

Preparation of analyte-sensitive polymeric supports for biochemical sensors

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Abstract

The preparation and use of multiple polymers attached to a surface plasmon resonance (SPR) sensor for optimization of signal enhancement and minimization of fouling during sensing of biological species has been achieved. These polymers are advantageous compared to the current practice of carboxymethylated-dextran (CM-dextran). The polymers offer a wide range of functionalities and different molecular weights. Using these polymers, the SPR sensors can be fabricated as fast or faster than the CM-dextran sensor. In this study, we investigated the use of nine polymers for SPR biosensors. Polysaccharides, including CM-dextran, CM-hyaluronic acid, hyaluronic acid, and alginic acid, were investigated. Humic acid, polylactic acid, polyacrylic acid, orthopyridyldisulfide-polyethyleneglycol-*N*-hydroxysuccinimide (OPSS-PEG-NHS) and a synthesized polymer; polymethacrylic-acid-co-vinyl-acetate (PMAVA), were also used. The polymers were chemically attached to a thiol monolayer on the SPR biosensor using carbodiimide chemistry. The polymers were functionalized for binding of anti-myoglobin (anti-MG). The sensor performance was measured using myoglobin (MG) at 25 ng ml⁻¹, a biologically relevant level for myocardial infarction detection. Most polymers offered similar performance to CM-dextran for MG detection in HEPES buffer saline pH 7.4 (HBS). In preliminary studies in bovine serum, each of the candidate polymers demonstrated better performance than CM-dextran.

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1. Introduction

The use of polymeric supports for surface plasmon resonance (SPR) sensors has been restricted mainly to carboxymethylated-dextran (CM-dextran) [1], with some studies using streptavidin [2], polylysine [3], polyethyleneglycol (PEG) [4], and polyvinylphenylboronic acid [5] as a support layer. Most recent SPR studies include the binding and adsorption interactions of polymers [6–16], the optical properties of polymers [17], the growth monitoring of polymers [18], the hydration properties [19], and the use of molecularly imprinted polymers as molecular recognition elements [20,21]. The work by Gauglitz demon-

strated the use of chitosan, dextran, poly(oxyethylene), poly(ethyleneimine), and poly(acrylamide) [22] as well as a high density PEG [23] for immunoprobes on a glass slide using contact angle measurements. Baldini investigated the use of carboxylated poly(vinylchloride), polystyrene, and chloropropyl-modified sol-gel for a direct evanescent wave immunoassay using total internal reflectance fluorescence [24]. However, these polymers by Baldini were only coated to a fiber optic instead of being covalently attached to the surface. Kusnezow and Hoheisel reviewed the use of different antibody attachment technique using solid supports for immunoassays [25]. As described in a communication by Smith and Corn [26], the polymer layers are subject to non-specific binding. There is clearly a need for polymeric supports that will enhance the SPR signal by increasing the number of adsorption sites and minimizing non-specific binding, allowing SPR sensors to be used in complex matrices like serum or blood.

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The major challenge to overcome before the use of SPR in complex solutions is to reduce or eliminate sensor fouling. SPR measures any change of refractive index at the probe surface, so non-specific binding will produce an undistinguishable signal from specific binding. In the case of SPR-based immunoassays, proteins and cells will create an overwhelming signal, 10–100 times more intense than the signal from the antigen. CM-dextran fails as a support when antigens are to be detected in bovine serum due to its inability to control non-specific binding [27]. Biocompatible polymers have been used to reduce cell and protein fouling on implantable devices. Cells can be filtered out using a mesh around the probe [28,29]. However, protein fouling is still present.

This work investigates the preparation and use of SPR sensors with different biocompatible polymers to eliminate non-specific fouling. The biocompatible polymers must have carboxylic acids on their backbone to allow antibody attachment and must be able to attach a sufficient amount of antibodies to allow the antigen detection at biologically relevant concentrations. Humic acid [30], hyaluronic acid [31–33], carboxymethylated hyaluronic acid (CM-hyaluronic acid), alginic acid [34], polyacrylic acid [6], orthopyridyldisulfide-polyethyleneglycol-*N*-hydroxysuccinimide (OPSS-PEG-NHS) [35], and polymethacrylic-acid-co-vinyl-acetate (PMAVA) are biocompatible and can attach antibodies. DL-poly-lactic acid (PLA) [36] is a biocompatible polymer without any carboxylic acids on the backbone.

Myocardial infarctions (MIs) are a leading cause of death in the United States. During a myocardial infarction, the cardiac muscles are damaged and proteins or cardiac markers are released from these muscles. Currently, multiple blood samples are collected at different time intervals and cardiac marker levels are monitored in vitro to detect MI. This approach requires several hours to provide a definitive diagnosis of infarction. A sensor that can monitor the cardiac markers myoglobin (MG) and cardiac Troponin I (cTnI) in less than 10 min would improve patient care by allowing a definitive diagnosis of MI in real-time. The detection of cardiac markers MG [37,38] and cTnI [38] has been achieved at biological levels using SPR. This was done in less than 10 min in HBS, pH 7.4.

