Surface Analysis of Polydimethylsiloxane Fouled with Bovine Serum Albumin

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***Abstract*—**Polydimethylsiloxane (PDMS) is prone to bio-fouling because of its hydrophobic surface. This results in the non-specific adsorption of proteins and other bio materials. The adsorption causes modification on the PDMS surface which becomes a problem in applications which require hydrophobic surface and the use of proteins. PDMS blocks were treated with a solution of 50mg/ml bovine serum albumin (BSA) for 10 minutes, and then washed with phosphate buffer saline (PBS). The contact angle for both water and BSA was measured on both native and fouled devices. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy was performed to show the chemical modifications. A Skuur drop shape analyzer (DSA 100) was used to determine the critical micelle concentration (CMC) of BSA. The results showed that the PDMS loses its hydrophobicity when in contact with BSA. FTIR spectra also showed that there was a chemical modification on the PDMS surface at the 1655 and 1543 cm^{-1} region. The DSA showed through measurement of the surface tjension that the CMC for BSA is 50mg/ml.

***Keywords*—**Adsorption, Bsa, Hydrophobicity, Pdms.

I. Introduction

THE interest in the analysis of biological materials surfaces has grown both academically and industrially. This is because the applications for surfaces in contact with biological materials have also increased. These applications include optimization of surfaces and surface coatings in order to either enhance or minimize adsorption of biomaterials[1]. Polydimethylsiloxane (PDMS) elastomer is a preferred material in life science applications such as microfluidics[2]. This is due to its ease of fabrication, low cost, inertness and biocompatibility. Normally, PDMS has a hydrophobic surface, forming a contact angle of about 107-110° with de-ionized water. It is due to its hydrophobic nature that the elastomer is prone to bio-fouling, such as non-specific adsorption of proteins[3]. The non specific adsorption becomes a limitation in applications that require clean hydrophobic surfaces and the use of proteins. This paper therefore investigates the changes in the surface of PDMS after being in contact with bovine serum albumin (BSA). BSA is a standard protein[5] which has been widely investigated in the field of biomaterials. It has also been reported to adsorb on hydrophobic surfaces[6] which causes fouling. However, the effects caused by the adsorption of BSA on PDMS are not well understood. The effects of BSA combined with surfactants (surface active molecules) have been studied but not the effects of BSA independently, even though some proteins can act as surfactants. It is therefore important to know whether a protein is a surfactant or not and also understand its effects. This work will therefore analyze the changes on the PDMS surface after it has interacted with a BSA solution

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II. Materials and Methods

A. Sample preparation

PDMS elastomer blocks were prepared by mixing a 10:1 ratio of Sylgard 184® silicone base to curing agent, purchased from Dow Corning. The fluid mixture was cast into moulds of 15 by 15mm. It was then degassed in a vacuum for 30 minutes to remove the bubbles that formed during the mixing. After all the bubbles were removed, the mixture was cured in a conventional oven at 60°C for 2 hours. The blocks were removed from the moulds and treated in a beaker with a 50mg/ml BSA solution for 10min. A 50mg/ml solution was used because with higher concentrations it was difficult to wash off the BSA since too much of it accumulates on the PDMS surface. The treatment was done by dipping the blocks in a beaker with the BSA solution, putting the beaker on a stirrer plate and stirring at 600rpm. The BSA powder used was purchased from Sigma-Aldrich. The PDMS blocks were washed with phosphate buffer saline (PBS), purchased from Sigma-Aldrich, at the same stirring rate and time. This was done in order to remove the excess BSA on top of the PDMS surface and also to see that the changes on the PDMS were permanent or just visible because of the excess BSA crystals.

B. Contact angle measurements

A drop shape analyzer (Kruss DSA 100) was used to measure the contact angles of de-ionized water and 50mg/ml BSA, on both the clean and fouled devices using the sessile drop method. In this method, the drop is squeezed out of a needle, using a syringe and put on the surface of the sample as shown in fig. 1. This was done in order to see if the PDMS surface loses its hydrophobicity or gains it further when in contact with BSA. A lost in hydrophobicity is seen by a decrease in contact angle.

