COMPACT AND MULTIPLE SURFACE-PLASMON-RESONANCE IMMUNOSENSOR
FOR SUB-PPB-LEVEL SMALL MOLECULES

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Abstract

Highly sensitive and selective analysis of benzo[a]pyrene and 2-hydroxybiphenyl was realized by using an indirect competitive immunoreaction based on surface plasmon resonance (SPR) detection. A compact and multiple SPR immunosensor designed for this method was developed in the dimensions of 160 mm x 90 mm x 60 mm.

Keywords: SPR, conjugate antigen, competitive immunoreaction

1. Introduction

Biosensing methods based on immunoreactions are very attractive for the analysis of low-concentration toxic pollutants in the environment because of their high selectivity in complex matrices. An enzyme-linked immunosorbent assay (ELISA) method has been used commonly for detecting the extent of an immunoreaction. In optical detection techniques, an SPR-based sensor is versatile with the salient features such as in-situ determination, real-time response and reusability. We have already reported a new immunosensing method for small molecules with sub-ppb sensitivity: an indirect competitive immunosensing method [1]. In this paper, we present the development of a compact and multiple SPR sensor by using the indirect competitive immunosensing and microchannel fabrication techniques. The simultaneous analysis of biologically harmful molecules such as benzo[a]pyrene (BaP) and 2-hydroxybiphenyl (HBP) is demonstrated. The \(\mu\)TAS approaches for this immunosensing technique should be very useful because of rapid responses in the microchannels, low consumptions of the expensive biochemical reagents and portability for on-site analysis.

2. Indirect competitive immunosensing

The principle of the indirect competitive immunosensing is shown schematically in Figure 1. The small molecules such as BaP and HBP themselves do not induce the synthesis of the antibodies against them. In order to bring them into an immunoassay...
system, a conjugated target molecule with bovine serum albumin (BSA) and a monoclonal antibody for the conjugate are prepared. This antibody can bind both the conjugate and the target small molecule. A standard solution containing the antibody at an appropriate concentration flows on the gold thin film on which the conjugate antigen is physically immobilized. The immunoreaction on the gold film produces a large angle shift in the SPR measurement because the molecular weight of the antibody is very large, ca. 150,000. If the target molecule exists in the standard solution, the antibody could react with the target molecule and a part of the conjugate on the gold film remains free. Therefore, the angle shift becomes smaller than that for the standard solution. An analytical curve was obtained for BaP and HBP in the concentration range of 0.1 - 1000 ppb by using a conventional SPR apparatus, as shown in Figure 2 [2].

3. Compact and multiple SPR sensor

Figure 3 shows a prototype of the SPR immunosensor equipped with a multi- and micro-flow cell (500 μm x 50 μm x 4 channel) which consists of a cover glass with SPR active gold surface, a PDMS plate with the microchannels and PMMA plates with inlet and outlet channels. The multichannel scheme might be useful for simultaneous detection for several analytes and improvement of sensitivity and accuracy by using reference channels. The SPR signals were two-dimensionally analyzed from Fourier transform images of the total reflection light on a c-MOS camera chip in the Kretschmann-configuration. In order to make the apparatus compact (160 mm x 90 mm), we used a specially designed trapezoid prism and a diode laser which emitted a good parallel and polarized light. A
laptop computer provided electric power and acquired the data from the c-MOS camera via a USB interface. A snapshot of running software for the SPR image analysis is shown in Figure 4. The SPR angle shift could be determined with the resolution of 0.01 degree and in the range of 5 degree wide. The sensitivity and response were evaluated in comparison with the conventional SRP apparatus. A development of practical protocols by using the reference is in progress.

Figure 3. Compact SPR immunosensor and interior elevation of the multichannel cell.

Figure 4. A snapshot of SPR image on the c-MOS camera and analysis software.

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References

