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Development of a biosensor microarray towards food screening, using imaging surface plasmon resonance

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ABSTRACT

In this study we examined the possibilities of implementing direct and competitive immunoassay formats for small and large molecule detection on a microarray, using IBIS imaging surface plasmon resonance (iSPR) system. First, IBIS iSPR optics performance was evaluated. Using a glycerol calibration curve on underivatized surface we observed high baseline variability, but uniform and robust sensitivity between hundred regions of interest. Further on, a direct immunoassay for bovine IgG detection and a competitive immunoassay for gentamicin and neomycin were developed. The direct immunoassay for bovine IgG detection in a microarray format showed poor sensitivity in comparison to the assay performed in Biacore 3000, due to low immobilization efficiency on spots. The competitive immunoassay for parallel gentamicin and neomycin detection in a microarray format displayed sensitivity in the ng mL^{-1} range, comparable with the sensitivity achieved in Biacore 3000 and in the range of maximum residue limits in milk, established in the European Union. We expect that, utilization of the IBIS iSPR system for food analysis, by screening high and low molecular weight compounds, will allow rapid and simultaneous detection of various ingredients and contaminants, providing the end-user with a detailed food profile. However, assay transfer from conventional SPR biosensors to the imaging microarray platform also presents new challenges, such as sufficient immobilization on spots, that must be addressed in future studies.

1. Introduction

Surface plasmon resonance (SPR) biosensors are applied in a wide range of disciplines and become an increasingly acceptable analytical tool in general and in food analysis specifically (Phillips and Cheng, 2007; Rich and Myszka, 2000,2004; Robert, 2004; Mullett et al., 2000). The majority of the advantages offered by the SPR biosensors can be summarized to sensitive, non-invasive and continuous real time measurements. They do not require an electrical signal or any kind of labeling and are not disturbed by electromagnetic properties of the analyzed sample. SPR biosensors can provide both qualitative (epitope mapping, binding specificity, compound screening) and quantitative (concentration analysis, kinetic and thermodynamic constants) information. Usually, SPR biosensors for high molecular weight compound detection are based on direct assays, whereas inhibition assays are particularly useful for detection of small molecules. For example, they have been successfully used for specific detection and quantification of several molecules in food, e.g. vitamins, antibiotics, toxins and plant proteins (Indyk et al., 2000, 2002; Ferguson et al., 2002; Baxter et al., 2001; Homola et al., 2002; Haasnoot et al., 2001). To meet the requirements of the current "-omic era", SPR technology developed in the direction of high-throughput systems and multi-analyte measurements. One of such systems is imaging SPR (iSPR), which measures spatial refractive index changes on the surface (Steiner, 2004; Homola et al., 2005; Palumbo et al., 2003; Berger et al., 1998; Campbell and Kim, 2007). There are several commercial iSPR instruments available, e.g. Biacore Flexchip (GE Healthcare), SPRi-Plex™ (Genoptics Bio interactions), ProteOn™ XPR36 (Bio-Rad laboratories), SPRimager®II ARRAY system (GWC Technologies) and IBIS iSPR (IBIS Technologies B.V.). The instruments differ in optics, fluidics, sample handling and surface preparation. In this study we used the IBIS iSPR system, which is based on angular modulation of monochromatic plane-polarized light. In this system a 25 mm^2 surface area is illuminated with incident light at different angles and the images of the surface are captured by

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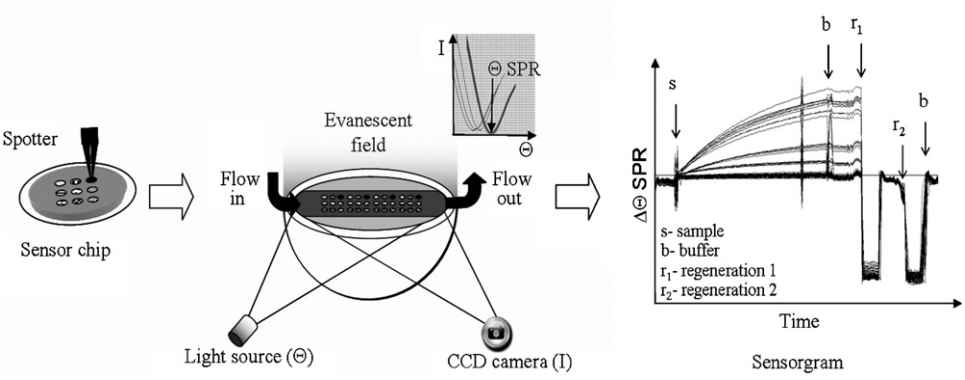

