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F_{ab} fragments imprinted SPR biosensor for real-time human immunoglobulin G detection

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ABSTRACT

F_{ab} fragments imprinted surface plasmon resonance (SPR) chip was prepared for the real-time detection of human immunoglobulin G (IgG). In order to attach polymerization precursor on SPR chip, the SPR chip surface was modified with allyl mercaptan. F_{ab} fragments of the IgG molecules were prepared by papain digestion procedure and collected by fast protein liquid chromatography (FPLC) system using Hi-Trap_r Protein A FF column. The collected F_{ab} fragments were complexed with histidine containing specific monomer, N-methacryloyl-L-histidine methyl ester (MAH). Molecular imprinted polymeric nanofilm was prepared on SPR chip in the presence of ethylene glycol dimethacrylate and 2-hydroxyethylmethacrylate. The template molecules, F_{ab} fragments, were removed from the polymeric nanofilm using 1 M NaCl solution (pH: 7.4, phosphate buffer system). The molecular imprinted SPR chip was characterized by contact angle, atomic force microscopy and Fourier transform infrared spectroscopy. By the real-time IgG detection studies carried out using aqueous IgG solutions in different concentrations, the kinetics and isotherm parameters of the molecular imprinted SPR chip–IgG system were calculated. To show selectivity and specificity of the molecular imprinted SPR chip, competitive kinetic analyses were performed using bovine serum albumin (BSA), IgG, F_{ab} and F_c fragments in singular and competitive manner. As last step, IgG detection studies from human plasma were performed and the measured IgG concentrations were well matched with the results determined by enzyme-linked immunosorbent assay (ELISA). The results obtained with the molecular imprinted SPR chip were well fitted to Langmuir isotherm and the detection limit was found as 56 ng/mL. In the light of the results, we can conclude that the proposed molecular imprinted SPR chip can detect IgG molecules from both aqueous solutions and complex natural samples.

1. Introduction

Surface plasmon resonance (SPR) is an excellent method for measuring the refractive index changes at the surface of a metal (Homola, 2008). SPR based biosensors are optical sensors and used for characterizing and quantifying biomolecular interactions with real time measurement, high sensitivity and specificity, no need to labeling (Ersoz et al., 2008; Uzun et al., 2009a; Kastl et al., 2010; Krishnamoorthy et al., 2010; Lautner et al., 2010; Scarano et al., 2010; Zhu et al., 2010; Wan et al., 2011). SPR based biosensor technology is used in biotechnology and biomedicine in order to evaluate the interaction models in monitoring, diagnosis, genotype analyzing, DNA sensing, serotyping, cellular response to osmotic stress, drug discovery, food safety and environmental interest (Mello and Kubota, 2002; Lakshmanan et al., 2007; Chavane et al., 2008; Peeters et al., 2008; Uzun et al., 2009a; Piliarik et al., 2010; Baumgarten and Robelek, 2011).

Molecular imprinting is a popular approach to create artificial counter parts having affinity constants as high as natural ones of the interested molecules. In this approach, interested molecules also called as template are complexed with functional monomers; then, high degree of crosslinking converts these complexes into solid matrix that have ability to recognize the template molecule. Although protein imprinting is one of intensively studied area to produce molecular imprinted analogs of the (bio)macromolecules, it has still some drawbacks such as tendency of proteins to denaturation and conformational change, probable non-specific interactions due to large number of binding sites on the protein surface (Ge and Turner, 2008). Surface and epitope imprinting approaches are promising alternatives to imprinting of whole

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protein molecules. In former situation, whole protein molecules are partially imprinted in/onto polymeric matrix. In latter situation, small epitope parts such as substrate, cofactor, activator and inhibitor binding sites of the enzymes, antigen binding fragments of antibodies, small polypeptide sequences are imprinted to create a polymeric matrix having ability to recognize whole protein molecules (Whitcombe et al., 2011). Molecularly imprinted polymers are extensively used in analytical separations, solid-phase extractions, chemical sensors, drug delivery, library screening tools, etc. (Asir et al., 2005; Li and Husson, 2006a; Uludag et al., 2007; Diltemiz et al., 2009; Ge and Turner, 2009; Uzun et al., 2009b; Asliyuce et al., 2010; Gultekin et al., 2010; Sener et al., 2010).

Immunoglobulins (IgG) are the most frequently used biorecognition elements employed in biosensors (Homola, 2008). IgGs are found in blood or other body fluids and used by the immune system to identify and neutralize foreign objects. IgG is a "Y" shaped molecule and the base of Y is called as F_c fragment because of its crystallizability and constancy. The arms of Y contain sites that bind to antigen and specifically recognize foreign molecules. This antigen-binding region of the molecule is called the F_{ab} (antigen binding fragment) region (Beale, 1987). Because of their smaller size as functional components of the whole molecule, F_{ab} fragments offer several advantages over intact antibodies for use in certain immunochemical techniques, experimental applications like molecular imprinting of antibodies (Fernandes et al., 2008).

