Microcontact-BSA imprinted capacitive biosensor for real-time, sensitive and selective detection of BSA

Gizem Ertürk 1, Dmitriy Berillo, Martin Hedström 2, Bo Mattiasson 2,*

Department of Biotechnology, Lund University, Lund, Sweden

A R T I C L E  I N F O

Article history:
Received 10 April 2014
Received in revised form 19 June 2014
Accepted 19 June 2014
Available online 25 June 2014

Keywords:
Microcontact imprinting
Capacitive biosensor
BSA detection

A B S T R A C T

An analytical method is presented, combining novel microcontact imprinting technique and capacitive biosensor technology for the detection of BSA. Glass cover slips were used for preparation of protein stamps. The microcontact-BSA imprinted gold electrodes were prepared in the presence of methacrylic acid (MAA) and poly-ethylene glycol dimethacrylate (PEGDMA) as the cross-linker by bringing the protein stamp and the gold electrode into contact under UV-polymerization. Real-time BSA detection studies were performed in the concentration range of 1.0 × 10^{-20}–1.0 × 10^{-8} M with a limit of detection (LOD) of 1.0 × 10^{-19} M. Cross-reactivity towards HSA and IgG were 5 and 3%, respectively. The electrodes were used for >70 assays during 2 months and retained their binding properties during all that time. The NIP (non-imprinted) electrode was used as a reference. The microcontact imprinting technology combined with the biosensor applications is a promising technology for future applications.

© 2014 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/);

1. Introduction

Molecular imprinting is the technology of creating artificial recognition sites complimentary in both form and function to the "template" molecule [1-4]. Molecularly imprinted polymers (MIPs) are formed by the polymerization of a functional monomer around the molecular template in the presence of cross-linker. MIPs have been used in solid-phase extractions, analytical separations, catalysis, drug delivery systems and as a biorecognition element in biosensors [5-11]. MIP technology is successfully used for the recognition of low molecular weight templates, but there are still some difficulties in the design of MIPs for macromolecular templates like proteins [12,13]. Due to this, many researchers have focused on imprinting the template protein directly onto a substrate, thus creating a substrate surface, which will be recognized by the target protein [14-16]. Microcontact imprinting is the surface coating technique used for employing recognition cavities for large molecules and assemblies [17-20]. The general procedure of the method depends on the polymerization between two surfaces – a protein stamp and a polymer support. In the first step, the protein stamp is formed by adsorption of the template protein onto the pre-cleaned glass surface. Then, the protein stamp is brought into contact with the second surface, monomer-coated substrate. By this way, thin polymer film is formed on the support via UV polymerization. As the last step, template protein is removed from the surface and specific protein recognition sites are formed only at the surface of the imprinted support [14-19]. This method has some advantages like reducing activity loss of the imprinted molecule and in addition the method requires very small amount of template molecule for the imprinting process [21,22]. Microcontact imprinting method has been used for various proteins [23-27].

BSA (bovine serum albumin) is a protein with the molecular weight of 66.5 kDa and it has many uses in biomedical applications and enzymatic reactions. It is used to prevent adhesion of enzymes during applications [28]. It is a generally used protein reagent in protein assays, like Bradford assay, to measure the concentration of a protein in solution. Furthermore, BSA has a structural homology with HSA (human serum albumin) [29]. Due to this, BSA is frequently studied as a model protein instead of HSA. Moreover, BSA is a commonly used target to analyze when designing new immunochemical assays.

Determination of micro-quantities of BSA is possible with methods like radioimmunooassay (RIA) or enzyme-linked immunosorbent assay (ELISA) [30]. There are also some determinations like FT-IR spectroscopy, polarographic and fluorimetric measurements used for BSA detection [28,31,32]. Some of the methods require a labelled reagent like a radioisotope or enzyme labeled antibody/antigen [30]. Some of them are really expensive and need time-consuming, complex procedures. Low selectivity and...
sensitivity is the another drawback of these methods [33]. Direct, label-free, fast and sensitive measurement of various analytes with biosensors has attracted considerable interest [34]. Highly sensitive biosensor concepts make it possible to assay biomacromolecules at concentrations below the limit of detection of conventional methods [35]. Capacitive biosensors are the electrochemical sensors that measure changes in the dielectric properties when an analyte interacts with a biorecognition element on the sensor surface, causing a decrease in the capacitance [36–41]. Capacitive biosensors have been used for the detection of various analytes like antigens, antibodies, proteins and heavy metal ions [42–47]. These types of biosensors have a lot of advantages like inherent rapidity, high sensitivity, simplicity, low cost, easy manipulation and real-time measurement without labeling.