Fig. 1. Scheme of the workflow with a microarray biosensor based on imaging SPR system. CMD sensor chip is spotted with different ligands using contact spotter. Then the sensor chip is mounted on a glass prism and assembled with the flow cell. The surface of the sensor chip is illuminated at different light angles and images of the surface are taken by a CCD camera. For each spot the SPR angle is determined from angle versus intensity plots. The change in SPR angle (sensorgram) is monitored in real time and simultaneously on all the spots during buffer, sample and regeneration solution injections.

a charge-coupled device (CCD) camera. Light reflectivity is determined from the gray values of the pixels and plotted as a function of the scanning angle (Beusink et al., 2008; Lokate et al., 2007). Data acquired from the camera are processed automatically by the software and the response is expressed as a shift of the SPR angle in millidegrees. SPR angles are monitored simultaneously on the entire imaged surface using pre-defined regions of interest (ROIs). Fluidics on the sensor chip surface is handled by a cuvette or a flow cell. The instrument is also capable of automatic sample handling from different containers and a microtiter plate. Spatial modification of the surface, by microarraying, in combination with iSPR allows multiplexing analyses of several different compounds in a single measurement (Fig. 1). The goal of this study was to develop a microarray biosensor using IBIS imaging SPR system and evaluate its performance and analytical applicability. First we considered the optical sensitivity and reproducibility of the IBIS iSPR instrument. Then, we developed on the microarray biosensor platform a direct immunoassay for detection of bovine IgG (bIgG), as a model for high molecular weight compounds in food. This is particularly useful for determining milk adulteration (Hurley et al., 2004). Further on, a competitive immunoassay for detection of neomycin and gentamicin, as models for low molecular weight compounds, was developed. We used neomycin and gentamicin as an example for broad-spectrum antibiotics, commonly used in veterinary. Due to the high risk for the consumer, their presence in food is closely monitored (Brander, 1986).

2. Materials and methods

2.1. Chemicals and materials

Round sensor chips with a low density, 200 nm carboxymethylated dextran (CMD) layer were purchased from Xantec bioanalytics (Muenster, Germany). Biacore CM5 sensor chip, 10 mM acetate buffer pH 4, amine coupling kit containing 0.1 M *N*-hydroxysuccinimide (NHS), 0.4 M *N*-ethyl-*N*-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 1 M ethanolamine hydrochloride pH 8.5, HBS-EP buffer containing: 10 mM 4-(2-hydroxyethyl) piperazine-1-ethanesulphonic acid pH 7.4, 150 mM sodium chloride, 3 mM EDTA, 0.005% (v/v) surfactant polysorbate (P20) were purchased from GE Healthcare (Uppsala, Sweden). Glycerol, sodium hydroxide, hydrochloric acid, acetic acid and Tween 20 were purchased from Fluka (Zwijndrecht, The Netherlands). Affinity purified polyclonal rabbit anti-bovine IgG, gamma-globulin fraction from bovine serum (bovine IgG), gentamicin sulphate powder and neomycin trisulphate powder were purchased from Sigma–Aldrich (Zwijndrecht, The Netherlands). Monoclonal anti-neomycin and anti-gentamicin antibodies were purchased from Biodesign (Huissen, The Netherlands). Round sensor chip holder, refractive index matching oil ($n = 1.518$), hemispheric prism (BK7), cuvette and flow cell were purchased from IBIS Technologies B.V. (Hengelo, The Netherlands).

