

# SPR Biosensor: Food Science Applications

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**T**here is a need in the food industry for methods that offer greater sensitivity, speed, accuracy, specificity and capability for on-line and real-time analyses. Such methods may be used for analysis of food composition of raw materials and end products, nutrients for proper labeling, for process control and product evaluation to ensure food safety, and as research tools for new and improved products. Biosensors can fulfill these analytical needs in the food industry.

A biosensor is a device that senses and transmits information about a biological process. It is composed of a biological or chemical recognition complex—such as antibody-antigen, enzyme-substrate, receptor-ligand—which is placed in close proximity to a transducer. The latter is a device that receives a signal in one form or type and converts it to a signal in another form. The transducers used in bioanalytical sensors transform electrochemical signals with electrodes, optical signals by optodes, thermal energy by thermistors, and molecular mass by piezoelectric, surface acoustic wave, or quartz crystal microbalance. Various biosensors utilized in food analysis are described by Wagner and Guilbault.<sup>1</sup> Biosensor instruments for label-free, real-time monitoring of intermolecular interactions allow direct and rapid determination of their binding properties and kinetics. These biosensors have detection systems based on surface plasmon resonance (SPR), optical evanescence—resonant mirror and optical evanescence—diffraction grating.<sup>2</sup>

During our research we have utilized an SPR biosensor, the BIAcore. This SPR biosensor consists of three major components: the coupling matrix covalently linked to a thin layer of gold coated onto a slide glass, a liquid handling system and an optical system to measure and report the interactions. The coupling matrix is a carboxylated dextran layer to which the capturing molecule (ligand) is covalently immobilized. A sensor chip containing four of these active surfaces is housed in a microfluidics cartridge

(IFC), allowing the transport of analytes and reagents to the sensor device. A polarized light from a light-emitting diode is focused into a line on the glass backing of the sensor chip and the reflected light is detected by a photodiode array (Figure 1). Its automated system includes sample handling, data acquisition, analysis and evaluation. Qualitative and quantitative analyses of the biospecific interactions are obtained.

The BIAcore system can analyze a biochemical interaction in real-time by allowing a direct analysis of the binding of any component of a biomolecular recognition pair to an immobilized recognition pair (ligand) without the use of labels. The binding interaction generates a change in mass resulting from the captured molecule. This promotes changes in the optical properties of the medium in the vicinity of the gold film. The evanescent wave, an electromagnetic field component of the light, probes this interaction by penetrating into the lower refractive index medium between the gold film and matrix. When the evanescent wave interacts with the cloud of electrons on the gold surface resonance occurs resulting in surface plasmon wave, which takes up the energy of the incident light resulting in a “dip” in intensity of the reflected light. The photodiode ray determines the angle at which SPR occurs and measures the resulting dip in intensity and these interactions are expressed in arbitrary resonance units (RU). The binding response (RU) is proportional to the mass (amount and molecular size) of adsorbed molecules on the sensor chip.<sup>3</sup> The binding interactions are monitored continuously and plotted in real-time as RU versus time (seconds), showing a binding curve that is displayed as a sensorgram in a monitor screen. A detailed description of the BIAcore and the principles of its analysis and operation are described in the literature.<sup>4,7</sup>

## DETECTION OF LOW MOLECULAR WEIGHT CHEMICAL COMPOUNDS

The use of the BIAcore for the analysis of low

molecular weight compounds were described for aminothephyllyne,<sup>8,9</sup> chemical residues, atrazine,<sup>10</sup> sulfamethazine,<sup>11</sup> and hygromycin B.<sup>12</sup> Since chemical residues have low molecular mass, these compounds (aminothephyllyne, sulfamethazine and hygromycin) were immobilized onto the coupling matrix via amino moiety and utilized as ligands (capturing molecules). Atrazine was modified by the addition of a free amino group prior to immobilization on the carboxylated dextran of the sensor chip. These compounds were detected indirectly by using a competitive assay; that is, the compound is mixed off-line with an excess but constant amount of the antibody. The excess, unreacted antibody was adsorbed by the sensor containing the immobilized compound, hygromycin B. The detection range for hygromycin B was 2.5 ng/mL to 5 mg/mL. Analysis of spiked milk samples showed a minimum detectability of 25 ppb, which can be attributed to interference by the binding of aminoglycosides with milk lactalbumin, a milk protein.<sup>13</sup> In contrast, atrazine was detected at 0.05 ng/mL in drinking water and sulfamethazine was detected in milk at 1 ng/mL.