In this study, we have produced F_{ab} fragments imprinted nanofilm on SPR chip to develop SPR based biosensor for real-time detection of IgG molecules. For this reason, in the first step, we prepared the functional monomer N-methacryloyl-L-histidine methylester (MAH). The monomer was selected for coordination of F_{ab} fragments because it is a polymerizable derivative of histidine amino acid. For a long time, histidine has been used as pseudo-specific ligand for IgG purification and the interaction between histidine and F_{ab} fragments were characterized and several studies were reported elsewhere (Uzun et al., 2005; Akgol et al., 2007; Elkak et al., 2008, 2009; Yilmaz et al., 2008). Then, papain digestion procedure was applied to break IgG molecules into F_{ab} and F_c fragments; then, they were collected by fast protein liquid chromatography (FPLC) system containing Protein A based column. After complexing of F_{ab} fragments with MAH, F_{ab} fragments imprinted nanofilm on SPR chip was prepared in the presence of 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA). HEMA, approved by FDA, was selected as basic monomer to prepare hydrophilic and biocompatible polymeric nanofilm. EGDMA was used as crosslinker and selected because of its structural similarity to basic monomer HEMA. By this way, we aimed to prepare chemically homogeneous polymeric film surface that is an important property to achieve repeatable, accurate and trustable sensor responses. IgG detection studies were carried out from aqueous IgG solutions in different concentrations and diluted human plasma samples as the natural IgG source. Specificity and selectivity of the sensor were determined and kinetics and isotherm parameters were calculated by applying association kinetics analysis, Scatchard, Langmuir, Freundlich and Langmuir–Freundlich isotherms.

2. Materials and methods

2.1. Materials

Human immunoglobulin G (IgG) (Sigma, cat. no. 160101), papain, ethylenediamine tetraacetic acid (EDTA), iodoacetamide, L-histidine methyl ester, methacryloyl chloride, allyl mercaptan and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (St. Louis, USA). HEMA, EGDMA and N,N′-azobisisobutyronitrile (AIBN) were purchased from Fluka A.G. (Buchs, Switzerland). HiTrap_r Protein A FF column was supplied from GE Healthcare Bio-Sciences (Uppsala, Sweden). All other chemicals were reagent grade and purchased from Merck A.G. (Darmstadt, Germany).

2.2. Preparation and collection of F_{ab} fragments

Preparation, collection and characterization of F_{ab} fragments were carried out according to literature (Erturk et al., 2011). Papain digestion of IgG molecules to produce F_{ab} fragments was performed in 20 mM phosphate buffer (pH: 7.0) containing 20 mM EDTA. The digestion was initiated by the addition of papain to the digestion solution containing 0.5 mg IgG/mL at an enzyme to antibody ratio of 1:20. The resulting solution was incubated at 37 °C for 24 h. Freshly prepared 30 mM iodoacetamide solution was used to stop the reaction. The digestion mixtures were analyzed by FPLC system (AKTA-FPLC, Amersham Bioscience, Uppsala, Sweden). Separation was achieved on a 2.5 cm × 0.7 cm Hi-Trap_r Protein A FF (GE, Healthcare) column. The unbound, F_{ab}, and bound, F_c, fragments were collected by the Frac 920 fraction unit of the system. The collected solutions were freeze-dried to remove solvent at −52 °C and under 20 mbar pressure (Christ Alpha LD 1-2 Plus, Osterode am Harz, Germany).

2.3. Synthesis of MAH monomer

The following experimental procedure was applied for the synthesis of MAH (Garipcan and Denizli, 2002; Uzun et al., 2005). L-Histidine methyl ester (5.0 g) and hydroquinone (0.2 g) were dissolved in 100 mL of dichloromethane solution. This solution was cooled down to 0 °C. 12.7 g of triethylamine was added to the solution. Methacryloyl chloride (5.0 mL) was poured slowly into this solution under nitrogen atmosphere and stirred magnetically at room temperature for 2 h. After that, hydroquinone and unreacted methacryloyl chloride were extracted with 10% NaOH solution. The residue (i.e., MAH) was crystallized in ethanol and ethyl acetate. MAH monomer was characterized by ^{1}H NMR. The obtained peaks in the ^{1}H NMR spectrum are listed as; ^{1}H NMR ($CDCl_3$): δ 1.85 (t; 3H, CH_3), 1.4 (m; 2H, CH_2), 3.42 (s; 3H, $-OCH_3$), 5.28 (s; 1H, vinyl H), 5.6 (s; 1H, vinyl H), 6.6–6.9 (m; 5H, aromatic); 7.42 (1H, NH); 7.47 (1H, NH).

2.4. Surface modification of the SPR chip with allyl mercaptan

The SPR chip was modified with allyl mercaptan (CH_2CHCH_2SH) as described before (Uzun et al., 2009a). SPR chip was immersed in 20 mL of alkaline piranha solution (3:1 NH_4OH:H_2O_2, v/v) for 5 min; then, washed with pure ethanol and dried in vacuum oven (200 mmHg, 40 °C) for 3 h. Later on, the chip was immersed in ethanol/water (4:1, v/v) solution containing 3.0 M allyl mercaptan for 12 h. Finally; it was rinsed with ethanol and dried with N_2 under vacuum (200 mmHg, 40 °C).

2.5. Polymer preparation on SPR chip surface

F_{ab} fragments imprinted polymeric nanofilm on allyl mercaptan modified SPR chip was prepared as follows: in the first step, F_{ab} fragments (template) and the MAH monomer, in the molar ratio of 1:1, were mixed with 400 μL distilled water and stirred at room temperature for 2 h. In the next step, AIBN (5 mg) was dissolved in HEMA (1 mL) and EGDMA (2 mL) and the F_{ab}–MAH complex was added into this solution to prepare stock monomer solution. As a third step, 2.5 μL aliquot was taken from the stock monomer solution and dropped onto the trimethylsilyl coated glass lamella surface. Gold surface of the SPR chip was placed into this solution. Polymerization was initiated by UV light at room temperature (100 W, 365 nm) and

continued for 30 min at room temperature under nitrogen atmosphere. At the end of 30 min, the glass lamella was removed from the SPR chip.

