Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 768–773
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical Methods

Sulfamethazine detection with direct-binding optical waveguide lightmode spectroscopy-based immunosensor

Namsoo Kim [a,*], Dong-Kyung Kim [a], Woo-Yeon Kim [a,b]

[a] *Food Function Research Division, Korea Food Research Institute, San 46-1, Baekhyun-dong, Bundang-gu, Songnam-si, Kyonggi-do 463-746, Republic of Korea*

[b] *Department of Biotechnology, Chung-Ang University, Ansung-si, Kyonggi-do 456-756, Republic of Korea*

Received 17 November 2006; received in revised form 11 June 2007; accepted 9 November 2007

Abstract

A direct-binding optical waveguide lightmode spectroscopy-based immunosensor detecting sulfamethazine (SMZ) was prepared, followed by the measurement of its specificity and sensitivity. System construction was undertaken with a peristaltic pump, an injector and the main unit comprising a sensor holder, two signal-harvesting photodiodes, a beam mirror, shutter and He–Ne laser source emitting a monochrome light ($\lambda = 632.8$ nm), plus a PC. Antibody immobilization was performed *in situ* by covalent binding of an anti-SMZ antibody over the surface of a glutaraldehyde-activated 3-aminopropyltriethoxysilane-treated sensor chip. The reaction buffer for the system was 4 mM Tris–HCl (pH 7.2) that showed a medium surface coverage and stable baseline. Sensor response was quite specific to antibody–antigen complexation, as judged from no sensor response caused by bovine serum albumin immobilization. The sensor responses according to SMZ concentrations from 10^{-8} to 10^{-2} M increased linearly in a semi-logarithmic scale, with the limit of detection of 10^{-8} M. The immunosensor was favorably reusable for SMZ screening.

Keywords: Optical waveguide lightmode spectroscopy-based immunosensor; Sulfamethazine detection; Specificity and sensitivity

1. Introduction

During the last decades, the use of antibiotics and chemotherapeutics in animal and fish husbandry has increased enormously. As a consequence, these residues occur in many types of food of animal and fish origin, resulting in a potential health hazard to consumers (Gehring et al., 2006; McCaughey, Elliott, & Crooks, 1990; Sternesjö, Mellgren, & Björck, 1995). Sulfonamides are a group of synthetic antibiotics with a broad action spectrum and are commonly used in animal and fish feedstuffs for prophylactic and therapeutic purpose (Saschenbrecher & Fish, 1980). Sulfamethazine (SMZ) that is a suspected carcinogen has been identified and determined in meat, fish, milk and cheese (Clark et al., 2005; Gehring et al., 2006; Pena et al., 2004; Wen, Zhang, Zhao, & Feng, 2005), and has been rendered as the major cause in approximately 95% of all violations involving sulfonamides in tissues (Ram et al., 1991). Due to its toxicity, a maximum residue level (MRL) of 25 ppb has been set in milk from the Codex Committee of FAO/WHO and a MRL of 100 ppb has also been established for the sum of sulfonamides in the EU (Situ, Crooks, Baxter, Ferguson, & Elliott, 2002; Sternesjö et al., 1995).

Apart from the quantitative analytical methods for SMZ possibly present in food (Maudens, Zhang, & Lambert, 2004; Salibury, Sweet, & Munro, 2004; Wen et al., 2005), the importance of rapid screening methods has been increased due to the elevated inspection demand for the quality control on food (Park, Kim, & Kim, 2004). A routine screening method for SMZ is based on growth inhibition of a sensitive test organism. This method, however,

* Corresponding author. Tel.: +82 31 780 9131; fax: +82 31 709 9876.
E-mail address: k9130sen@hanmail.net (N. Kim).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.11.018

detects very low concentrations of penicillin, whereas its sensitivity to sulfonamides is usually unsatisfactory, which might cause an ambiguity in the following identification step, using HPLC and other methods, for the inhibitory substance(s) in positive findings (Sternesjö et al., 1995). Therefore, it has been strongly required that a faster and more sensitive screening method for SMZ be developed to control this anti-microbial agent effectively in the food industry.

