# Resistance to protein sorption as a model of antifouling performance of Poly(siloxane-urethane) coatings exhibiting phase separated morphologies 

A. Santiago ${ }^{\text {a }}$, L. Irusta ${ }^{\mathrm{a}, *}$, T. Schäfer ${ }^{\mathrm{a}, \mathrm{c}}$, A. Corres ${ }^{\mathrm{a}}$, L. Martin ${ }^{\text {b }}$, A. González ${ }^{\text {a }}$<br>${ }^{\text {a POLYMAT, Department of Polymer Science and Technology, University of the Basque Country UPV-EHU, PO Box 1072, } 20080 \text { Donostia/San Sebastián, Spain }}$<br>${ }^{\mathrm{b}}$ Macrobehaviour-Mesostructure-Nanotechnology SGIker Service, Polytechnic School, University of the Basque Country UPV-EHU, Plaza Europa 1, 20018 Donostia/San Sebastián, Spain<br>${ }^{\text {c }}$ Ikerbasque, Basque Foundation for Science, Bilbao, Spain

## A R TICLE INFO

## Article history:

Received 16 December 2015
Received in revised form 4 May 2016
Accepted 13 May 2016

## Keywords:

Poly(siloxane-urethane) thermoset
copolymers
Phase separation
Protein adsorption
Qcm-d


#### Abstract

In this study, bovine serum albumin (BSA) adsorption measurements were used as a model test to investigate the anti-biofouling performance of hybrid poly(siloxane-urethane) coatings. Different coatings were obtained from isophorone diisocyanate trimer, polycaprolactone triol and hydroxy-terminated poly(dimethylsiloxane). The copolymers showed a phase separated structure that depended on the mixing time and casting temperature. Two types of adsorption measurements were performed: (a) static adsorption measurements, immersing the film in a BSA solution and determining the BSA concentration of the remaining solution by UV; (b) measuring the adsorption using a quartz crystal microbalance with dissipation monitoring (QCM-D). According to static adsorption measurements, the BSA adsorption was reduced when the coatings showed a phase separated structure. In addition, QCM-D measurements, and particularly the dissipation data, showed that in nanostructured coatings the protein adsorption occurred in a conformation that prevented water retention. The latter could be the origin of the fouling resistance ability of these copolymers.


© 2016 Elsevier B.V. All rights reserved.

## 1. Introduction

Biofouling is generally defined as the accumulation of living organisms including microorganisms, algae and animals on a wetted surface. This undesirable colonization has a serious impact that can be environmental, economic and/or ecological. Although toxic antibiofouling coatings containing tin, copper and other biocides have provided an effective control of many fouling species, they have a detrimental impact on the environment [1,2]. In order to lessen this impact there is a growing interest in the development of non-toxic antibiofouling coatings [3,4].

Traditionally, the fouling process has been divided into different stages: the initial stage is mainly due to the adsorption of molecules, such as polysaccharides, proteins and proteoglycans, and gives rise to the so-called conditioning film [1]. This initial stage is considered problematic as it subsequently triggers severe fouling. As a result, the adsorption of proteins is considered in some studies as a sim-

[^0]plified way of evaluating the antifouling activity of a surface [5-7]. According to this assumption, surfaces with low protein adsorption are supposed to have greater anti-fouling eficiency. However, as the protein adsorption absolute values are depending on the performed experiment, it is not easy to define an appropriate adsorption value for a surface being considered as antifouling and only comparative data can be addressed.

Poly(dimethylsiloxane) PDMS or silicone materials have been the focus of extensive research in the development of minimally adhesive surfaces [8]. These materials have also led to studies of their utility as potential antifouling materials for marine applications, among other things, owing to their good fouling-resistance performance. However, PDMS has some obvious disadvantages, such as poor adhesion to substrates, low mechanical strength and high cost.

Self-stratified poly(siloxane-urethane) coatings try to overcome some of these disadvantages, such as poor adhesion, while keeping the fouling-release properties. These kinds of novel non-toxic fouling-release coatings are used to combat biofouling [9-12]. Due to the thermodynamic incompatibility between the siloxane and
urethane components of the coating, the low surface energy siloxane component migrates to the surface, imparting hydrophobicity.

