A Surface Plasmon Resonance-based Immunosensor for Sensitive Detection of Bisphenol A

Matsumoto, Kiyoshi
Faculty of Agriculture, Kyushu Unviersity

Sakai, Takatoshi
Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu Unviersity

Torimaru, Akira
Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu Unviersity

Ishitobi, Sachiko
Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu Unviersity

https://doi.org/10.5109/4676

出版情報：九州大学大学院農学研究院紀要. 50 (2), pp.625-634, 2005-10-01. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **50** (2), 625–634 (2005)

A Surface Plasmon Resonance-based Immunosensor for Sensitive Detection of Bisphenol A

Kiyoshi MATSUMOTO*, Takatoshi SAKAI[1], Akira TORIMARU[1] and Sachiko ISHITOBI[1]

Laboratory of Food Analysis, Division of Food Biotechnology, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan
(Received June 10, 2005 and accepted July 26, 2005)

A method for the determination of bisphenol A (BPA), a representative endocrine-disrupting chemicals, was developed using a surface plasmon resonance (SPR) sensor. BPA-ovalbumin (BPA-OVA) conjugate was immobilized on an Au thin film of the SPR sensor chip by physical adsorption, and BPA determinations were performed by an indirect competitive immunoassay, where a BPA sample containing an anti-BPA antibody is introduced into the SPR sensor system. The addition of BPA into the anti-BPA antibody solution (0.8μg/ml, final concentration) was found to decrease the incidence angle shift sharply because of an inhibition effect of BPA. The RSDs (n=3) of each point were less than 4%. An evaluation of the affinity constant (K_1) between anti-BPA antibody and BPA-OVA conjugate on the chip was found to be 2.26×10^6 M^{-1}, and that (K_2) between anti-BPA antibody and free BPA (analyte) was 5.72×10^4 M^{-1}. The lowest detection limit for BPA by SPR was almost the same as that by ELISA, 10^{-9}g/ml (1 ppb).

INTRODUCTION

Bisphenol A (BPA) is used in the production of epoxy and polycarbonate resins, and as a stabilizer for polyvinyl chloride resin. Polycarbonate is widely used in polycarbonate baby bottles and in the epoxy resin coating of the lid and polyethylene terephthalate. As a result of such applications, very small amounts of BPA can migrate into food and drink. BPA represents a member of a class of endocrine-disrupting chemicals and it has been reported to have weak estrogenic properties (Krishnam *et al.*, 1993; Gould *et al.*, 1998) and an abnormal action for genital function, such as lack of sperm activity (Morrissey *et al.*, 1988). To evaluate the risk from BPA, many investigations have been undertaken to determine human exposure levels and carefully study the validity of the low-dose effects (Gray *et al.*, 2004). In order to determine BPA level in environment, such as river water, or food and drink as well as in human urine and serum, a sensitive and rapid method is desirable. Although high-performance liquid chromatography (HPLC) (Steinmetz *et al*, 1998) and gas chromatography-mass spectrometry (GC-MS) (Cervantes and Losada, 2003; Jeannot *et al.*, 2002; Zafra *et al.*, 2003; Braun *et al.*, 2003) can be used for the analysis of BPA, several time-consuming sample pretreatment steps are required. Therefore, they are not suited for rapid analyses, and also require a large volume of

[1] Laboratory of Food Analysis, Division of Food Biotechnology, Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenviromental Sciences, Kyushu University
* Corresponding author (E-mail: kmatsu@agr.kyushu-u.ac.jp)

sample. When dealing with serum or urine as the media of measurement, it would be very difficult to measure from the limited quantity available. To overcome this handicap, an enzyme-linked immunosorbent assay (ELISA) has been widely used for the determination of BPA due to its high selectivity, high sensitivity, and high sample throughput (Goda *et al.*, 2002; Zhao *et al.*, 2002; Ohkuma *et al.*, 2002), but it still requires over an hour to complete a single measurement.

