Steady-State Fluorescence Anisotropy Studies of Molecularly Imprinted Polymer Sensors

Yin-Chu Chen[a], Zheming Wang[b], Mingdi Yan[c], and Scott A. Prahl[a]
[a]Biomedical Engineering Department, Oregon Health Science University, Portland, OR
[b]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA
[c]Chemistry Department, Portland State University, Portland, OR

ABSTRACT

Molecularly imprinted polymers (MIPs) are used as recognition elements in biochemical sensors. In a fluorescence-based MIP sensor system, it can be difficult to distinguish the analyte fluorescence from the fluorescence of the polymer itself. We studied steady-state fluorescence anisotropy of anthracene imprinted in a polymer (polyurethane) matrix. Vertically polarized excitation light was incident on MIP films coated on silicon wafers; vertically and horizontally polarized emission was measured. We compared the fluorescence anisotropy of MIPs with imprinted molecules, MIPs with the imprinted molecules extracted, MIPs with rebound molecules, and non-imprinted control polymers. It is shown that differences in fluorescence anisotropy between the polymers and imprinted fluorescent molecules may provide a means to discriminate the fluorescence of analyte from that of the background polymer.

INTRODUCTION

Molecularly imprinted polymers (MIPs) used as the recognition elements in biochemical sensors are of great interest [1–3]. The advantages of MIPs include their stability in a wide range of environments, their facility in sensor micro-fabrication, and their ability to detect analytes that are difficult or impossible to sense by immunoassay. MIPs commonly rely on fluorescence of a bound analyte to monitor the presence of an analyte. Unfortunately, the MIP polymers themselves also have fluorescence properties. Background fluorescence from the polymer will contaminate the fluorescence signal from the analytes, thereby dramatically decreasing the sensitivity of this type of MIP sensor [4]. Fluorescence anisotropy has been used to investigate polymer processing and characterization [5–7], fluorescent molecules in various polymer environments [8, 9], and fluorescent MIPs [10]. Anisotropy methods are based on the principle of photoselective excitation of fluorophores by polarized light. Fluorescence anisotropy from the analyte bound to a rigid system reveals information about the analyte's local environment. In this study, we investigated the steady-state fluorescence anisotropy properties of imprinted polymers, and the analytes after they were bound to MIP recognition sites. The differences in anisotropy between the polymers themselves and the bound analytes were large enough to allow discrimination of the fluorescent analytes from the polymers.

EXPERIMENTAL DETAILS

Materials

The MIP system tested was polyurethane imprinted with anthracene [11]. Imprinted MIPs were made from mixture of anthracene, 1.25 M solutions of monomers (0.375 mmol bisphenol A

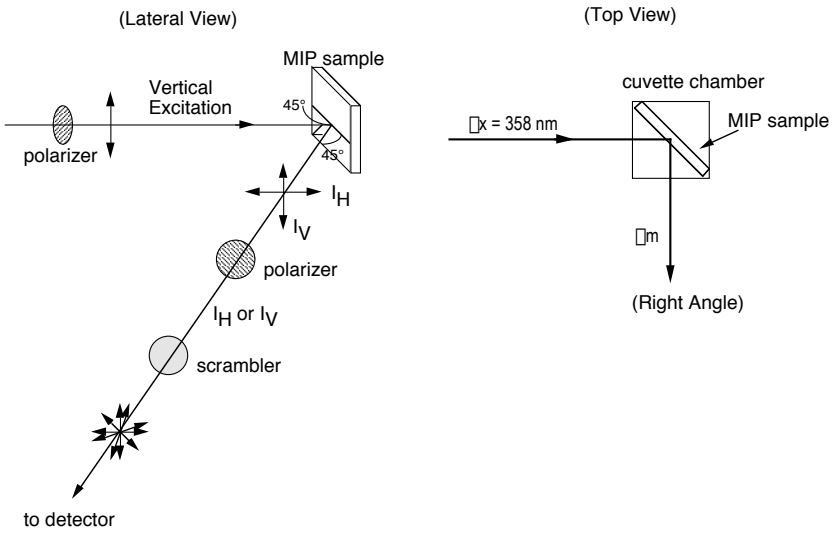

Figure 1: Set-up of MIP fluorescence anisotropy experiments. Excitation light was vertically polarized and incident at an angle of 45° relative to the plane of MIP samples. Fluorescence emission was collected at an angle of 90° relative to the incident light.