2.2. iSPR measurements

iSPR measurements were conducted using IBIS iSPR instrument. CMD sensor chip was assembled with the prism using refractive index matching oil in an appropriate holder. A flow cell (3 μL volume) or a cuvette (400 μL volume) depending on the experiment, was fixed on top of the surface. In the flow cell, the sample was delivered to a surface through a tubing and was pumped back and forth (10 $\mu L\,s^{-1}$) during the interaction. In the cuvette, the sample was delivered to the surface and mixed during the interaction by a double needle (200 $\mu L\,s^{-1}$). Prior to every measurement, the surface was equilibrated with the working buffer and ROIs in size of 150 μm × 150 μm were defined using IBIS software. The SPR angle was scanned on each pre-defined ROI in the range between −1.5 deg and +1.5 deg in steps of 50 mdeg. SPR curves were fitted automatically by IBIS software while curve parameters were limited to 20 points before and after the dip. All the measurements were performed in the "baseline mode", recording the SPR angle as a function of time. Subsequently, SPR data were analyzed using Scrubber2 software (BioLogic Software). Final responses were calculated from the difference in SPR angle before the start and the end of sample injection and referenced to the response on a blank surface.

2.3. IBIS iSPR optics evaluation

100 ROIs were defined in the middle of the imaged surface of a CMD sensor chip representing a 10 × 10 microarray. The surface was wetted with RO water and baselines on each ROI were measured. To eliminate flow pattern influence on the responses, filling and emptying the cuvette was done manually and without mixing. In order to construct a calibration curve of a change in the SPR signal as a function of increasing the refractive index of the solution, glycerol solutions in the following concentrations: 0.5%–5% (v/v) were used. Following baseline measurements, glycerol solutions were introduced to the surface and the responses on each ROI were measured. Before each new glycerol solution the fluid in the cuvette was replaced three times, to minimize the effect of

the previous solution on the measurement, and the baseline with water was re-measured. All the measurements were performed in duplicate and repeated on three different days and sensor chips. The responses, measured on each ROI, were plotted as a function of glycerol percentage. From the slope of the described glycerol calibration curve we calculated the optical sensitivity of the IBIS iSPR per ROI and averaged 100 ROIs to obtain the average sensitivity over the microarray. Variation in sensitivity between ROIs was derived from the standard deviation in sensitivity and inter-experimental variation in sensitivity was derived from standard deviation in average sensitivity between experiments performed on three different days and sensor chips. Limit of detection (LOD) was calculated from the average baseline noise plus three standard deviations.

2.4. X-ray photoelectron spectroscopy (XPS)

The XPS analysis of surfaces was performed using a JPS-9200 Photoelectron Spectrometer (JEOL, Japan), on underivatized Xantec CMD sensor chip. The high-resolution spectra were obtained under UHV conditions using monochromatic Al Kα X-ray radiation at 12 kV and 25 mA, using an analyzer pass energy of 10 eV. $Au_{4f7/2}$ peak was used for the gold layer characterization and C_{1s} peak was used for the dextran layer characterization. The atomic percentage of C and Au was measured on 12 spots on the surface, using a 200 μm spot size, diagonally across an area of 9 mm^2 in the exact middle of the sensor disk.

2.5. Microarray manufacturing

A Xantec CMD sensor chip was activated with a freshly prepared mixture of 0.1 M NHS and 0.4 M EDC for 10 min at room temperature. The EDC and NHS mixture was washed away with ice-cold 5 mM acetic acid, the sensor chip was dried under a stream of nitrogen for 4 min and immediately spotted using Microgrid II contact arrayer (ApogenDiscoveries, UK) equipped with SMP3 Telechem stealth pins. The ligands were prepared beforehand in appropriate pH spotting buffer: bIgG in 10 mM acetate buffer pH 4.5, anti-bIgG in 10 mM acetate buffer pH 4, gentamicin and neomycin in 10 mM carbonate buffer pH 8.5. The activated sensor chip was spotted with the ligands at 80% relative humidity and was left inside the instrument for 1 h after the spotting; subsequently the surface was blocked with 1 M ethanolamine pH 8.5 and rinsed with HBS-EP buffer.