An immunosensor based on surface plasmon resonance (SPR) has been receiving increasing attention in recent years, due to its potential as a label-free, real-time, rapid, and high-selective immunoassay technique (Deckert and Legay, 1999; Vikinge *et al.*, 1998; Iwasaki *et al.*, 2001; Soh *et al.*, 2003). These methods are able to perform an assay in 5–30 min. Shimomura *et al.* (2001) reported an SPR sensor for determining 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin, PCB, and atrazine using an SPR sensor chip, on which antibodies for these chemicals were immobilized by an amine coupling method. In the report, an antibody for analyte was immobilized on the sensor chip, and the analytes and the labeled analytes were reacted competitively (direct competitive immunoassay). On the other hand, an indirect competitive immunoassay using an SPR has been shown as a highly sensitive and selective method. Sakai *et al.* (1999) reported an SPR sensor for determining methamphetamine and benzopyrene using an SPR sensor chip, on which the analytes (antigen) conjugated with bovine serum albumin (BSA) was immobilized, and the analytes and the antibodies for analyte were reacted competitively. Recently, Soh *et al.* (2003) also determined BPA based on an SPR sensor by using an indirect competitive immunoassay technique. The limit of detection of their sensor was about a few 10 ppb.

In this paper, we report on an SPR immunosensor for BPA using a sensor chip, on which BPA-ovalbumin (BPA-OVA) was immobilized. The mixture of BPA and anti-BPA antibody was flowed on the sensor chip, and BPA and anti-BPA antibody were coupled competitively to BPA-OVA on the chip. The sensitivity for BPA was about 1 ppb. The affinity constants of the anti-BPA antibody to free BPA in a sample solution as well as to BPA-OVA in the SPR chip were also evaluated from the concentration dependency of the SPR sensor responses.

MATERIALS AND METHOD

Materials

Anti-rabbit immunoglobulin G (anti-rabbit IgG, from goat, affinity purified, polyclonal antibody) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma (St. Louis, MO, USA). *p,p'*-Isopropylpyridinediphenol (bisphenol A, BPA), 2-bromoacetic acid ethyl ester and diethyleneglycoldimethyl ether (dry glyme) were from Wako Pure Chemicals Ind., Ltd (Osaka, Japan). Bovine serum albumin (BSA) and N-hydroxysuccinimide (NHS) were from Nacalai Tesque, Inc. (Kyoto, Japan). Econo-Pac column was from Bio-Rad, Lab. (CA, USA). Anti-BPA antibody (anti-BPA Ab, from rabbit, anti serum) and horseradish peroxidase labeled BPA (BPA-HRP) were obtained from Fukube Pharmaceuticals, Inc. (Kanagawa, Japan). All other chemicals were of analytical reagent grade. All buffer solutions were prepared using water purified with a Milli-Q (Millipore, Bedford, UK) system.

Apparatus

SPR measurements were performed using the Spreeta™ evaluation module kit (Texas Instruments, Inc., Texas, USA) attached to a sensor chip, a flow-through cell (18×2×0.2 mm) and a microtube pump (EYELA, SMP-23, Tokyo, Japan). ELISA measurements were performed using 96-well immunoplates (NUNC, No. 446612, Roskilde, Denmark) and a microplate reader (Spectra 1, Wako, Osaka, Japan). Spectrophotometric measurements were performed by the use of Shimadzu Multi Spec 1500 (Kyoto, Japan). Thin layer chromatography (TLC) was performed by using TLC plate Silica gel 60 F254, 20×20 cm (Merck, Darmstadt, Germany).

Preparation of BPA-OVA conjugate

Preparation of BPA-OVA conjugate was performed by the method reported by Ohkuma *et al.* (2002) and Nishii *et al.* (2000) with a little modification.