In the study reported here, a capacitive biosensor with an automated-flow injection system was used for BSA detection. BSA is most commonly used model protein in the macromolecular imprinting studies. However, to our knowledge, this is the first microcontact-BSA imprinting study for the detection of BSA with the capacitive biosensor. Microcontact imprinting method was applied for the imprinting of BSA onto the pre-modified gold electrode surface. After modification of the gold electrode surface with poly-tyramine and acryloyl chloride, the protein stamp was brought together with a mixture of monomer and cross-linker in contact with the electrode. Thus, the microcontact BSA imprints were introduced to the electrode surface via UV-polymerization. After optimization of the conditions such as pH, ionic strength and buffer type, BSA detection studies were performed using standard solutions of BSA at different concentrations. In between each injection, regeneration of the BSA sensor was performed using a low-pH buffer [48]. Selectivity of the developed BSA-imprinted electrode was tested together with other proteins (HSA, IgG) and selectivity as a result of imprinting efficiency was indicated with the comparison of the results obtained from NIP (non-imprinted) electrode.

2. Materials and methods

2.1. Materials

BSA, tyramine (99%, HOC₆H₄(CH₂CH₂NH₂) and human serum albumin (HSA) were obtained from Sigma (Steinheim, Germany). Acryloyl chloride and 1-dodecanethiol were obtained from Aldrich (Deisenhofen, Germany). Glutaraldehyde 50% (w/v), triethyamine, 3-aminopropyl-triethoxysilane (APTES) and α-α′-azoisobutyronitrile (AIBN) were purchased from Fluka (Buchs, Switzerland). Human gamma globulin (human IgG) was purchased from Octapharma AB (Stockholm, Sweden). Glass microscope cover slips (24 × 40 mm) were used as the base for protein stamp in microcontact imprinting. All other chemicals used were of analytical grade. All buffers were prepared with water processed using a reverse osmosis step with a Milli-Q system from Millipore (Bedford, MA, USA). Prior to use, all buffers were filtered through a Millipore filter (pore size 0.22 µm) and degassed for 1 h.

2.2. Preparation of the microcontact-bovine serum albumin (BSA) imprinted electrodes

The microcontact-BSA imprinted capacitive electrodes were prepared in three steps:

(a) Preparation of the glass cover slips (protein stamps):

Glass cover slips (24 × 40 mm) were used for the preparation of protein stamps in this procedure. In the first step, cover slips were cleaned in 10 mL of 1 M HCl, de-ionized water, 1 M NaOH, de-ionized water and ethanol, respectively in ultrasonic cleaner for 10 min in each step. After cleaning, the cover slips were dried with nitrogen gas.

The cleaned cover slips were immersed in 10% (v/v) APTES (3-aminopropyl-triethoxysilane) in ethanol at room temperature for 1 h to introduce amino groups on the surface. Subsequently, the electrodes were rinsed with ethanol to remove any unbound APTES molecules. For the activation of the amino groups on the APTES modified cover slip surface, they were immersed in 5% (v/v) glutaraldehyde (GA) solution in 10 mL phosphate buffer (pH 7.4) at room temperature for 2 h. Then, the cover slips were rinsed with phosphate buffer to remove excess GA and dried with nitrogen gas. In the last step, the cover slips were immersed in 0.1 mg/mL BSA solution (in phosphate buffer, 10 mM, pH 7.4) at 4°C for 24 h for the immobilization of BSA onto the surface. Finally, the cover slips were washed with phosphate buffer and then, dried with nitrogen gas. They were kept at 4°C in a closed Petri dish until use.

