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Indirect Competitive Immunoassay for Bisphenol A, Based on a Surface Plasmon Resonance Sensor

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A method for the determination of bisphenol A (BPA), a representative endocrine-disrupting chemical, was developed using a surface plasmon resonance (SPR) sensor. The method is based on an indirect competitive immunoassay, where a BPA sample containing an anti-BPA antibody is introduced into the SPR sensor system. A sensor chip immobilized with a 2AET layer/ BPA layer membrane was prepared by depositing 2-aminoethanthiol (2AET) on a gold film on the sensor chip, followed by reacting the 2AET layer with esterified BPA. The resulting sensor chip was placed into the SPR sensor of a flow system, which consisted of a syringe pump and an injector. The sensor response, in the form of a resonance angle shift, was measured as a function of time before and after injecting different concentrations of BPA in a sample solution that contained the anti-BPA antibody at a constant concentration. In order to estimate the affinity constant of the anti-BPA antibody to BPA, which was immobilized on the sensor chip, the SPR angle shift was first measured by injecting an anti-BPA antibody solution at different concentrations into the SPR system. The affinity constant of the anti-BPA antibody to BPA immobilized on the sensor chip (K_1) was calculated to be 9.3×10^5 M^{-1} from the SPR angle shift data, assuming a Langmuir adsorption isotherm. BPA sample solutions (1–100 ppb) containing 40 ppm anti-BPA antibody were then injected into the SPR system, and the SPR angle shift was determined for each of the sample solutions. A conventional sigmoidal calibration curve, which was typically observed in a competition immunoassay, was obtained when the SPR angle shift was plotted against the BPA concentration. The affinity constant of the anti-BPA antibody to the free BPA (K_2) in the sample solution was estimated to be 1×10^7 M^{-1} by fitting the observed calibration curve to the theoretical one for a competitive inhibition assay. The BPA detection limit of the method was determined to be approximately 10 ppb.

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