Folic acid and biotin were analyzed using the SPR biosensor, BIAquant. It also utilizes a competitive inhibition immunoassay wherein folic acid or biotin are immobilized on the sensor chip. Monoclonal antibodies were added to samples that had been homogenized and clarified. The mixtures were then injected onto the BIAquant system and the excess, unbound antibodies were determined. Unlike the BIAcore research model, the signals generated reported results as concentrations of the vitamins, 3-100 ng/mL biotin and 2-100 ng/mL folic acid.<sup>14</sup> In addition, this model is simpler and less expensive than the BIAcore.

#### ANTIBODY KINETICS, SPECIFICITY

The BIAcore was used to assess antigen-antibody interactions, including binding and dissociation kinetics, specificity, and antibody concentrations.<sup>5,15-18</sup> Our group reported the binding and dissociation rate constants of anti-hygromycin antibody and its antigen. Utilizing the BIAevaluation system, the affinity constants of the antibody bound to the hygromycin sensor were determined as  $1.45 \times 10^{10}$  and  $1.84 \times 10^{10}$  at 12.5 and 25 ng/mL IgG in buffer.<sup>12</sup> The binding crossreactivity of the antibody with other aminoglycoside drugs, neomycin, gentamicin, spectinomycin,

streptomycin and dihydrostreptomycin was evaluated using the same sensor chip immobilized with hygromycin B. The aminoglycosides were mixed with the anti-hygromycin off-line and injected into the BIAcore system. The unreacted anti-hygromycin was captured by the sensor chip and measured. A high antibody binding response indicated a low or negligible antibody crossreactivity. Results from this study indicated that cross-reactivity was less than 10% when the drugs were tested at concentrations of at least 1000 times greater than action levels. This system can be used to monitor antibody production during the immunization process or to select monoclonal antibodies with desired binding properties and specificities.

The binding epitopes on the sensor can be regenerated after each sample analysis by use of appropriate regeneration agents. HCl (0.1M) regenerated the hygromycin-

polyclonal, polyclonal-bacteria-monoclonal, monoclonal-bacteria-polyclonal and monoclonal-bacteria-monoclonal. The best response was obtained with the monoclonal-bacteria-polyclonal combinations with detection limits of  $2.1 \times 10^6$  CFU taken from a cell suspension of  $7 \times 10^7$  CFU/mL. The use of Protein A as the sensor capturing molecule did not improve the sensitivity. Injection of a  $5 \times 10^9$  CFU/mL suspension generated lower signals than with an antibody sensor. In this assay, the use of 6M guanidine-HCl as regenerating agent allowed over 50 repeat analyses.

In an attempt to further improve *E. coli* detection with the SPR biosensor, a competitive assay was performed similar to the method used in the detection of the small molecules described. *E. coli* antibody (5 ug/mL) and bacterial cells were mixed off-line. The supernatant containing the unreacted IgG separated by centrifugation was injected into the BIAcore. The bound IgG were desorbed with 0.1M HCl from the Protein A sensor surface. An inhibition curve (CFU/mL vs % inhibition) was plotted to determine the minimum number of cells detected. This indirect competitive assay showed an enhanced detection of  $10^6$  CFU/mL compared to the "sandwich assay."<sup>19</sup> These approaches are less sensitive compared with standard enzyme-linked immuno-sorbent assays (ELISA), which can detect  $10^3$ - $10^4$  CFU/mL.<sup>18</sup> However, Haines and Patel reported that *Salmonella* was detected at  $10^4$  CFU/mL in a similar off-line competitive assay.<sup>20</sup> The bacteria suspension in an enrichment medium was mixed with an antibody, and the excess antibody was separated from the unbound and antibody bound bacteria by filtration. The filtrate was then injected into the BIAcore system and the *Salmonella* antibody was captured by an anti-Fab immobilized on the sensor surface.