2.6. Template removal from the SPR chip surface

In order to remove the F_{ab} fragments 1 M NaCl solution (pH: 7.4, phosphate buffer) was used as the desorption agent. F_{ab} fragments were desorbed via batch system setup. F_{ab} fragments imprinted SPR chip was immersed into desorption solution (20 mL) and shaked in water bath (200 rpm) at room temperature for 1 h. The SPR chip was washed with deionized water and dried with N_2 under vacuum (200 mmHg, 25 °C) after removing of F_{ab} fragments.

2.7. Surface characterization of SPR chip

2.7.1. Contact angle measurements

Contact angle measurements of the SPR chip were determined with KRUSS DSA100 (Hamburg, Germany) instrument. Contact angle of SPR chip was measured with sessile drop method using water as liquid phase. Ten separate photos were taken from the different parts of the SPR chip and contact angle values were measured for each drop. The reported contact angle values were calculated as average of the left contact angle, the angles from the left contact point of the droplet with solid, and the right contact angle, the angles from the right contact point of the droplet with solid.

2.7.2. Atomic force microscopy studies

In order to characterize the surface of the SPR chip, atomic force microscope (AFM) was used in tapping mode (Nanomagnetics Instruments, Oxford, UK). AFM system can perform measurements in high resolution (4096 × 4096 pixels) because of the cantilever interferometer. SPR chip was attached on a sample holder by using double-sided carbon strip. Oscillation frequency (341.30 Hz), vibration amplitude (1 V_{RMS}) and free vibration amplitude (2 V_{RMS}) were the experimental parameters. Imaging studies were made in 2 μm/s scanning rate and 256 × 256 pixels resolution.

2.7.3. FTIR-ATR spectrophotometer analysis

FTIR-ATR spectrophotometer (Thermo Fisher Scientific, Nicolet iS10, Waltham, MA, ABD) was also used for the surface characterization of the F_{ab} fragments imprinted SPR chip. Total light reflection from the surface was measured in a wave number range of 400–4000 cm^{-1} at 2 cm^{-1} resolution. Eighteen replicated FTIR-ATR spectra were obtained and baseline correction was done due to Ge window.

2.8. Kinetic studies with SPR chip

After the preparation and characterization of the SPR chip, the chip was used for real time detection of F_{ab} fragments and IgG molecules from aqueous solution by attaching it to SPR system (GenOptics, SPRiLab, Orsay, France). The SPR chip was washed with 20 mM phosphate buffer (pH: 7.4, 50 mL, 2.0 mL/min flow rate) and deionized water (50 mL, 2.0 mL/min flow rate). Then, the aqueous solutions including F_{ab} fragments and IgG molecules in different concentrations, in the range of 2–15 mg/mL for F_{ab} fragments and 0.02–0.5 mg/mL for IgG solutions, were applied to SPR system (10 mL, 2.0 mL/min flow rate). Reflectivity (%) changes in resonance frequency were monitored instantly and reached to plateau value in 40 min for F_{ab} and in 50 min for IgG, approximately. In the next step, desorption was done by applying 1 M NaCl (pH: 7.4, 20 mM phosphate buffer, 2.0 mL/min flow rate). The SPR chip was washed with phosphate buffer (pH: 7.4, 20 mM) and deionized water (50 mL, 2.0 mL/min flow rate) at the end of the desorption step. For each sample, adsorption–desorption-cleaning steps were repeated. SPR1001 software (GenOptics, Orsay, France) was used to analyze the kinetic data obtained. In order to show the specificity and selectivity of the SPR chip, the response of the sensor was monitored while applying BSA, F_{ab}, F_c and IgG solutions in singular manner and mixed solutions including IgG/F_c/BSA and F_{ab}/F_c/BSA in competitive manner.

Subsequently, the kinetic studies from human plasma were also performed as second confirmation of the specificity of the F_{ab} fragments imprinted SPR chip. For this purpose, the blood sample was taken into EDTA containing tubes and, then, centrifuged at 3800 rpm at room temperature for 30 min. Later on, the sample was passed from 3 μm filter and stored in a deep freeze at −20 °C. The plasma samples diluted with isotonic solution (0.9% NaCl) in different ratios between 1/800 and 1/20,000 were applied to SPR system and kinetics measurements were performed as mentioned above.