BPA (100 mg) was dissolved in 400 μl of N,N'-dimethylformamide (DMF) in the presence of 200 mg of potassium carbonate, and 100 μl of 2-bromoacetic acid ethyl ester was added. After addition, the reaction mixture was stirred for 20 min at 65 °C. The mixture was completely extracted with ethyl acetate, and 3 ml of H_2O was added. The pH of the mixture was adjusted to 3.5 with 5 M HCl. After sufficient stirring, 3 ml of H_2O was added again, and then the pH was adjusted to 2.0. The aqueous phase was washed out and the organic phase was dried over anhydrous sodium sulfate. The organic phase thus obtained was subjected to a TLC with the developing solvent being chloroform. The spot of monocarboxymethyl ether was collected and the product was eluted with ethanol. The eluate was centrifuged and the supernatant was evaporated to a solid powder with a centrifugal evaporator (EYELA, CVE-100D, Tokyo, Japan) *in vacuo*. The BPA-monocarboxymethyl ether (BPA-$OCH_2COOC_2H_5$) was dissolved in 70% ethanol solution and was saponified using 85% potassium hydroxide, and the reaction mixture was stirred for 1 h at 80 °C. After the saponification, 3 ml of H_2O was added to the mixture and pH was adjusted to 1.2. The mixture was completely extracted with ethyl acetate, and then washed three times with H_2O. The organic phase was dried over anhydrous sodium sulfate, and then evaporated to a solid powder with a centrifugal evaporator. BPA-monocarboxymethyl ether (BPA-monoacetate) was thus obtained as above.

BPA-monoacetate (25 mg) and NHS (13 mg) were dissolved in 1 ml of dry glyme, 50 mg of sodium sulfate was added and the mixture was cooled in an ice bath. After addition of 34 mg of EDC, the mixture was stirred over night at room temperature. Then, the mixture was slowly dropped 3 times of 62 μl each at interval of 30 min to 1 ml of 0.1 M borate buffer (pH 8.0) containing 10 mg of OVA. The mixture was stirred for 2 h at room temperature, and then subjected to a gel filtration using an Econo-Pac column. The eluate of BPA-OVA was lyophilized. Figure 1 shows the scheme of the preparation of BPA-OVA.

Direct competitive ELISA for BPA

The measurements for BPA by direct competitive ELISA was performed as follows. Ninety-six-well immunoplates were coated with 100 μl of anti-rabbit IgG (10 μg/ml) in 50 mM carbonate buffer (pH 9.6) were left overnight at room temperature. The following day, the plates were washed three times with phosphate buffered saline (PBS) containing

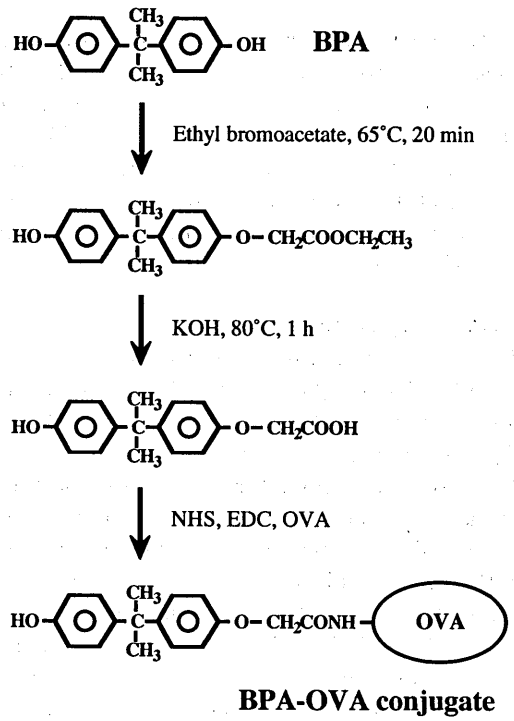

Fig. 1. Preparation scheme of the bisphenol A–ovalbumin conjugate.

0.05% Tween 20 (PBST) and treated with 1% gelatin for 1 h at room temperature. The plates were washed three times again with PBST and added 100 μl of the equivalent mixtures of BPA–HRP (4 μg/ml, diluted with PBS) and serial diluted BPA (diluted with PBS containing 10% methanol). Then, 50 μl of anti–BPA antibody (2000–fold diluted with PBS) was added and reacted for 1 h at room temperature. After washing three times with PBST, the substrate solution was added and reacted for 30 min at 37 °C. The results were expressed as absorbance at 405 nm. The substrate solution was a 10:9:1 mixture (v/v) of 0.006% H_2O_2 dissolved in 0.2 M citrate buffer (pH 4.0), H_2O, and a solution of 6 mg/ml of 2,2'–azinobis–(3–ethylbenzotiasoline–6–sulfonic acid, diammonium salt).