According to literature [5], it is clear that the surfaces presenting water contact angles between $60-80^{\circ}$ give rise to high protein adsorption. However, surfaces with contact angles lower than $20^{\circ}$ and approaching $120^{\circ}$ exhibit a reduction in protein adsorption. Therefore, in order to obtain an antifouling coating the surfaces with contact angles between $60-80^{\circ}$ must be avoided. Poly(siloxane-urethane) coatings present water contact angles between $100-110^{\circ}$ and are therefore good candidates to be applied as fouling release materials, as has been addressed in literature [11].

Alternatively to siloxane based polymers, amphiphilic structures containing both hydrophilic and hydrophobic structures such as perfluoropolyether surfaces have been proposed as potential fouling release coatings. The latter materials have many features in common with silicones and some papers are very relevant concerning protein-surface interaction mechanism. A segregated surface is suggested to resist biofilm formation by presenting an "ambiguous" surface to the protein [13-15].

In previous works [16,17], we reported the synthesis and surface hydrophobicity of a series of poly(siloxane-urethane) copolymers with potential anti-fouling applications. It is interesting to note that the addition of small quantities of poly(dimethylsiloxane) increased the water contact angle and substantially. In addition, higher contact angles were obtained when the systems presented a phase separated morphology. According to some literature results, the anti-fouling ability of block copolymers is also related to the phase separated morphology [18,19]. Bearing this in mind, the present work aims at determining the fouling-release capacity of these phase separated copolymers through protein adsorption measurements. Following the methodology used in previous work [16], acetyl acetone was added to the formulation in order to control the phase separation of the copolymer. Three different formulations containing $5 \%, 10 \%$ and $15 \%$ of siloxane were synthesized using different mixing times to control sample morphology. The adsorption of the protein bovine serum albumin (BSA) was evaluated by using a colorimetric method [20] and by quartz crystal microbalance with dissipation monitoring (QCM-D) [21]. The adsorption data were correlated with the sample morphology.

## 2. Experimental part

### 2.1. Materials

Aliphatic polyisocyanate Vestanat T 1890 E (IPDI trimer, 70 wt-\% in butyl acetate) was obtained from Evonik Industries. Poly(dimethylsiloxane) terminated in polyethylene glycol (PDMS, $\mathrm{Mn} 1000 \mathrm{~g} \mathrm{~mol}^{-1}, 20 \mathrm{wt}-\%$ non siloxane component) was supplied by Gelest Inc. Trifunctional polyol (polycaprolactone, PCL, Mn $900 \mathrm{~g} \mathrm{~mol}^{-1}$ ), dibutyltin dilaurate (DBTDL), butyl acetate (BA) and acetylacetone (AA) were supplied by Sigma-Aldrich. Bovine serum albumin protein (BSA) was supplied by Sigma-Aldrich. Dye reagent concentrate to perform the protein assay was obtained from BioRad.

### 2.2. Coating preparation

In order to prepare the coating formulation, PCL and PDMS solutions in butyl acetate ( $33 \mathrm{wt}-\%$ of solids) were introduced in a 100 mL erlenmeyer at room temperature and mixed for 1 min under magnetic stirring. The equivalent ratio of both polyols changed between 95:05 to 85:15 PCL:PDMS. Then, the required amount of IPDI trimer, NCO:OH equivalent ratio 1.1:1.0, and $10 \%$ of AA were added to the reaction mixture. Finally, DBTDL ( $19 \mathrm{mg}, 0.03 \mathrm{mmol}$ ) was added to start the reaction. At a variety of mixing times,
between 60 min and 24 h , the solutions ( 4 mL ) were cast over aluminium pans of 43 mm diameter following two methods. In the first, the coating was kept under room temperature for 24 h . In the second, the coating was kept at $50^{\circ} \mathrm{C}$ for 24 h on a hot plate in a fume hood. In both cases, this was followed by oven curing at $80^{\circ} \mathrm{C}$ for 45 min .

### 2.3. Contact angle measurements

The static and dynamic contact angle measurements were performed in an OCA20 Instrument at controlled temperature and humidity ( $25^{\circ} \mathrm{C}$ and $55 \%$, respectively). In the static experiments, the volume of the deionised water droplets was $5 \mu \mathrm{~L}$. Advancing and receding contact angles were measured dispensing/withdrawing liquid ( $5 \mu \mathrm{~L}$ ) over a liquid drop ( $10 \mu \mathrm{~L}$ ) placed on the surface under equilibrium conditions. For each composition, three films were analyzed and the contact angle measurements were made with five replicates for each film.

