Materials Science and Engineering C 33 (2013) 3609–3614

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Microcontact imprinted surface plasmon resonance sensor for myoglobin detection

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ARTICLE INFO

Article history:
Received 20 June 2012
Received in revised form 12 April 2013
Accepted 20 April 2013
Available online 26 April 2013

Keywords:
Molecularly imprinted polymer
Microcontact imprinting
SPR sensor
Myoglobin
Nanofilm

ABSTRACT

In this study, we prepared surface plasmon resonance (SPR) sensor using the molecular imprinting technique for myoglobin detection in human serum. For this purpose, we synthesized myoglobin imprinted poly(hydroxyethyl methacrylate-N-methacryloyl-L-tryptophan methyl ester) [poly(HEMA-MATrp)] nanofilm on the surface of SPR sensor. We also synthesized non-imprinted poly(HEMA-MATrp) nanofilm without myoglobin for the control experiments. The SPR sensor was characterized with contact angle measurements, atomic force microscopy, X-ray photoelectron spectroscopy, and ellipsometry. We investigated the effectiveness of the sensor using the SPR system. We evaluated the ability of SPR sensor to sense myoglobin with myoglobin solutions (pH 7.4, phosphate buffer) in different concentration range and in the serum taken from a patient with acute myocardial infarction. We found that the Langmuir adsorption model was the most suitable for the sensor system. The detection limit was 87.6 ng/mL. In order to show the selectivity of the SPR sensor, we investigated the competitive detection of myoglobin, lysozyme, cytochrome c and bovine serum albumin. The results showed that the SPR sensor has high selectivity and sensitivity for myoglobin.

1. Introduction

Molecularly imprinted polymers (MIPs) are artificial receptors with the ability to recognize and to bind specifically with the target molecules [1–4]. Due to their stability, ease of preparation and low cost, the researchers have assessed using these MIPs as substitutes for antibodies or enzymes in chemical sensors, catalysis, and separations [5–9]. Although creating MIP for all molecules is straightforward now, the imprinting of large structures, such as proteins, is still a challenge [10]. The major problem associated with the imprinting of large structures is their restricted mobility within highly cross-linked polymer networks and their poor efficiency in rebinding. Up to now, imprinting on the surface seemed to be the most promising way to overcome these difficulties [11,12]. Recently, Chou et al. [13] reported a microcontact imprinting (μCIP) method to prepare nanofilm with C-reactive protein on a glass surface. Using the μCIP approach, the researchers imprinted polymeric films with ribonuclease A [14], lysozyme [15], and myoglobin [16] on a glass surface and obtained promising results for potential sensor applications. Several studies have shown the potential of MIPs in combination with surface plasmon resonance (SPR) sensors that enable the analysis and detection of chemical and biological compounds without the need for biomolecule labeling [17–21]. But, MIP-based SPR sensors prepared for proteins are more rare than those prepared for small molecules, probably because of the challenge of protein imprinting. Matsunaga et al. [21] successfully prepared MIP-based SPR sensor for lysozyme detection. Uzun et al. synthesized hepatitis B surface antibody (HBsAb) imprinted nanofilm on a SPR sensor gold surface for the diagnosis of HBsAb in human serum [22].

Acute myocardial infarction (MI) remains the leading cause of mortality in the world and represents an enormous cost to the health care system. Cardiac markers have long been the cornerstone of diagnosis and continue to play an important role, especially in the group of patients with low to medium risk. A single marker that meets all of the criteria has yet to be found, therefore, a multi-analyte diagnostic approach would be the best. Biochemical markers used to diagnose acute MI are myoglobin, creatine kinase-myocardial band (CK-MB), and cardiac troponins. Myoglobin is a 17.6 kDa heme protein in the cytosol of skeletal and cardiac muscle but not smooth muscle. Because of its small size, myoglobin is rapidly released from the areas of ischemic injury. It is significantly elevated as early as 1 h after the onset of MI with peak levels occurring at 3–15 h [23]. At presentation and within the first few hours after chest pain, the sensitivity of myoglobin is greater than that of CK-MB and troponins (T and I) [24]. However, myoglobin lacks specificity. Patients with renal failure, skeletal muscle injury, or trauma may have abnormal concentrations of myoglobin in the absence of acute MI. This limitation may be resolved by the combined measurement of myoglobin and a skeletal specific marker (carbonic anhydrase III) or a cardiac specific marker (troponin I) [25].