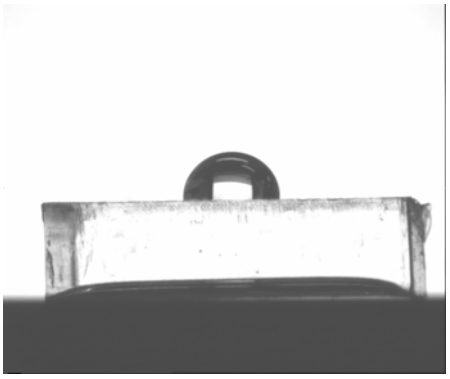

Fig. 1 Water droplet on a hydrophobic PDMS surface

C. Spectroscopic analysis

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) has been used in many biological experiments in order to analyze the surfaces of solids and liquids of biomaterials. FTIR works by identifying chemical bonds (functional groups) which are present in a molecule by producing an infrared absorption spectrum (peak). The wavelength absorbed is a characteristic of the chemical bond present. PDMS blocks were analyzed with a Perkin Elmer FT-IR spectrometer under three different conditions. The first condition was a native PDMS. The second and third conditions were BSA-coated PDMS, where the BSA was washed off in one of them and left on in the other, respectively. Each block was placed over an ATR crystal and a force gauge at 149N was applied, using the slip-clutch. All spectra were collected from 4000-1000cm^{-1}.

D. Determination of critical micelle concentration

An important measure for the characterization of surface active agents is the critical micelle concentration (CMC).
This is the concentration of the surfactant; above which micelles are spontaneously formed, a process called micellization [4] (see fig. 2). It is important to measure the CMC of proteins in order to know if they can act as surfactants on their own. This is seen by their ability to decrease the surface tension of water or any other liquid that they interact with. The CMC of BSA was determined using the pendant drop method (fig. 3); where by the surface tension of varying BSA concentrations was measured until a point was reached where an increase in concentration had no effect on the surface tension. The surface tension is measured by squeezing out a drop of the solution through a needle, the DSA measures the surface tension over time, giving an average of all the data points.

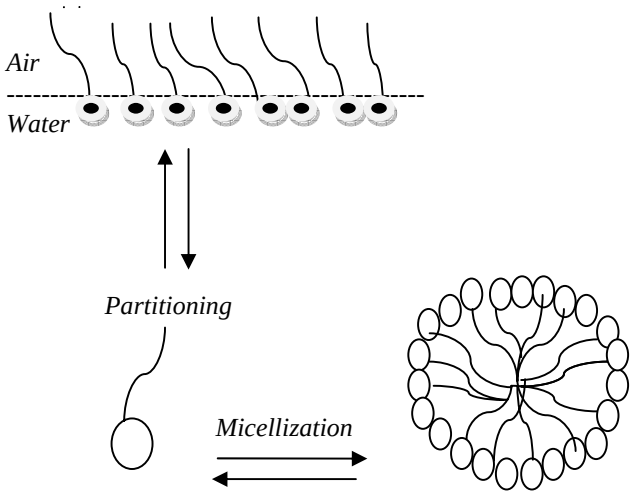

Fig. 2 CMC phenomenon (showing the arrangement of surfactant in water and the micellization which happens above the CMC value. When a surfactant is in water, its hydrophilic part stays in water while the hydrophobic tail stays in the air or another hydrophobic liquid. When the surfactant is added, it starts partitioning into separate structures, which then combine to form one structure with the hydrophilic parts inside and the hydrophobic ones on the outermost.

Fig. 3 (a): Water pendant drop, hanging from a needle during surface tension measurements. The droplet has attached more on the needle because the surface tension is very high.

Fig. 3 (b): Pendant drop from a 50mg/ml solution of BSA. The droplet has attached less on the needle due to the fact that the surface tension has decreased. This causes the droplet to fall off easily from the needle.

RESULTS & DISCUSSION

Table 1: Contact angle measurements on pure and fouled PDMS (The below table shows the differences in contact angles of a pure PDMS surface and a BSA-coated surface. The results show that the contact angle decreases, which means that the PDMS surface loses its hydrophobicity. The contact angle of a BSA droplet on a BSA-coated surface could not be measured because the droplet just slipped off, hence the N/A).