An immunosensor based on antibody–antigen complexation is a strong candidate for screening SMZ in various types of food due to its intrinsic high-sensitivity and versatility in system set-up, together with possible reuse of some expensive reagents like antibody and real-time demonstration of sensor response on a computer screen (Park & Kim, 2006). Normally, transducers used for immunosensing are piezoelectric, electrochemical and optoelectronic by nature and a wide difference in sensor sensitivity and reproducibility takes place according to the used transducers. An optical waveguide lightmode spectroscopy (OWLS)-based biosensor is a recently developed device in the field of integrated optics, and exploits the science of light guided in structures smaller than the wavelength of the light. As a method for studying processes at the solid/liquid interface such as protein–DNA interaction, environmental monitoring and interaction with cell (Vörös et al., 2002), it has been reported to push the sensitivity to levels even higher than the already reported achievements of the former techniques of ellipsometry, scanning angle reflectometry and surface plasmon resonance (Elwing, 1998; Homola, Yee, & Gauglitz, 1999; Huetz et al., 1995; Kim, Park, & Kim, 2007). The immobilization of a bioligand to an OWLS-based biosensor occurs by the silanization of waveguide layer and the coupling of it to the silanized sensor surface. In this case, the normally used coupling procedures are immobilization via epoxy or aldehyde group, immobilization via carboxymethylated dextran and immobilization via biotin–avidin linkage (Polzius, Schneider, Bier, Bilitewski, & Koschinski, 1996).

The aim of this study is to develop a direct-binding OWLS-based immunosensor that is applicable to the screening for SMZ in food samples due to its high sensitivity and specificity. For this purpose, the reaction buffer for the sensor system was first determined, followed by the elucidation of sensor properties.

2. Experimental

2.1. Reagents and transducer

An anti-SMZ antibody that was prepared from rabbit serum was purchased from Accurate Chemical and Scientific Corporation (NY, USA). A standard sample of SMZ and 3-aminopropyltriethoxysilane (APTS) that was the amino-terminal silane compound used for antibody immobilization in this study were obtained from Sigma Chemical Co. (MO, USA). Glutaraldehyde that was used for the preparation of an activated intermediate during antibody binding and the other chemicals for reagent preparation were also from Sigma Chemical Co, and a doubly distilled water was used throughout this study.

An optical grating coupler (OGC) sensor chip (OW 2400) as the transducer of the immunosensor of this study was the product of MicroVacuum Ltd. (Budapest, Hungary). The diffraction grating of it has the surface relief depth ~20 nm, the grating periodicity of 2400 lines/mm, and the grating area dimensions of ~2 mm (length) and 16 mm (width). The refractive index (n_F) and thickness (d_F) of the waveguide film under diffraction grating were 1.77 and 170–220 nm, respectively. Substrate glass slide has the dimensions of 48 mm (length), 16 mm (width) and 0.55 mm (thickness), and the refractive index (n_S) of 1.53.

2.2. Measuring principle of OWLS-based immunosensor

Structure of the OGC sensor chip and direct-binding measuring principle of the immunosensor of this study are shown in Fig. 1. The panel A of Fig. 1 indicates ray-optic representation of a coupled and guided wave through waveguide layer of the sensor chip. That is, He–Ne laser ($\lambda = 632.8$ nm) is diffracted by a grating at the interface and starts to propagate via the total internal reflection inside waveguide layer. At a well-defined incident angle (α), the phase shift during one internal reflection equals

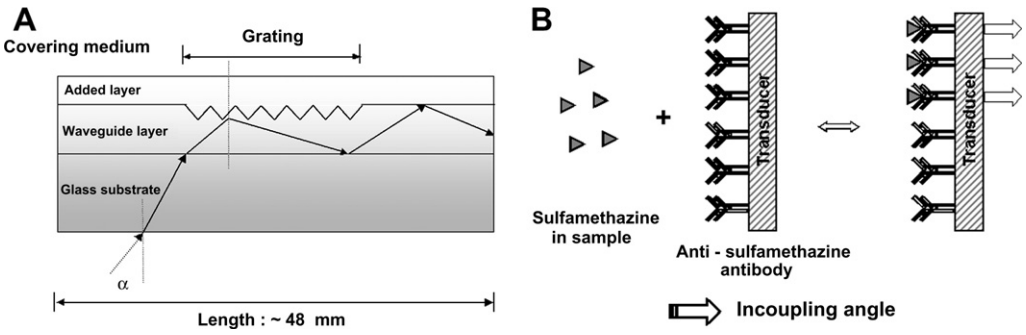

Fig. 1. Ray-optic representation of coupled and guided wave indicated by arrows inside OGC sensor chip (panel A) and measuring principle of direct-binding immunosensor detecting SMZ (panel B).