and 0.455 mmol p,p′-diisocyanatodiphenylmethane) and crosslinkers (0.250 mmol trihydroxybenzene and 0.195 mmol p,o,p′-triisocyanatodiphenylmethane) in dimethylformamide (DMF). Non-imprinted polymers were prepared in a similar manner as the imprinting solution except the anthracene solution was omitted. The silicon wafers were first cleaned with piranha solution. Additional silanization with an amino-silane was required to covalently attach the polyurethane to the wafers [12]. Freshly prepared mixtures of the imprinting or non-imprinting solutions were spin-coated onto the silanized silicon wafers at 1000 rpm. One day after the MIPs films were formed, the imprinted anthracene was extracted by soaking the imprinted polymer samples in toluene. The non-imprinted polymer samples were not treated in this step. Rebinding of anthracene was conducted by soaking each of the imprinted/non-imprinted MIP-wafer samples separately in a 10 mL of 0.5 mM anthracene solution in DMF, sealed with aluminum foil and shaken for two days. Afterwards, the samples were rinsed with DMF, and dried for one day.

Methods

The polarized fluorescence of anthracene solutions and MIP samples were measured using a fluorimeter (Fluorolog II, SPEX) with two polarizers and a scrambler, as shown in Fig. 1. One quartz polarizer was placed at the window of excitation monochromator; the other polarizer was located at the emission window, 90° relative to the excitation light. A light scrambler was placed after the second polarizer to depolarize the light to avoid bias in the detection of vertical or horizontal fluorescence. An anthracene solution in DMF (0.1 mM) inside a cuvette was measured at room temperature as calibration. MIP samples were placed at the diagonal position of a cuvette chamber as shown in Fig. 1. Excitation scans used vertically polarized (relative to the plane of the table) excitation light from 310 to 380 nm; vertical (I_V) and horizontal (I_H) polarized states of

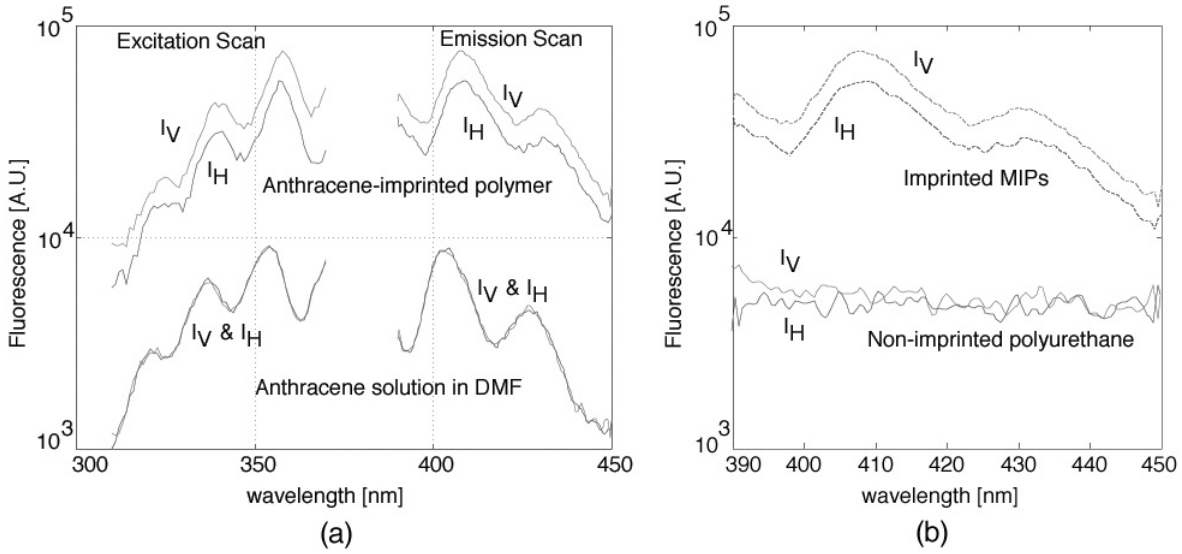

Figure 2: (a) The horizontally and vertically polarized fluorescence of MIPs and anthracene solution in DMF for excitation scan and emission scan. The horizontal and vertical fluorescence scans for the anthracene solution in DMF overlap. (b) The horizontally and vertically polarized fluorescence of non-imprinted polyurethane films, and imprinted MIPs (emission scan).

emission at 405±2.5 nm were recorded. Emission scans used 358±2 nm vertically-polarized excitation light; both I_V and I_H were scanned from 380 to 450 nm. The anisotropy (r) of the samples was calculated as

$$r = \frac{I_V - I_H}{I_V + 2I_H}$$

RESULTS AND DISCUSSION

Figure 2 (a) shows the vertical and horizontal components of excitation and emission of anthracene imprinted MIPs and the reference anthracene solution in DMF. Fluorescence of anthracene in MIPs shows about 6 nm stoke shift to the anthracene solution in DMF. Theoretically, the horizontal and vertical fluorescence curve of the anthracene solution in DMF, $I_{V(DMF)}$, and $I_{H(DMF)}$, should overlap since the steady-state anisotropy of anthracene molecules in a rotation-free media is zero [13]. Therefore, in our experiment, the ratio, $I_{V(DMF)}/I_{H(DMF)}$, was used to calibrate the horizontal and vertical fluorescence curves of all other measurements.