In this study, a SPR sensor for myoglobin detection was prepared using the microcontact imprinting technique. For this purpose, myoglobin-imprinted poly(hydroxyethyl methacrylate-N-methacryloyl-L-tryptophan methyl ester) [poly(HEMA-MATrp)] nanofilm was synthesized on a gold surface of a SPR sensor. First, we modified the glass surface

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0928-4931/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.msec.2013.04.041

of lamella and the gold surface of SPR sensor and carried out the polymer film formation. The changes on the surfaces were determined using atomic force microscopy (AFM), contact angle (CA) and X-ray photoelectron spectroscopy (XPS) and ellipsometry. We studied the myoglobin detection from aqueous myoglobin solutions. In order to show selectivity of microcontact-imprinted poly(HEMA-MATrp) myoglobin (μCIP) SPR sensor, we used bovine serum albumin (BSA), lysozyme, and cytochrome c as competitor proteins. We calculated the kinetic and isotherm parameters and investigated the myoglobin detection capability of the prepared SPR sensor in human serum.

2. Experimental

2.1. Materials

Myoglobin was supplied by Sigma (Steinheim, Germany). We obtained L-tryptophan methyl ester, methacryloyl chloride, and allyl mercaptan from Sigma Chemical Co. (St. Louis, USA). We purchased hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA) and N,N′-azobisisobutyronitrile (AIBN) from Fluka A.G. (Buchs, Switzerland). We distilled HEMA and EGDMA under reduced pressure in the presence of hydroquinone inhibitor and stored at 4 °C until use. The gold surfaces (gold coated SF10 glass) for SPRimager II instrument were purchased from GWC Technologies (Madison, ABD). All other chemicals were of reagent grade and purchased from Merck A.G. (Darmstadt, Germany).

2.2. Synthesis of N-methacryloyl-L-tryptophan methyl ester (MATrp) monomer

The synthesis and characterization of functional monomer N-methacryloyl-L-tryptophan methyl ester (MATrp) were reported previously [26,27]. In the synthesis reaction, L-tryptophan methyl ester (5.0 g) and hydroquinone (0.2 g) were dissolved in 100 mL of dichloromethane. This solution was cooled to 0 °C. Triethylamine (12.7 g) was added into the solution. Methacryloyl chloride (5.0 mL) was poured slowly into this solution and then stirred magnetically at room temperature for 2 h. At the end of the chemical reaction, hydroquinone and unreacted methacryloyl chloride were extracted with a 10% NaOH solution. The aqueous phase was evaporated in a rotary evaporator. The residue (i.e., MATrp) was recrystallized in ethanol.

2.3. Preparation of the μCIP SPR sensor

The following procedures were used to prepare the poly(HEMA-MATrp) nanofilm on the gold surface of SPR sensor: (1) cleaning of lamellas used to make the protein stamp, (2) modifying glass surface with aminopropyl triethoxysilane (APTES) and glutaraldehyde (GA), followed by covalent immobilization of the myoglobin and pretreatment of monomer mixture, (3) allylmercaptan modification of the gold surface of the SPR sensor, (4) bringing the protein stamp into contact with the modified gold surface of the SPR sensor, allowing microcontact imprinting of the protein into the monomer solution [16], (5) UV irradiation to initiate polymerization and fix the imprinted orientation of monomers, and (6) removing the protein template.

2.3.1. Preparation of protein stamps

To prepare the protein stamps, we modified cover glasses in batches (each consisting of approximately 10 pieces) and cleaned them sequentially (30 min each step) in 100 mL of neutral cleaner (2 vol%; SoDosil® 02), deionized water, isopropanol, ethanol, and, finally, with deionized water in an ultrasonic bath at 55 °C. Each cover glass was then dried with nitrogen gas [15].

Prior to use, the cleaned glass slides were immersed in a 70/30 (v/v) mixture of H_2SO_4 and 30% H_2O_2 and dried in a vacuum oven for 2 h at 80 °C followed by washing with deionized water. The slides were immersed in 5% (v/v) APTES in acetone for 30 min at room temperature to introduce amino groups onto the surfaces of slides, then rinsed thoroughly with acetone and deionized water to remove any non-bound silane compounds. The prepared slides were dried in a vacuum oven at 80 °C for 2 h. The amino groups on the APTES silanized surfaces of the slides were activated via GA to covalently immobilize myoglobin. Specifically, the silanized slides were immersed in 2.5% (v/v) GA solution in a 0.1 M phosphate buffer (pH 7.4) and kept quiescent for 2 h at room temperature. Then, the slides were rinsed thoroughly with a phosphate buffer (pH 7.4) and dried with nitrogen gas. After that, the cover glasses were then incubated with 0.01 mg/mL myoglobin solution (pH 7.4, phosphate buffer) at 4 °C for 24 h and then dried with nitrogen gas. The cover glasses used for preparing non-imprinted nanofilm were incubated with phosphate buffer (pH 7.4) and dried with nitrogen gas.