SPR theory has been extensively described [39,40]. Light undergoing total internal reflection exhibits an evanescent wave. This evanescent wave can excite a standing charge on a thin gold film (Fig. 1). The gold film is typically 50 nm thick. In order for the standing charge excitation on the gold film to occur, it must be in contact with a sample of lower refractive index than the waveguide. In order for this to occur, the wavevector of the standing charge k_{sp} and the wavevector of the evanescent wave k_x must be equal (Eq. (1)).

$$k_{sp} = k_o \sqrt{\frac{\epsilon_m \epsilon_s}{\epsilon_m + \epsilon_s}} \quad (1a)$$

$$k_x = k_o \eta_D \sin \theta_{inc} \quad (1b)$$

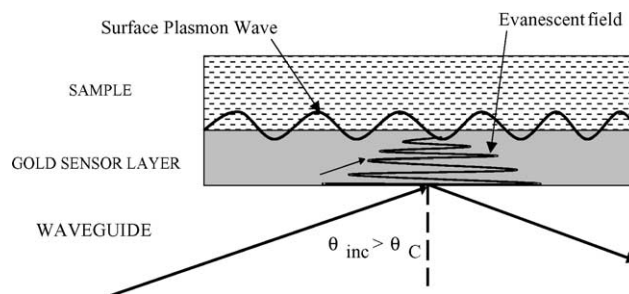


Fig. 1. SPR theory, Light excitation of a standing charge on a thin metal film from the surface.

where k_o is the wavevector of the incident light, ϵ_m and ϵ_s are the complex dielectric constants of the metal and the sample, respectively, η_D is the refractive index of the waveguide, θ_c is the critical angle of the light in the optical fiber and θ_{inc} is the incident angle of the light. Multiple combinations of incident light angles and wavelengths can excite the standing charge. When this occurs, the photon is absorbed, shown by a minimum in the reflection spectra (Fig. 2). The position of the minimum (λ_{SPR}) is indicative of the dielectric constant or the refractive index within 100–200 nm of the gold film. SPR is most sensitive for processes occurring at the surface. The sensitivity decreases exponentially for processes occurring further from the surface.

In this present study, the sensor performance for quantitative MG sensing using different biopolymers is explored. The sensor preparation is explained. The biopolymer attachment has been monitored using SPR and FTIR. Antibodies for MG have been attached to every biopolymer, except for polylactic acid, which acts as a reference. The performance of the sensors during the detection of MG from a 25 ng ml^{-1} MG solution in HBS pH 7.4 are also compared. A preliminary study to demonstrate the fouling reduction in bovine serum is presented for the polysaccharides CM-dextran, CM-hyaluronic acid, hyaluronic acid, and alginic acid. The analytical performance, sensitivity, precision, and limits of detection will be discussed in a later article currently in preparation.

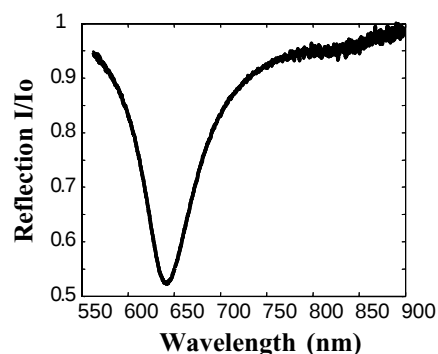


Fig. 2. SPR signal at constant angle.

2. Experimental

2.1. Sensor system construction

The manufacture of the SPR sensors used in this study has been described previously [41,42]. Here 400 μm diameter multimode fiber optics were employed for the sensor tip. However, multimode fibers as narrow as 50 μm could be used. In the current configuration, fibers 45 mm in length are cleaved. An 11 mm long piece of the buffer protecting the fiber is removed and 5 mm is replaced to protect the mirror on the distal end (Fig. 3). The distal end is polished with 5 and 1- μm -lapping films. The distal end is then washed with isopropanol and the sensor is dried at 100 °C for 10 min. A 5-nm adhesion layer of Cr is sputtered on the distal end of the sensor and a 50-nm layer of Au is deposited to form a mirror. The mirror is sealed using oven-cured epoxy. 10–15 mm of the buffer on the other end of the fiber is removed. The fiber is installed on the connector and fixed in place using oven-cured epoxy. The connector end is polished using 9, 5, and 1- μm -lapping films. The cladding on the sensing area is removed using acetone. The sensor is visually inspected using a microscope objective to insure that all the cladding has been removed. Five nanometers of Cr and 50 nm of Au is deposited on the sensing area. The sensor is rotated while being sputtered to ensure an even layer of Au. The probe performance is tested in ethanol. Fig. 3 presents one of the fiber optic probe tips to scale. Two 200 μm diameter fibers are fitted into the custom design adaptor; one fiber brings light from the white LED employed as a source, the other returns the reflected light to the spectrometer and CCD detector. A Jobin-SPEX 270M spectrometer with an 1800 g mm^{-1} grating was used to narrow the spectral range to 42.8 nm. The spectra were collected with an Andor CCD camera. A resolution of 0.0421 nm per pixel is obtained.