zero (constructive interference) and a guided mode is excited, which generates an evanescent field penetrating into covering medium (Vörös et al., 2002). The change in refractive index at the surface (e.g., formation of an added layer by antibody–antigen complexation, as shown by the panel B of Fig. 1) can be monitored *on-line* by precise measurement of incoupling angle as a function of time with the operating software of the immunosensor system.

2.3. Construction of immunosensor system

The immunosensor system was operated in a stop-flow mode during antibody immobilization and maintained at a flow mode during sample measurement. System construction was undertaken with a Reglo digital pump (ISM 832A, Ismatec Co., Switzerland), an injector (Rheodyne 7225, Supelco Co., USA), the main unit (OWLS 110, MicroVacuum Ltd.) which was composed of a sensor holder attached with a flow-through cuvette over the OGC sensor chip, two photodiodes, a beam mirror, shutter and He–Ne laser emitting a monochrome light of 632.8 nm, plus a PC. The individual parts of the system were connected with capillary tubing from Ismatec Co. (Kim et al., 2007).

2.4. Antibody immobilization

The antibody protein of this study was immobilized *in situ* over the surface of the OGC sensor chip referring to APTS protocol of Kim et al. (2007) as follows. In this instance, APTS protocol was used because of its high concentration-dependency, resulting in a good limit of detection (LOD), baseline stability and simple procedure of antibody immobilization (Kim et al., 2007). The OGC sensor chip was first dipped into piranha solution (H_2SO_4: $H_2O_2 = 3{:}1$, v/v) for 10 min with extensive care to avoid heat generation, followed by washing with distilled water for 5 min and hydrating with hot water around 90 °C for 1 h in sequence. The washed sensor chip was immersed into 10% acetonic APTS solution (pH 3.5) in a cap tube at 75 °C for 3 h, afterward it was heat-treated at 100 °C overnight in a convection oven. The amino-silanized sensor chip was inserted into correct position of the sensor holder and then a flow-through cuvette was attached over the sensor chip, followed by fitting the resulting sensor holder into the main unit of the immunosensor system. After washing the flow line with distilled water around 20 min at the flow rate of 194 μl/min, 200 μl of 2.5% glutaraldehyde solution was injected into the system, followed by turning off Reglo digital pump for 6 min to activate the diffraction grating of waveguide layer. Then, the pump was turned on to wash flow line with distilled water until a stable baseline was obtained. The eluent of the system was changed to the reaction buffer, 4 mM Tris–HCl (pH 7.2), and a new steady-state baseline was established by the elution of it around 20 min. At this moment, 200 μl of the antibody solution which was prepared by diluting the anti-SMZ antibody with the reaction buffer by 10-fold was added into flow line to the sensor surface at the flow rate of 194 μl/min, followed by stopping flow for 7 min to immobilize the antibody. After switching the system to the flow mode, the sensor surface bound with the antibody was washed by injecting 500 μl of 10 mM HCl, followed by flowing the reaction buffer until sample measurement.

2.5. Analytical procedure

Five hundred microliters of different concentrations of SMZ solution were separately injected into the immunosensor system in the flow mode to induce immune response. The flow rate of the reaction buffer was maintained at 194 μl/min and a sensor response was regarded as a surface mass change (ng/cm^2), caused by SMZ, in the reaction buffer before and after a sample addition. A change in the surface mass during a measurement was automatically displayed on the computer screen using the operating software based on the measured incoupling angles comprising the transverse electric mode (α_{TE}) and transverse magnetic mode (α_{TM}), via a three-step calculation on the effective refractive indices for electric and magnetic mode, N_{TE} and N_{TM}, and the refractive index (n_A) and thickness (d_A) of the added layer. The regeneration of the OGC sensor chip was performed in the flow mode by the addition of 500 μl of 10 mM HCl after each measurement.