2.3.2. Allyl mercaptan modification of the SPR sensor

The surface of the gold SPR sensor was modified with allyl mercaptan (CH_2CHCH_2SH). Before its modification, the SPR sensor was cleaned with alkaline piranha solution (3:1, NH_4OH:H_2O_2, v/v). The SPR sensor was immersed in 20 mL of alkaline piranha solution for 5 min. Then, it was washed with pure ethyl alcohol and dried in a vacuum oven (200 mm Hg, 40 °C) for 3 h. After that, the SPR sensor was immersed in an ethanol/water (4:1, v/v) solution containing 3.0 mM allyl mercaptan for 12 h. Finally; it was thoroughly rinsed with ethanol and dried with nitrogen gas under vacuum (200 mm Hg, 40 °C).

2.3.3. Preparation of myoglobin-imprinted nanofilm on SPR sensor

Myoglobin imprinted nanofilm on allyl mercaptan modified SPR sensor was prepared using the following steps: First, HEMA, MATrp, and EGDMA (in mol ratio of 1:1:5) were mixed at room temperature for 2 h. Then, AIBN (5 mg) was dissolved in a monomer mixture to prepare the stock monomer solution. After that, 5 μL aliquot was taken from the stock monomer solution and dropped onto the surface of the protein stamp, which was stored at 4 °C for 30 min to pre-organize the template (myoglobin) with functional monomer (MATrp). Then, the gold face of the SPR sensor was placed into this solution. Polymerization was initiated using UV light at room temperature (100 W, 365 nm) and was continued for 4 min at room temperature under nitrogen atmosphere. After the polymerization process, the protein stamp was stripped from the SPR sensor surface. The nanofilm coated SPR sensor was then washed, first with 0.1 M HCl/methanol (1:1, v/v) and then with methanol. Finally, it was dried in a vacuum oven.

2.4. Surface characterization studies

2.4.1. Contact angle measurements

KSV Attention Tetha instrument (Hamburg, Germany) was used to determine the contact angles (CA) of the SPR sensor and glass surfaces. The contact angles of the surfaces were measured via using taking 40 separate photos from the different parts of surfaces. In order to determine the surface free energy (SFE) of the SPR sensors, water and ethylene glycol were used by applying the Owens–Wendt method.

2.4.2. Atomic force microscopy studies

In order to characterize the SPR sensor and glass surfaces, the non-contact mode atomic force microscope (AFM) (Nanomagnetics Instruments, Oxford, England) was used. Visualization studies were carried out in non-contact mode. Sample area of $1 \times 1\ \mu m^2$ was displayed with a 2 μm/s scanning rate and a resolution of 256×256 pixels.

2.4.3. X-ray photoelectron spectroscopy studies

We analyzed the chemical composition of the bare glass and protein stamp using an XPS apparatus (PHI-5000, USA). The energy of excitation source, monochromatic Al K_α radiation, was 1486.6 eV,

and the survey scan range was 0–1100 eV. The electron take-off angle was fixed at 45°. After scanning the spectrum for 2–3 min, the peaks were recorded over narrow ranges for C1s, O1s, N1s, S2p, and Si2p for 4–5 min.

2.4.4. Ellipsometry

Ellipsometer measurements were carried out using an auto-nulling imaging ellipsometer (Nanofilm EP3, Germany). All thickness measurements were performed at a wavelength of 532 nm with an angle of incidence of 62°. In the layer thickness analysis, a four-zone auto-nulling procedure integrating a sample area of approximately 50 μm × 50 μm was followed by a fitting algorithm. Measurements were carried out as triplicate at 6 different points of SPR sensor surface, and the results were reported as mean value of the measurements with standard deviations.

2.5. Kinetic, isotherm and selectivity studies with SPR sensor

SPR sensors prepared from myoglobin-imprinted (μCIP) and non-imprinted poly(HEMA-MATrp) nanofilms (NIP) were used for kinetic analysis by SPRimager II (GWC Technologies, Madison, ABD). Firstly; the sensor surface was washed with 50 mM NaOH solution (50 mL, 150 μL/min flow-rate) and deionized water (50 mL, 150 μL/min flow-rate). Then, the surface was washed with a pH 7.4 phosphate buffer for 30 min. After that, the mirror system was arranged in a resonance angle, and kinetic studies were done at this angle value. Sequentially, the buffer circulation using the SPR system continued for 2 min more. Then, the sample solution was applied to the SPR system (25 mL and 150 μL/min flow rate). The ΔR% values were monitored instantly and reached a plateaue value in approximately 25 min. After that, the sensor surface was washed again with phosphate buffer (pH 7.4) to remove non-specifically bound protein. The desorption from the sensor surface was done by applying 1 M ethylene glycol solution (25 mL and 150 μL/min flow-rate). Finally, the SPR sensor surface was washed with deionized water followed by phosphate buffer washing.

