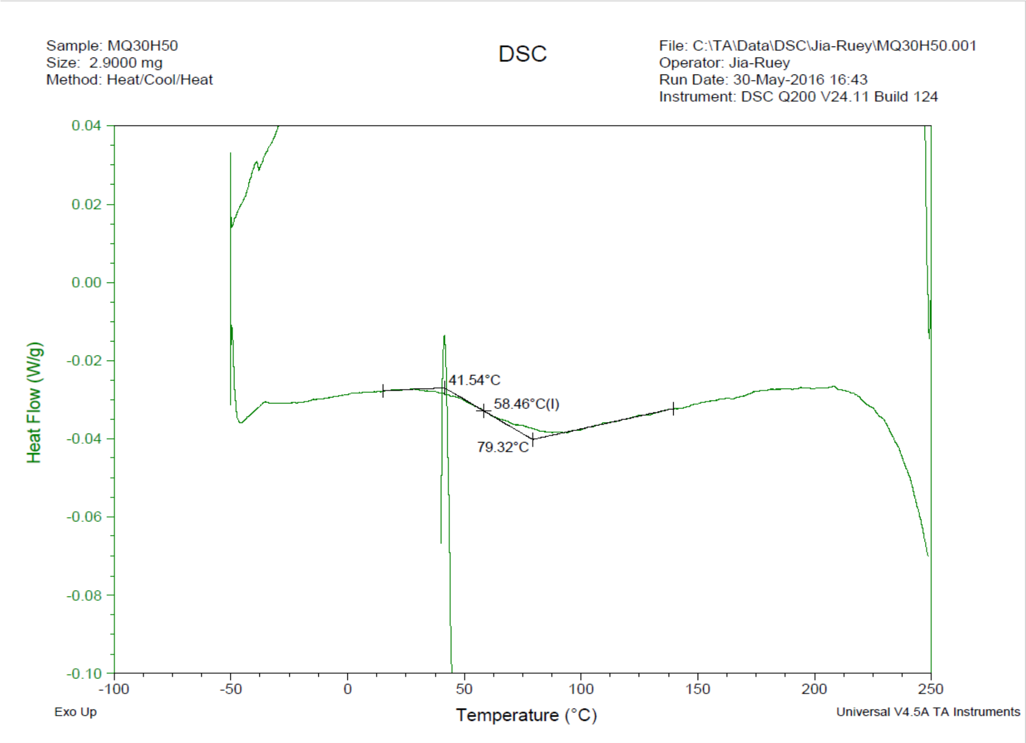

Fig 8. DSC analysis for M30H50 formulation

For three formulations studied, their glass transition temperature is ML30H50 20.82℃ (with long counter ion), MS30H50 (with short counter ion) is 39.64℃ and M30H50 (without counter ion) is 58.46, respectively. The only difference of these three formulations is the counter ion used:

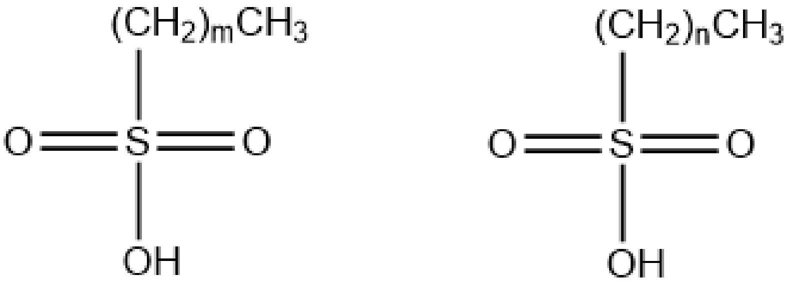

Fig 9. Different counter ions. (note: m>n)

As can be seen, the glass transition temperature for copolymer becomes higher and higher as system has more charged as expected. The long carbon chain or small molecule helps to increase volume between main chains, which enhance the mobility of main chains.

Contact angle

After hydrolysis, the surface of coating should be more hydrophilic. We observed lower contact angle after hydrolysis as expected. However, most of formulations hydrolyze after soaking and have similar contact angle. With the increment of the soft component, the contact angle becomes higher, indicating that the soft component provides hydrophobicity to coating. There is a possibility that the surface goes through rearrangement while drying. Logically, the higher hydrophilic component, the better non-fouling property the coating is. M70H20 with cross-linker such as 1, 4-butanediol diglycidyl ether, ML50H25 and MS30H50 were chosen for studies.

