Progress in Organic Coatings 99 (2016) 110–116

Contents lists available at ScienceDirect

Progress in Organic Coatings

journal homepage: www.elsevier.com/locate/porgcoat

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

A. Santiago [a], L. Irusta [a,*], T. Schäfer [a,c], A. Corres [a], L. Martin [b], A. González [a]

[a] POLYMAT, Department of Polymer Science and Technology, University of the Basque Country UPV-EHU, PO Box 1072, 20080 Donostia/San Sebastián, Spain
[b] Macrobehaviour-Mesostructure-Nanotechnology SGIker Service, Polytechnic School, University of the Basque Country UPV-EHU, Plaza Europa 1, 20018 Donostia/San Sebastián, Spain
[c] Ikerbasque, Basque Foundation for Science, Bilbao, Spain

ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form 4 May 2016
Accepted 13 May 2016
Available online 6 June 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.

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 simplified 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

* Corresponding author.
E-mail address: lourdes.irusta@ehu.es (L. Irusta).

http://dx.doi.org/10.1016/j.porgcoat.2016.05.011
0300-9440/

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° give rise to high protein adsorption. However, surfaces with contact angles lower than 20° and approaching 120° exhibit a reduction in protein adsorption. Therefore, in order to obtain an antifouling coating the surfaces with contact angles between 60–80° must be avoided. Poly(siloxane-urethane) coatings present water contact angles between 100–110° 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, Mn 1000 g mol^{-1}, 20 wt-% non siloxane component) was supplied by Gelest Inc. Trifunctional polyol (polycaprolactone, PCL, Mn 900 g 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 Bio-Rad.

2.2. Coating preparation

In order to prepare the coating formulation, PCL and PDMS solutions in butyl acetate (33 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 mg, 0.03 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 °C for 24 h on a hot plate in a fume hood. In both cases, this was followed by oven curing at 80 °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 °C and 55%, respectively). In the static experiments, the volume of the deionised water droplets was 5 μL. Advancing and receding contact angles were measured dispensing/withdrawing liquid (5 μL) over a liquid drop (10 μL) 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 μm X 20 μm 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 mg/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 cm^2 and 200 μm of thickness were immersed in 100 mL BSA/water solution (0.45 mg/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 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 °C for 45 min.

QCM measurements were performed on a Q-SENSE E1 system operating at 23 °C. Prior to the experiments, the sensors were stabilized overnight under a constant water flux of 100 μL/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/L.

QCM-D technique detects changes in the resonance frequency (Δf), and dissipation (ΔD). 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