3. Results and discussion

3.1. Determination of reaction buffer for immunosensor system

In the OWLS-based immunosensor of this study, the changes in refractive index due to eluent change, chemical reaction, antibody binding and antibody–antigen complexation cause symmetric shifts of TE and TM peaks (Kim et al., 2007). From the absolute incoupling angles of TE and TM peaks calculated by averaging the negative and positive incoupling angles, the surface coverage (surface mass), expressed as ng/cm^2, at a specific time is measured by the operating software and displayed on a PC screen (data not shown).

For the operation of the OWLS-based immunosensor system, Tris–HCl buffer with the molarity of 4 mM was chosen (MicroVacuum Ltd., 2002). When the time-courses of sensor response were measured at different pHs (Fig. 2), surface coverage increased abruptly during the initial phase of elution and then converged to the individual steady-state values. Taking into account obtained steady-state surface coverage values and baseline stability, 4 mM Tris–HCl (pH 7.2) was finally selected as the reaction buffer for the immunosensor system.

3.2. Specificity of immunosensor

The specificity of the direct-binding OWLS-based immunosensor in measuring SMZ was determined by add-

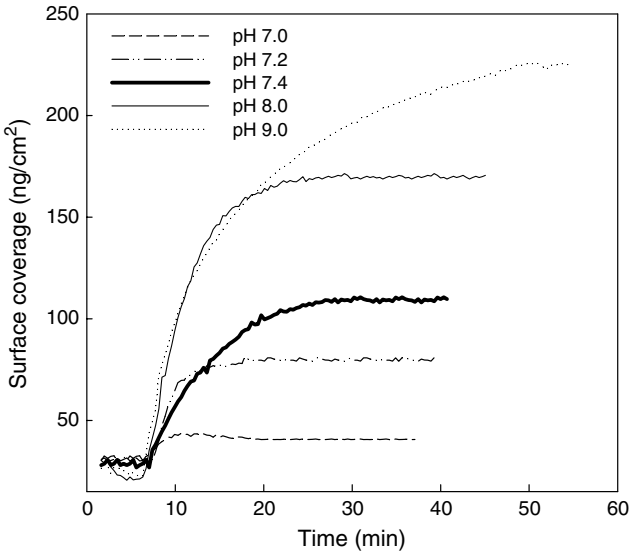

Fig. 2. Time-dependent surface coverage profiles of SMZ sensor according to pHs of 4 mM Tris–HCl.

ing 0.1 mM of SMZ solution to the sensor system and comparing the changes in surface coverage of two OGC sensor chips which were separately immobilized with the anti-SMZ antibody and bovine serum albumin (BSA). As shown in Fig. 3, no sensor response was found in the case of BSA immobilization because BSA evidently has no binding affinity with SMZ (Kim, Park, & Kim, 2004; Kim et al., 2007). On the other hand, the averaged five values of sensor response at adjacent measuring time, obtained with the immobilization of the anti-SMZ antibody, corresponded to 20.6 ± 1.86 ng/cm^2. This fact evidently indicated that the current sensor holds a high specificity to SMZ (Park & Kim, 1998).

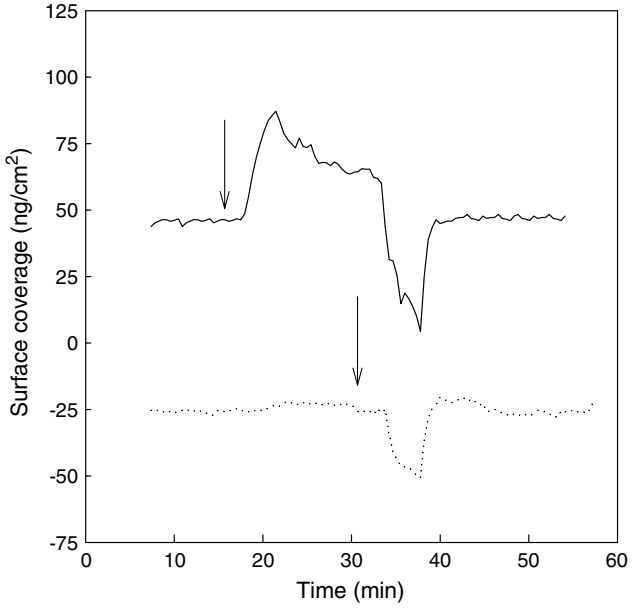

Fig. 3. Comparison of time-dependent surface coverage profiles of SMZ sensor immobilized with BSA (.....) and anti-SMZ antibody (—). Arrows indicate the injection points of 10^{-4} M SMZ.