(b) Preparation of the capacitive gold electrodes:

In the first step, gold electrodes were washed with ethanol, de-ionized water, acetone, de-ionized water and piranha solution (3:1, H₂SO₄:H₂O₂, v/v), respectively for 10 min in each step in ultrasonic cleaner. Then, the electrodes were plasma cleaned (Mod. PDC-3XC, Harrick, NY) for 30 min. Prior to modification of the surface of the electrodes, electropolymerization of tyramine was performed by cyclic voltammetry (CV) in ethanolic solution of 10 mM tyramine with a set potential range of 0–1.5 V (vs. Ag/AgCl) and a scan rate of 50 mV s⁻¹ for 15 scans, as described before [45]. By this way, polyyamine was deposited on the electrode, and free primary amino groups were introduced on the surface of the electrode. The coated electrodes were rinsed with water and dried with nitrogen gas.

In the second step, the electrodes were immersed in a solution containing 30 mM acryloyl chloride and 30 mM triethylamine (in toluene) overnight, at room temperature. Hence, the reaction of the acryloyl chloride with the amino groups on the surface of the electrode generated amide groups. After modification, the electrode was rinsed with distilled water and dried with nitrogen gas.

Cyclic voltammetry (CV) is used to evaluate the degree of insolation of the electrode surface after each step.

(c) Microcontact imprinting of BSA onto the capacitive gold electrode:

The monomer solution containing MAA (methacrylic acid) and PEGDMA (Poly ethylene glycol dimethacrylate) (1 mM: 1.5 mM) was prepared and the initiator (AIBN) was added to this solution. Monomer solution (1.5 µL) was pipetted onto the electrode surface. Then, the protein stamp (the glass cover slip) was brought into contact with this monomer solution. The polymerization was initiated under UV light (365 nm, 400 W) and continued for 15 min. After polymerization, the cover slip was removed and any template protein (BSA) that got stuck on the electrode surface was eluted away (Fig. 1). This elution/washing was done as a security step since the print protein was immobilized to the glass plate and would in principle stay on that plate and thus be removed when the plate was taken away. Finally, the electrode was immersed in 1-dodecanethiol (10 mM in ethanol) for 20 min in order to cover bare parts of the gold surface. When not in use, electrodes were kept at 4°C in a closed Petri dish filled with nitrogen gas.

Non-imprinted (NIP) electrodes were prepared with the same procedure without immobilization of the template protein, BSA, onto the glass cover slips.
2.3. Real time BSA detection with the microcontact-BSA imprinted electrode

2.3.1. Capacitive measurement:

The capacitive measurements were performed with the automated flow-injection system, as described by Erlandsson et al. [43]. The BSA imprinted electrode was inserted in the electrochemical flow cell and connected to the platinum auxiliary and reference electrodes. The capacitance measurement was performed via current pulse method, as described previously. The capacitance was calculated as a function of time from the resulting potential profile (Fig. 2).

Prior to analysis, a regeneration solution (25 mM glycine–HCl, pH 2.5) was injected to the system to clean the surface, and it was repeated between each analyte injections. After a stable baseline was recorded, the standard solutions of different concentrations of BSA (1.0 × 10^{-8}–1.0 × 10^{-4} M) were injected sequentially. The binding of the target protein (BSA) to the imprinted cavities on the surface of the electrode resulted in a decrease of the registered capacitance and the change was calculated automatically by CapSenze Smart Software (CSS). In all of the analysis, the flow rate was 100 μL/min and the injected sample volume was 250 μL.

The effects of type (phosphate and Tris–HCl buffers, 10 mM), pH (6.0–8.0) and ionic strength of the running buffer to the BSA detection were evaluated by monitoring the change of capacitance signal at the same standard concentration of BSA (1.0 × 10^{-10} M).

2.3.2. Selectivity of the microcontact-BSA imprinted electrode

In order to show the selectivity of the BSA imprinted electrode, the responses of the capacitive system against the competitive proteins HSA and IgG were monitored. The protein solutions were applied in singular manner and also, mixed solutions of HSA, IgG and BSA were studied in competitive manner. The protein concentration was 1.0 × 10^{-10} M for each protein during the analysis. Samples of solutions of the individual proteins were also analyzed using NIP-electrodes.