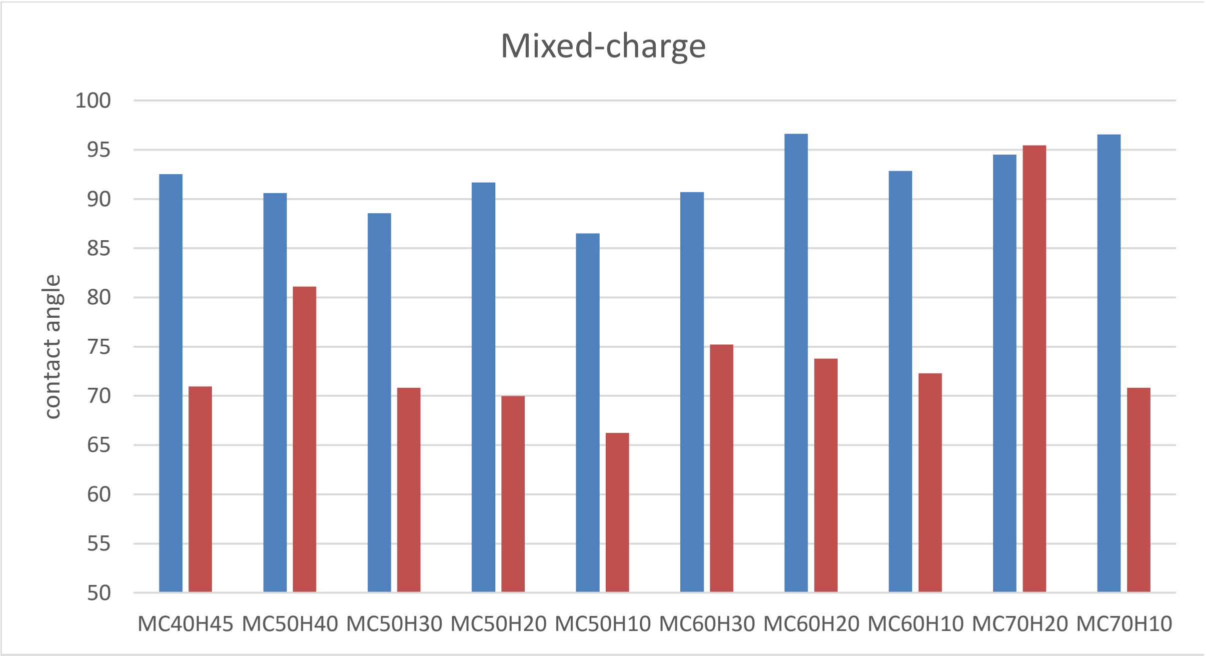

Fig 10. Contact angle data for mixed-charge formulations

Copolymer molecular weight

The GPC helps us to understand the molecular weight of copolymer. This example is one of formulation.

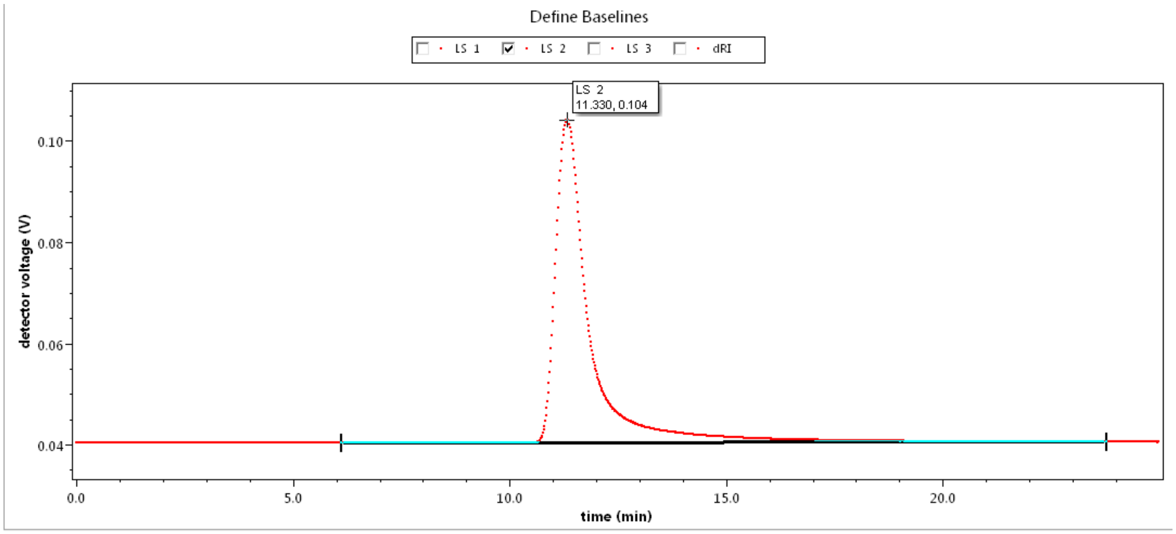

Fig 11. Example of GPC data for previous formulation sample as reference.