3.3. Detection of SMZ with immunosensor

The responses of the OWLS-based immunosensor at increasing concentrations of SMZ were measured after each regeneration step with 10 mM HCl (Fig. 4). The sensorgrams at all concentrations were characterized by an initial steep increase followed by a gradual decrease in surface coverage to a steady-state value, which indicated a washing of the unbound SMZ molecules. This kind of time-dependency is typical for the sensorgrams obtained using flow-type optoelectronic biosensors based on affinity-binding principle (Hug, Prenosil, & Morbidelli, 2001; Kim et al., 2007; Leonard, Hearty, Quinn, & O'Kennedy, 2004). In this instance, the time required to obtain a steady-state response was less than 5–10 min when SMZ concentration was lower than 10^{-4} M. Over this concentration, however, more than 10 min was required to obtain a steady-state response. An addition of the reaction buffer itself to the system did not cause sensor response as shown in the arrow a in Fig. 4. On the other hand, the changes in surface coverage increased from 1.2 to 31.0 ng/cm^2 at SMZ concentrations from 10^{-8} to 10^{-2} M.

The relationship between SMZ concentration and surface coverage change was plotted in a semi-logarithmic scale for the maximum data fitting of whole SMZ concentrations. As shown in Fig. 5, a linear relationship was obtained in the SMZ concentrations of 10^{-8}–10^{-2} M with the r value of 0.9620. This type of behavior has also been reported in the label-free QCM and OWLS-based immunosensor which normally measure sensor response in the wide concentration range of target analytes (Kuhlmeier, Rodda, Kolarik, Furlong, & Bilitewski, 2003; Park et al., 2004).

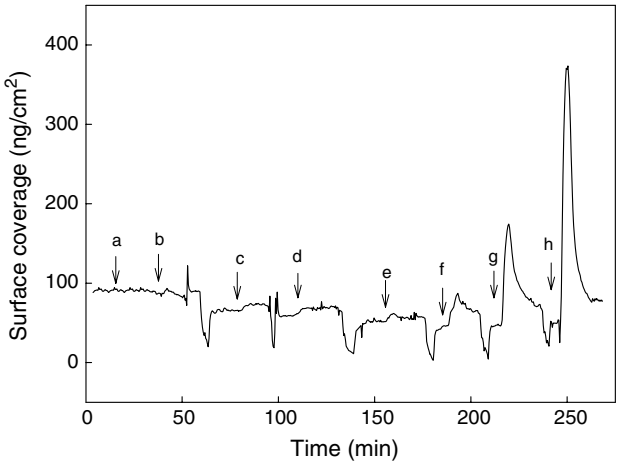

Fig. 4. Concentration-dependent responses of SMZ sensor. Five hundred microliters of the reaction buffer (a), and SMZ solutions at the concentrations of 10^{-8} (b), 10^{-7} (c), 10^{-6} (d), 10^{-5} (e), 10^{-4} (f), 10^{-3} (g) and 10^{-2} M (h) were individually injected into the immunosensor system after baseline stabilization following 10 mM HCl treatment.