2.2. Ge attenuated total reflection Fourier transform infra-red spectroscopy (GATR-FTIR) system

The polymer attachment on the gold surface was monitored using GATR-FTIR. The analysis of the polymer coated glass slides was performed using a Bruker IFS66v/s FTIR with an MCT detector cooled by liquid nitrogen. A

Harrick GATR attachment was also used. The germanium crystal was washed with methyl ethyl ketone and the coated glass slides were placed face down on the crystal. The GATR attachment was placed in the FTIR and the compartment was evacuated to 1 mbar. Each transmission spectra comprised of the average of 1024 scans with the background subtracted. Precleaned glass slides were washed with acetone. A 5 nm layer of Cr and 50 nm layer of Au were deposited on the glass slide. The slides were modified chemically as described in Section 2.3. Upon completion of the reactions, the polymer coated gold slides were washed with ethanol and dried with compressed air. The slides were then analyzed by GATR-FTIR.

2.3. Preparation of the polymer layers

2.3.1. CM-dextran, CM-hyaluronic acid, and hyaluronic acid layer preparation

The synthesis of these layers is based on the CM-dextran chemistry used elsewhere for protein immobilization on an SPR surface [43,44]. All reactions occur in aqueous solution without any stirring or shaking. The bare gold surface on the SPR probe is contacted overnight with 0.005 M 11-mercaptoundecanol (Aldrich, Milwaukee, WI) in an 80:20 solution of ethanol and water to form a self-assembled monolayer (SAM). This SAM is reacted with 0.6 M epichlorohydrin in a 1:1 mixture of diglyme and 0.4 M NaOH for 4 h. This layer is washed with water, ethanol, and water again. The surface is reacted for 20 h with an aqueous solution containing 0.3 g ml^{-1} dextran (Spectrum, Gardena, CA) or 0.3 g ml^{-1} hyaluronic acid (Fisher, Hampton, NH) and 0.1 M NaOH. Stopping at this stage is producing a hyaluronic acid layer on the sensor when using hyaluronic acid. The resulting matrix is modified to a carboxymethylated matrix by reaction with 1 M bromoacetic acid in 2 M NaOH for 16 h.

2.3.2. Alginic acid, humic acid, DL-polylactic acid, and polyacrylic acid layer preparation

The bare gold surface on the SPR probe is reacted with 11-mercaptoundecanol, with epichlorohydrin, and washed as described in Section 2.3.1. The probe is contacted with a 1 M ethanolamine solution at pH 8.5 for 20 h. The sensor is then equilibrated for 15 min in water. Meanwhile, a 1:1 solution of 0.4 M EDC (*N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride) and 0.01 M NHS (*N*-hydroxysuccinimide) is reacted for 5 min. A 10 mg ml^{-1} solution (alginic acid, Aldrich, Milwaukee, WI; polyacrylic acid, Polysciences, Warrington, PA), or 5 mg ml^{-1} solution (DL-polylactic acid, Polysciences, Warrington, PA), or 2 mg ml^{-1} (humic acid, Aldrich, Milwaukee, WI) is mixed 1:1 with the EDC-NHS solution and equilibrated for 10 min. The sensor is reacted with the polymer-EDC-NHS solution at 50 °C for 16 h for alginic acid, humic acid, and polyacrylic acid or 20 min at 50 °C for polyacrylic acid.

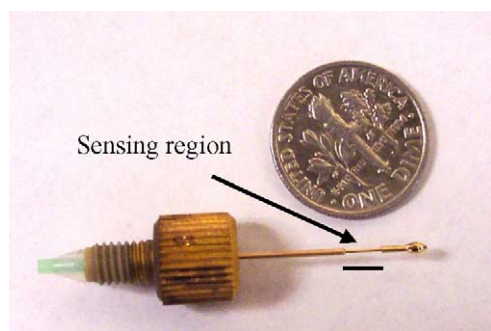


Fig. 3. Optical fiber SPR sensor.

2.3.3. PMAVA layer preparation [45]

The bare gold surface on the SPR probe is reacted with 11-mercaptoundecanol, with epichlorohydrin, and washed as described in Section 2.3.1. The probe is contacted with a 1 M ethanolamine solution at pH 8.5 for 20 h. The sensor is then equilibrated for 15 min in water. Meanwhile, a 1:1 solution of 0.4 M EDC and 0.01 M NHS is reacted for 5 min. A 10 mg ml⁻¹ solution of 4,4' Azobis(4-cyanovaleric acid) (AIBN) is mixed 1:1 with the EDC-NHS solution and equilibrated for 10 min. AIBN does not fully dissolve in water. The suspension is used as it is. Then the sensor is reacted with the AIBN-EDC-NHS solution at room temperature for 20 min. The sensor is washed in water for 5 min. The sensor is placed in a hot solution, 60 °C, of 0.5 ml of methacrylic acid, 0.5 ml of vinyl acetate, and 1 ml of ethanol. Then the temperature is increased to 80 °C and maintained until the polymerization begins in the solution and the solution boils. This process requires 5 min to occur. These phenomena occur simultaneously. The probe is finally rinsed in ethanol.