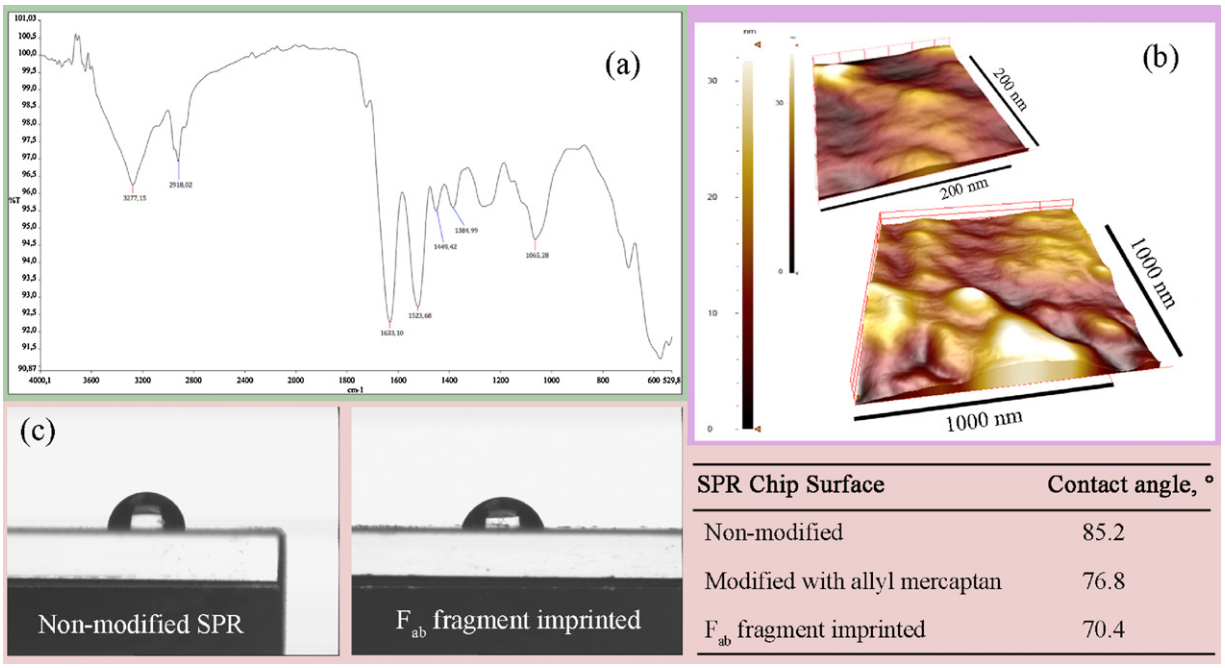
3. Results and discussion

3.1. Surface characterization of F_{ab} fragments imprinted SPR chip

Before preparation of F_{ab} fragments imprinted nanofilm on SPR chip, papain digestion of whole IgG molecules into subfragments, F_{ab} and F_c, was applied. The obtained fragments were collected with FLPC system containing Protein A separation column (Supplementary information, Fig. SI1) (Erturk et al., 2011). After creating the F_{ab} fragments imprinted nanofilm on SPR chip, the SPR chip was characterized by FTIR-ATR, AFM and contact angle measurements. The specific bands of the polymeric structure were determined as carbonyl band at 1633 cm^{-1}, amide bands at 1523 cm^{-1}, –NH stretching band at 3277 cm^{-1} and the aliphatic –CH stretching bands at 2918 cm^{-1} (Fig. 1a). The band at 1065 cm^{-1} was stemmed from imidazole ring of the MAH. AFM image of the SPR chip was given in Fig. 1b. As clearly seen in the figure, due to polymerization process, a rough polymeric surface was formed on the SPR chip. The surface deepness of the SPR chip was increased from 7.37 nm, the surface deepness of non-modified SPR chip, to 30.0 nm determined by AFM measurements. Contact angle measurements were also performed to characterize the surface (Fig. 1c). Because of the allyl mercaptan modification, the contact angle value of the chip surface decreased from 85.2° to 76.8°, respectively. This significant decrease in contact angle value showed that hydrophilicity and/or polarity of surface increased. After producing F_{ab} fragment imprinted polymeric nanofilm on the allyl mercaptan modified surface, the contact angle value decreased to 70.4°. This indicates that polymeric nanofilm formed on the allyl mercaptan modified SPR chip has hydrophilic character stemmed from the hydrophilic structures of both HEMA and MAH monomers.

3.2. Kinetic studies with F_{ab} fragments imprinted SPR chip

F_{ab} fragments imprinted SPR chip was used for real time monitoring of the interactions between the molecular imprinted nanofilm and F_{ab} fragments, also IgG molecules, from aqueous solutions. The SPR chip was interacted with aqueous solutions of F_{ab} fragments and IgG molecules in different concentration ranges of 2–15 mg/mL for F_{ab} fragments and 0.02–0.50 mg/mL for IgG molecules. As seen in Fig. 2, all steps including equilibration–adsorption–desorption–regeneration were almost completed in 50 min for F_{ab} fragments and 70 min for IgG molecules. Increase in concentration caused also increase in sensor response. In our study, the change in reflectivity increased from 0.5 to 3.5 while F_{ab} concentration increased from 2.0 mg/mL to 15 mg/mL. Similarly the change in reflectivity increased from



SPR Chip Surface	Contact angle, °
Non-modified	85.2
Modified with allyl mercaptan	76.8
F_{ab} fragment imprinted	70.4

Fig. 1. Characterization of F_{ab} fragments imprinted SPR chip. (a) FTIR-ATR spectrum; (b) AFM image and (c) contact angle measurement of F_{ab} fragments imprinted SPR chip.