### 2.4. Atomic force microscopy studies

Atomic Force Microscopy (AFM) studies were performed in a Multimode Nanoscope IV of Digital Instruments. Experiments were operated under tapping mode in air at ambient conditions. Samples for AFM studies were prepared by casting over a glass surface. Topographical and phase images of $20 \mu \mathrm{~m} \times 20 \mu \mathrm{~m}$ were obtained.

### 2.5. Protein sorption studies

The protein adsorption onto the polymer surface was analyzed using two different techniques. The first one was the Bio-Rad Protein Assay, which is a dye-binding assay where a differential colour change of a dye occurs in response to various concentrations of protein [22]. The standard procedure advised by Bio-Rad was followed. A standard curve for the Bio-Rad Protein Assay of bovine serum albumin (BSA) between $0.2-0.9 \mathrm{mg} / \mathrm{mL}$ was generated in order to determine the protein sorption behaviour of the films. UV-vis transmittance spectra were obtained using a spectrophotometer Shimadzu UV-VIS-NIR 3600 using a photomultiplier tube detector. Samples with an outer surface area of $10 \mathrm{~cm}^{2}$ and $200 \mu \mathrm{~m}$ of thickness were immersed in 100 mL BSA/water solution $(0.45 \mathrm{mg} / \mathrm{mL})$. At different times, 0.1 mL of the BSA/water solution were taken and after mixing with 5 mL of the dye reagent the concentration of BSA was calculated by UV-vis absorption at 595 nm . The amount of BSA adsorbed by the sample was calculated by a mass balance using initial and final concentration of solutions measured by UV-vis. Triplicate experiments were carried out for all systems studied.

The second technique used to determine the protein sorption behaviour was a quartz crystal microbalance with dissipation monitoring (QCM-D). Polymer-coated sensors were obtained by spin-coating the solutions onto a gold sensor (diameter $=14 \mathrm{~mm}$, Q-SENSE, Sweden) at a rate of 2500 rpm for 30 s using a Lot Oriel SCC 200 spin-coater. After the spin coating, the samples were cured at $80^{\circ} \mathrm{C}$ for 45 min .

QCM measurements were performed on a Q-SENSE E1 system operating at $23^{\circ} \mathrm{C}$. Prior to the experiments, the sensors were stabilized overnight under a constant water flux of $100 \mu \mathrm{~L} / \mathrm{min}$. Subsequently, the respective sensors were put in contact with different concentrations of BSA in aqueous solution up to a maximum of 100 mg BSA $/ \mathrm{L}$.

QCM-D technique detects changes in the resonance frequency $(\Delta \mathrm{f})$, and dissipation ( $\Delta \mathrm{D}$ ). During the adsorption/desorption cycle, the resonance frequency of the crystal changes according to changes in the mass. If the mass forms an evenly distributed, rigid layer whose mass is small compared to that of the crystal, then the


Fig. 1. AFM phase images ( $20 \mu \mathrm{~m} \times 20 \mu \mathrm{~m}$ ) for films obtained at room temperature with different amounts of PDMS at different mixing times.
mass per unit area can be calculated from the Sauerbrey equation [23]
$\Delta \mathrm{m}=-\frac{\mathrm{C} \Delta \mathrm{f}}{\mathrm{n}}$
In this equation, C is a constant ( $17.7 \mathrm{ng} \mathrm{cm}^{-2} \mathrm{~s}^{-1}$ in this equipment) and $n$ is the resonance overtone number. In this work, $n=5$ was used.

## 3. Results and discussion

### 3.1. Morphology and hydrophobicity of the coatings

The phase separation degree of the Poly(siloxane-urethane) copolymers depends on the reaction conversion as described by Webster et al. [24]. In a previous paper [16] we presented similar results to those obtained by Webster using a copolymer containing $10 \%$ of siloxane. In that paper, the conversion was controlled by varying the mixing time, and the highest phase separated morphol-
ogy was obtained at intermediate mixing times. Following the same methodology, in the present paper a series of films were prepared varying the siloxane content.

Topographical and phase images of the cured films were obtained by AFM. The phase images of samples generated at room temperature are shown in Fig. 1.