The fluorescence spectrum of non-imprinted polyurethane films was compared with MIP films (imprinted with 12 mM anthracene) in Fig. 2 (b). Note that polyurethane has fluorescence in the same wavelength range as anthracene. According to our experiments, the fluorescence intensity of non-imprinted polyurethane is about the same as that of MIPs imprinted with 1 mM anthracene for the same thickness of films. This means that it is difficult to discriminate between the fluorescence signal from anthracene in MIPs and the background fluorescence signal from polyurethane when the concentration of anthracene in MIPs is lower than 1 mM. However, Fig. 2 (b) shows that imprinted MIPs have distinguishable horizontally and vertically polarized fluorescence, while the

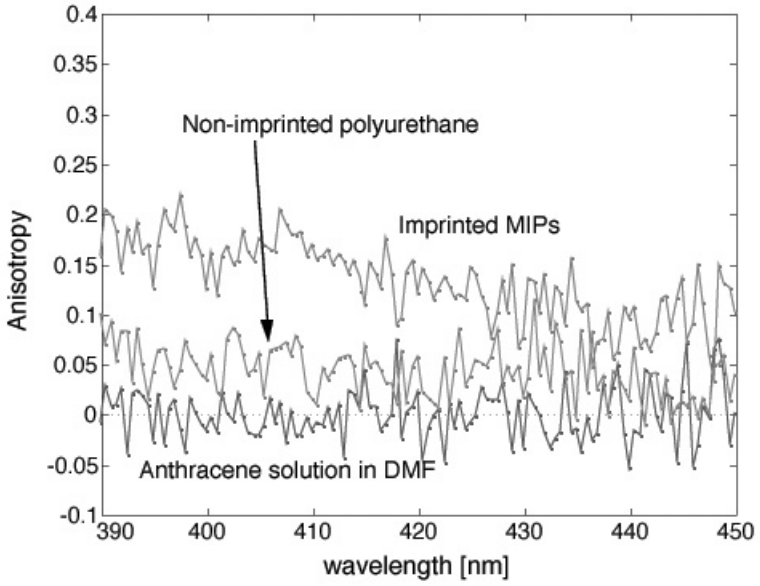

Figure 3: The fluorescence anisotropy for imprinted MIPs, non-imprinted polyurethane, and anthracene in DMF solution.

horizontal and vertical fluorescence of non-imprinted polyurethane overlap. Their calculated fluorescence anisotropy as a function of wavelength is shown in Fig. 3. As we can see, the anisotropy of non-imprinted polyurethane is lower than that of imprinted MIPs from 390 to 450 nm.

Figure 4 compares the anisotropy at 407$\pm$5 nm of four different samples: 0.18$\pm$0.05 for imprinted MIPs, 0$\pm$0.02 for extracted MIPs, 0.14$\pm$0.04 for rebound MIPs, and 0.03$\pm$0.03 for non-imprinted polyurethane. Generally, MIP samples with anthracene bound in polymer matrix have higher anisotropy values than those without anthracene.

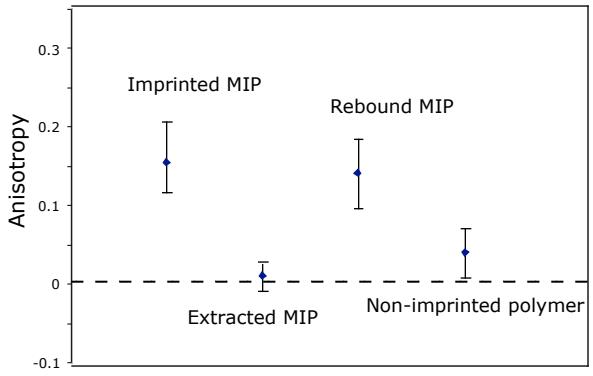

Figure 4: The comparison of imprinted, extracted, rebound MIP, and non-imprinted polyurethane in steady-state emission fluorescence anisotropy value at 407$\pm$5 nm.

CONCLUSIONS

We have investigated the fluorescence anisotropies of MIPs templated with anthracene. It is difficult to distinguish the fluorescence from polymers themselves from the imprinted fluorescent molecules. Nevertheless, our results showed that the imprinted analytes had higher fluorescence anisotropy values than the polymers themselves. This suggests that it is possible to use fluorescence anisotropy measurements to distinguish the fluorescence signals from the analytes and the signals from the polymers.

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