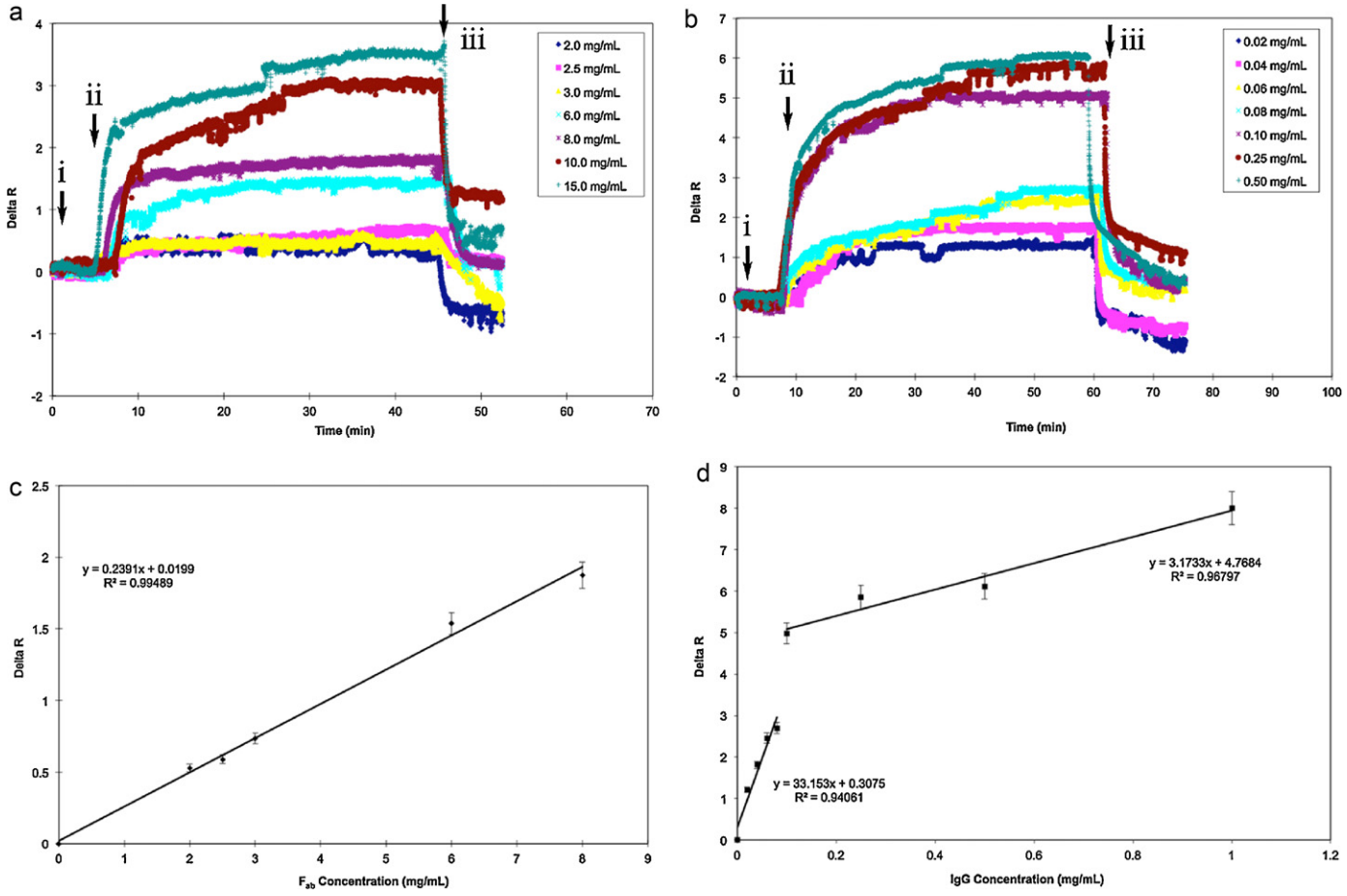


Fig. 2. The real-time analyte detection and concentration dependency of the SPR chip response. (a) The real-time F_{ab} detection; (b) the real-time IgG detection; (c) reflectivity vs F_{ab} concentration; and (d) reflectivity vs IgG concentration [(i) equilibration with phosphate buffer (pH 7.4); (ii) injection of the analyte solutions prepared in phosphate buffer (pH 7.4); and (iii) elution with phosphate buffer (pH 7.4) including 1 M NaCl].

Table 1
Kinetic and isotherm parameters for F_{ab} fragments imprinted SPR chip.

Association kinetic analysis		Scatchard	
k_a, μg/mL s	2.419	ΔR_{max}, Reflectivity%	9.515
k_d, 1/s	0.161	K_A, mg/mL	6.3659
K_A, mg/mL	15.025	K_D, mL/mg	0.1571
K_D, mL/mg	0.0666	R^2	0.7016
R^2	0.9722		

Langmuir		Freundlich		Langmuir–Freundlich	
ΔR_{max}, Reflectivity%	7.67	ΔR_{max}, Reflectivity%	9.52	ΔR_{max}, Reflectivity%	27.43
K_A, mg/mL	8.929	$1/n$	0.1893	K_A, mg/mL	0.334
K_D, mL/mg	0.112	R^2	0.8940	K_D, mL/mg	2.99
R^2	0.9624			R^2	0.9456

1.25 to 6.0 while IgG concentration increased from 0.02 mg/mL to 0.50 mg/mL (Fig. 2).

Fig. 2 also shows the concentration dependency of F_{ab} fragments imprinted SPR chip based on both aqueous solutions of F_{ab} fragments and IgG molecules. As expected, the increase in concentration caused also increase in sensor response. The F_{ab} fragments imprinted nanofilm has high affinity and ability to recognize F_{ab} fragments (Fig. 2c). Here, it should be mentioned that the linearity of the sensor response in the studied concentration range is very high and has linear regression constant as high as 99.49%. As seen in Fig. 2d, F_{ab} fragments imprinted SPR chip has two different regions for aqueous IgG solutions. The result can be explained by binding via two interaction pathways. As one of them, IgG molecules bind onto F_{ab} fragments imprinted SPR chip through two different orientations by using their F_{ab} regions randomly with high affinity. As a secondly, IgG molecules bind onto the SPR chip through using all F_{ab} regions in case of lower IgG concentrations. The increase in concentration causes a competition between F_{ab} fragments. Therefore, IgG molecules can only use single F_{ab} region to bind onto the SPR chip. But, we should lay emphasis on that both binding tendencies have high linearities and affinities with regression constants as 94.1% for former situation and 96.8% for latter one, respectively.