TABLE I CONTACT ANGLE MEASUREMENTS

PDMS block	Droplet	Contact angle
Clean	de-ionized water	105°
Clean	bsa	76.4°
Coated	de-ionized water	67.8°
Coated	bsa	n/a

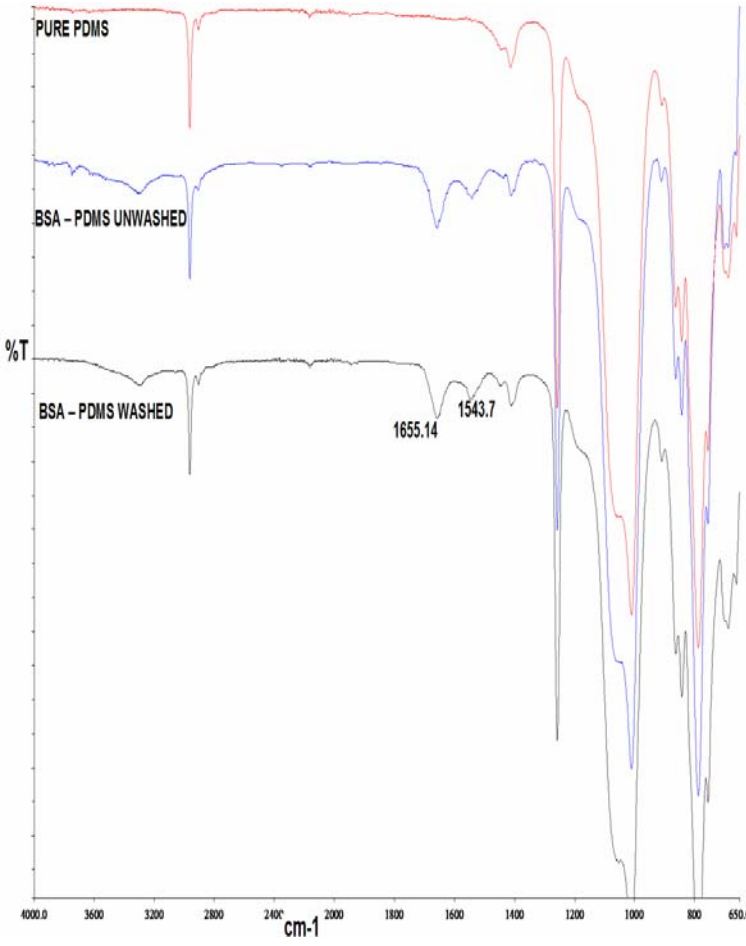

Fig. 4 ATR-FTIR spectral showing the modification on the PDMS surface. The 2 amide peaks (1655.14 & 1543.7) show the wavelength at which the PDMS surface was modified. The 2 amide peaks are normally found on a native BSA spectra and they appeared on the PDMS spectra after it was in contact with the BSA. The peaks were still observed even after the PDMS was washed, this means that the PDMS surface was modified. All three spetral graphs were taken at a percentage transmittance (%T) of 100.

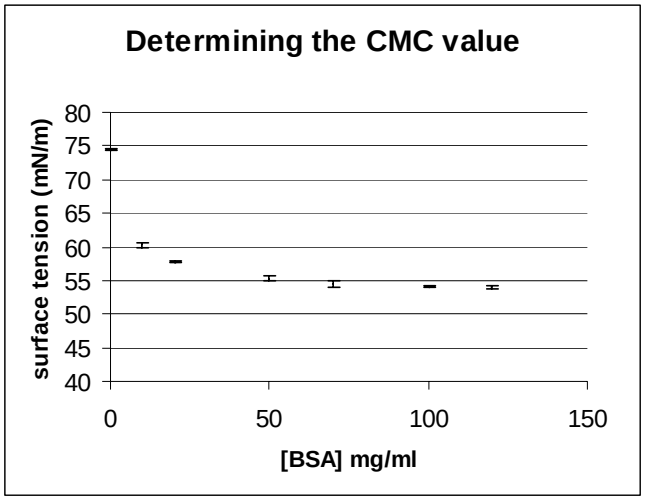

Fig. 5: Surface tension graph showing the CMC value for BSA. This figure shows the decrease in surface tension of the de-ionized water as BSA is added. The surface tension decreases until a concentration of 50mg/ml. Above 50mg/ml, the surface tension remains the same. This means that 50mg/ml is the CMC value for BSA, above this concentration BSA starts forming micelles.

III. CONCLUSION

The contact angle of the PDMS surface coated with BSA is lower than that of the native PDMS surface, which means that BSA reduces the hydrophobicity of PDMS. BSA modifies the chemical structure of PDMS; this is seen by the appearance of amide peaks on the PDMS surface. The CMC value for BSA is 50mg/ml. The next step of the project is to analyze the micelles formed by the BSA through microscopic methods.

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