Indirect competitive SPR measurements for BPA

For highly sensitive detection, we used an indirect competitive method, which was effective for detecting chemical substances with low molecular weight (Miura *et al.*, 1993, 2003; Sakai *et al.*, 2003). The refractive index or resonance angle of Au thin films modified with antigen–protein conjugate depends on the concentration of the antigen, and this small change can be detected using the SPR. The schematic diagram of the indirect inhibition SPR measurement is shown in Fig. 2. In this method, antigen proteins were immobilized on the sensor chip (Au thin film) by circulating the BPA–OVA solution (1 mg/ml in

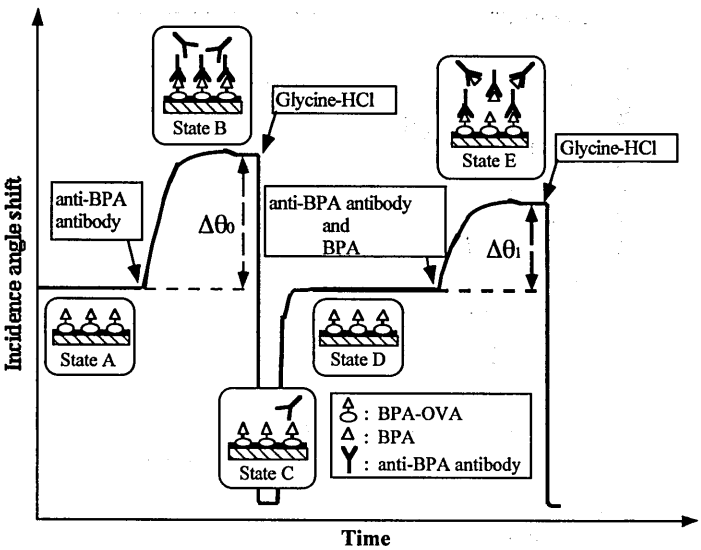

Fig. 2. Response transients of the BPA–OVA immobilized sensor to the anti–BPA antibody without antigen ($\Delta\theta_0$) and with antigen ($\Delta\theta_1$).

PBS) for 1 h (physical adsorption). The sensor chip was washed by circulation of the carrier buffer (PBS) for 5 min. A BSA solution (2 mg/ml in PBS) was circulated for 1 h to reduce nonspecific adsorption of the antibody. After washing with carrier buffer (State A in Fig. 2), the anti–BPA antibody solution (appropriate concentration) was allowed to flow for 3 min, and the change in incidence angle shift ($\Delta\theta_0$) caused by the association of the anti–BPA antibodies to the immobilized antigens (BPA–OVA) was measured (State B). After 3 min, the flowing solution was changed to carrier buffer and allowed to flow for another 3 min, and then the sensor chip was regenerated by circulation of glycine–HCl buffer (10 mM, pH 2.2) for 2 min (State C). After the chip was washed with carrier buffer and the signal base line was recovered (State D), 1 ml of an equivalent mixture of anti–BPA antibody (1.6 μg/ml depending on the condition) and an appropriate amount of BPA was allowed to flow to realize a decreased incidence angle shift ($\Delta\theta_1$) with inhibition by BPA (State E). This cycle was performed for the mixture of anti–BPA antibody and serial diluted BPA. Quantitative determination of the antigen concentration can be made from the difference of $\Delta\theta_0$ and $\Delta\theta_1$. The flow rate was constantly maintained at 300 μl/min, and all the procedures were carried out at room temperature.