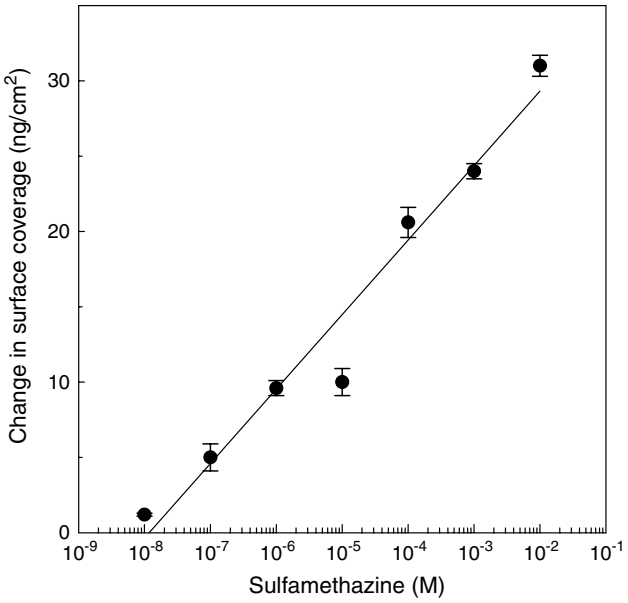

Fig. 5. Calibration curve for SMZ. Seven values of change in surface coverage at adjacent measuring time were determined and error bars were inserted.

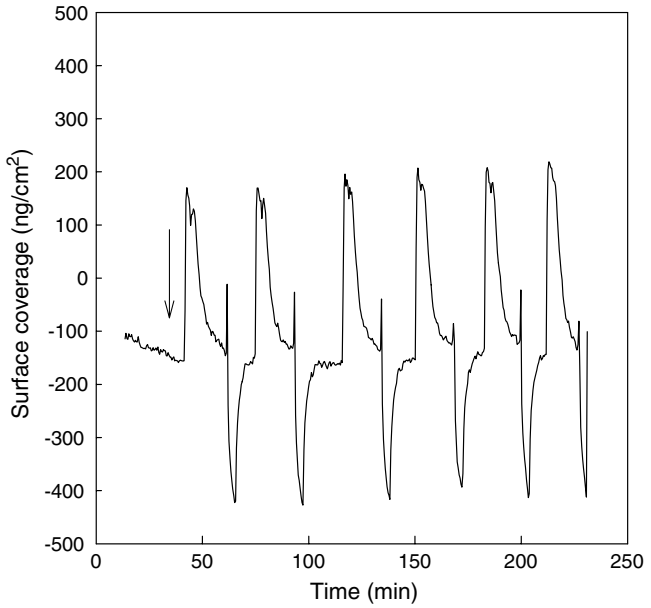

Fig. 6. Sensor responses during six repetitive measurements after regeneration with 10 mM HCl. Arrow indicates start of the first measurement.

The LOD of the OWLS-based immunosensor of this study for SMZ detection was presumed as 10^{-8} M, considering the normally accepted criterion of three times of the standard deviation for baseline drift. The sensitivity of SMZ detection obtained by our study was as comparable as that of a previous report on the monitoring of sulfonamide residues by an in-tube solid-phase micro-extraction coupled to HPLC (Wen et al., 2005), and was superior to those of the former reports on SMZ detection by a high-volume enzyme immunoassay, a *Bacillus stearothermophilus* tube test and a biosensor-based immunochemical screening assay (Bjurling et al., 2000; Ram et al., 1991; Shitandi, Oketch, & Mahungu, 2006). Considering the LOD and analysis time around 10 min found for the OWLS-based immunosensor of this study, it might be applied to a sensitive and rapid screening for SMZ that is possibly present in food. Whereas, the detection range of the current immunosensor was present from 10^{-8} to 10^{-2} M SMZ, as judged from the linear response obtained at this concentration range.

3.4. Reusability of immunosensor

It is a cautious procedure accompanying an inevitable partial loss of antibody layer to dissociate the bound analyte molecules selectively from the surface of an antibody-coated immunosensor (Park et al., 2004; Pyun, Beutel, Meyer, & Ruf, 1998). In a previous report, 10 mM HCl was successfully used as a regenerant for repeated use of the OWLS-based immunosensor detecting *Salmonella typhimurium*, a hygienic index in the food industry (Kim et al., 2007). Based on this fact, 10 mM HCl was also used as the regenerant in this study.