2.6. bIgG detection by direct immunoassay based microarray biosensor in IBIS iSPR

Xantec CMD sensor chip was spotted with 12 spots of 1 mg mL^{-1} anti-bIgG as described in Section 2.5. The spotted sensor chip was assembled with the cuvette in IBIS iSPR and ROIs were defined on each spot. bIgG calibration curve was constructed from responses generated by injections of different bovine IgG concentrations (0.025, 0.05, 0.1, 0.2, 0.4 mg mL^{-1}) in HBS-EP buffer to the sensor chip surface in duplicate. Regeneration conditions were optimized to be 100 mM HCl followed by 50 mM NaOH.

2.7. bIgG detection by direct immunoassay in Biacore 3000

Biacore CM5 sensor chip was coated with anti-bIgG antibody via amine coupling procedure in Biacore 3000, as follows. Prior to immobilization, the antibody was diluted in 10 mM acetate buffer pH 4 to a final concentration of 25 μg mL^{-1} and the sensor chip was pre-conditioned by serial injections of 100 mM HCl, 50 mM NaOH and 0.5% (v/v) Tween 20. The sensor surface was activated with a freshly prepared mixture of 0.1 M NHS and 0.4 M EDC for 10 min, followed by injections of the antibody until the desired immobilization level was reached. Subsequently, the surface was blocked with 1 M ethanolamine pH 8.5 for 10 min and stabilized by serial injections of 50 mM NaOH. Regeneration conditions were optimized to be 50 mM HCl followed by 30 mM NaOH. bIgG calibration curve was constructed from responses generated by injections of different bIgG concentrations (0.025, 0.05, 0.1, 0.2, 0.4 mg mL^{-1}) in HBS-EP buffer to the sensor chip surface in duplicate. The response was calculated as a difference in SPR signal (measured in RU) before and after the sample injection. To compare between Biacore 3000 and IBIS iSPR responses, we used a conversion factor of 10.8 RU = 1 mdeg, as provided by the IBIS iSPR manufacturer.

2.8. Gentamicin and neomycin detection by a competitive immunoassay based microarray biosensor in IBIS iSPR

A Xantec CMD sensor chip, spotted with four spots of 3 mg mL^{-1} gentamicin and four spots of 3 mg mL^{-1} neomycin, was assembled with the flow cell in IBIS iSPR and ROI was defined on each spot. Neomycin and gentamicin calibration curves were constructed from responses generated by injections of different antibiotic concentrations, ranging from 0.02 to 2000 ng mL^{-1}, in HBS-EP buffer to the sensor chip surface in triplicate. To each concentration 300 fold diluted anti-neomycin and anti-gentamicin antibodies were added. This antibody dilution was chosen as a good compromise between maximal responses measured with each antibiotic concentration and assay sensitivity. Between each sample injection the surface was regenerated with 100 mM HCl and 50 mM NaOH. The percentage of binding for each point in the calibration curve was calculated relatively to the response measured in a sample without antibiotics. To construct a calibration curve, the binding percentage was plotted as a function of the antibiotics concentration. Half maximal inhibitory concentration (IC_{50}) was calculated from the inhibition curve using four-parameter fit in BIAevaluation software (GE Healthcare).

3. Results and discussion

3.1. IBIS iSPR optics performance

In order to investigate the basic optical performance of the instrument we used a template that would resemble a 100 spots microarray, by defining 100 ROIs on underivatized CMD surface. We evaluated the sensitivity and reproducibility of the IBIS iSPR instrument and compared SPR measurements obtained from individual ROIs by introducing glycerol solutions at different concentrations. First, we observed baseline variation between different ROIs, ranging from −50 to 350 mdeg. ROIs closer to the center of the imaged surface display the highest and the lowest signals, with an average of 50% variation between the different ROIs. Baseline variation over the surface may result from surface heterogeneity or, alternatively, from the optical settings of the instrument. Since we used sensor chips with a gold layer coated by a CMD layer, surface heterogeneity of both should be taken into consideration. Whereas variation in the gold layer thickness is known to influence the broadness, but not the position of the SPR dip, the surface heterogeneity must have originated from the dextran layer (Salamon et al., 1997; Wang et al., 2005). However, XPS analysis of the surface showed only a small variation in the C_{1s} atomic composition (2.7%), thus, the source of the baseline variation is probably related to the optical settings of the instrument and not to the surface heterogeneity. In spite of the observed differences in baselines, the sensitivity on each ROI was found to be uniform over the imaged surface. Glycerol calibration curves measured on each of the 100