Assay of anti–BPA antibody concentration by ELISA

Ninety–six–well immunoplate was coated with 50 μl of anti–rabbit IgG β–galactosidase conjugate (1000–fold diluted with 50 mM carbonate buffer, pH 9.8) overnight. The plate was treated with 1% gelatin for 1 h. Fifty μl of serial diluted anti–BPA antibodies (from rabbit) or serial diluted rabbit IgGs were added and reacted for 1 h. Then, 50 μl of alkaline phosphatase labeled anti–rabbit IgG was added and reacted for 1 h. The

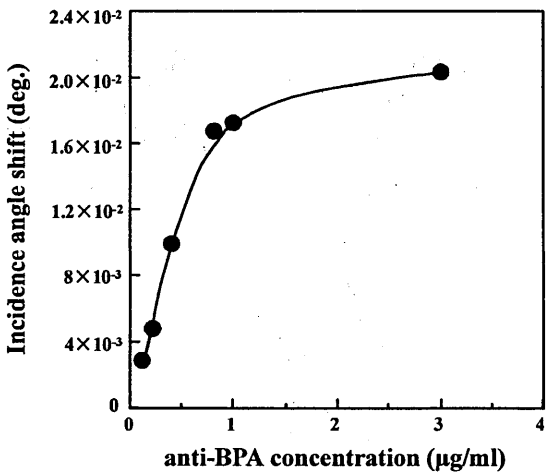

Fig. 3. Effect of anti-BPA antibody concentration on the incidence angle shift of the BPA–OVA immobilized sensor.

substrate, *p*–nitrophenylphosphate (50 μl, 2 mg/ml), was reacted for 30 min and the absorbance at 405 nm was measured. Wells were rinsed three times with PBST between each step. All treatments were done at room temperature. The concentration of anti–BPA antibody was represented as that of rabbit IgG as a standard.

RESULTS AND DISCUSSION

Optimization of the BPA assay by SPR

A BPA–OVA immobilized chip was exposed to the flow of anti–BPA antibody at various concentrations (0.1–3.0 μg/ml). The incidence angle shifts were plotted agaist the concentration of anti–BPA antibody; the plots are shown in Fig. 3. The incidence angle shift increased rapidly with increasing concentration of anti–BPA antibody up to approximately 0.8 μg/ml, and then increased meagerly above this concentration. From this figure, the use of an approximately 0.8 μg/ml anti–BPA antibody solution in the final concentration is considered to be the best for BPA sensing experiments, considering the use of the indirect competitive method.

Detection of BPA with SPR and a comparison with ELISA

Anti–BPA antibody solutions (1.6 μg/ml) were mixed with equal volumes of BPA of various concentrations, and incubated for 5 min at room temperature prior to flowing over the BPA–OVA immobilized sensor chip. The percent inhibition, which was the decrease in the response relative to that of no BPA antigen, $(\Delta\theta_0 - \Delta\theta_i)/\Delta\theta_0 \times 100$, was plotted against the concentration of BPA, where $\Delta\theta_i$ stands for the incidence angle shift of the i–th mixture of anti–BPA antibody and BPA; the plots are shown in Fig. 4. The percent inhibition was increased rapidly with an increase in the concentration of BPA higher than

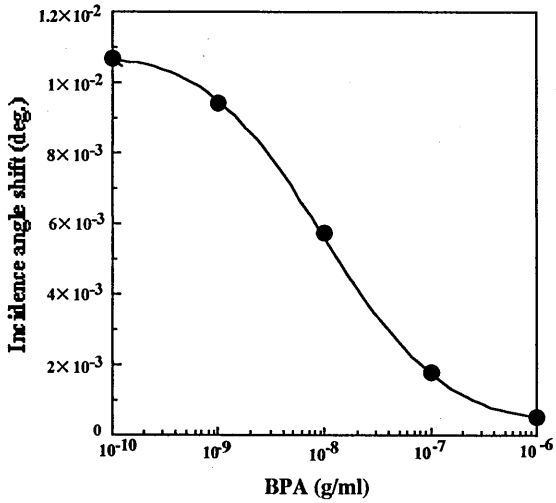

Fig. 4. Inhibition curve for BPA by indirect competitive SPR method.