2.3.4. OPSS-PEG-NHS layer preparation

OPSS-PEG-NHS is a custom synthesis from Nektar (Huntsville, AL, USA). It was used as described by Hirsch et al. [35]. OPSS-PEG-NHS was reacted overnight at 4 °C with anti-MG in 100 mM NaHCO₃ at pH 8.5. The concentration of anti-MG and PEG-NHS was 1.2 mg ml⁻¹. Two hundred microliters of the OPSS-PEG-anti-MG solution was diluted to 2 ml with 1.8 mM K₂CO₃. The gold probes were reacted for 24 h at 4 °C with the OPSS-PEG-anti-MG K₂CO₃ solution.

2.4. Anti-MG attachment to the sensor

After the polymers are immobilized on the probes, their surfaces are activated by immersion in 1:1 aqueous solutions of 0.4 M EDC and 0.01 M NHS for 10 min. An amine coupling is performed on this activated surface by reaction with a 700 µg ml⁻¹ solution of human anti-MG (ICN Biochemicals, polyclonal rabbit antiserum to human MG, K_A and k_A are not available) at pH 4 (10 mM sodium acetate buffer) and 37 °C for 20 min. Next, non-specifically bound proteins are washed away and the non-reacted sites on the polymers are deactivated by rinsing the probes with an aqueous solution of 1 M ethanolamine at pH 8.5 for 10 min. Finally, the probes are dipped in 25 ng ml⁻¹ buffered aqueous solutions of MG to test their performance. The measurement is done in a static solution at 25 °C. The temperature is controlled to about 0.5 °C using a water bath.

2.5. Sensor fouling

The technique used to measure serum fouling has been previously described [27]. The sensors with CM-dextran, CM-hyaluronic acid, hyaluronic acid and alginic acid are prepared as described above. Anti-MG functionalized sen-

sors are then placed in a bovine serum solution at 0 °C and measurement of λ_{SPR} is made daily for 14 days.

3. Results

3.1. Polymer attachment monitoring

The polymer attachment on the SPR sensor was monitored in two different ways. First, a near real-time analysis of the SPR signal can provide useful information on the polymer binding on the sensor. A second approach is to prepare a gold-coated glass slide and analyze the final sensor using GATR-FTIR. The sensor preparation time varies from 1 to 4 days. The CM-dextran sensor takes the longest to prepare, 4 days. Most of the other sensors take 3 or 4 days to prepare except for the sensors with OPSS-PEG-NHS, which takes only 1 day.

3.1.1. SPR analysis

The polymers bound to the probes have a higher refractive index than the water into which the probes are immersed. They will induce a red-shift when they attach to the surface compared to the signal of a stable intermediate in water alone. The amount of red-shift can be related to the surface coverage of the polymer. Larger red-shifts signify larger amounts of polymer on the surface. Therefore, the reaction conditions in Section 2.3 were optimized to maximize this shift. Table 1 includes the shift for five polymers used in this experiment. The shift is used to compare the antibody binding to the sensor instead of the surface coverage because the relative performance is compared. The polymers are three-dimensional structures, so using a two-dimensional coverage would not include the polymer thickness. However, calculating the three-dimensional coverage of the antibodies in the polymer matrix is next to impossible. The shifts for the polysaccharides were not monitored because of the reaction not allowing an easy intermediate and stable step before the polymer attachment to the sensor. The shift varies from 1.8 to 9.5 nm. The smallest shift is for humic acid. Humic acid induces a smaller shift than the other polymers because only a small fraction of the humic acid reacts with the surface. Upon reaction, a precipitate can be found in the vial, which comes from a large fraction of humic acid agglomerating at the high reaction temperature of 50 °C. OPSS-PEG-NHS has a similar molecular weight to humic acid, but the reaction sequence requires the antibody reaction prior to the polymer attachment to the sensor. Therefore, the shift reported in

Table 1
SPR shift resulting from polymer attachment on the probe

Polymer	Shift (nm)	Polymer	Shift (nm)
OPSS-PEG-NHS	6.6 ^a	Humic acid	1.8
Polyacrylic acid	2.2	Polylactic acid	9.5
PMAVA	9.5		

^a Includes the shift from the antibody binding on the polymer.

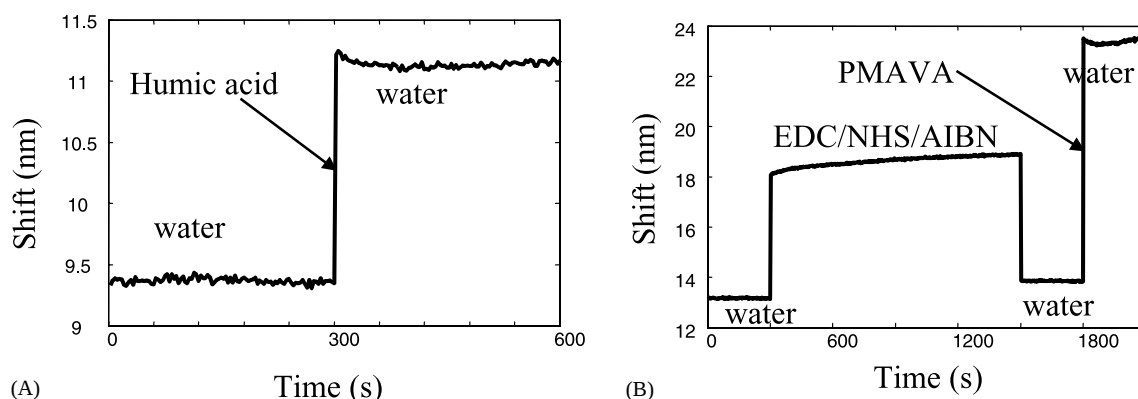


Fig. 4. Sensorgram for the polymer attachment to the SPR sensor: (A) Humic acid attachment to the free amine and (B) PMAVA reaction sequence.