In order to determine the concentration dependency of the μCIP SPR sensor, myoglobin solutions with varying concentrations in the range of 0.1 μg/mL–10 μg/mL (phosphate buffer, pH 7.4) were applied to the SPR system.

In order to determine the kinetic and equilibrium isotherm parameters, we examined SPR sensor data using a pseudo- first-order kinetic analysis and four different equilibrium isotherm models, Scatchard, Langmuir, Freundlich, and Langmuir–Freundlich, were applied to μCIP SPR sensor data [22,28,29]. The linear form of the applied model can be given as:

$$\text{Equilibrium Kinetic Analysis } d\Delta R/dt = k_a C\Delta R_{max} - (k_a C + k_d)\Delta R \quad (1)$$

$$\text{Scatchard } \Delta R_{ex}/C = K_A \Delta R_{max} - K_A \Delta R_{eq} \quad (2)$$

$$\text{Langmuir} \Delta R = \{\Delta R_{max}[C]/K_D + [C]\} \quad (3)$$

$$\text{Freundlich} \Delta R = \Delta R_{max}[C]^{1/n} \quad (4)$$

$$\text{Langmuir}-\text{Freundlich} \Delta R = \left\{\Delta R_{max}[C]^{1/n}/K_D + [C]^{1/n}\right\}. \quad (5)$$

ΔR is the response measured with binding; C is myoglobin concentration (μg/mL); 1/n is Freundlich exponent; k_a (μg/mL s) and k_d (1/s) are forward and reverse kinetic rate constants; K_A (μg/mL) and K_D (mL/μg) are forward and reverse equilibrium constants; subscripts ex, max, and eq indicate experimental, maximum, and equilibrium, respectively. When applying the equilibrium kinetic analysis, we calculated slopes of curves and plotted concentration vs. slope curve to determine k_a and k_d, respectively [30].

Lyzozyme, BSA, and cytochrome c were used to investigate the selectivity of the SPR sensor. The solutions of competitor proteins with a concentration of 1.0 μg/mL (pH 7.4) were applied to both μCIP and NIP SPR sensors. The responses of the μCIP and the NIP SPR sensors were determined as ΔR%.

The distribution coefficient is utilized to evaluate the binding specificity of imprinted polymers:

$$K_d = [(C_i - C_f)/C_f] \times V/m \quad (6)$$

where; K_d (mL/g) is the distribution coefficient, C_i (μg/mL) is the concentration of protein in solution, and C_f (μg/mL) is the concentration of protein in solution after binding; V (mL) is the volume of protein solution; and m (g) is the mass of polymer. In SPR sensor applications, the concentration and mass parameters are modified because there is no significant difference between the initial and final concentration of protein solutions [28]. In addition, the mass of the polymer is not accurately determined and the relationship between ΔR and concentration is linear [31]. Therefore, the selectivity coefficient k is described by the following equation.

$$k = \Delta R_{template}/\Delta R_{competitor} \quad (7)$$

The equation for relative selectivity coefficient, k′ can be written:

$$k' = k_{MIP}/k_{NIP}. \quad (8)$$

We also investigated the interaction of μCIP SPR sensor with the human serum. The blood from a patient who had suffered an acute MI was put into tubes containing EDTA. In order to remove cells, the blood sample was centrifuged at 4000 *g* for 30 min at room temperature. Then, the serum sample was passed through a 3 μm filter and stored in a deep freeze at −20 °C. Before it was used, the serum sample was thawed at 37 °C for 1 h. The standard addition method was used to determine the response of the μCIP SPR sensor. First, the serum sample was diluted in the ratio of 1/15 for blank solution preparation, and then the samples spiked with 0.3, 0.5, 0.7 and 1.0 μg/mL myoglobin in the same dilution ratio were prepared. An isotonic solution (0.9% NaCl solution) was used for the dilution of samples. The myoglobin concentration in the serum (with a dilution of 1/15) was determined with ELISA (Myoglobin EIA test kit, BioChek, Inc. Foster City, CA, USA).