2.3.3. Reproducibility

BSA was detected repeatedly, using the assay cycle; equilibration-injection-regeneration, for 70 times. The reproducibility of the assay was evaluated by monitoring the change in capacitance at the same concentration of standard BSA solution, 1.0 × 10^{-10} M.
3. Results and discussion

3.1. Electrochemical characterization of the biosensor surface

Proper insulation of the electrode surface is an important step in the capacitive biosensor assay [40–47]. Cyclic voltammetry (CV) is the generally used method in the presence of a permeable redox couple to evaluate the degree of insulation of the electrode surface. As shown in Fig. 3, the degree of insulation increased after modification of the electrode surface with tyramine and acryloyl chloride. The density of the surface after each step increased, compared to that of the bare surface. Finally, treatment with 1-dodecanethiol reduced the redox currents substantially and the surface was completely blocked. The cyclic voltammetry results show that the surface of the electrode is insulated well and it can be used in the subsequent capacitive measurements.

3.2. Optimization of the capacitive biosensor

The BSA imprinted electrode was placed in the electrochemical flow cell and it was connected to the automated flow-injection system.

The operating conditions of the capacitive system were optimized for type, pH and ionic strength of the running buffer.

For the influence of type of buffer; 10 mM phosphate and 10 mM Tris–HCl were tested. The pH of the buffer solution was investigated in the range of 6.0–8.0. Standard BSA solutions of 1.0 × 10⁻¹⁰ M were prepared in each of these buffers and injected.

Fig. 2. Schematic diagram showing the capacitive immunosensor with an automated flow injection system.

Fig. 3. Cyclic voltammograms recorded in a solution of 100 mM KCl containing 100 mM K₃[Fe(CN)₆] using: bare electrode (red-a), gold electrode after electropolymerization of tyramine (green-b), gold electrode after surface modification with acryloyl chloride (black-c), after treatment with 1-dodecanethiol (blue-d). The scan range was from −0.3 to 0.8 V (vs. Ag/AgCl), at a scan rate of 0.1 V s⁻¹.
into the system. There was no significant capacitance difference between these buffers in the studied BSA concentration (Fig. 4(A)). However, phosphate buffer at pH 7.4 gave a more stable baseline and thus, the capacitance change was more clear. Due to the results, 10 mM phosphate buffer (pH 7.4) was used as the running buffer in the subsequent studies.

The effect of ionic strength of the running buffer was also investigated for the optimization of the conditions. The effect of ionic strength was studied by adding different concentrations of NaCl to the running buffer for the standard BSA solution of 1.0 × 10⁻¹⁰ M. As shown in Fig. 4(B), the change in the capacitance decreased with the increasing ionic strength of the medium. Thus, maximum capacitance change was observed in the running buffer which did not contain any salt.

3.3. Capacitive measurements-real time BSA detection

After optimization of BSA detection conditions, real-time BSA detection studies from aqueous BSA solutions were carried out with the automated flow-injection capacitive system as described in Section 3.2.

The BSA imprinted electrode was placed in the electrochemical flow cell and it was connected to the automated flow injection system.

The running buffer was continuously passed through the flow system by the pump at a flow rate of 100 µL/min. Standard solutions of BSA in the concentration range of 1.0 × 10⁻¹⁰ to 1.0 × 10⁻⁶ M were prepared in the same running buffer and sequentially injected into the system. Phosphate buffer (10 mM, pH 7.4) was used as running buffer. Each solution was injected for 3 times through the flow system. After injection and equilibration periods, in total 15 min, regeneration buffer was injected during 2.5 min before running buffer was used for reconditioning until the baseline signal was achieved. The decrease in capacitance increased with the increasing concentrations of BSA, as expected (Fig. 5(A)). In order to obtain a reliable analytical signal, an average of the last five capacitance readings was calculated. The graph was obtained by plotting the capacitance change (−pF cm⁻²) versus the logarithm of BSA molar concentration (Fig. 5(B)). An almost linear relationship was obtained between 1.0 × 10⁻¹⁸ and 1.0 × 10⁻¹⁶ M and the limit of detection (LOD) was determined to be 1.0 × 10⁻¹⁹ M, based on IUPAC guidelines. Due to the results, the capacitance change as a function of log concentration of the analyte in the studied concentration range was linear with the regression equation of y = 52.27x + 1805.2 (R² = 0.9477). When not in use, the electrodes were stored at 4°C in a closed Petri dish.