Table 1 for OPSS-PEG-NHS includes the shift induced by the antibody. Fig. 4A shows the kinetic sensorgram for a water sample before and after the reaction of humic acid and the surface. The binding reaction cannot be monitored in real-time due to the absence of an in-line temperature controller other than room temperature control exerted with a water bath. Therefore, water is used as a reference point, and the shift presented in Table 1 was calculated using water as a reference point. The stability of the water signal indicates that the polymer attaching to the surface caused the shift. Fig. 4B shows the sensorgram for the preparation of the PMAVA sensor. An on-sensor polymerization technique has been developed recently [45]. The initiator is attached to the surface as shown in the EDC/NHS/AIBN part of the sensorgram. Then, the sensor is immersed in the polymerization solution including the monomers, vinylacetate, methacrylic acid, and ethanol solvent. The polymerization occurs at high temperature by the radical breaking of the initiator. These data are not shown, since it is not possible to distinguish the exothermic polymerization reaction from the temperature fluctuations at the sensor's surface. A sensor without the initiator was prepared, and no difference in the SPR signal as well as any polymerization was noted. Finally, the sensor signal is measured again in the reference water solution to measure the shift. The polymerization on the probe seeds polymerization in the bulk solution. The polymer created by this process was collected and analyzed using Raman spectroscopy to confirm the reaction.

3.1.2. GATR-FTIR characterization

The GATR-FTIR experiment was performed on gold coated glass slides instead of the fiber-optic based sensors described in Section 3.1.1. Fig. 5 shows the GATR-FTIR spectra for every polymer attached to the SPR sensor. The GATR-FTIR was performed on eight of the nine coatings. This technique was not needed to verify the polymer attachment for polyacrylic acid because the polymer was visible on the surface. Two regions of interest were monitored. Each polymer shares similar bands from the presence of carboxylic acids on their backbone. The uniqueness lies in the band position and relative intensity.

The regions of C=O vibration, around 1650 and 1750 cm^{-1} , were analyzed to see the carboxylic acid, amide, and ester bands of the polymer. The C–H region around 3000 cm^{-1} was also analyzed. Finally, a comparison to ensure the uniqueness of the fingerprint region between 1400 and 1000 cm^{-1} was done. A close comparison of some similar polysaccharides, e.g. alginic acid, CM-dextran, CM-hyaluronic acid, and hyaluronic acid (Fig. 5B–E, show distinctive differences in the regions of interest. Although they have the characteristic bands at 1700 cm^{-1} , the relative intensity is different for each polymer, and the fingerprint regions differ greatly. OPSS-PEG-NHS, PMAVA, humic acid, and polylactic acid (Fig. 5A, F–H) all have distinct spectra. They also differ from the thiol-amine linker used to attach the polymer to the gold surface (Fig. 5I).

3.2. Analyte-sensitive properties

Two different approaches are taken to monitor the sensor's performance. First, the degree of shift caused by the antibody attachment is an indication of the surface coverage of the antibody on the sensor. Next, the amount of shift caused by probe immersion in a 25 ng ml^{-1} saline solution of MG is used to monitor the sensor's performance.

3.2.1. Antibody binding on the sensor

The antibody loading was performed as previously optimized [38]. The reaction was performed at pH 4 and 37 °C. It was optimized for the CM-dextran polymer. The shift reported in Table 2 was calculated from the sensors' response in a reference media, HEPES buffer saline pH 7.4 (HBS), before and after the reaction with the antibody. Fig. 6 shows how the shift was calculated. The shift for OPSS-PEG-NHS was calculated for OPSS-PEG-anti-MG. Therefore, it contains the shift induced by anti-MG and the polymer. There is no trend relating the molecular weight and the amount of anti-MG binding to the polymer. This can be explained by the fact that the layer preparation can induce some aggregation for alginic acid, humic acid, polyacrylic acid, and polylactic acid. Usually, the number of binding sites for the anti-MG increases using larger polymers as previously

