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Adsorption of polysorbate 20 and proteins on hydrophobic polystyrene surfaces studied by neutron reflectometry

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ABSTRACT

Understanding the adsorption of protein and surfactant molecules on hydrophobic surfaces is very important for storage stability and delivery of pharmaceutical liquid formulations as many commonly-used devices, such as drug containers and syringes, have hydrophobic surfaces. Neutron reflectometry is used here to investigate the structure information of the adsorption process of non-ionic surfactant (polysorbate 20) and proteins (monoclonal antibody (mAb) and lysozyme) on polystyrene surfaces. Thickness of adsorbed polysorbate 20 thin film is observed to be ≈21 Å, comparable to the radius of gyration of polysorbate 20 micelles in solution. Although no lysozyme adsorption is observed on the polystyrene surface in low solution pH condition, the mAb can be strongly absorbed on the polystyrene surface with a layer thickness of ≈145 Å. The mAb concentration near the surface is about 135 mg/ml significantly larger than the bulk protein concentration. The differences in adsorption behavior are attributed to different protein interactions with a hydrophobic surface. Further, both surfactants and proteins adsorbed on the polystyrene surfaces can not be rinsed off using pure water.

1. Introduction

Monoclonal antibodies (mAbs) based therapeutic drugs are now the fastest growing section of the pharmaceutical industry due to their excellent target selectivity with fewer side effects compared with traditional small-molecule drugs [1]. To maintain long-term stability of mAbs in solutions, stable buffers containing saccharides, surfactants, and salts, are typically used to provide favorable physio-chemical environment for mAbs, and impede irreversible-aggregate formation during storage and drug administration. Non-ionic surfactant polysorbate 20, also commercially known as Tween™ 20, has been widely used in mAbformulations due to its bio-compatibility, low toxicity, and stabilizing properties.

Despite all the efforts, irreversible aggregations remain a vexing problem for the industry, and may lead to adverse biological consequences [2–4]. Among many possible routes [5–7], irreversible aggregate formation can be initiated through the protein adsorption at solid-liquid and air-liquid interfaces [8]. Therefore, understanding the protein adsorption at different interfaces is important for the pharmaceutical industry to prevent the adsorption from happening. At the same time, the protein adsorption can be also very useful for some other applications since well-controlled protein layers on surfaces can be used as a new generation of reactor beds for biosensor as well as disease diagnostics [9,10]. Because of these interests from both industrial and academic fields, the study of protein adsorption at interfaces has attracted much research attention.

Surface adsorption of proteins and excipients has long been studied on both hydrophilic and hydrophobic surfaces by many methods such as microscopy [11–13], quart crystal microbalance – dissipation (QCM-D) [14,15], ellipsometry [16,17], X-Ray photoelectron spectroscopy (XPS) [13,16], and X-ray/neutron reflectometry [18–21]. Many studies have focused on hydrophobic surfaces, because medical devices, such as infusion tubes or

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prefilled syringes, are typically either comprised of hydrophobic polymeric materials or are coated with a layer of hydrophobic oil on glass surfaces to lubricate injection process [22–24]. However, the adsorption behavior of proteins depends on the properties of proteins and surfaces. mAb adsorption on hydrophilic silica surface has been studied with atomic force microscopy (AFM). At relatively low protein concentration, mAb proteins are observed to take predominately a flat-on orientation on the surface [12]. In contrast, a different mAb in solutions with a similar concentration range is believed to take a mixture of orientations including flat-on, side-on, and end-on on 1-docecanethiol surface [15]. A recent study on mAbadsorption in the presence of non-ionic surfactants (polysorbate 20 and polysorbate 80) showed that the amount of protein adsorption depends on the protein and surfactants exposure method on (3, 3-trifluoropropyl) chloromethylsilane surface. In this study, it is observed that the adsorbed protein can only be partially rinsed off by the surfactants [14]. In contrast, a different study showed that complete removal of proteins on the octadecyltrichlorosilane (OTS) surface can be achieved by rinsing the surface with surfactant solutions [11]. The protein adsorption behavior on the OTS surface is found to be sensitive to the relative concentration between proteins and surfactants molecule [11]. The problem is even more complicated when proteins can penetrate the surface. Adsorption of plasma proteins on copolymer hydrogel surfaces have been investigated by measuring thickness changes of hydrogel films with ellipsometry as well as changes in detected surface elements by XPS [17]. The results indicate that absorption of plasma proteins is due to hydrophobic interactions between plasma proteins and copolymer hydrogel. In addition, adsorption is found to be a transport limited process that depends on both the size of proteins and pores formed by hydrophobic sections of the copolymer. Despite many studies of protein adsorption on liquid-solid interfaces, the adsorption mechanism seems still system specific for many cases [8,25].