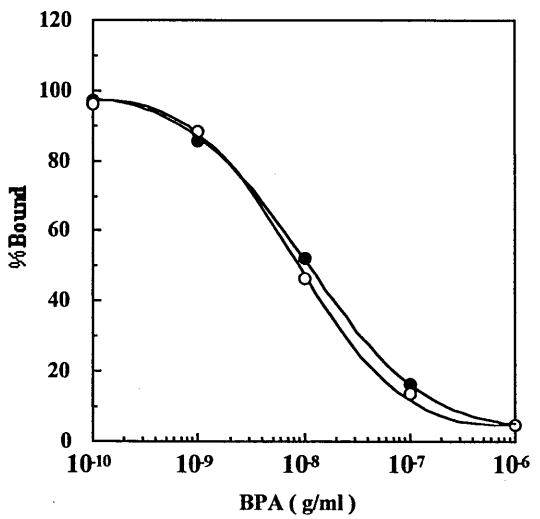

Fig. 5. Comparison of the results of bisphenol A measurements using indirect competitive SPR immunosenosor with those obtained by direct competitive ELISA.
Symbols: ●, SPR immunosensor, ○, ELISA

10^{-9}g/ml (1 ppb). The RSDs (n=3) of each point were less than 4.0%. The direct competitive ELISA was also done for the detection of BPA; the results observed by both SPR and ELISA methods are compared in Fig. 5. The lowest detection limit for BPA by SPR was almost the same as that of ELISA, 10^{-9}g/ml (1 ppb). However, the RSDs (n=3) of the ELISA method were higher than those of SPR method.

Evaluation of affinity constants

The determination of an affinity constant, K_A, or its reciprocal, the dissociation constant, K_D, is frequently useful in the study of antigen–antibody interactions.

We attempted to evaluate the affinity constants of the anti–BPA antibody to immobilized BPA–OVA and BPA using SPR data (Sakai *et al.*, 1999, 1998; Sakai *et al.*, 2003). The analyses were performed according to the method reported by Sakai *et al.* They evaluated affinity constants relevant to an immunoassay system by assuming a Langmuir–type adsorption model for the immunoreaction. The present system involves two competitive immunoreactions as follows:

$$\mathrm{Ab} + \mathrm{BPA\text{–}OVA_{bound}} \xrightarrow{K_1} \mathrm{Ab\text{–}BPA\text{–}OVA_{bound}} \quad (2)$$

$$\mathrm{Ab} + \mathrm{BPA} \xrightarrow{K_2} \mathrm{Ab\text{–}BPA} \quad (3)$$

Here, Ab indicates anti–BPA antibody, and K_1 and K_2 are affinity constants. Three assumptions are required to correlate the incidence angle shift with each immunoreaction. First, the antigen–antibody reaction proceeds by monovalency. Second, a Langmuir–type adsorption model can be adopted. Third, the incidence angle shift is proportional to the coverage of adsorption sites (immobilized BPA–OVA) by anti–BPA antibody. Based on these assumptions, we obtain the following equation for the first immunoreaction.

$$[\mathrm{Ab}]/\Delta\theta_0 = [\mathrm{Ab}]/\Delta\theta_{0,\max} + 1/\Delta\theta_{0,\max} K_1 \quad (4)$$

Here, [Ab] is the molar concentration of anti–BPA antibody, $\Delta\theta_0$ is the equilibrium incidence angle shift for a given concentration of antibody, and $\Delta\theta_{0,\max}$ is the maximum incidence angle shift at full coverage. Assuming the latter immunoreaction to also be equilibrium, eq. (5) can be derived.

$$1/\Delta\theta = 1/\Delta\theta_0 + K_2[\mathrm{BPA}]/\Delta\theta_{0,\max} K_1[\mathrm{Ab}]_0 \quad (5)$$

Here, $\Delta\theta$ and $\Delta\theta_0$ are the angle shifts in the presence and absence of BPA, respectively. $[\mathrm{Ab}]_0$ is the initial molar concentration of anti–BPA antibody.