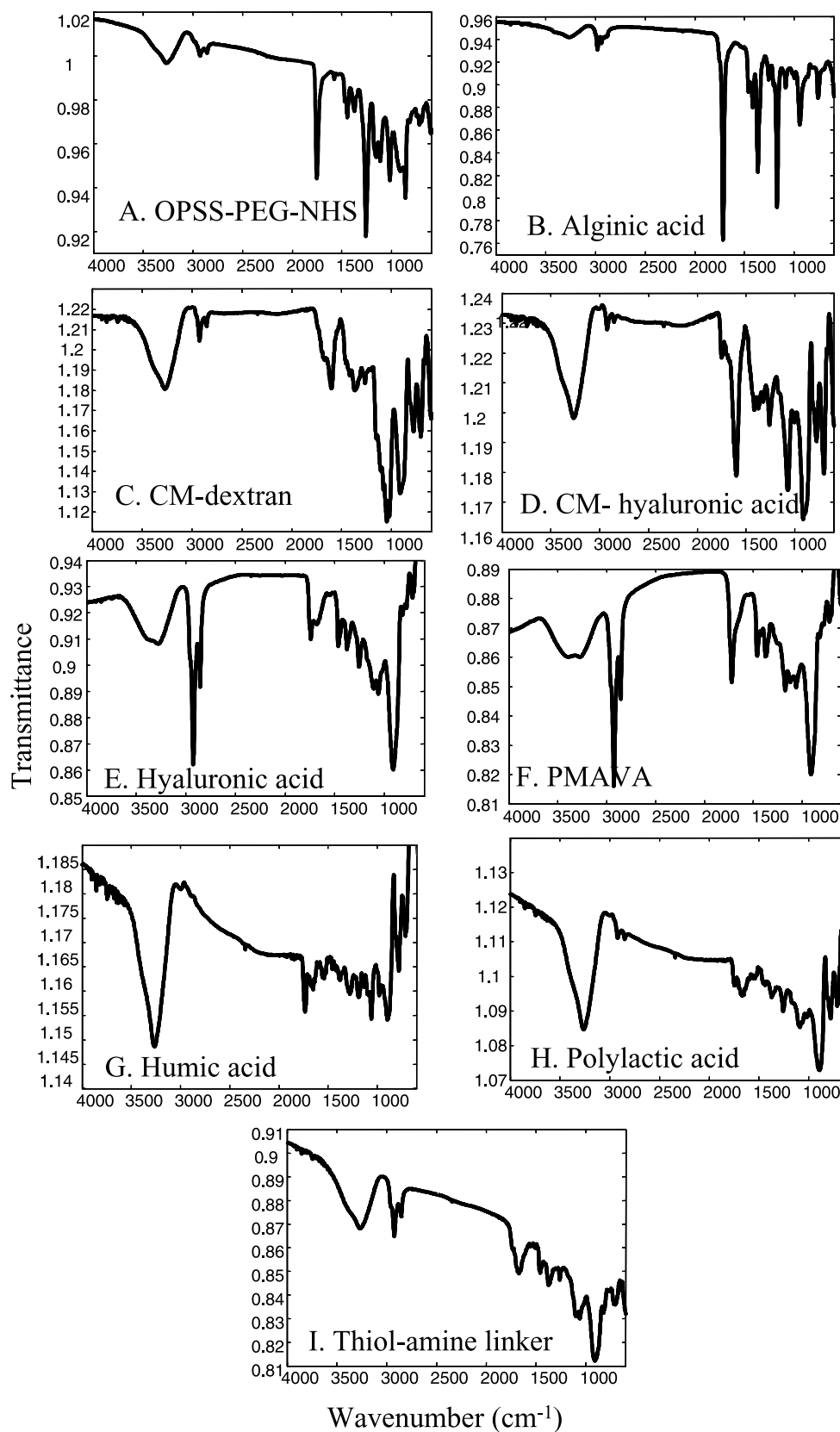


Fig. 5. GATR-FTIR spectra for (A) OPSS-PEG-NHS, (B) alginic acid, (C) CM-dextran, (D) CM-hyaluronic, (E) hyaluronic, (F) PMAVA, (G) humic acid, (H) polylactic acid, and (I) thiol-amine linker.

Table 2

Sensor performance for anti-MG binding and MG detection with different biocompatible polymers

Polymer	Molecular weight (Da)	Antibody shift (nm)	MG shift (nm)
OPSS-PEG-NHS	2000	6.6 ^a	0.082
Polyacrylic acid	50,000	6.8	0.050
CM-dextran	500,000	10.4	0.132
CM-hyaluronic acid	>1,000,000	6.1	0.082
Hyaluronic acid	>1,000,000	3.0	0.020
Alginic acid	12,000–80,000	10.6	0.138
PMAVA	N/A	5.0	0.050
Humic acid	2,000–500,000	3.2	0.041
Polylactic acid	330,000–600,000	4.9	0.056

^a Includes the shift from the polymer.

demonstrated by Masson et al. [38]. An increase in sensitivity using larger CM-dextran up to 500,000 kDa was shown. The molecular weights for polymers used in this study range from 2000 Da to larger than 1,000,000 Da, but the shift does not correspond to the molecular weight. For example, alginic acid has the same shift as CM-dextran, but a molecular weight about ten times smaller. Attempts to measure the molecular weight for PMAVA by mass spectrometry and gel-permeation chromatography were not successful. Every polymer showed a shift for the anti-MG binding. The shift for polylactic acid is believed to come from non-specific binding of anti-MG. Specifically, anti-MG can be trapped in the polymer.