3.3. Mathematical analysis of kinetic data

In order to describe the detection system, F_{ab} fragments imprinted nanofilm and analyte molecules, and to analyze the interaction kinetic, five models including association kinetic analysis and Scatchard, Langmuir, Freundlich and Langmuir–Freundlich models were applied to biosensing data.

$$\text{Association kinetic analysis} \quad \frac{d\Delta R}{dt} = k_a C(\Delta R_{max} - \Delta R) - k_d \Delta R \tag{1}$$

$$\text{Scatchard} \quad \frac{\Delta R_{ex}}{[C]} = K_A(\Delta R_{max} - \Delta R_{eq}) \tag{2}$$

$$\text{Langmuir} \quad \Delta R = \left\{ \frac{\Delta R_{max}[C]}{K_D} + [C] \right\} \tag{3}$$

$$\text{Freundlich} \quad \Delta R = DR_{max}[C]^{1/n} \tag{4}$$

$$\text{Langmuir–Freundlich} \quad \Delta R = \left\{ \frac{\Delta R_{max}[C]^{1/n}}{K_D} + [C]^{1/n} \right\} \tag{5}$$

where $d\Delta R/dt$ is the rate of change of the SPR response signal, ΔR and ΔR_{max} are experimental and theoretical maximum sensor responses measured while binding of analyte molecule (Reflectivity%/s), C is the injected concentration (mg/mL), k_a is the association rate constant (mL/mg s), k_d is the dissociation rate constant (1/s), $1/n$ is Freundlich heterogeneity index. Binding constant, i.e., association constant K_A, may be calculated as $K_A = k_a/k_d$ (mL/mg) and dissociation constant, K_D (mg/mL), is equal to $1/K_A$.

The adsorption models can be used to define the recognition ability, interaction selectivity and surface homogeneity of the biosensors (Lin et al., 2005; Li and Husson, 2006b). Association kinetic analysis is an approach based on pseudo-first order adsorption kinetic (Uzun et al., 2009a; Krishnamoorthy et al., 2010). Scatchard model is generally used to analyze the data for freely reversible host/guest binding interactions and calculate the total number of binding sites the host has in equilibrium situation (Sener et al., 2010). Langmuir model depends on the acceptation of homogeneous distribution of interaction points with equal energy and no lateral interactions (Sari et al., 2011). Freundlich model is well fitted to heterogeneous surfaces (Wei et al., 2005). Mixed model, Langmuir–Freundlich can be applied to a system that is full fitted to both systems, provides heterogeneity information on adsorption behavior over wide concentration regions (Sener et al., 2010). The parameters calculated for all models were summarized in Table 1. The best fitted model to explain the interaction between the SPR chip and the analyte molecules is Langmuir isotherm ($R^2 = 0.9624$) (Supplementary information, Fig. SI4). The linear fit with the Langmuir equation means that the binding of analyte molecules onto SPR chip is monolayer. Due to the results, K_A and K_D values were determined as 8.929 mg/mL and 0.112 mL/mg, respectively. Detection limit, defined as the concentration of analyte causing frequency shift equivalent to three standard deviations of the blank, was calculated as 56 ng/mL.

3.4. Selectivity and specificity of F_{ab} fragments imprinted SPR chip

In order to indicate the selectivity and specificity of the F_{ab} fragments imprinted SPR chip, the real time monitoring of interactions between the aqueous solutions of BSA, F_{ab}, F_c, IgG molecules and pre-mixed protein solution having IgG/F_c/BSA and F_{ab}/F_c/BSA molecules and the SPR chip was also performed (Fig. 3). Therefore, BSA, F_{ab}, IgG and F_c solutions (1.0 mg/mL, pH: 7.4, phosphate buffer) were applied to the molecular imprinted SPR chip. As seen from the figure, the SPR chip did not give any response to BSA and F_c solutions while giving specific responses to F_{ab} fragments and IgG molecules with higher affinity. As a second confirmation of selectivity of SPR chip, we applied the pre-mixed protein solutions containing IgG (1.0 mg/mL)/F_c (1.0 mg/mL)/BSA (1.0 mg/mL) and F_{ab} (1.0 mg/mL)/F_c (1.0 mg/mL)/BSA (1.0 mg/mL). Although pre-mixed protein solutions cause lower SPR response stemmed from competitive and antagonistic effects of BSA and F_c molecules, F_{ab} fragments imprinted SPR chip has still high affinity to template (F_{ab}) and interested (IgG) molecules under competitive conditions. Fig. 3 also shows that F_{ab} fragments imprinted SPR chip specifically detects IgG molecules in not only singular manner but also competitive one. The selectivity coefficients for IgG and F_{ab} are

