

## Contact Lens Biocompatibility Studies – Measuring Protein-Polymer Interactions using Dual Polarisation Interferometry

### Introduction

Dual Polarisation Interferometry (DPI) is an important enabling tool for the study of polymer deposition processes and the molecular mechanisms behind subsequent interactions of proteins and surfactants with the polymer layer. This application note focuses on one area where such studies are of great relevance, the biocompatibility behaviour of polymers commonly used in contact lenses. The system under investigation was HEMA-MA polymer and its behaviour when challenged by the protein lysozyme.

We were interested in using DPI to gain a real time understanding of the interfacial molecular changes taking place during the interaction between polymer and protein and their consequent structural implications, underpinned by the quantitative data provided by the DPI technique.

### Experimental

The DPI experiments were performed on a Farfield **AnaLight**<sup>®</sup> instrument. The surface used in all studies was an amine functionalised silicon oxynitride **AnaChip**<sup>™</sup>. The temperature of the samples was controlled throughout to 20°C. Water used in buffer and reagent preparation was deionised and free from organic impurities. All buffers and reagents were analytical grade or higher, and solutions were degassed prior to use.

**Polymer Deposition:** Phosphate buffer (PBS, 10mM, 150mM NaCl, pH7.4) running buffer was flowed over the amine functionalised **AnaChip**<sup>™</sup> surface at 100µl/min. A 19:1 mix of 2-hydroxyethyl methacrylate and methacrylic acid, (HEMA-MA, 0.01 mg/ml) in a 1:1 mix of ethanol and water was added to the flow for 3 minutes injection, followed by a rinse with PBS running buffer until steady state was reached in the measurements.

**Polymer - Protein Interactions:** Once the deposited HEMA-MA polymer layer was stable in PBS running buffer, lysozyme (50µg/ml in PBS) was added to the flow at 100µl/min for 3 minutes. The system was then returned to PBS buffer flow at 100µl/min for 10 minutes for observation of the interfacial behaviour.

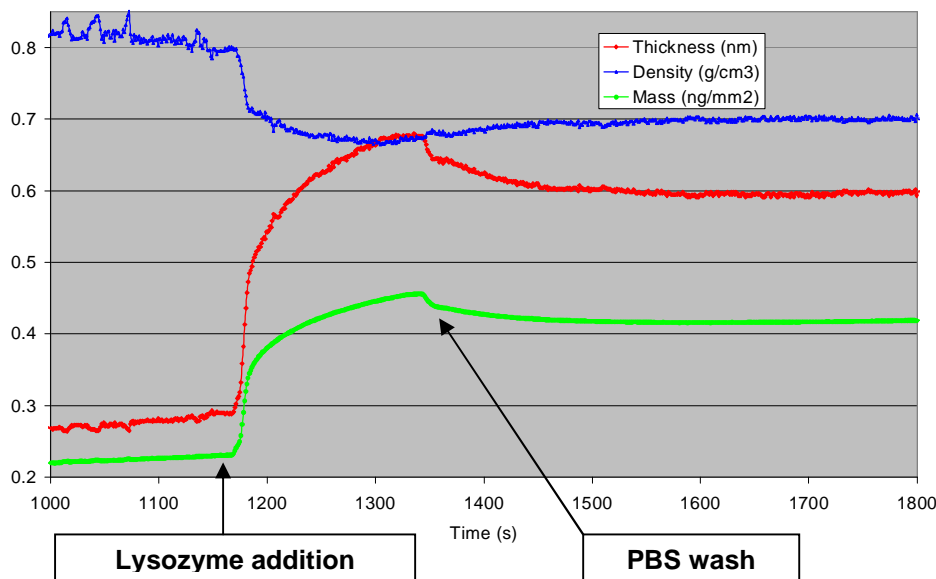
### Results and Discussion

**Polymer Deposition:** The deposition of HEMA-MA contact lens polymer onto the amine functionalised surface was repeated six times, resulting in average values for the polymer layer as having a thickness of 0.37nm (SD = 0.102 nm) and a density of 0.65g/cm<sup>3</sup> (SD = 0.115 g/cm<sup>3</sup> - see **Figure 2**).

**Polymer - Protein Interactions:** This stage of the experiment was also repeated six times on the six polymer deposition surfaces prepared above. **Figure 1** shows a typical result for the thickness, density and mass changes taking place during the HEMA-MA polymer interaction with the lysozyme protein.

**Figure 1** shows clearly that a biphasic process is taking place when the protein interacts with the polymer. During the first 10 seconds following lysozyme addition there is a sharp increase in the thickness and deposited mass of protein and a concurrent decrease in density of the layer. During the remaining 170 seconds of lysozyme addition, the results indicate that a second, different process is taking place. There is a decrease in the rate of mass deposition and thickness increase and the density of the layer remains constant. Once the protein addition ends (1350 seconds in **Figure 1**) and the layer is washed with PBS running buffer for 10 minutes, a small mass loss and decrease in thickness take place as material is washed from the surface, whilst the density of the layer remains essentially constant.

**Figure 2** summarises the data for the six repeat polymer deposition and protein-polymer interaction experiments. The layer table and the bar chart of **Figure 3** show values averaged over the six experiments. The phases of the experiment are summarised as “**HEMA**” for polymer deposition, “**fast**” for the values at the end-point of the rapid density drop, “**slow**” for the values for the values at the end of lysozyme addition phase, and “**end**” for the values after 10 minutes PBS buffer wash.