3.2.2. Sensor performance to detect MG

The sensor's performance in 25 ng ml⁻¹ MG saline solution was measured for every polymer. Fig. 7 shows an example sensorgram of antigen binding using CM-dextran. It also shows how the shift was calculated for the MG binding. A larger shift with MG denotes a more sensitive sensor. The polymers showing the larger shift were CM-dextran and alginic acid. However, every polymer showed a detectable signal for this solution. Only hyaluronic acid has a very weak signal of 0.02 nm, which is the detection limit with the system used. The signal for polylactic acid comes from the non-specific binding of anti-MG. Table 2 summarizes the results obtained for anti-MG and MG performance. There is an interesting and predictable correlation between

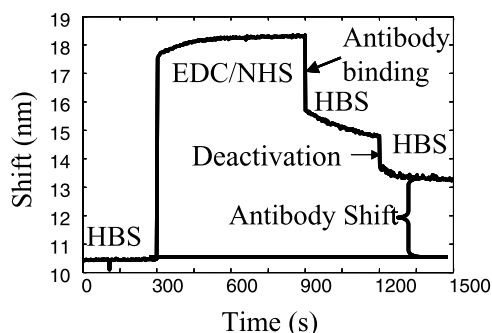


Fig. 6. Example of a sensorgram for the antibody binding to the polymers.

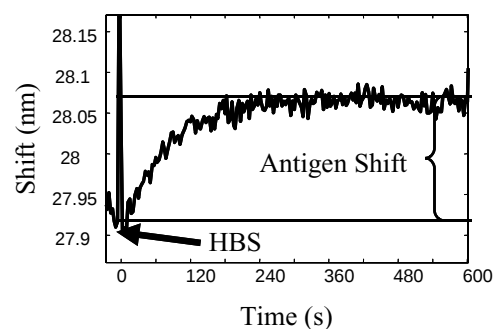


Fig. 7. Antigen binding curve.

the anti-MG shift and the MG shift. The MG shift is directly proportional to the anti-MG shift (Fig. 8). This demonstrates that the antibodies are reacting similarly regardless of the polymer used to make the SPR sensor. Every polymer used is able to detect a biologically relevant level of MG. The polymers have different molecular weights, eliminating the need for very large polymers to achieve the desired detection levels. This means that CM-dextran can be replaced, which will have a great deal of interest for large scale manufacturing of the sensors. It will eliminate the dextran solution that is very viscous and hard to manipulate. Many different polymers can be used for biosensors in general. Use of the polymers is not limited to SPR, it can also be used in electrochemistry, localized surface plasmon resonance (LSPR) or evanescent field fiber-optic fluorescence. It also shows that these specific polymers do not interfere differently with the performance of the antibodies.

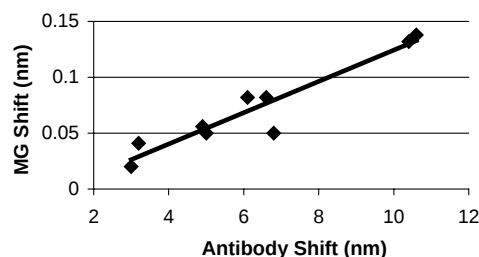


Fig. 8. Correlation between the Antibody shift and the MG shift.

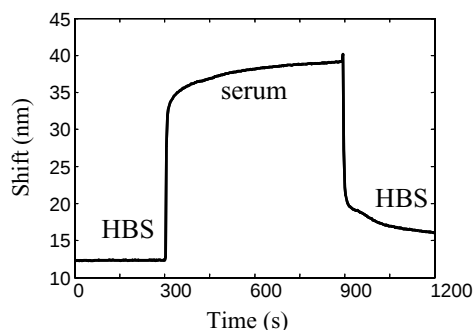


Fig. 9. CM-dextran sensor fouling in a bovine serum solution.

3.3. Preliminary results for biofouling

3.3.1. Dextran

In order to use the SPR sensors in complex solutions, the signal from serum or blood must be negligible. As shown in CM-dextran sensors will foul quickly in a complex solution (Fig. 9). To make this plot, a sensor was placed in a bovine serum solution for 10 min and the output was monitored. The signal for the bovine serum is around 10–100 times the signal of cTnI or MG at the low ng ml^{-1} concentration range. Therefore, the signal from the antigen cannot be detected in a serum solution. Bovine serum was used for its low cost and because its protein concentration is similar to that found in human serum. To further check the possibility that one can distinguish the antigen signal from the signal due to non-specific binding of serum proteins, a dual sensor system was assembled with a reference sensor to account for serum fouling. One of the sensors had antibodies on its surface (sensing) and the other had CM-dextran only (reference). However, the signal from a serum solution spiked with the antigen was so large that simple probe to probe variance will be large enough to “mask” the signal from antigen binding. A reference probe cannot be used to eliminate the background signal from serum. The sensor was also placed in contact with HBS for 14 days in the same conditions as with serum. No difference in the signal was noted with HBS after 14-day exposure [38].

A set of sensors without immobilized antibodies was prepared to compare the amount of fouling relative to the set of sensors with immobilized antibodies. The signal is statistically the same for the sensors with or without antibodies. This rules out any possibility of a localized fouling on the antibodies. Fig. 10 shows the signal from serum is the same for both anti-MG functionalized sensors and CM-dextran only sensors. Therefore, using this method to investigate the fouling of the polymer gives a correlation independent of the amount of antibodies bounded to the surface.