As can be seen, some of the images showed a phase separated structure with microtopographical surfaces, the dispersed phase being composed of siloxane domains. For each composition, no phase separated images were obtained at low and high mixing times and domain formation was observed at intermediate times. According to these results, the formation of microtopographical surfaces only occurred when the films were cast at intermediate conversion. When the conversion was higher (higher than $50 \%$, according to previous results [16]), total miscibility between the urethane and siloxane components was obtained. The behaviour of the samples containing 10 and $15 \%$ of PDMS was similar. However, in the sample containing 5\% of PDMS, the phase separated structures and the final mixing was obtained at lower mixing times.


Fig. 2. Contact angle values for the coatings generated at room temperature (left) and at $50^{\circ} \mathrm{C}$ (right).

In the case of the images obtained for films generated at $50^{\circ} \mathrm{C}$ (data not shown), the behaviour was the same as that obtained for the room temperature cast samples. However, the microdomain formation process took place within a shorter time interval. This result could be explained on the basis of a faster evaporation of acetyl acetone at $50^{\circ} \mathrm{C}$. As described in a previous paper [16] acetyl acetone slowed down the reaction rate and therefore the evaporation of this compound accelerated the phase formation process.

The hydrophobicity of the samples obtained at different mixing times was calculated by means of water contact angle measurements. The results of the water static contact angle are summarized in Fig. 2.

As can be observed, all the coatings showed contact angles higher than $90^{\circ}$ indicating that all surfaces were hydrophobic. In addition, regardless of sample composition and casting temperature, the maximum contact angles were obtained at intermediate mixing times. In relation to the sample composition, and for both temperatures, the maximum contact angles of samples containing 10 and $15 \%$ of siloxane were of the same order, although slightly higher values were obtained for the sample containing $15 \%$ of siloxane. Lower contact angle values were obtained for samples with $5 \%$ of siloxane. Finally, it must be pointed out that for samples cast at $50^{\circ} \mathrm{C}$, the maximum of the contact angles was obtained at mixing times lower than in the samples cast at room temperature.

Dynamic water wettability measurements with evaluation of advancing/receding components and hysteresis, were performed for a better correlation with functional performances. All the samples showed hysteresis values lower than $5^{\circ}$ (the data for all compositions can be found in the supplementary material). In literature, higher hysteresis values were obtained for similar urethane/siloxane copolymers [19] and for ether/Fluorated copolymers [13,15]. The high hysteresis values obtained in these works were attributed to surface chemical heterogeneity. According to this, the samples obtained in the present work exhibited a more homogenous surface, probably related with the lower siloxane concentration and/or a lower difference between the surface energy of the copolymer components. However, our results clearly showed that higher contact angles were obtained for samples generated at intermediate mixing times where the samples presented nanostructured morphologies. According to this, the surface morphology plays an important role in the surface wetting behaviour.

### 3.2. Evaluation of the antifouling performance

Protein adsorption measurements were performed as a simple way to evaluate the antifouling performance.
a) Static adsorption tests

Typical adsorption profiles of BSA for samples generated at room temperature and measured using the dye-binding assay are shown in Fig. 3. In all cases, the data of a reference polyurethane without siloxane are included for comparison purposes.

The adsorption rate of BSA is influenced by the surface affinity and diffusion rate of the protein through the solution. At 300 min , nearly full coverage of each of the surfaces was observed.

For the samples containing 5\% of siloxane, regardless the mixing time, the adsorption values were similar to those obtained for the reference polyurethane. However, reduced protein adsorption was obtained for samples containing 10 and $15 \%$ of siloxane. This result proved that the siloxane reduced the adsorption of the protein. Therefore, these coatings could be interesting candidates as fouling release coatings. Similar conclusions have been reported using different Bio Assay measurements for poly(siloxane-urethanes) [25] and for polyester/polysiloxane coatings [26].

The BSA adsorption curves of the samples obtained by casting at $50^{\circ} \mathrm{C}$ showed very similar behaviour to the data obtained from samples cast at room temperature. Table 1 summarizes the BSA adsorption values at 300 min for samples cast at different temperatures and times.

As can be observed, for each composition and casting temperature the adsorption of BSA was lower in the samples obtained at 240 min where the samples presented nanostructured morphology and higher contact angles.