The retention indicates that the 11.33 min for the highest peak. We use this value and put it into

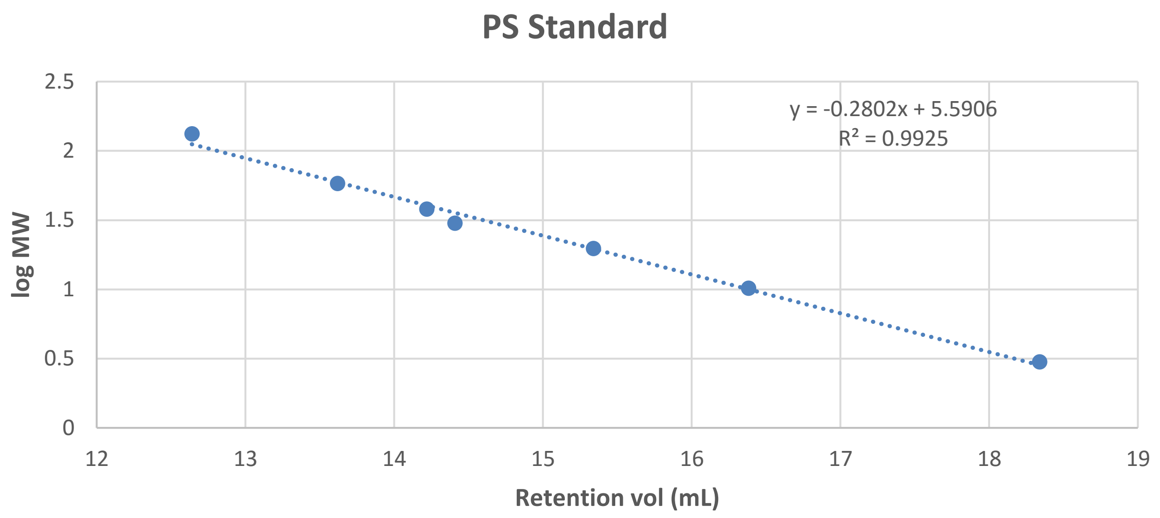

Fig 12. Polystyrene retention time relation with molecular weight

PS standard. Finally the molecular weight is roughly around 260 kDa. This molecular weight we set as a reference for each formulation studied.

Fouling test

The ELISA assay is used to determine non-fouling property of coating. Although the protein that microorganism use to adhesive on surface is not the fibrinogen, we want to see any correlation between ELISA assay and field test. If there is a correlation, we can easily predict non-fouling property under ELISA assay for a quick screen. One of the formulation was test under ELISA assay:

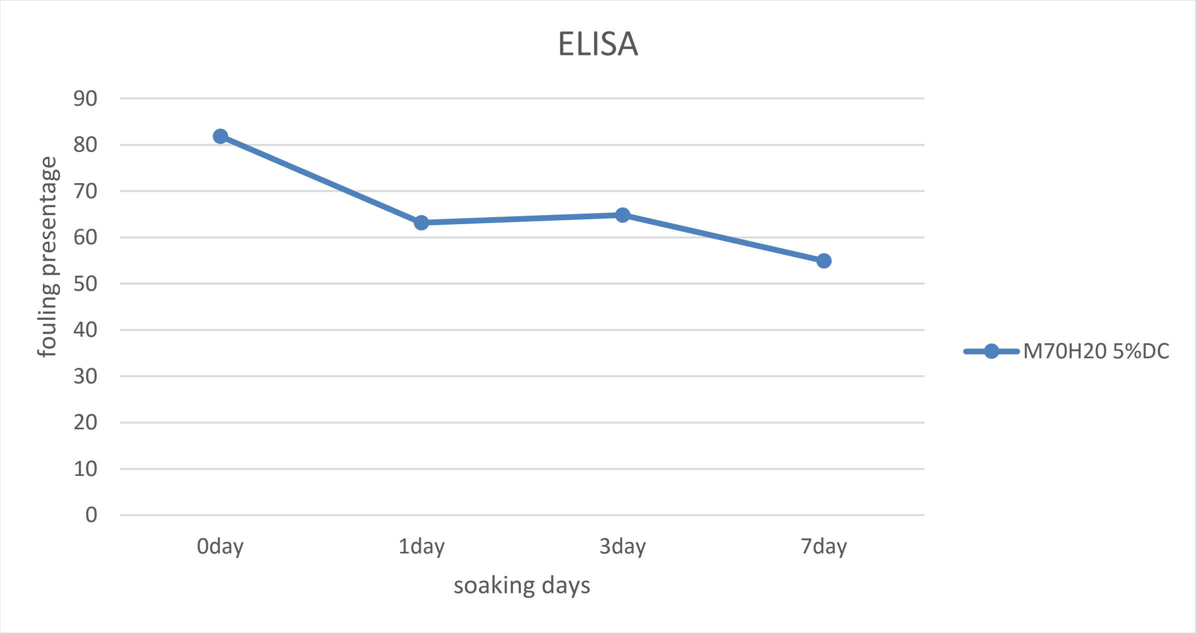

Fig 13. ELISA fouling data for M70H20

CONCLUSIONS

Results from fibrinogen protein fouling show that these marine coatings can reduce protein adsorption by more than 50% after hydrolysis. Result of contact angle indicates that the monomer indeed hydrolyzes after soaking. However, this process introduces tremendous hydrophilicity to the copolymer. Thus, we introduced hard and soft components and cross-linkers to enhance mechanical property. By adjusting the ratio of hard to soft components, we could provide toughness and avoid cracking. By increasing the degree of cross-linking, the coating would have higher stability, better non-fouling and lower self-polishing rate. By adding counter ions, the coating can be further stabilized. The next step is to perform field tests of these coatings.

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