From eq. (4), $[\mathrm{Ab}]/\Delta\theta_0$ should be linear to [Ab], the slope and intercept giving the values of $\Delta\theta_{0,\max}$ and K_1, respectively. The data points in Fig. 3 are replotted in this way, and a linear correlation (correlation coefficient r=0.986) was obtained, giving $\Delta\theta_{0,\max}$= 0.0257 deg. and $K_1 = 2.26 \times 10^6$ M^{-1}. From eq. (5), the inverse $\Delta\theta$ should be linear to [BPA], K_2 being obtained from the slope and the known values of $\Delta\theta_{0,\max}$, K_1 and $[\mathrm{Ab}]_0$.

The data points of the SPR method in Fig. 4 are replotted, giving $K_2 = 5.72 \times 10^4\ M^{-1}$.

With consideration of the values of K_1 and K_2, the anti-BPA antibody has higher affinity to the bound BPA-OVA on the sensor chip than that to free BPA. In the indirect competitive immunoassay, it is desirable that K_1- and K_2-values are almost the same, or K_2-value should be a little higher than K_1-value. In this case, K_1-value is about 40 times higher than K_2-value. If the K_2-value has the order of $10^6\ M^{-1}$, the detection limit will be improved to some extent.

Thus, it can be concluded that an indirect competitive immunoassay using SPR, as described above, is a useful method for detection of BPA, which requires only 11 min for one cycle measurement.

REFERENCES

Braun P., M. Moeder, St. Schrader, P. Popp, P. Kuschk and W. Engewald 2003 Trace analysis of technical nonylphenol, bisphenol A and 17α-ethinyestradiol in wastewater using solid-phase microextraction and gas chromatography- mass spectrometry. *J. Chromatogr. A*, **988**(1): 41–51

Cervantes J. L. and P. P. Losada 2003 Determination of bisphenol A in, and its migration from, PVC stretch film used for food packaging. *Food Addi. Contam.*, **20**: 596–606

Dekert F. and F. Legay 1999 Development and Validation of an Immunoreceptor Assay for Simulect Based on Surface Plasmon Resonance. *Anal. Biochem.*, **274**(1): 81–89

Goda Y., A. Kobayashi, K. Fukuda, S. Fujimoto, M. Ike and M. Fujita 2000 Development of the ELISAs for detection of hormone-disrupting chemicals. *Water Sci. Technol.*, **42**(7/8): 81–88

Gould J. C., L. S. Leonard, S. C. Maness, B. L. Wagner, K. Conner, T. Zacharewski, S. Safe, D. P. McDonnell and K. W. Gaido 1998 Bisphenol A interacts with the estrogen receptor α in a distinct manner from estradiol. *Mol. Cell. Endocinol.* **142**(1/2): 203–214

Gray G. M., J. T. Cohen, G. Cunha, C. Hugher, E. E. McConnel, L. Phomberg, I. G. Sipes, D. Mattison 2004 Weight of the evidence evaluation of low-dose reproductive and developmental effects of bisphenol A. *Human & Ecolog. Risk Asses.*, **10**(5): 875–921

Iwasaki Y. T. Horiuchi and O. Niwa 2001 Detection of Electrochemical Enzymatic Reactions by Surface Plasmon Resonance Measurement. *Anal. Chem.*, **73**(7): 1595–1598

Jeannot R., H. Sabik, E. Sauvard, T. Dagnac and K. Dohrendorf 2002 Determination of endocrine-disrupting compounds in environmental samples using gas and liquid chromatography with mass spectrometry. *J. Chromatogr. A*, **974**(1/2): 143–159

Krishnam A. V., P. Stathis, S. F. Permuth, L. Tokes and D. Feldman 1993 Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinol.*, **132**(6): 2279–2286

Miura N. H. Higobashi, G. Sakai, A. Takeyasu, T. Uda and N. Yamazoe 1993 Piesoelectric crystal immunosensor for sensitive detection of methamphetamine (stimulant drug) in uman urine. *Sens. Actuators B*, **13**(1/3): 188–191