3.4. Selectivity of the BSA imprinted electrode against competing proteins

In order to test the selectivity of the BSA imprinted electrode, HSA and IgG were selected as competing proteins. For this purpose, the interactions between the aqueous solutions of BSA, HSA and IgG molecules and pre-mixed protein solutions having BSA/HSA, BSA/IgG, BSA/HSA/IgG and the BSA imprinted electrode were also investigated. As seen from Fig. 6(A), the change in capacitance was very low for the standard HSA (1.0 × 10⁻¹⁰ M, 10 mM phosphate buffer, pH 7.4) and IgG solutions (1.0 × 10⁻¹⁰ M,
10 mM phosphate buffer, pH 7.4) compared to that from the standard BSA solution (1.0 × 10⁻¹⁰ M, 10 mM phosphate buffer, pH 7.4). As a second confirmation of the selectivity, the pre-mixed protein solutions of BSA (1.0 × 10⁻¹⁰ M)/HSA (1.0 × 10⁻¹⁰ M), BSA (1.0 × 10⁻¹⁰ M)/IgG (1.0 × 10⁻¹⁰ M) and BSA (1.0 × 10⁻¹⁰ M)/HSA (1.0 × 10⁻¹⁰ M)/IgG (1.0 × 10⁻¹⁰ M) were injected into the capacitive system. Pre-mixed protein solutions caused a lower capacitance change compared to the singular standard BSA solution. This difference could be stemmed from the competitive effects of HSA and IgG proteins. However, it could be clearly observed that the BSA imprinted electrode showed high affinity for the template protein (BSA) and the electrode could detect BSA in singular manner and also under competitive conditions. The calculated selectivity coefficients are summarized in Table 1. Due to the results, the BSA imprinted capacitive electrode exhibited good selectivity for the template protein, BSA, compared to other proteins with cross-reactivities of 5 and 3% against HSA and IgG, respectively.

3.5. Real time BSA detection with the NIP (non-imprinted) electrode

Real time BSA detection was also performed with NIP-electrodes. Standard BSA solutions in the concentration range of 1.0 × 10⁻²⁻¹.0 × 10⁻⁶ M were prepared in the running buffer (10 mM phosphate, pH 7.4) and the analyses were identical to that with the imprinted electrodes. No change in the capacitance could be observed for the lower BSA concentrations. The limit of detection (LOD) was determined to be 1.0 × 10⁻¹⁰ M, based on IUPAC recommendations.

To evaluate the analytical efficiency of the imprinting procedure, standard BSA (1.0 × 10⁻¹⁰ M), HSA (1.0 × 10⁻¹⁰ M) and IgG (1.0 × 10⁻¹⁰ M) solutions were injected to the capacitive system in a serial manner (Fig. 6(B)). It was observed that, there was no significant difference in the capacitance change with the changing proteins for the NIP electrode. The change in capacitance was almost in the same value for all three. The calculated selectivity coefficients for NIP electrode were 1.07 and 0.376 for BSA, compared to HSA and IgG, respectively (Table 1). There was a big difference in the selectivity coefficients of NIP and BSA imprinted electrode. These results indicate that, the imprinting of the protein onto the electrode surface generates cavities highly specific for the template protein. In addition, the imprinting efficiency values were calculated and the results are summarized in Table 1. The enhanced selectivity coefficients of the BSA imprinted capacitive sensor according to competing proteins are approximately 21 and 85 for BSA against HSA and IgG, respectively.