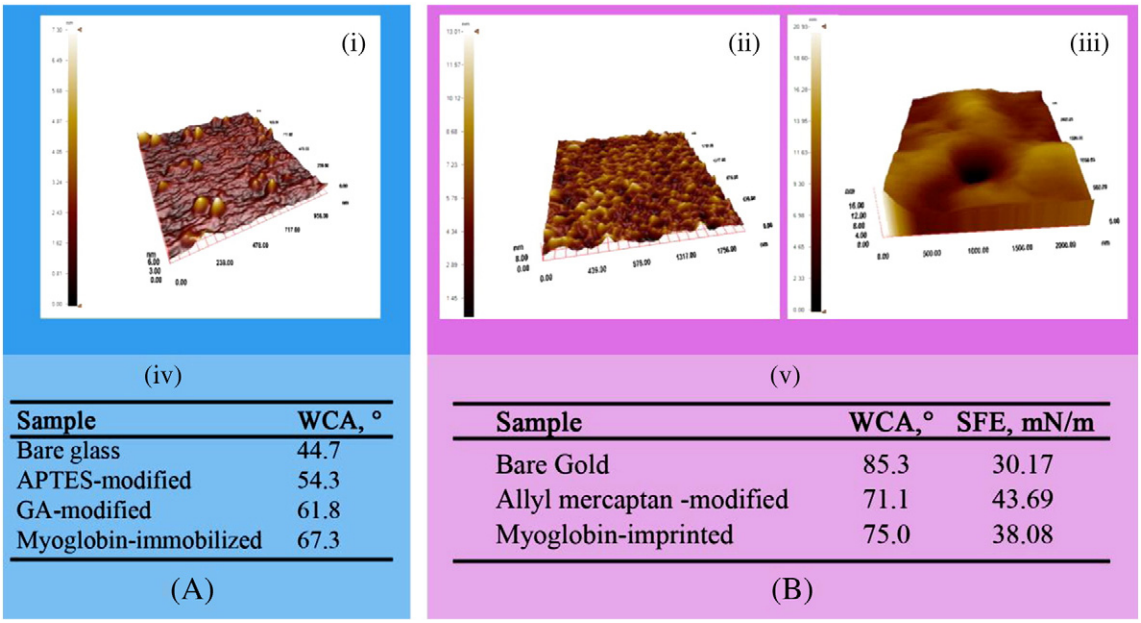
3. Results and discussion

3.1. Characterization of the surfaces

For microcontact imprinting of myoglobin onto the SPR sensor, two surfaces, glass lamellas and SPR sensor, were modified concurrently. Both surfaces were characterized after all modification steps by CA, AFM, XPS and ellipsometry measurements. The results were summarized in Fig. 1 and detailed discussions were given in Supplementary data. According to the results, microcontact imprinting of myoglobin onto the SPR sensor was successfully performed.

3.2. Real-time monitoring of μCIP SPR sensor response

In this study, μCIP SPR sensor was prepared for real-time myoglobin detection in serum. For this purpose, the SPR sensor was first interacted with aqueous myoglobin solutions in the concentration range of 0.1 μg/mL–10 μg/mL (Fig. 2). As seen in figure, the sensor had a quick response when the myoglobin solution reached the sensor surface. The increase in myoglobin concentration also caused an increase in SPR sensor response. After 25 min (1500 s) a plateau was reached and phosphate buffer was injected on to the SPR sensor for flushing of non-specifically bound molecules. At first, the sensor



(A)

Sample	WCA, °
Bare glass	44.7
APTES-modified	54.3
GA-modified	61.8
Myoglobin-immobilized	67.3

(B)

Sample	WCA,°	SFE, mN/m
Bare Gold	85.3	30.17
Allyl mercaptan -modified	71.1	43.69
Myoglobin-imprinted	75.0	38.08

Fig. 1. Surface characterization of lamella and SPR sensor during modifications (A) Glass lamella and (B) SPR sensor. AFM images of (i) myoglobin immobilized lamella; (ii) bare SPR sensor; (iii) myoglobin imprinted SPR sensor via microcontact approach; contact angle changes of (iv) lamella and (v) SPR sensor during modification steps.

response increased linearly, and then saturation started at a plateau value of a relatively high myoglobin concentration (4.0 μg/mL) because of the saturation of accessible imprinted cavities. The sensor response goes almost constantly after a 7.5 μg/mL concentration value.