When sensors foul in serum, there is an electrostatic attraction between the proteins and the negatively charged polymer. The polymer molecular weight influences the fouling, such that larger polymers will show more fouling because they can physically trap more serum proteins than

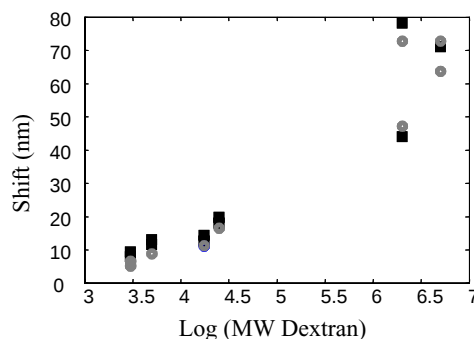


Fig. 10. Serum fouling measured by the shift (nm) after 14-day exposure for sensor with CM-dextran (gray) and CM-dextran with anti-MG on the surface (black).

smaller proteins (steric interactions). Non-specific binding to the antibodies is also possible but this is a minor fouling effect compared to the interactions with the polymer. This work demonstrates that changing the polymeric support can have a significant effect on probe fouling by proteins in solution.

3.3.2. Polysaccharides biopolymers

The sensors were prepared as described above. Also as described above, anti-MG functionalized sensors were placed in a bovine serum solution at 0°C and measurement of λ_{SPR} is made daily for 14 days. Every sensor was measured once a day. The time required to measure the signal for each sensor is about 30 s. Measuring every sensor takes around 10 min, therefore the measurement is considered to be simultaneous. The sensor to sensor variability is 0.5 nm. The serum in this experiment comes from a single batch.

As shown in Fig. 11, CM-dextran shows the worst fouling performance. Alginic acid produces results very similar to those produced when CM-dextran is applied. The fouling of each candidate polymer was normalized to that observed when CM-dextran was applied, and the amount of fouling decreases from CM-dextran (100%) > alginic acid (97%) > CM-hyaluronic acid (44%) > hyaluronic acid (41%). This demonstrates clearly that a sensor's fouling in serum can be greatly reduced. CM-hyaluronic acid demonstrates 41%

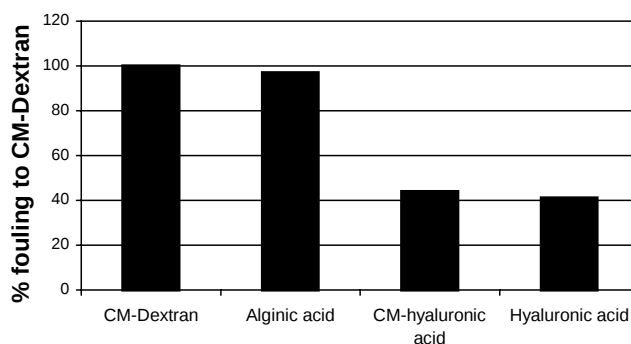


Fig. 11. Sensor fouling using different polysaccharides.

of the fouling of CM-dextran, and is 62% as sensitive as the CM-dextran. As a result, the overall performance of the sensor is improved using CM-hyaluronic acid in place of CM-dextran. CM-hyaluronic acid has fewer carboxylic acids on the sugar structure than CM-dextran, which explains the reduced sensitivity toward MG (fewer antibodies) but also explains the better performance (reduced fouling) in serum. CM-dextran has six carboxylic acids per two sugar subunits, while CM-hyaluronic acid has five carboxylic acids per two subunits, and hyaluronic acid only has one carboxylic acid per two subunits. However, since the signal due to fouling from serum proteins should be as low as possible, more experimentation is needed to optimize the polymers for minimal fouling and optimal sensitivity.

4. Conclusions

A variety of polymers were evaluated as replacements for CM-dextran as polymeric supports for biosensors. CM-hyaluronic acid, hyaluronic acid, alginic acid, humic acid, polylactic acid, polyacrylic acid, OPSS-PEG-NHS, and PMAVA were synthesized and were chemically attached to the SPR sensors. The SPR signal from the sensors was monitored to ensure that the polymers were attached to the surface. Glass slides coated with Au were treated in the same fashion as the SPR sensors. GATR-FTIR was performed on the slides to confirm the polymer attachment to the gold surface of the SPR sensors. Antibodies for MG were chemically bonded to the polymers and the sensors were immersed in 25 ng ml⁻¹ MG saline solution. The best performance to detect MG was obtained for alginic acid and CM-dextran. Every polymer was able to bind anti-MG and detect biologically relevant levels of MG. Probes fabricated using CM-dextran to bind anti-MG to the sensors were unable to detect MG in serum. A series of polysaccharides were used in place of CM-dextran, and the responses of the resulting probes were monitored in serum. These showed less fouling than probes fabricated using CM-dextran. This indicates that changing the polymer supporting the antibodies on the SPR sensor can improve the sensor's performance in serum. CM-hyaluronic acid and hyaluronic acid decreased by about 60% the amount of non-specific binding on the SPR sensor. To minimize the serum fouling, the polymer must reduce the electrostatic interactions and the steric interaction between the polymer and the serum proteins. This work is in progress and more coating will be tested to optimize sensor performance and minimize sensor fouling.

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