3.6. Reproducibility

The BSA imprinted electrodes were evaluated in terms of reproducibility by monitoring the capacitance change (−pF cm⁻²) at the same concentration of standard BSA solution (1.0 × 10⁻¹⁰ M) for 70 times. After injection and equilibration periods, in total 15 min, regeneration buffer was injected during 2.5 min before running buffer was used for reconditioning until the original baseline signal was achieved. The capacitance of the BSA imprinted sensor versus the number of injections is shown in Fig. 7. The BSA detection activity of the sensor retained about the same level during the injections. Microcontact imprinting method has an advantage of reducing activity loss of the imprinted molecule during the application [21,22]. In this study, the same electrode was used in whole experiments and triplicate injections were made for each analysis. The results show that the BSA imprinted electrode can be reused for BSA detection with good reproducibility without any significant loss in the activity. A total of 80 assays during a period of 2 months were carried out on the same electrode, still with retained performance.

| Protein | Capacitance change, Δ | Selectivity coefficient, k | Relative selectivity coefficient, k' |
|---------|-----------------------|---------------------------|--------------------------------------|
| BSA     | 987                   | 32                        | -                                    |
| HSA     | 44                    | 30                        | 22.43                                | 107 | 20.96 |
| IgG     | 31                    | 85                        | 31.84                                | 0.376 | 84.68 |
The study was carried out to evaluate the possibility to use microcontact imprinting of protein molecules on electrodes for capacitive biosensor measurements. As model target, the acidic BSA protein was chosen. With the acidic functional monomer MAA chosen in the study, it could be expected that some repulsion might occur which could reduce the surface affinity in the binding step. However, since the electrode should be utilized for repetitively analytical cycles, this system was chosen to facilitate regeneration (complex dissociation) of the surface rather than optimizing binding strength. In fact, both selectivity and stability proved to be at an acceptable level.

This is a promising method that can be utilized for the creation of biorecognition imprints exhibiting high selectivity and operational stability for the target using the biosensor technology. In the future, the capacitive biosensor technology combined with the microcontact imprinting method can be used in various applications, including the diagnosis of diseases where real-time, rapid, highly selective and very sensitive detection of a known biomarker is required.

4. Conclusion

Acknowledgements

GE was supported by a fellowship from Hacettepe University, Turkey. The support from Prof. Adli Denizli and Prof. M. Aşkin Tümer, both at Hacettepe University, is gratefully acknowledged.

The project was also supported by the Swedish Research Council and the instrument used for analysis was a loan from CapSenze HB, Lund, Sweden.