**Figure 1: Thickness, density and mass changes to the HEMA-MA polymer layer on challenge with lysozyme**

	Thickness (nm)	Density (g/cm <sup>3</sup> )	Mass (ng/mm <sup>2</sup> )
<i>HEMA</i>	0.374	0.652	0.236
<i>Fast</i>	0.538	0.604	0.316
<i>Slow</i>	0.688	0.607	0.411
<i>End</i>	0.634	0.613	0.379

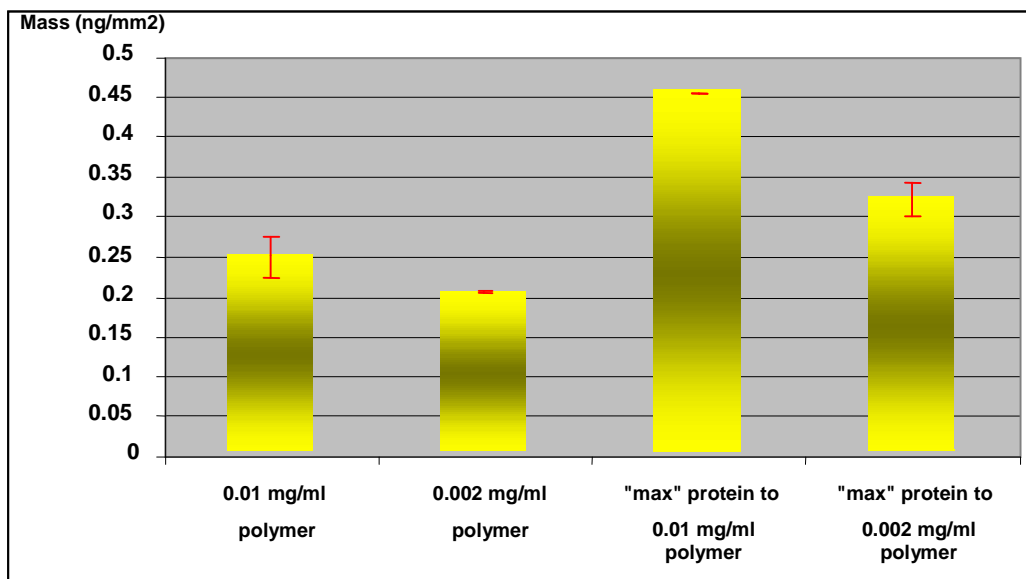
**Figure 2: Quantitative layer values at key points throughout the experiment (average values over six repeats)**

The data in **Figure 2** gives average values over six repeat experiments, but within this data set the polymer loading was varied in two of the runs. In these cases, the polymer was added to the **AnaChip™** surface at lower concentration (0.002mg/ml) for a shorter period of time. This variation did not have a significant effect on the mass of HEMA-MA polymer deposited on the **AnaChip™** surface, but the mass of lysozyme adsorbed by the polymer layer significantly decreased in these cases, as illustrated in **Figure 3**.

The DPI data shows that lysozyme interactions with the polymer HEMA-MA takes place in two distinctive phases. In the first, more rapid phase the thickness and deposited mass in the layer increase rapidly with a concurrent sharp drop in the density of the layer. These observations imply that significant structural changes are taking place in the HEMA-MA layer as it forms a polymer-protein complex on contact with the initial lysozyme molecules.

The second, slower phase is characterized by a slow thickness and mass increase whilst the density remains constant. This is indicative of the slower, non-specific adsorption of further lysozyme molecules onto the surface of the polymer-protein layer formed in the rapid, early phase.

The final buffer wash after protein addition shows a small loss of both thickness and mass whilst the density remains constant. These decreases in mass and thickness are brought about by the removal through washing of non-specifically bound lysozyme from the surface of the polymer-protein complex. The polymer-protein complex itself remains unchallenged by the buffer wash, and this is reflected in the constant density measurements throughout this phase of the experiment.



**Figure 3: Mass values for different HEMA-MA polymer loadings and the resultant effects on mass of lysozyme protein adsorbed**

**Figure 3** demonstrates that although the deposition of polymer is largely equivalent at the different polymer concentrations, the behaviour in terms of lysozyme physisorption is clearly different. In the case of polymer deposition at higher (0.01 mg/ml) concentration there is clearly a greater propensity for protein physisorption than is the case at the lower (0.002 mg/ml) polymer concentration. This clearly has implications in terms of processing the polymer and the likely efficacy of post-processing treatments in terms of the biofouling properties of the polymer.

### Conclusions and Benefits

DPI enables the study of the interfacial behaviour and structure of a diverse range of molecular systems. These experiments show DPI can be successfully used to give key insights into the biocompatibility behaviour of polymers interacting with proteins. In this specific example, a polymer used in contact lenses has been studied in terms of how it interacts with a protein commonly found in the eye. These early studies show the applicability of DPI can be extended to help design polymer composition for maximum biocompatibility of contact lenses, as well as optimising cleansing formulations for fouled contact lenses.

The **AnaLight**<sup>®</sup> instruments and their experimental protocols give the researcher a unique combination of high-resolution data in real time on thickness, density (refractive index) and surface coverage from a bench top technique. The **AnaLight**<sup>®</sup> is an important enabling tool for biocompatibility studies on materials such as contact lens polymers, giving the researcher the ability to:

- Clearly understand the molecular mechanisms underlying polymer-protein interactions
- Distinguish between absorption and adsorption processes in real-time, using the additional information provided by DPI and not available through other, more established approaches
- Tailor polymers and other materials for maximum biocompatibility and effective lifetime
- Avoid the limitations and ambiguities that are inherent in other techniques for such studies
- Screen materials for biocompatible behaviour in a fraction of the time and at higher throughput than other established methods

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