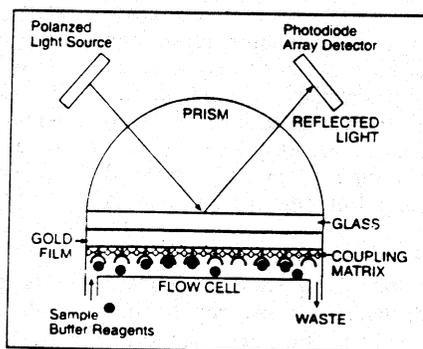


Figure 1. Surface plasmon resonance sensor, optical detection system and liquid flow channel.

sensor surface by desorbing the bound immunoglobulin (IgG) and allowed over a 100 analyses. The hygromycin sensor was stable for 8 months when stored in buffer containing 0.05% sodium azide, but the sensor was not tested beyond this period. The automated system also allowed analysis of 96 samples a day for a contact time (binding) of 10 minutes.

#### DETECTION OF FOOD PATHOGENS

SPR-immunochemical methods to detect *E. coli* O157:H7 were reported by Fratamico, et al.<sup>19</sup> Anti-*E. coli* monoclonal or polyclonal antibodies were immobilized on the sensor chip. A suspension of live *E. coli* cells were injected into the BIAcore system. The direct detection of the bacteria generated a low response. A second anti-*E. coli* antibody was injected to probe the presence of the bacteria, which were captured by the anti-*E. coli* sensor and also to enhance the response. This "sandwich assay" was tested using different combinations: polyclonal-bacteria-

#### KINETIC ANALYSIS OF ANTI-*E. COLI* ANTIBODY WITH IMMOBILIZED CELLS

The BIAcore system was utilized to determine the association and dissociation rates of the antibody against *E. coli* O157:H7. The kinetic values derived can be utilized in the development of immuno-based diagnostic biosensors.<sup>21</sup> Irradiated, whole bacterial cells were immobilized on the sensor chip using standard techniques for coupling free amino groups. The optimum binding pH was also determined and indicated a maximum capture of IgG at pH 5. Various concentrations of anti-*E. coli* IgG were

injected and allowed to react with the bacteria for 30 minutes. The sensor chip was utilized many times, but regeneration of the bacterial surface with 6M guanidine-HCl resulted in gradual loss of the binding surface (near 20% after 12 cycles).

In later studies, a 1M guanidine-HCl effectively regenerated the sensor surface, allowing more than 50 analyses on the same sensor surface. Binding analysis of 42 and 84 nM IgG indicated an average association rate constant ( $k_a$ ) of  $1.27 \times 10^4$  ( $M^{-1} s^{-1}$ ); dissociation rate constant ( $k_d$ ) of  $4.12 \times 10^{-5}$  ( $s^{-1}$ ); and affinity constant ( $K = k_a/k_d$ ) of  $3.09 \times 10^8$  ( $M^{-1}$ ). These studies demonstrated that the binding and dissociation kinetic properties of bacterial antibodies can be determined using immobilized cells. Such kinetic values would be impractical to determine using traditional methods. Results from this research also provided a model system to study the interactions of extracellular matrix components with immobilized bacterial surface.<sup>22</sup>

## CONCLUSIONS

These studies demonstrate that the BIAcore can be used in diagnostic analysis of chemical residues and other small molecules. It provides high sensitivity and is capable of analyzing four different analytes by immobilizing different ligands on the sensor chip. With an upgraded model, different analytes can be simultaneously detected. With the use of appropriate regeneration agents the sensor chips can be utilized more than 100 times. It is best utilized for binding and kinetic studies, and to determine association and dissociation rates, as well as affinity constants of molecular interactions. The use of the research model BIAcore instrument requires high technical skills and it requires a large capital outlay, however.

Our research indicates that many of the food testing and analytical field's needs can be met with a simple instrument consisting of the sensing device, detector, readout or output for results, and automated handling for samples and reagents. The BIAquant is an example of this "stripped-down" model, although it is a "dedicated" instrument for folic acid and biotin. This model might be expanded to analyze other food nutrients, chemical contaminants and toxicants.

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