References

[1] R. Arshady, K. Mosbach, Synthesis of substrate-selective polymers by host-guest polymerization, Macromol. Chem. 182 (1981) 687–692.
[2] G. Wulf, A. Sarhan, K. Zabrocki, Enzyme-analogue built polymers and their use for the resolution of racemates, Tetrahedron Lett. 14 (1973) 4329–4332.
[3] G. Wulf, Enzyme-like catalysis by molecularly imprinted polymers, Chem. Rev. 102 (2002) 1–27.
[4] K. Haupt, Molecurally imprinted polymers, the next generation, Anal. Chem. 1 (2003) 376–383.
[5] L.T. Andersson, Molecular imprinting: developments and applications in the analytical chemistry field, J. Chromatogr. B Biomed. Sci. Appl. 745 (2000) 3–13.
[6] C. Alexander, L. Davidson, W. Hayes, Imprinted polymers: artificial molecular recognition materials with applications in synthesis and catalysis, Tetrahedron 59 (2003) 2025–2057.
[7] X. Li, S.M. Husson, Two-dimensional molecular imprinting approach to produce optical biosensor recognition elements, Langmuir 22 (2006) 9658–9663.
[8] G. Ertürk, L. Uzun, M.A. Tümer, R. Say, A. Denizli, Fmol fragments imprinted SPR biosensor for real-time human immunoglobulin G detection, Biosens. Bioelectron. 28 (2011) 97–104.
[9] K. Haupt, K. Mosbach, Molecularly imprinted polymers and their use in biomimetic sensors, Chem. Rev. 100 (2000) 2495–2504.
[10] K. Haupt, Molecularly imprinted polymers in analytical chemistry, Analyst 126 (2001) 747–756.
[11] L. Uzun, R. Say, S. Uhal, A. Denizli, Production of surface plasmon resonance based array kit for hepatitis diagnosis, Biosens. Bioelectron. 24 (2009) 2878–2884.
[12] D.R. Kyscio, N.A. Peppas, Surface imprinting thin polymer film systems with selective recognition for bovine serum albumin, Anal. Chim. Acta 718 (2012) 109–115.
[13] N. Bergmann, N.A. Peppas, Molecularly imprinted polymers with specific recognition for macromolecules and proteins, Prog. Polym. Sci. 33 (2008) 271–288.
[14] H.D. Inerowicz, S. Howell, F.E. Regnier, R. Reifenberger, Multiprotein immunosay arrays fabricated by microcontact imprinting, Langmuir 18 (2002) 5263–5268.
[15] Y.W. Chen, J. Rick, T.C. Chou, A systematic approach to forming micro-contact imprints of creatine kinase, Org. Biomol. Chem. 7 (2009) 488–494.
[16] P.C. Chou, C-reactive protein thin-film molecularly imprinted polymers formed using a micro-contact approach, Anal. Chim. Acta 542 (2005) 20–25.
[17] P.C. Liao, Y.C. Yang, J.F. Hsu, T.C. Chou, H.Y. Lin, Assessing the binding selectivity of molecularly imprinted polymer artificial antibodies by mass spectrometry-based profiling system, J. Biomed. Mater. Res. A 91A (2009) 597–604.
[18] H.Y. Lin, C.Y. Hsu, J.L. Thomas, S.E. Wang, H.C. Chen, T.C. Chou, The microcontact imprinting of proteins: the effect of cross-linking monomers for lysozyme, ribonuclease A and myoglobin, Biosens. Bioelectron. 22 (2007) 534–543.
[19] J.B. Foley, E. Fu, L.J. Gamble, P. Vager, Microcontact printed antibodies on gold surfaces: function, uniformity and silicone contamination, Langmuir 24 (2008) 3628–3635.
[20] L. Chen, S. Xu, J. Li, Recent advances in molecular imprinting technology: current status, challenges and highlighted applications, Chem. Soc. Rev. 40 (2011) 2922–2942.
[21] G. Şener, E. Ozgur, A.R. Rad, L. Uzun, R. Say, A. Denizli, Rapid real-time detection of procalcitonin using a microcontact imprinted surface plasmon resonance biosensor, Analyst 138 (2013) 6422–6428.
[22] B. Osman, L. Uzun, N. Beşirli, A. Denizli, Microcontact imprinted surface plasmon resonance sensor for myoglobin detection, Mat. Sci. Eng. C 33 (2013) 3695–3704.
[23] H.Y. Lin, J. Rick, T.C. Chou, Optimizing the formulation of a myoglobin molecularly imprinted thin-film polymer formed using a micro-contact imprinting method, Biosens. Bioelectron. 22 (2007) 3293–3301.
[24] C.Y. Hsu, H.Y. Lin, J.L. Thomas, T.C. Chou, Synthesis of and recognition by ribonuclease A imprinted polymers, Nanotechnology 17 (2006) 77–83.
[25] P.J. Yoo, S.J. Choi, J.H. Kim, D. Suh, S.J. Baek, T.W. Kim, H.H. Lee, Unconventional patterning with a modulus-tunable mold: from imprinting to microcontact imprinting, Chem. Mater. 16 (2004) 5000–5005.
[26] A.P. Quist, E. Pavlovic, S. Ocarinza, Recent advances in microcontact imprinting, Anal. Bional Chem. 381 (2005) 591–600.
[27] H.W. Chien, W.B. Tsai, Fabrication of tunable micropatterned substrates for cell patterning via microcontact printing of polydopamine with poly(ethylene imine)-grafted copolymers, Acta Biomater. 8 (2012) 3678–3686.
[28] Y. Jinghua, W. Fuwei, Z. Congrong, Y. Mei, Z. Xiaona, W. Shaowei, Molecularly imprinted polymer microspheres for determination of bovine serum albumin based on flow injection chemiluminescence sensor, Biosens. Bioelectron. 26 (2010) 632–637.
[29] J.S. Mandeville, H.A. Tajmir-Riahi, Complexes of dendrimers with bovine serum albumin, Biomacromolecules 11 (2010) 465–472.
[30] J.Y. Lin, C-H. Hu, T-C. Chou, Determination of albumin concentration by MIP-QCM sensor, Biosens. Bioelectron. 20 (2004) 75–81.
[31] D.B. Luo, J.G. Lan, C. Zhou, C.X. Luo, Polarographic behaviour of Co(II)-BSA or HSA complex in the presence of a guanidine modifier, Anal. Chem. 75 (2003) 6346–6350.
[32] A. Wittmann, M. Ballauf, Secondary structure analysis of proteins embedded in spherical polyelectrolyte brushes by FT-IR spectroscopy, Anal. Chem. 76 (2004) 2813–2819.
[33] C. Berggren, B. Bjarnason, G. Johansson, Capacitive biosensors, Electroanalysis 13 (2001) 173–180.
[34] B. Mattiasson, K. Teerapursapun, M. Hedström, Immunochemical binding assays for detection and quantification of trace impurities in biotechnological product, Trends Biotechnol. 28 (2010) 20–27.
[35] P. Bataillard, F. Gardies, N. Jaffrezic-Renault, C. Martelet, Direct detection of immunospecies by capacitance measurements, Anal. Chem. 60 (1998) 2374–2379.
[36] W. Limbut, M. Hedström, P. Thavarrungkul, P. Kanatharana, B. Mattiasson, Capacitive biosensor for detection of endotoxin, Anal. Bioanal. Chem. 389 (2007) 517–525.
[37] M. Steltze, E. Sackmann, Sensitive detection of protein adsorption to supported lipid bilayers by frequency-dependent capacitance measurements and microelectrolysis, Biochim. Biophys. Acta 981 (1989) 135–142.
[38] V. Billard, C. Martelet, P. Bindet, J. Therasse, Toxin detection using capacitance measurements on immunospecies grafted onto a semiconductor substrate, Anal. Chim. Acta 249 (1991) 367–372.
[39] M. Labib, M. Hedström, M. Amin, B. Mattiasson, A novel competitive capacitive glucose biosensor based on concanavalin A-labeled nanogold colloids assembled on a polytyramine-modified gold electrode, Anal. Chim. Acta 659 (2010) 194–200.