Fig. 2B depicts the linear range of the μCIP SPR sensor. The sensor shows linearity in the range of 0.1 μg/mL–1.0 μg/mL. The data obtained from this concentration range were used to calculate the limit of detection (LOD) and limit of quantitation (LOQ) values of the μCIP SPR sensor. LOD and LOQ values were calculated to be 26.3 ng/mL and 87.6 ng/mL, respectively. The myoglobin levels in human serum ranged from 12–100 ng/mL. In the case of a person having a heart attack, the myoglobin level quickly increases depending on the muscle destruction [32]. The useful clinical range for myoglobin determination was reported as 80–800 ng/mL [33]. When viewed from this aspect, μCIP SPR sensor can be applicable to determine myoglobin in serum samples, not just in aqueous solutions.

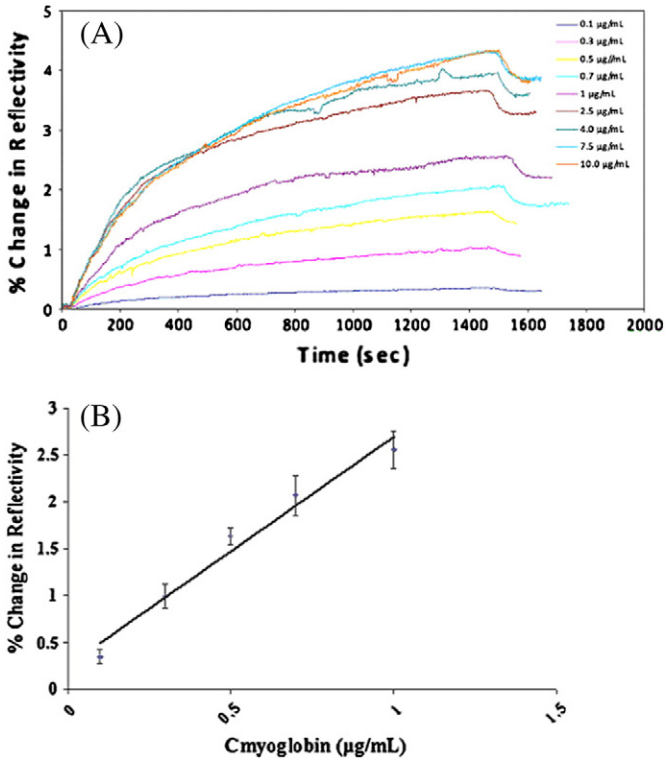


Fig. 2. (A) Real-time detection of myoglobin with μCIP SPR sensor. (B) Concentration dependency of μCIP SPR sensor in the range of 0.1 μg/mL–1.0 μg/mL.

3.3. Kinetic and isotherm analysis of μCIP SPR sensor data

For testing the experimental data the Langmuir, the Freundlich and the Langmuir–Freundlich isotherm models were evaluated. Langmuir model is generally used to fit MIP binding isotherms. Freundlich model is used to show multilayer binding of analyte molecules. Langmuir–Freundlich can be applied to a system that is not fitted to both systems, provides heterogeneity information adsorption behavior over wide concentration regions. Among the applied models, Langmuir is the best-fitted isotherm model with a high regression coefficient ($R^2 = 0.9971$). Both models, Freundlich ($R^2 = 0.9118$) and Langmuir–Freundlich ($R^2 = 0.9256$), have poor regression coefficients. These results show that the binding of myoglobin molecules onto μCIP SPR sensor is monolayer [34]. The calculated parameters for all models were given in Table 1. The ΔR_{max} value obtained from the Langmuir model was very close to the experimental one (4.33). The K_A and K_D values were calculated as 0.594 μg/mL and 1.681 mL/μg, respectively.

3.4. Selectivity of μCIP SPR sensor

In order to show the selectivity of the μCIP SPR sensor, lyzozyme, cytochrome c, and BSA were used as competitor proteins. Lyzozyme and cytochrome c were preferred for similar molecular weight and isoelectric point to myoglobin. BSA was selected because differing from myoglobin in dimensions and charge. The selectivity coefficients for the μCIP and the NIP SPR sensors and the relative selectivity coefficients were given in Table 2. As seen in table, myoglobin recognition sites have been created during the microcontact imprinting process. Although the NIP SPR sensor may contain enough functional groups, no defined cavities exist to provide selective binding. The selectivity coefficients for lyzozyme, cytochrome c, and BSA were determined to be 6.71, 10.68 and 39.16, respectively. The relative selectivity

Table 1
Kinetic and isotherm parameters.