To evaluate the reusability of the OWLS-based immunosensor of this study, a SMZ solution with a concentration of 10^{-3} M was added six times to the immunosensor system after the regeneration with 10 mM HCl. As shown in Fig. 6, the sensor responses were quite reproducible during six cycles of measurement with the mean surface coverage change of 23.00 ± 2.24 ng/cm^2 and the coefficient of variability of 9.70%. This value was as comparable as that of a biosensor assay on sulfadiazine and SMZ that showed the precision between runs less than 8% (Bjurling et al., 2000). Also, the sensor response at the sixth measurement corresponded to 87.0% of that at the first measurement. This detection sensitivity was better than that of an OGC sensor chip showing 50% of the initial sensor response in *on-line* monitoring for a monoclonal antibody in animal cell culture (Polzius et al., 1996). It is expected that the repeatability of measurement for SMZ detection will be improved better, possibly due to their uninjured antibody layers, when six new OGC sensor chips immobilized with the anti-SMZ antibody are used. Considering that the primary application field of the current OWLS-based immunosensor will be an initial screening for SMZ in real food matrices, it is expected that the immunosensor of this study might be used at least six times for screening purpose because the stringent reproducibility needed for a quantification is, in most cases, not required for a screening (Park et al., 2004).

4. Conclusion

An OWLS-based immunosensor that is applicable to a sensitive detection for SMZ in various types of food was developed by *in situ* immobilization of an anti-SMZ antibody, according to APTS protocol, over the surface of an OGC sensor chip throughout this study. As the sensor

measures SMZ with high sensitivity and reusability, it might be used in an initial screening for the presence of SMZ in food matrices with real-time scale. An application of the present sensor to the SMZ detection in various livestock products such as meat and milk will be required in the future.

Acknowledgement

This work was supported by a Grant No. R01-2003-000-10307-0 from the Basic Research Program of the Korea Science and Engineering Foundation, Republic of Korea.

References

Bjurling, P., Baxter, G. A., Caselunghe, M., Jonson, C., O'Connor, M., & Persson, B. (2000). Biosensor assay of sulfadiazine and sulfamethazine residues in pork. *Analyst, 125*, 1771–1774.

Clark, S. B., Turnipseed, S. B., Madson, M. R., Hurlbut, J. A., Kuck, L. R., & Sofos, J. N. (2005). Confirmation of sulfamethazine, sulfathiazole, and sulfadimethoxine residues in condensed milk and soft-cheese products by liquid chromatography/tandem mass spectrometry. *Journal of the Association of Official Analytical Chemists International, 88*, 736–743.

Elwing, H. (1998). Protein absorption and ellipsometry in biomaterial research. *Biomaterials, 19*, 397–406.

Gehring, T. A., Griffin, B., Williams, R., Geiseker, C., Rushing, L. G., & Siitonen, P. H. (2006). Multiresidue determination of sulfonamides in edible catfish, shrimp and salmon tissues by high-performance liquid chromatography with postcolumn derivatization and fluorescence detection. *Journal of Chromatography B: Analytical Technologies in Biomedical Life Sciences, 840*, 132–138.

Homola, J., Yee, S. S., & Gauglitz, G. (1999). Surface plasmon resonance sensors: Review. *Sensors and Actuators B: Chemical, 54*, 3–15.

Huetz, P., Schaaf, P., Voegel, J. C., Mann, E. K., Miras, B., Ball, V., et al. (1995). Reactivities of antibodies on antigens adsorbed on solid surfaces. *Proteins Interfaces II, 602*, 334–349.

Hug, T. S., Prenosil, J. E., & Morbidelli, M. (2001). Optical waveguide lightmode spectroscopy as a new method to study adhesion of anchorage-dependent cells as an indicator of metabolic state. *Biosensors and Bioelectronics, 16*, 1865–1874.

Kim, N., Park, I.-S., & Kim, D.-K. (2004). Characteristics of a label-free piezoelectric immunosensor detecting *Pseudomonas aeruginosa*. *Sensors and Actuators B: Chemical, 100*, 432–438.

Kim, N., Park, I.-S., & Kim, W.-Y. (2007). *Salmonella* detection with a direct-binding optical grating coupler immunosensor. *Sensors and Actuators B: Chemical, 121*, 606–615.

Kuhlmeier, D., Rodda, E., Kolarik, L. O., Furlong, D. N., & Bilitewski, R. (2003). Application of atomic force microscopy and grating coupler for the characterization of biosensor surfaces. *Biosensors and Bioelectronics, 18*, 925–936.

Leonard, P., Hearty, S., Quinn, J., & O'Kennedy, R. (2004). A generic approach for the detection of whole *Listeria monocytogenes* cells in contaminated samples using surface plasmon resonance. *Biosensors and Bioelectronics, 19*, 1331–1335.