[40] M. Labib, M. Hedström, M. Amin, B. Mattiasson, A capacitive immuno sensor for detection of cholera toxin, Anal. Chim. Acta 634 (2009) 255–261.

[41] M. Hedström, I.Y. Galaev, B. Mattiasson, Continuous measurements of a binding reaction using a capacitive biosensor, Biosens. Bioelectron. 21 (2005) 41–48.

[42] M. Labib, M. Hedström, M. Amin, B. Mattiasson, A multipurpose capacitive biosensor for assay and quality control of human immunoglobulin G, Biotechnol. Bioeng. 104 (2009) 312–320.

[43] D. Erlandsson, K. Teeparuksapun, B. Mattiasson, M. Hedström, Automated flow-injection immuno sensor based on current pulse capacitive measurements, Sens. Actuat. B 190 (2014) 295–304.

[44] S. Bacheer, D. Samyn, M. Hedström, M.S. Thakur, B.L. Persson, B. Mattiasson, A membrane protein based biosensor: use of a phosphate-H⁺ symporter membrane protein (Pho84) in the sensing of phosphate ions, Biosens. Bioelectron. 27 (2011) 58–63.

[45] K. Teeparuksapun, M. Hedström, E.Y. Wong, S. Tang, L.K. Hewlett, B. Mattiasson, Ultrasensitive detection of HIV-1 p24 antigen using nano-functionalized surfaces in a capacitive immuno sensor, Anal. Chem. 82 (2010) 8406–8411.

[46] K. Teeparuksapun, M. Hedström, P. Kanatharana, P. Thavarungkul, B. Mattiasson, Capacitive immuno sensor for detection of host cell proteins, J. Biotechnol. 157 (2012) 207–213.

[47] M. Labib, Hedström M, Amin M, Mattiasson B, A capacitive immuno sensor for detection of staphylococcal enterotoxin B, Anal. Bioanal. Chem. 393 (2009) 1539–1544.

[48] S. Loyprasert, M. Hedström, P. Kanatharana, P. Thavarungkul, B. Mattiasson, Sub-attomolar detection of cholera toxin using a label-free capacitive biosensor, Biosens. Bioelectron. 27 (2010) 41–48.