ROIs displayed very similar slopes with high linearity to glycerol concentration (R^2 = 0.9997) with 1.5% variation. Knowing that the sensitivity on each ROI is the same, the main drawback of baseline variation is that subtraction of the response on a reference spot from a response on a measuring spot does not yield reliable quantification of the immobilized amount. Descriptive figures of the results reported above are provided in the Supplementary information. The sensitivity of the instrument was calculated from the glycerol calibration curves and averaged over the 100 ROIs. One percent glycerol change in solution produced 140.13 mdeg shift in SPR angle. This value is close to the expected theoretical shift, 140 mdeg per Δ 1% glycerol, which was calculated using Hansen's N-phase method adapted to four-phase reflectivity calculation, see Supplemented information, (Corn; Hansen, 1968). The average sensitivity did not vary much (1%), neither between different days, nor between different sensor chip batches (data not shown). To estimate the minimal amount of protein on the surface that is needed to produce an SPR signal in this instrument, the LOD was calculated. Using 10.8 pg mm^{-2} = 1 mdeg relationship, supplied by the manufacturer, the LOD was calculated to be 43 pg protein mm^{-2}. This conversion factor was checked by performing exactly the same glycerol experiment in Biacore 3000, using 1 RU = 1 pg protein mm^{-2} relationship for comparison (data not shown) (Stenberg et al., 1991). This LOD implies that there should be at least 0.96 pg of protein molecules bound to the surface in order to detect the binding on a 150 μm × 150 μm ROI that approximately fits a 200 μm diameter spot. For IgG molecules, the distance between the binding sites at the LOD would be 100 nm within this ROI, and is eight times lower then the distance reported for the high-density immobilized CMD surface (12 nm) (Steve Howell, 1998). This indicates that the LOD of the system is sufficiently lower than the spot capacity, and thus should be suitable for bimolecular interaction measurements in a microarray format. Recently Beusink et al. (2008) reported 2.5 times higher LOD in IBIS iSPR system of 1.62 attomole (2.43 pg) IgG per 150 μm × 150 μm ROI. The difference originates in the procedure to determine the LOD value. Beusink et al. derived the LOD value from responses generated by binding of an anti-biotin antibody to biotinylated groups of antibodies and peptides immobilized to the surface. Although this LOD value is expressed in accumulated mass per mm^2, it is dependent on the affinity of the biomolecular interaction and immobilized ligand properties, whereas we derived the LOD value based singularly on the IBIS iSPR system properties. The LOD value presented in this paper is invariant to the ability of the surface to capture analyte molecules and thus is application independent. It should be noted that 10.8 pg mm^{-2} = 1 mdeg relationship, that we used for the calculations, is true for proteins, but is expected to be different for low molecular weight compounds (Davis and Wilson, 2000). The summary of the IBIS iSPR optics performance is presented in Table 1. It is difficult to compare between currently commercially available imaging SPR sensors, due to different implementations of the technology. Nevertheless, we would like to point out two main advantages of the IBIS iSPR that in our opinion make it stand out. Firstly, the IBIS iSPR sensor may be especially useful for multi-analyte measurements in complex matrixes, such as food, because the light does not pass through the analyzed sample, as opposed to Flexchip system. Presence of the large particles in food samples is quite common and may interfere with the SPR measurements by light scattering. Secondly, IBIS iSPR system is neither restricted to a specific surface chemistry, as opposed to systems like Genoptics and Flexchip, nor to measurement at pre-defined positions on the surface, as opposed to GWC system. The flexibility of the IBIS iSPR system in terms of surface chemistry and immobilization techniques, applicable to the sensor chip, offers a broader range of applications.