Maudens, K. E., Zhang, G. F., & Lambert, W. E. (2004). Quantitative analysis of twelve sulfonamides in honey after acidic hydrolysis by high-performance liquid chromatography with post-column derivatization and fluorescence detection. *Journal of Chromatography A, 1047*, 85–92.

McCaughey, W. J., Elliott, C. T., & Crooks, S. R. H. (1990). Determination of sulphadimidine in animal feedstuffs by an enzyme-linked immunoassay. *Food Additives and Contaminants, 7*, 259–264.

MicroVacuum Ltd. (2002). In *Optical waveguide lightmode spectroscopy system owls 110 & biosense 2.2 software user's manual* (pp. 25–26).

Park, I.-S., & Kim, N. (2006). Development of a chemiluminescent immunosensor for chloramphenicol. *Analytica Chimica Acta, 578*, 19–24.

Park, I.-S., & Kim, N. (1998). Thiolated *Salmonella* antibody immobilization onto the gold surface of piezoelectric quartz crystal. *Biosensors and Bioelectronics, 13*, 1091–1097.

Park, I.-S., Kim, D.-K., & Kim, N. (2004). Characterization and food application of a potentiometric biosensor measuring β-lactam antibiotics. *Journal of Microbiology and Biotechnology, 14*, 698–706.

Pena, A., Serrano, C., Reu, C., Baeta, L., Calderon, V., Silveira, I., et al. (2004). Antibiotic residues in edible tissues and antibiotic resistance of faecal *Escherichia coli* in pigs from Portugal. *Food Additives and Contaminants, 21*, 749–755.

Polzius, R., Schneider, Th., Bier, F. F., Bilitewski, U., & Koschinski, W. (1996). Optimization of biosensing using grating couplers: Immobilization on tantalum oxide waveguides. *Biosensors and Bioelectronics, 11*, 503–514.

Pyun, J. C., Beutel, H., Meyer, J.-U., & Ruf, H. H. (1998). Development of a biosensor for *E. coli* based on a flexural plate wave (FPW) transducer. *Biosensors and Bioelectronics, 13*, 839–845.

Ram, B. P., Singh, P., Martins, L., Brock, T., Sharkov, N., & Allison, D. (1991). High-volume enzyme immunoassay test system for sulfamethazine in swine. *Journal of the Association of Official Analytical Chemists, 74*, 43–46.

Salisbury, C. D., Sweet, J. C., & Munro, R. (2004). Determination of sulfonamide residues in the tissues of food animals using automated precolumn derivatization and liquid chromatography with fluorescence detection. *Journal of the Association of Official Analytical Chemists International, 87*, 1264–1268.

Saschenbrecher, P. W., & Fish, N. A. (1980). Sulphamethazine residues in uncooked edible tissues of pork following recommended oral administration and withdrawal. *Canadian Journal of Comparative Medicine, 44*, 338–345.

Shitandi, A., Oketch, A., & Mahungu, S. (2006). Evaluation of a *Bacillus stearothermophilus* tube test as a screening tool for anticoccidial residues in poultry. *Journal of Veterinary Sciences, 7*, 177–180.

Situ, C., Crooks, S. R. H., Baxter, A. G., Ferguson, J., & Elliott, C. T. (2002). On-line detection of sulfamethazine and sulfadiazine in porcine bile using a multi-channel high-throughput SPR biosensor. *Analytica Chimica Acta, 473*, 143–149.

Sternesjö, Å., Mellgren, C., & Björck, L. (1995). Determination of sulfamethazine residues in milk by a surface plasmon resonance-based biosensor assay. *Analytical Biochemistry, 226*, 175–181.

Vörös, J., Ramsden, J. J., Csúcs, G., Szendrö, I., De Paul, S. M., & Textor, M. (2002). Optical grating coupler biosensors. *Biomaterials, 23*, 3699–3710.

Wen, Y., Zhang, M., Zhao, Q., & Feng, Y. Q. (2005). Monitoring of five sulfonamides antibacterial residues in milk by in-tube solid-phase microextraction coupled to high-performance liquid chromatography. *Journal Agricultural and Food Chemistry, 53*, 8468–8473.