Miura N. M. Sasaki, K. V. Gobi, S. Kataoka and Y. Shoyama 2003 Highly sensitive and selective surface plasmon resonance sensor for detection of sub-ppb levels of benzo[a]pyrene by indirect competitive immunoreaction method. *Biosens. Bioelectron.*, **18**(7): 953–959

Morrissey R. E., J. C. Lamb, B. A. Schwetz, J. L. Teague and R. W. Morris 1988 Association of sperm, vaginal cytology, and reproductive organ weight data with results of continuous breeding reproduction studies in Swiss (CD-1) mice. *Fundam. Appl. Toxicol.*, **11**(2): 359–371

Nishii S., Y. Soya, K. Matsui, T. Ishibashi and Y. Kawamura 2000 Determination of bisphenol A by ELISA using an organic solvent-resistant anti-bisphenol A monoclonal antibody. *Bunseki Kagaku*, **49**(12): 969–975

Ohkuman H. K. Abe, M. Ito, A. Kokado, A. Kmbegawa and M. Maeda 2002 Development of a highly sensitive enzyme-linked immunosorbent assay for bisphenol A in serum. *Analyst*, **127**: 93–97

Sakai G., S. Nakata, T. Uda, N. Miura and N. Yamazoe 1999 Highly selective and sensitive SPR immunosensor for detection of methamphetamine. *Electrochim. Acta*, **44**(21/22): 3849–3854

Sakai G., K. Ogata, T. Uda, N. Miura and N. Yamazoe 1998 A surface plasmon resonance-based

immunosensor for highly sensitive detection of morphine. *Sens. Actuators B*, **49**(1/2): 5–12

Sakai T., A. Torimaru, K. Shinahara, N. Miura, T. Imato, K. Toko and L. Matsumoto 2003 Preparation of a Polyclonal Antibody and a Bioassay for Nitroaromatic Compounds by an Enzyme–Linked Immunosorbent Assay Technique and a Surface Plasmon Resonance Biosensor. *Sens. Mater.*, **15**(8): 439–452

Shimomura M., Y. Nomura, W. Zhang, M. Sakino, K–H. Lee, K. Ikebukuro and I. Karube 2001 Simple and rapid detection method using surface plasmon resonance for dioxins, polychlorinated biphenylx and atrazine. *Anal. Chim. Acta*, **434**(2): 223–230

Soh N., T. Tokuda, T. Watanabe, K. Mishima, T. Imato, T. Masadome, Y. Asano, S. Okutani, O. Niwa and S. Brown 2003 A surface plasmon resonance immunosensor for detecting a dioxin precursor using a gold binding polypeptide. *Talanta*, **60**(4): 733–745

Soh N., T. Watanabe, Y. Asano and T. Imato 2003 Indirect Competitive Immunoassay for Bisphenol A, Based on a Surface Plasmon Resonance Sensor. *Sens. Mater.*, **15**(8): 423–438

Steinmetz R., N. A. Mitchner, A. Grant, D. L. Allen, R. M. Bigsby and N. Ben–Jonathan 1998 The Xenoestrogen Bisphenol A Induces Growth, Differentiation, and *c–fos* Gene Expression in the Femal Reproductive Tract. *Endocrinol.* **139**(6): 2741–2747

Vikinge T. P., A. Askendal, B. Liedberg, T. Lindahl and P. Tengvall 1998 Immobilized chicken antibodies improve the detection of serum antigens with surface plasmon resonance (SPR). *Biosens. Bioelectron.*, **13**(12): 1257–1262

Zafra A., M. Olmo, B. Suarez, E. Hontoria, A. Navalon and J. L. Vilchez 2003 Gas chromatographic–mass spectrometric method for the determination of bisphenol A and its chlorinated derivatives in urban wastewater. *Water Res.*, **37**(4): 735–742

Zhao M., Y. Li, Z. Guo, X. Zhang and W. Chang 2002 A new competitive enzyme–linked immunosorbent assay (ELISA) for determination of estrogenic bisphenols. *Talanta*, **57**(6): 1205–1210