Table 1
Summary of the IBIS iSPR optics performance

Sensitivity	Δ*n* 0.1429 = Δ140.13 mdeg
Sensitivity variation between ROIs	1.5%
Inter-experimental variability	1%
Baseline variation between ROIs	50%
Limit of detection (LOD)	43 pg mm^{-2}*

Δ: Change; *n*: refractive index of solution; variation = (standard deviation/average response) × 100; LOD: baseline noise plus three standard deviations.
* Based on 1 mdeg = 10.8 pg mm^{-2}.

3.2. bIgG detection by a microarray biosensor

We used a bIgG assay as a model system to study the performance of a direct immunoassay for detection of high molecular weight compound using a microarray biosensor. Anti-bIgG antibody was spotted on the sensor chip and responses with different concentrations of bIgG were measured. For comparison, we set up the same assay using a four-flow channel (4FC) biosensor in Biacore 3000. In Biacore 3000, the sensor chip was first coated with anti-bIgG antibody at different levels in four FCs and then allowed to react with different concentrations of bIgG. The SPR images of both sensor chips are shown in Fig. 2. The responses obtained from Biacore 3000 were converted to millidegrees (10.8 RU = 1 mdeg) and plotted together with the responses obtained from the microarray in IBIS iSPR (Fig. 3). In a microarray based assay, the responses did not increase much with increasing bIgG concentration in solution, whereas in Biacore 3000 based assay, dose response was observed in two of the flow channels. From the measurements conducted

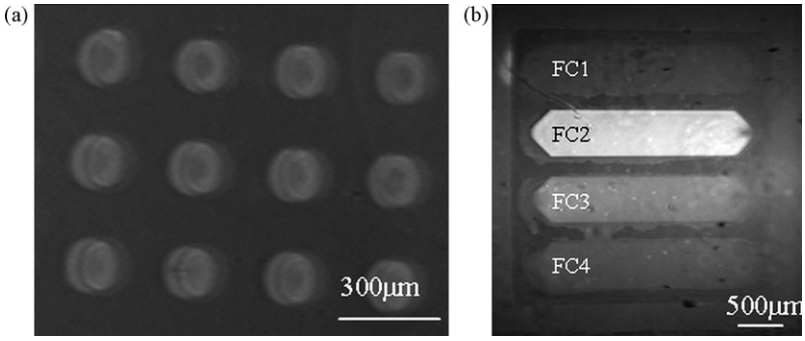

Fig. 2. SPR images of sensor chips with immobilized anti-bIgG antibody. CMD sensor chip spotted in Microgrid II with 1 mg mL^{-1} anti-bIgG (a). CM5 sensor chip with different immobilization levels (FC1: 0 RU, FC2: 8892 RU, FC3: 2078 RU and FC4: 543 RU) of anti-bIgG, coated in Biacore 3000 at 0.025 mg mL^{-1} concentration (b).

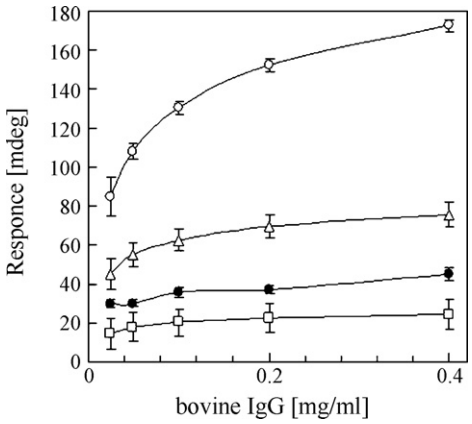

Fig. 3. Direct immunoassay for bIgG detection performed in a microarray format using IBIS iSPR and in conventional four FCs format using Biacore 3000. CMD sensor chip was spotted with 1 mg mL^{-1} anti-bIgG and bIgG calibration curve was measured in duplicate on each spot using IBIS iSPR (black). CM5 sensor chip was coated with different levels of anti-bIgG, and bIgG calibration curve was measured in duplicate using Biacore 3000 (white, FC2: round, FC3: triangle, FC4: square series).