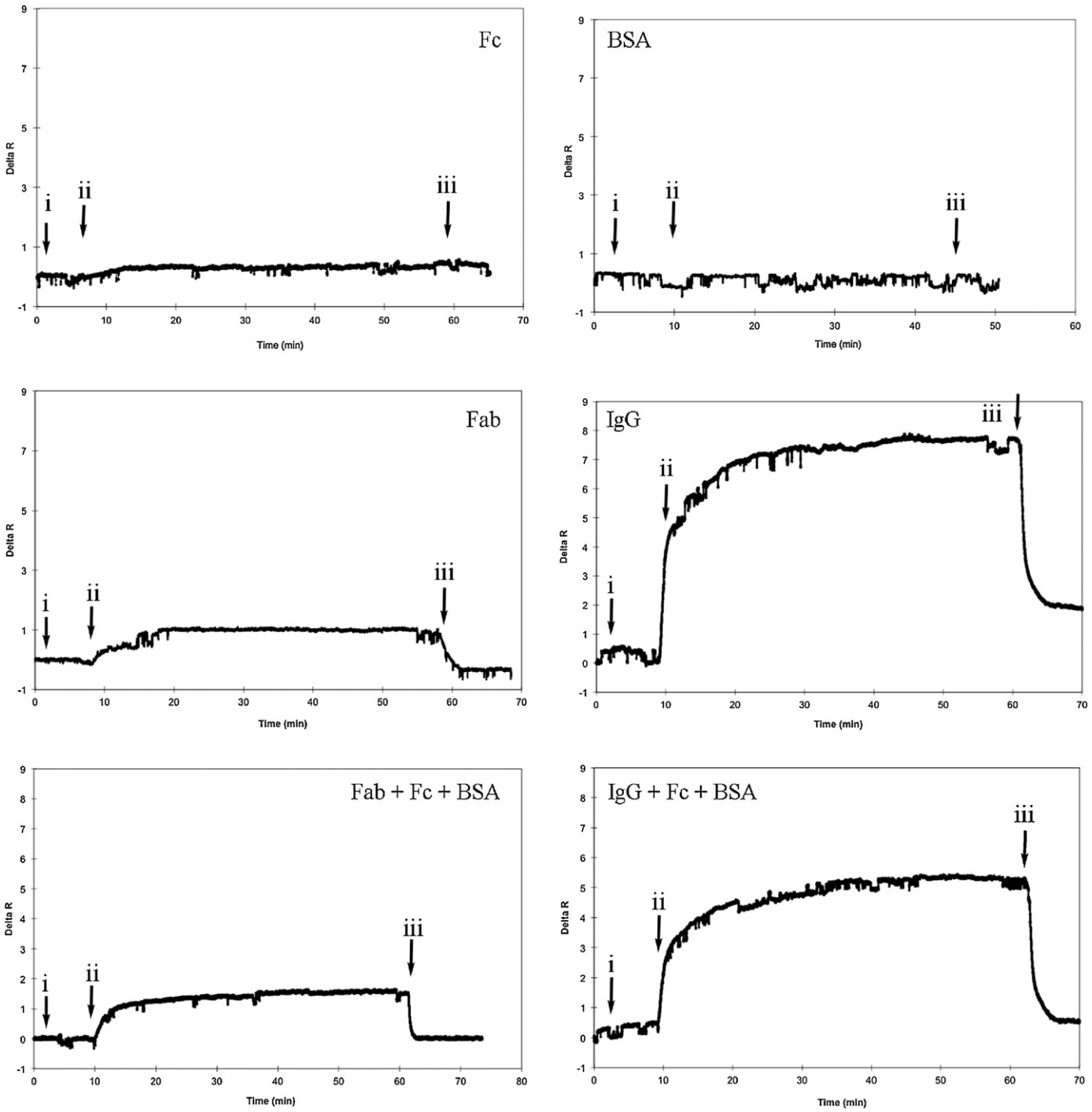


Fig. 3. Evaluation of the selectivity and specificity of F_{ab} imprinted SPR chip. The real-time monitoring of protein interactions from aqueous solutions in singular and competitive manner (protein concentrations for all is 1.0 mg/mL) [(i) equilibration with phosphate buffer (pH 7.4); (ii) injection of the analyte solutions prepared in phosphate buffer (pH 7.4); and (iii) elution with phosphate buffer (pH 7.4) including 1 M NaCl].

Table 2
Selectivity coefficients (SC) of F_{ab} fragments imprinted SPR chip for IgG molecules and F_{ab} fragments.

	F_{ab} vs competitor			IgG vs competitor	
Protein	ΔR	SC, $\Delta R_{F_{ab}}/\Delta R_{competitor}$	Protein	ΔR	SC, $\Delta R_{IgG}/\Delta R_{competitor}$
F_{ab}	1.1028	–	IgG	7.1668	
BSA	0.3763	2.9306	BSA	0.3763	21.0032
F_c	0.5797	1.9024	F_c	0.5797	13.6338