Association kinetic analysis		Equilubrium analysis (Scatchard)			
k_a, μg/mL s	2.0×10^{-4}	ΔR_{max}, μg/mL	5.19		
k_d, 1/s	2.6×10^{-3}	K_A, μg/mL	0.667		
R^2	0.9565	K_D, mL/μg	1.499		
		R^2	0.9575		
Langmuir		Freundlich		Langmuir–Freundlich	
ΔR_{max}, μg/mL	6.31	ΔR_{max}, μg/mL	1.95	ΔR_{max}, μg/mL	39.68
K_A, μg/mL	0.594	1/n	0.5619	1/n	0.5619
K_D, mL/μg	1.681	R^2	0.9118	K_A, μg/mL	0.1174
R^2	0.9971			K_D, mL/μg	8.154
				R^2	0.9256

coefficients of μCIP SPR sensor for myoglobin/lyzozyme, myoglobin/cytochrome c, and myoglobin/BSA were 3.19, 3.81, and 5.59, respectively. In this study, functional monomer MATrp, which is a amino acid monomer, assemble around the template by interacting with functional groups on myoglobin structure, leaving behind a selective binding site after polymerization. The relative selectivity coefficients obtained for myoglobin/lyzozyme, myoglobin/cytochrome c, and myoglobin/BSA systems show that the cavities created in the imprinted nanofilm recognize myoglobin preferentially and have structural memory for and amazing molecular size matching with myoglobin. The μCIP approach for proteins uses only a minimal mass of protein, which is effectively presented as a monolayer to the recognition substrate in which the imprint will be formed. In addition, the μCIP is advantageous because little or no template remains "trapped" under the polymer film's surface on the completion of polymerization, resulting in a material that is potentially homogenous and selective.

3.5. Serum analysis with μCIP SPR sensor

The μCIP SPR sensor was also used to detect myoglobin in serum. For this purpose, a blood sample taken from a patient shortly after an acute MI was used. After the removal of cells, a blank solution with a 1/15 dilution and the samples spiked with 0.3, 0.5, 0.7 and 1.0 μg/mL myoglobin in the same dilution ratio were interacted with the μCIP SPR sensor. Fig. 3 makes it obvious that the increase in concentration caused the increase in the response as expected. A large proportion of the sensor response obtained for blank solution results from the non-specific interaction of other biomolecules in serum. However, the spiked solutions are in the same dilution ratio and the increase in the sensor response with increasing myoglobin concentration results from myoglobin molecules in serum sample. The μCIP SPR sensor shows linearity in a range of 0.3 μg/mL–1.0 μg/mL. The myoglobin concentration in the diluted serum with a ratio of 1/15 (blank) was determined as 42.6 ng/mL. To further demonstrate the relevance of the μCIP SPR sensor, the myoglobin concentration in serum sample with the same dilution ratio was determined with ELISA (Myoglobin EIA test kit, BioChek, Inc. Foster City, CA, USA). The myoglobin concentration was 58.3 ng/mL. The results showed that the prepared μCIP SPR sensor can determine the myoglobin concentration with high accuracy when the ELISA method was taken as reference method.

Table 2
The selectivity and relative selectivity coefficients for competitor proteins.

Protein	MW, kDa	pI	MS, nm × nm × nm	MIP		NIP		k′
				ΔR	k	ΔR	k	
Myo	17.6	7.3	2.5 × 2.5 × 4.2	2.35	–	0.42	–	–
Lyz	14.3	11.3	3.0 × 3.0 × 4.5	0.35	6.71	0.20	2.10	3.19
Cyt c	12.3	10.2	2.6 × 3.2 × 3.0	0.22	10.68	0.15	2.80	3.81
BSA	66.4	4.7	5.0 × 7.0 × 7.0	0.06	39.16	0.06	7.00	5.59

Myo: myoglobin; Lyz: lysozyme; Cyt c: cytochrome c; BSA: bovine serum albumin.
MW: molecular weight; pI: isoelectric point; MS: molecular size.
Experiments were performed with phosphate buffer solution (pH 7.4) including 1 μg/mL of each protein species.