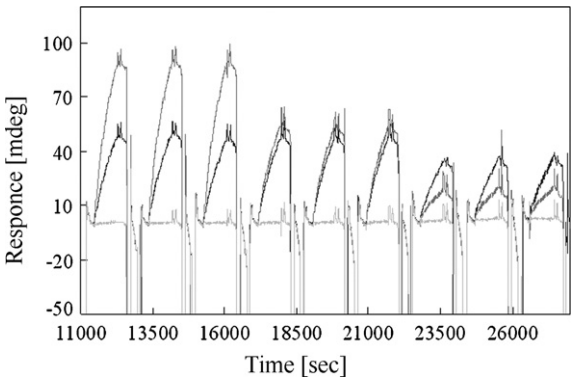

Fig. 4. Sensorgrams recorded during competition immunoassay for gentamicin and neomycin, in a microarray format, using IBIS iSPR. CMD sensor chip was spotted with 3 mg mL^{-1} gentamicin and neomycin on different spots. Gentamicin and neomycin at several concentrations were mixed in solution with anti-gentamicin and anti-neomycin antibodies and introduced to the sensor chip in triplicate. Sensorgrams measured simultaneously on gentamicin (dark grey), neomycin (black) spots and a reference spot (light grey) during triplicate injections of 1, 5 and 10 ng mL^{-1} of antibiotics.

in Biacore 3000, it can be seen that the responses are decreasing with lower immobilized amounts of anti-bIgG. As expected, FC2, with the highest amount of immobilized antibody, produces the most sensitive calibration curve, whereas in FC3 and FC4 the curve flattens. Additionally, from the SPR image of the sensor chips it can be seen that the intensity of the spots on the microarray is in between the intensities of FC3 and FC4, which is reflected in the responses obtained from the spots. These observations suggest that the amount of immobilized antibody on the spots is most probably the cause of the non-dose-dependent calibration curve. Since the concentration of anti-bIgG antibody used for spotting is forty times higher than the concentration of anti-bIgG antibody used for the immobilization in Biacore, it is evident that the immobilization efficiency on the microarray is significantly lower. This can be expected due to the fact that during spotting only a drop of 0.7 nL is delivered to a dry, pre-activated surface, whereas immobilization conditions in Biacore flow cell are much more favorable, including: flow over the surface, well wetted surface and higher ligand volume. Low immobilization efficiency on the spots may be overcome by increasing the molarity of the molecules in the immobilization solution; however, it may be difficult with compounds of high molecular weigh, such as antibodies. These results stress the importance of sufficient immobilization of molecules per spot and its effect on the performance of microarray biosensor detection assays based on direct immunoassay format. Since many biosensor based assays for food analysis have been already developed in Biacore 3000, comparison between the assay performances in the two instruments is interesting. Although the comparison between the two systems is not straightforward and may be complicated, it can help to estimate the outcome of an assay transfer from Biacore 3000 system to IBIS iSPR. The immobilization step would probably present the biggest challenge in the assay transfer in cases similar to the described above, such as direct immunoassay.

3.3. Gentamicin and neomycin detection by a microarray biosensor

We used gentamicin and neomycin assays as a model system to study the performance of a competitive immunoassay for detection of low molecular weight compound using a microarray biosensor. A sensor chip was spotted with gentamicin and neomycin and solutions containing gentamicin and neomycin at different concentrations with the antibodies against them were injected in a serial manner. Sensorgrams measured simultaneously on gentamicin, neomycin and reference spots during injections of 1, 5 and 10 ng mL^{-1} antibiotics in triplicate are shown in Fig. 4. The sensorgrams show decreased responses on gentamicin and neomycin spots with raising antibiotics concentration in solution. The measurements performed in triplicate show good reproducibility. Responses on the gentamicin spots decrease more rapidly than on the neomycin spots, indicating higher sensitivity of the assay to gentamicin. The spikes at maximal responses, that are present in all the cycles, are caused by the double air plug, which passes through the flow cell after the sample injection has ended. Since the measurements are taken 10 s before the end of the injection they do not influence the results. The baseline remained stable throughout the measurements and the sensor chip was proven to be robust for 200 cycles (data not shown), measured over a period of two months. Percentage of binding was plotted against antibiotics concentration in solution to construct inhibition curves and to derive IC_{50} values (Fig. 5). Both assays showed good sensitivity, IC_{50} of