Comparing the data of different composition containing samples, it became clear that $5 \%$ of siloxane was not enough to

Table 1
BSA Protein adsorption at 300 min for coatings obtained at room temperature and at $50^{\circ} \mathrm{C}$.

| PDMS\% | Mixing time <br> $(\mathrm{min})$ | Adsorption <br> $\left(25^{\circ} \mathrm{C}\right)\left(\mu \mathrm{g} / \mathrm{cm}^{2}\right)$ | Adsorption <br> $\left(50^{\circ} \mathrm{C}\right)\left(\mu \mathrm{g} / \mathrm{cm}^{2}\right)$ |
| :--- | :--- | :--- | :--- |
| 0 | 240 | 2.1 | 2.2 |
| 5 | 60 | 1.5 | 1.4 |
|  | 240 | 1.4 | 1.5 |
|  | 1440 | 2.4 | 3.2 |
| 10 | 60 | 1.2 | 1.0 |
|  | 240 | 0.6 | 0.2 |
|  | 1440 | 0.9 | 0.5 |
| 15 | 60 | 0.6 | 0.8 |
|  | 240 | 0.2 | 0.6 |
|  | 1440 | 0.6 | 0.8 |


 with $10 \%$ of PDMS (right). C) Adsorption for films with $15 \%$ of PDMS (below).
significantly reduce the protein adsorption. The data obtained for 10 and $15 \%$ of siloxane were not conclusive. Thus, for the samples cast at room temperature, the best results were obtained for samples containing $15 \%$ of siloxane. For those cast at $50^{\circ} \mathrm{C}$, the sample containing $10 \%$ of siloxane showed less protein adsorption.

## b) Quartz Crystal Microbalance (QCM-D) Measurements

The data obtained from the static adsorption test showed that the nanostructurated samples gave rise to lower BSA adsorption values. In order to obtain information about the reason for this behaviour, the adsorption of BSA of samples containing $10 \%$ of siloxane cast at room temperature was studied by QCM-D. Samples obtained at 60, 120 and 240 min of mixing time were selected in order to study the behaviour of a non-nanostructured sample ( 60 min ) and two nanostructured samples containing different morphologies ( 120 and 240 min ) (See Fig. 1).

Prior to the experiments, the sensors were conditioned overnight under a constant water flux. In order to understand if there was a general difference between the three samples as regards interaction with water, we represented the experimental data from conditioning through plotting dissipation (D) as a function of frequency. Whilst time is eliminated as an explicit parameter in this type of representation, it can implicitly be understood for these studies that time increases along with frequency as adsorption proceeds.

As can be seen from Fig. 4, for all samples an initial increase in the frequency, that can be related to a water sorption, went along with an increase in dissipation. However, the frequency change of the
nanostructured sample obtained after 240 min mixing was clearly les than that of the other two samples, indicating that the water sorption ability of this sample was lower (Fig. 5, detail).

Samples obtained at 60 and 120 min showed a similar behaviour up to about 40 Hz . At higher frequencies (water uptake), the sample of less mixing time showed a non-linear and very pronounced increase in dissipation, followed by a decrease in frequency (water loss) but maintaining high dissipation values. This result evidenced that the polymer underwent a strong swelling up to a point where it rearranged significantly and water desorption occurred. As this was the only sample that was not nanostructured, it may be concluded that the initial higher swelling owing to the water uptake was related to the lower hydrophobicity of the sample. After swelling, however, the polymer rearranged, promoting phase separation and therefore in turn reducing water sorption. It could be thought that the particular behaviour of the sample of 60 min could be due to a partial conversion of the coating with consequent leaching phenomena. However, the infrared spectrum of this coating (see supplementary material) did not show any isocyanate band what meant that the conversion of the reaction was high.

These data, hence, already indicated that the nanostructuration of the polymer modulated the interaction with water, based on either the overall hydrophilicity of the polymer, its morphology, or both. This is in line with results from respective contact angle measurements, where the nanostructured samples presented higher values than the homogeneous ones.

Subsequently, the respective sensors were put into contact with different concentrations of BSA in aqueous solution up to a maximum of 100 mg BSA/L. Samples were exposed to a successive


Fig. 4. Dissipation (D) versus frequency (F) for the samples containing $10 \%$ of siloxane obtained at 60,120 and 240 min of mixing time cast at room temperature during water conditioning (left), and detail of the graph (right).