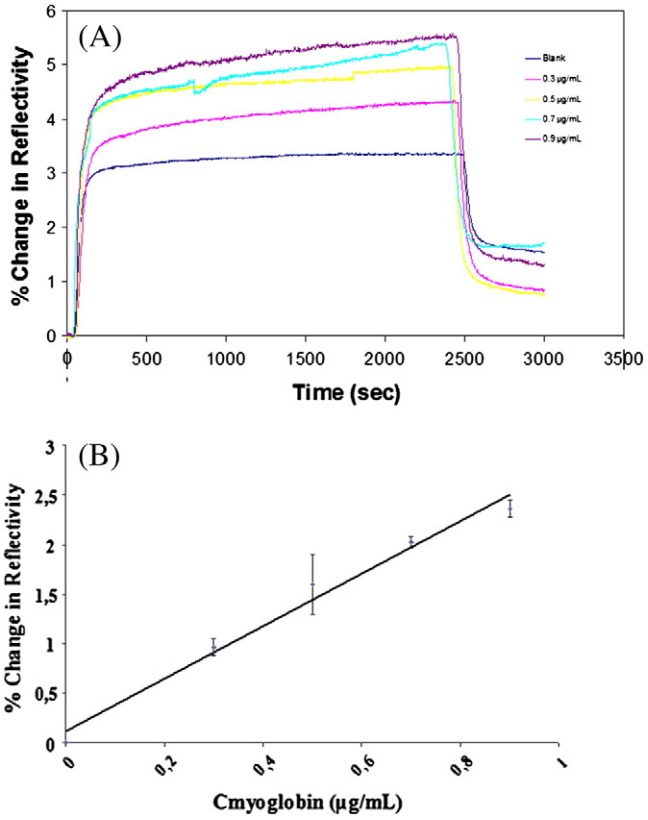


Fig. 3. (A) Sensorgram for real-time myoglobin detection in 1/15 diluted serum samples. (B) SPR sensor response in the range of 0.3 μg/mL–1.0 μg/mL myoglobin serum samples.

3.6. Reusability

In order to show the reusability of the μCIP SPR sensor, four equilibration–adsorption–regeneration cycles were repeated using aqueous myoglobin solutions. For this purpose, 0.1, 0.5, 0.7, and 0.1 μg/mL myoglobin solutions were interacted with the μCIP SPR sensor consecutively. The ΔR% values obtained for the four cycles are given in Fig. 4. As seen in the figure, the μCIP SPR sensor can be reused successfully with reproducible results for consecutive myoglobin solution supplement.

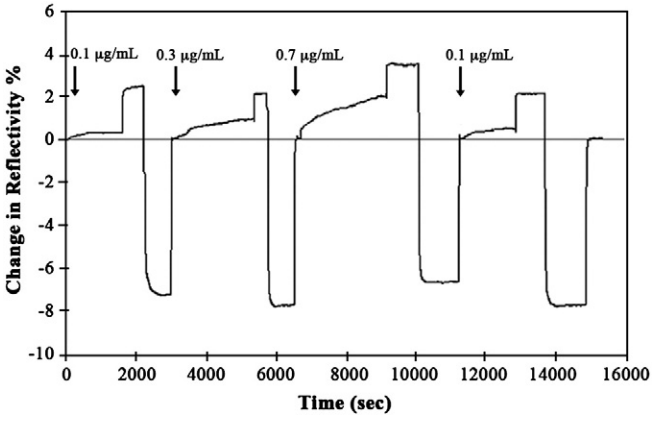


Fig. 4. Reusability/repeatability of μCIP SPR sensor.

4. Conclusions

New biochemical diagnostic methods and strategies for assessing acute MI are being investigated in response to the need for greater diagnostic accuracy, speed, cost control, and for improvements in management of patients with suspected acute MI. In this study, myoglobin-imprinted nanofilm was prepared on the gold surface of a SPR sensor via microcontact imprinting (μCIP) approach. The prepared μCIP SPR sensor was used for real-time myoglobin detection in aqueous myoglobin solutions and serum sample. LOD and LOQ values of the SPR sensor were determined to be 26.3 ng/mL and 87.6 ng/mL, respectively. The sensor data fit well with the Langmuir adsorption model. The selectivity studies show that the imprinted cavities formed in the nanofilm recognize myoglobin preferentially rather than lyzozyme, cytochrome c, and BSA with a relative selectivity coefficient 3.19, 3.81, and 5.59, respectively. The prepared μCIP SPR myoglobin sensor enables (1) high sensitivity (2) label-free detection, (3) real-time monitoring, (4) low volume sample consumption, (5) quantitative evaluation, and (6) determination of kinetic rate constants, unlike the other methods such as ELISA. In addition, SPR based myoglobin sensor is easy to perform and can be a cost-effective solution due to the reuseability of the prepared sensor. Furthermore, storage stability will be longer than antibody-based detection methods. In the light of obtained data, the μCIP SPR sensor can be classified as potential alternative for myoglobin sensing during acute MI cases.

Acknowledgement

This study was financially supported by the Scientific and Technological Research Council of Turkey and was assigned project number 110 T 785.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.msec.2013.04.041.

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