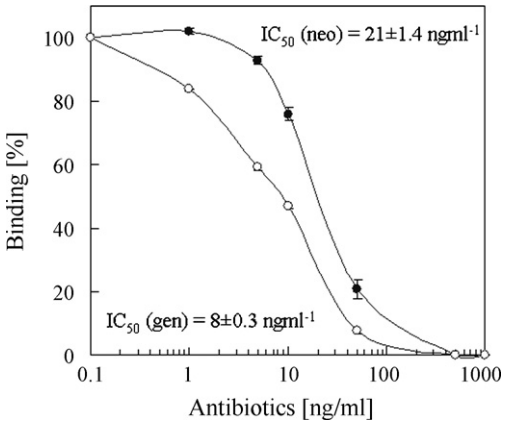

Fig. 5. Competition immunoassay for gentamicin and neomycin detection performed in a microarray format using IBIS iSPR. Gentamicin and neomycin at several concentrations were mixed in solution with anti-gentamicin and anti-neomycin antibodies and introduced to the CMD sensor chip spotted with gentamicin and neomycin. Inhibition curves for gentamicin (white) and neomycin (black) were constructed from binding percentage for each antibiotics concentration and IC_{50} values were calculated.

8 ± 0.3 and 21 ± 1.4 ng mL^{-1} for gentamicin and neomycin, respectively, which are five and three times more sensitive than previously described Biacore 3000 based biosensor immunoassays (40 and 70 ng mL^{-1} for gentamicin and neomycin, respectively) (Haasnoot et al., 2003). However, one measurement cycle in Biacore 3000 was much shorter: 7 min versus 30 min in IBIS iSPR. Longer measurement time may be a disadvantage when a rapid screening method is required, nevertheless simultaneous detection of several compounds in one measurement may compensate for that. More over, the maximum residue limits (MRLs) in milk, established in the European Union, are 100 ng mL^{-1} for gentamicin and 500 ng mL^{-1} for neomycin, requiring much lower assay sensitivity than the assay described in this paper. Thus, in principle, the sensitivity of the assay in IBIS iSPR may be reduced, for instance, by shortening the sample injection time and by so lowering maximal response measured per concentration. Coupling of the two assays on one sensor chip did not have an effect on the assay performance; we did not observe any differences in comparison to assays conducted separately (data not shown). These results show that detection of low molecular weight compounds using competitive immunoassay work as well in microarray format with imaging SPR as in conventional SPR format. Competitive immunoassay performance is better than direct immunoassay performance in the microarray format due to the fact that it is easier to immobilize a large amount of low molecular weight ligands per spot than high molecular weight ligands.

4. Conclusions

Although SPR biosensors have been used in many analytical applications, commercially available imaging SPR biosensors only recently emerged on the market and, therefore, limited information about these types of biosensors exists. Prior to assay development, intrinsic sensor properties are usually studied. IBIS iSPR optics demonstrated uniform sensitivity across the microarray, suitable LOD for biomolecular interaction measurements and robustness. However, estimation of immobilized amount, under current optical settings of the instrument, was not possible due to high baseline variability. Since in concentration measurements, based on SPR biosensors, maximum load of the sensor surface is preferred, the immobilization efficiency plays a crucial role. In microarray iSPR format, immobilization of a sufficient number of molecules with high molecular weight per spot proved to be difficult, due to the spotting procedure. It was especially problematic when the detection was based on a direct immunoassay format. iSPR microarray biosensor for detection of small molecules, based on a competitive immunoassay format, was sensitive at ng mL^{-1} level and robust. It displayed higher sensitivity than already established assay in Biacore 3000 and may be suitable for application in milk analysis in accordance with required MRLs. Overall, we found IBIS iSPR sensor to be a promising tool for concentration measurements. Immobilization of high molecular weight compounds in spotting format needs additional attention. Further studies will concentrate on the utilization of simultaneous multi-compound analysis in food, by combining different immunoassays on one sensor chip. Such a device will be highly relevant for multi-analyte screening of various food contaminants and will combine the advantages of both – an SPR biosensor and a high-throughput analytical system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.05.010.

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