Fig. 5. Dissipation (D) versus frequency (F) for the samples containing $10 \%$ of siloxane obtained at 60,120 and 240 min of mixing time casted at room temperature during adsorption of BSA in water. The values represented were taken from the fifth harmonic.
increase in BSA concentration without intermediate flushing with water or removal of the already adsorbed BSA. The data obtained were then again represented independent of time in a dissipationfrequency plot, with the results shown in Fig. 5. The frequency represents the quantity of BSA adsorbed on the surface while the dissipation reflects the "rigidity" with which BSA adsorbs. Low frequency values naturally represent the beginning of the experiments with low BSA concentration in the water, and high frequencies represent data toward the end of the experiment where the BSA concentration was maximum.

As can be seen, for a frequency up to about 2 Hz , all samples showed a similar dependence of dissipation on frequency. Hence, during a first contact with BSA, no difference was seen in the degree of adsorption. Minor variations amongst the dissipation values were statistically not significant. With increasing BSA concentration, however, distinct adsorption behaviour was observed for the
sample of less mixing time as it adsorbed as much as the one mixed for $120 \mathrm{~min}(\mathrm{~F}=7-8 \mathrm{~Hz}$ ), but with a significantly higher dissipation. This higher dissipation of the non nanostructured sample (mixing 60 min ) indicated a more viscoelastic adsorption "film" of BSA and was most probably due to higher water content resulting in a more "loosely" adsorbed BSA.

The dissipation of the two nanostructured samples (120 and 240 min of mixing, respectively) followed an almost identical trend as a function of frequency. It may be speculated that on nanostructured samples BSA was adsorbed in a different conformation that did not retain water as much as it did in the non-nanostructured one. This result would be in line with literature data, which indicate that the disruption of protein adsorption requires compositional heterogeneity to create a mismatch between the nanodomains and the anchoring sites of the protein [13,18,21].

It should be noted that the sample obtained after 240 min mixing presented a reduced adsorption of BSA: compared to the other two samples the maximum frequency reached was only half. This result revealed that while BSA adsorbed in the same manner and conformation on the two nanostructured samples, the amount of interaction sites decreased by $50 \%$ for the one with longer mixing times. We do not really know if this reduction in protein sorption is sufficient to have an impact on the subsequent fouling. However, other authors have showed that the nanostructured surfaces presented lower adhesion strength of barnacles [19] which suggests that the protein sorption test can be used as a qualitative measurement of the antifouling performance of these coatings.

Finally, using the Sauerbrey equation described in the experimental section [23], the absolute protein sorption values were calculated in order to compare the QCM adsorption results with the data obtained in static experiments. The results showed that the QCM gave rise to values that were one order of magnitude lower than those obtained in the static experiment. This result was obviously related with the different modes the experiments were performed. However if the results are compared in terms of relative adsorption reductions both techniques gave rise to similar values and therefore, the reduction in the protein adsorption obtained in this work is not a function of the employed measurement method.

## 4. Conclusions

As shown on previous works, Poly(siloxane-urethane) copolymers with low siloxane content presented a phase separated
morphology that can be tailored during the synthesis process varying the mixing time and casting temperature. Surfaces obtained at intermediate mixing times presented higher phase separation, and slightly higher values of water static contact angle. However, no significant differences were obtained in the water contact angle hysteresis.

Comparing static and dynamic BSA adsorption data higher values were obtained in static mode. However, both techniques showed that a $50 \%$ of reduction in the protein adsorption was produced in the nanostructured samples. This result showed that although the absolute protein sorption data depended on the experimental conditions, the reduction in the protein adsorption can be used in order to establish differences between formulations. In addition, QCM-D measurements, and particularly the dissipation data, showed that in nanostructured coatings the protein adsorption occurred in a conformation that prevented water retention. The latter could be the origin of the fouling resistance ability of these copolymers.

## Acknowledgements

The authors acknowledge the University of the Basque Country UPV/EHU (UFI 11/56) and, the Basque Government (Ayuda a grupos de investigación del sistema universitario vasco IT618-13 and PhD scholarship) and the Ministerio de Economia y competitividad (CTQ2013-4113-R) for the funding received to develop this work. Technical and human support provided by Macrobehaviour-Mesostructure-Nanotechnology SGIker Service of UPV/EHU is also gratefully acknowledged.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.porgcoat.2016. 05.011 .