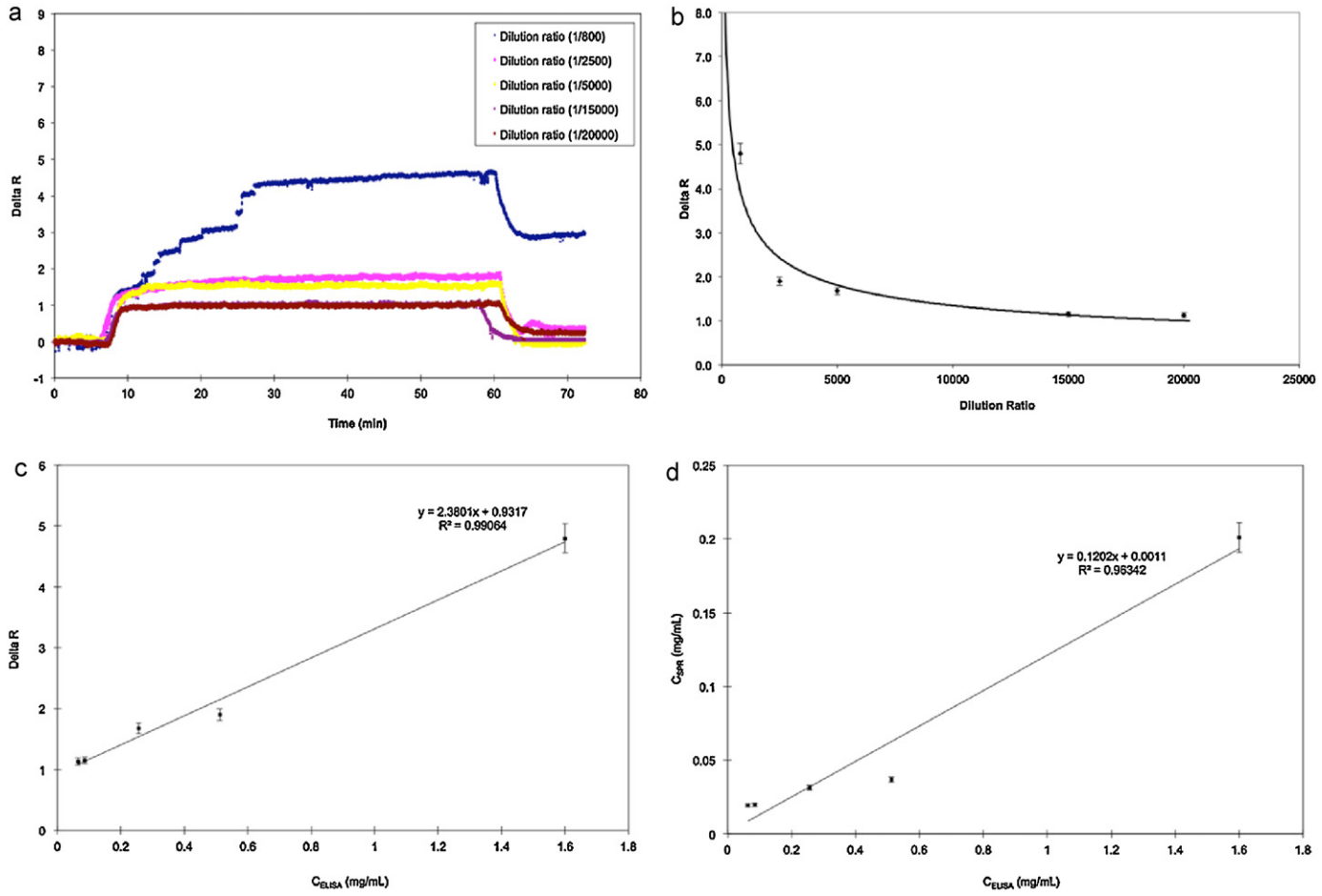


Fig. 4. The real-time IgG detection from (a) human plasma. (b) Relation between reflectivity and dilution ratio; (c) accuracy between reflectivity and concentration determined by ELISA; and (d) accuracy between concentrations determined by SPR and ELISA.

calculated and the results are summarized in Table 2. As summarized in Table 2, the F_{ab} fragments imprinted SPR chip specifically recognizes the F_{ab} fragments and IgG molecules. The selectivity coefficients calculated as $\Delta R_{F_{ab}}/\Delta R_{competitor}$ of the SPR chip for F_{ab} fragments are 2.93 and 1.90 according to BSA and F_c fragments, respectively. By the same approach, the selectivity coefficients for IgG molecules are calculated as 21.00 and 13.63 according to BSA and F_c fragments. Since IgG has approximately three times higher molecular weight than F_{ab} fragments and the sensor response is directly proportional to molecular weight, the SPR chip has higher response for IgG than F_{ab} fragments.

3.5. IgG detection from human plasma

F_{ab} fragments imprinted SPR chip was also used for real-time detection of IgG molecules from human plasma. For this purpose, plasma samples were diluted in the range of 1/800–1/20,000 (Fig. 4). As seen in Fig. 4b, the decrease in dilution ratio, in other words the increase in IgG concentration, caused an increase in sensor response as expected. The F_{ab} fragments imprinted SPR chip has a response even if 20,000 times diluted human plasma sample, IgG concentration was approximately 0.64 μg/mL, was applied to the sensor. As a conclusion, F_{ab} fragments imprinted SPR chip has the ability to detect IgG molecules from human plasma (91% water, 7% proteins, 2% ions, gases, wastes, hormones) and can be classified as a promising alternative to conventional detection systems.

Further demonstration of the relevance of the prepared F_{ab} fragments imprinted SPR chip, the results were compared with enzyme-linked immunosorbent assay (ELISA) measurements. Correlations between two methods were linear (Fig. 4c and d). As seen in these figures, ΔR values increased with higher IgG concentrations that were also determined by ELISA. When the ΔR data determined for the concentrations between 0.02 mg/mL and 1 mg/mL are taken under consideration, the curve equation is determined as $y = 2.3801x + 0.9317$ with linear regression constant as 99.1%. In other words, the prepared SPR chip is able to detect the IgG molecules from human plasma with 99.1% precision in the studied concentration range.

4. Conclusion

Surface plasmon resonance (SPR) is a type of optical biosensor and it is used mainly for the analysis of affinity interactions between biomolecules (Piletsky and Turner, 2002). SPR based biosensors have attracted considerable attention because of their properties like real-time measurement, high sensitivity, specificity, reproducibility and especially no need to any labeling (Uzun et al., 2009a; Sener et al., 2010). Due to capability of high specific recognition of molecular imprinted polymers to analyte molecules interested, molecular imprinted polymer based biosensors are extensively studied and several reviews have already been published (Haupt, 2003; Uludag et al., 2007; Ge and Turner, 2009; Whitcombe et al., 2011). Because of the tendency of proteins to denaturation and conformational change due to their molecular size, flexible and complex structure, and lower solubility in organic monomer phase,

the researchers try to imprint the some parts of the proteins instead of whole structure (Ozcan et al., 2006; Turner et al., 2006; Ge and Turner, 2008, 2009; Sener et al., 2010).

In this study, we have focused our attention on combining the epitope imprinting and SPR biosensor approaches for producing F_{ab} fragments imprinted SPR based IgG sensor for real time IgG detection. Hereby, we tried to overcome the drawbacks encountered during macromolecule imprinting, to facilitate the molecular imprinting procedure and enhance the sensor's efficiency by using F_{ab} fragments that are the main functional components and antigen binding regions of the whole IgG molecule. The characterization and kinetic studies carried out indicated that the F_{ab} fragments imprinted SPR chip has biorecognition ability to both F_{ab} fragments and whole IgG molecules in singular and competitive manner. In addition, F_{ab} fragments imprinted SPR chip can detect IgG molecules from complex real sample such as human plasma with a high linearity to conventional detection method ELISA. As a conclusion, we can say that F_{ab} fragments imprinted SPR chip is an encouraging alternative for the detection of IgG molecules in a wide concentration range of both aqueous solutions and plasma samples.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.07.004.

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