Amongst the many material choices available for medical devices, polystyrene and polystyrene-coated surfaces, such as STYRON™ 2678 MED, are also used on many devices. However, few studies focus on the adsorption of proteins and excipients on polystyrene surfaces [13,26,27]. Browne et al. studied adsorption of human albumin on polystyrene surfaces using XPS and AFM. They found that human albumin can be irreversibly adsorbed on the surface [13]. Kim et al. studied the adsorption of lysozyme, fibrinogen, and bovine serum albumin on polystyrene surfaces and found that protein structure is more random on hydrophobic polystyrene surfaces compared with that on hydrophilic silica surfaces [28]. In this paper, we investigate adsorption behavior of the non-ionic surfactant, polysorbate 20, and two proteins (mAb-X and lysozyme) by neutron reflectometry on polystyrene surface. Structure of the polysorbate 20 and protein adsorption layer is determined, such as layer thickness, the density distribution, and concentrations near surfaces. Further, we observe large differences in the adsorption behavior of mAb-X compared to lysozyme. While an adsorption layer of mAb-X is observed on the polystyrene surface, lysozyme is not adsorbed on the polystyrene surface at our solution conditions.

2. Materials and methods

2.1. Materials

Pharmacopoeial grade polysorbate 20 (CMC = 8.04×10^{-5} mol/L at 21 °C) [29] and monoclonal antibody mAb-X (pI = 6.75, MW = 150 KDa) were kindly provided by Genentech Inc. Lyophilized lysozyme was purchased from MilliPore(Catalog# 100831), and further purified at CNMS, ORNL following the previous method to remove most salts [30,31]. Polystyrene with molecular weight of 390 KDa ($M_w/M_n < 1.1$ Pressure Chemical Company) was kindly provided by Dr. Kalman Migler from Polymer and Complex Fluids Group at National Institute of Standard and Technology. 99% grade D_2O was purchased from Cambridge Isotope Laboratory. Deionized (DI) water was filtered through 18.2 MΩ before using. Reagent grade Toluene was purchased from J.T. Baker. The substrate made of 3 inches diameter silicon wafers was purchased from Institute of Electronic Material Technology™.

2.2. Preparation of polystyrene surface

Polystyrene was coated on a silicon wafer by spin casting using a solution of 0.5% mass fraction polystyrene in toluene that was mixed with a magnet stirring bar on a hot plate at 120 rpm and 50 °C overnight. The silicon wafer was washed by Micro90 and DI water three times to remove oil from the surface, and then treated in UV/Ozone oven for 1 h to remove any remaining organic contaminants. The wafer was then spin washed by DI water followed by a toluene wash before spin casting polymer solutions. After filtering the solution through a PTFE filter (0.2 μm), the casting solution was slowly dropped onto the surface of a stationary silicon wafer until the wafer surface was fully covered by the solution. The wetted wafer was spin cast at 2500 rmp for 60 s, and then covered by a clean glass petri dish at room temperature for about 30 min. Then the coated wafer with the petri dish was carefully moved into a vacuum oven at 150 °C. Note that the glass transition temperature of polystyrene is between 100 °C and 107 °C [32]. The glass petri dish was used during the annealing process to avoid the contamination of the wafer surface [33]. After annealing the film for 2 h, the sample was cooled down to 25 °C while maintaining a high vacuum of the oven. The coated wafer was subsequently assembled into the NR flow cell [34] and ready for neutron reflectometry measurements. The contact angle of water on the polystyrene coated surface was measured to be approximately 90°, consistent with a previous report [35].

2.3. Sample solutions preparation

1) 20 mM HisOAc buffer with pH = 5.5 preparation: 310.3 mg Histidine and 106 uL Acetic acid were dissolved into 100 mL H_2O and D_2O respectively to make buffers. 2) monoclonal antibody sample preparation: the excipient free monoclonal antibody(mAb-X) was originally provided in liquid state with concentration of 100 mg/ml. To prepare the sample in H_2O, the mAb-X solution was directly diluted into H_2O HisOAc buffer with final concentration of 5 mg/ml. To prepare mAb-X solution in D_2O, 1 mL of mAb-X solution at 100 mg/ml in H_2O buffer was injected into 15 mL (10 K MWCO) centrifugal filter unit and mixed with 14 mL D_2O buffer. The unit was centrifuged at 4000 rpm for 60 min, then it was topped off with D2O buffer. After three iterations, the solution inside the unit was collected and further diluted to 5 mg/ml with D_2O buffer. 3) lysozyme sample preparation: 5 mg/ml of lysozyme sample in D_2O was prepared by reconstituting lyophilized lysozyme powder with D_2O. The resulting solution is at pD = 4.8. 4) polysorbate 20 sample preparation: 1% mass fraction of polysorbate 20 sample was prepared by dissolving 250 mg polysorbate 20 into 25 g D_2O. 50 μL and 520 μL of 1% mass fraction stock solution were pipetted into 10 mL D_2O or H_2O to make solution with a final concentration of 4.075×10^{-5} M (pH = 6.71) and 4.075×10^{-4} M (pH = 6.96) respectively. All solutions were degassed at 635 mm Hg pressure using a TA Degassing Station for 20 min before any measurements.

2.4. Neutron reflectometry measurement and data analysis

Neutron reflectometry measurements were performed at the NG-7 horizontal reflectometer at NIST Center for Neutron Research

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