## References

[1] D.M. Yebra, S. Kiil, K. Dam-Johansen, Prog. Org. Coat. 50 (2004) 75-104.
[2] T. Vladkova, Surface modification approach to control biofouling, in: Springer Series on Biofilms, Springer-Verlag, Berlin, Heidelberg, 2016, http://dx.doi. org/10.1007/7142_2008_22135135.
[3] A.M. Brzozowska, F.J. Parra-Velandia, R. Quintana, Z. Xiaoying, S.S.C. Lee, L. Chin-Sing, D. Janíczewski, S.L.M. Teo, J.G. Vancso, Langmuir 30 (2014) 9165-9175.
[4] D.L. Schmidt, R.F. Brady, K. Lam, D.C. Schmidt, M.K. Chaudhury, Langmuir 20 (2004) 2830-2836.
[5] C.P. Stallard, K.A. McDonnell, O.D. Onayemi, J.P. O’Gara, D.P. Dowling, Biointerphases 7 (2012) 31-43.
[6] S.M.G. Demneh, B. Nasernejad, H. Modarres, Colloids Surf. B 88 (2011) 108-114.
[7] P.A. George, B.C. Donose, J.J. Cooper-White, Biomaterials 30 (2009) 2449-2456.
[8] E. Yilgör, I. Yilgör, Prog. Polym. Sci. 39 (2014) 1165-1195.
[9] P. Majumdar, A. Ekin, D.C. Webster, Smart coatings 957 (2007) 5-61.
[10] A. Ekin, D.C. Webster, J.W. Daniels, S.J. Stafslien, F. Cassé, J. Callow, J. Coatings Technol. Res. 4 (2007) 435-451.
[11] S. Sommer, A. Ekin, D.C. Webster, S.J. Stafslie, J. Daniels, L.J. VanderWal, Biofouling 26 (2010) 961-972.
[12] R.B. Bodkhe, S.E.M. Thompson, C. Yehle, N. Cilz, J. Daniels, S.J. Stafslien, J. Coatings. Technol. Res 9 (2011) 235-249.
[13] Y. Wang, L.M. Piter, J.A. Finlay, L.H. Brewer, G. Cone, D.E. Betts, M.E. Callow, J.A. Callow, D.E. Wendt, M.A. Hillmyer, J.M. DeSimone, Biofouling 27 (2011) 1139-1150.
[14] S. Kwon, H. Kim, J.W. Ha, S.Y. Lee, J. Ind. Eng. Chem. 17 (2011) 259-263.
[15] E. Molena, C. Credi, C. De Marco, M. Levi, S. Turri, G. Simeone, Appl. Suf. Sci. 309 (2014) 160-167.
[16] A. Santiago, L. Martin, J.J. Iruin, M.J. Fernández-Berridi, A. González, L. Irusta, Prog. Org. Coat. 77 (2014) 798-802.
[17] A. Santiago, A. González, J.J. Iruin, M.J. Fernández-Berridi, M.E. Muñoz, L. Irusta, Macromol. Symp. 321-322 (2012) 150-154.
[18] C.A. Amadei, R. Yang, M. Chiesa, K.K. Gleason, S. Santos, ACS Appl. Mater. Interfaces 6 (2014) 4705-4712.
[19] P. Majumdar, S. Stafslien, J. Daniels, D.C. Webster, J. Coat. Technol. Res. 4 (2007) 131-138.
[20] F. Li, J. Meng, J. Ye, B. Yanga, Q. Tiana, C. Deng, Desalination 344 (2014) 422-430.
[21] S.H. Baxamusa, K.K. Gleason, Adv. Funct. Mater. 19 (2009) 3489-3496.
[22] M.A. Bradford, Anal. Biochem. 72 (1976) 248-254.
[23] G. Sauerbrey, Z. Phys. 155 (1959) 206-222.
[24] P. Majumdar, D.C. Webster, Macromolecules 38 (2005) 5857-5859.
[25] S. Sommer, A. Ekin, D.C. Webster, S.J. Stafslien, J. Daniels, L.J. Vander Wal, S.E.M. Thompson, M.E. Collow, J.A. Collow, Biofouling 26 (2010) 961-972.
[26] F. Azemar, F. Fäy, K. Réhel, I. Linossier, Prog. Org. Coat. 87 (2015) 10-19.


[^0]:    * Corresponding author.

    E-mail address: lourdes.irusta@ehu.es (L. Irusta).
    http://dx.doi.org/10.1016/j.porgcoat.2016.05.011 0300-9440/© 2016 Elsevier B.V. All rights reserved.

