Development of a Quartz Crystal Microbalance with Impedance Measurement with Bio-Gold Nanoparticles for Enhanced Sensitivity

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***Abstract*—Immunosensor methods are worldwide applied methods for detecting and quantifying food- and feed contaminants, toxicants or pathogens in food matrices. Immunosensor methods are rapid and cost-effective methods that could reach high sensitivity and selectivity. In this study, chemically and biologically produced gold nanoparticles (AuNPs) were utilized to enhance signal sensitivity in case of a Quartz Crystal Microbalance with Impedance (QCM-I) measurement system. Biological production of AuNPs is a green technology; therefore its importance relies on less environmental impact. Results showed that biologically produced AuNPs are comparable with chemically produced nanoparticles in terms of signal sensitivity enhancement. Several biologically produced AuNPs were used with different origin and different median particle size. Therefore each of them has different physical properties. Results showed that bio AuNPs can enhance sensor sensitivity and also can enhance the accuracy of the measurement by decreasing the standard deviation of the signals; the effect depends on the physical properties of the applied bio AuNP.**

***Index Terms*—immunosensor, quartz crystal microbalance, gold nanoparticle, green technology, sensitization**

I. Introduction

According to the FAO/WHO regulations risk management in the field of food-supply chain has a high importance. [1] Implementation of the risk management decision involves regulatory food safety measurements. Developing more sensitive and selective measurement methods is necessary to reduce the risk in the food-chain system. [2] Immunosensors appeared at the scientific field in the 1960s and since then have been used in a very wide range of scientific fields, such as: health care system, industrial process control, environmental monitoring. [3] Immunosensors are a type of biosensors. They rely on immunoanalytical reactions, such as antibody-antigen reaction. The antigen or the antibody is bound onto the surface of a signal transducer. This phenomenon is selective only for the specific antigen/antibody. If the examined sample contains the antigen/antibody, it can bind onto the surface of the transducer. The signal transducer can measure a physical change due to the binding event. [4] The transducer detects a change in resistance, pH, heat, light, or mass and then converts that data to an electrical signal to be collected and processed. The Quartz Crystal Microbalance (QCM) is a piezoelectric mass-sensing device. QCM technology is based on a piezoelectric quartz crystal. [5] In case of an applied mechanical stress, the piezoelectric quartz crystal is forming an electric potential and vice versa. Therefore, in case of an applied alternating current, the piezoelectric quartz crystal starts to oscillate. The fundamental frequency depends on the crystals physical parameters. [6] The QCM then measures the frequency of oscillation in the crystal. When used as an immunosensor, the QCM can detect changes in frequency of the crystal due to changes in mass on the surface of the crystal. The QCM method has proven itself as time effective rapid measurement method for measuring various biomolecules (proteins, vitamins,

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antibodies, DNA), polymers and even cells. [7] QCMI technology has numerous advantages: high sensitivity, high stability, fast measurement and low cost. Moreover, QCM technology provides a label-free detection. Due to their high specific surface area, nanoparticles can significantly enhance the sensitivity of the applied biosensor. [8] Nanoparticles can be used to increase the surface of the signal transducer (binding onto the sensors surface) or in sandwich assays (binding with the antibody/antigen in the sample). Therefore, nanoparticles can increase the surface of the sensor or the mass of the immune complex in the sample. In both ways, the sensitivity is enhanced. [9] In this study, nanoparticles were bind onto the sensors surface in order to increase its specific surface area. Such methodology was used in the diagnosis Mycobacterium tuberculosis. [10] In this study, chemically- and biologically produced gold nanoparticles (AuNPs) were used to enhance the sensitivity of quartz crystal microbalance sensors. Model measurements with bovine serum albumin (BSA) and anti-BSA were implemented and the parameters were investigated during the experiments. A subsequent topic will be the appliance of the method for aflatoxin measurements in food sample.

II. Materials and Methods

A. Applied Chemicals

For the measurements, TRIS (tris(hydroxymethyl)aminomethane), ethanolamine, HCl, NaOH, L-cysteine, EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide), NHS (N-Hydroxysuccinimide), BSA (bovine serum albumin), anti-BSA, tetrachloroauric acid trihydrate ($HAuCl_4 \times 3H_2O$), acetic acid, sodium acetate, tetramethylammonium hydroxide, dichloromethane, 11-mercaptoundecanoic acid, chemically produced gold nanoparticles from Sigma-Aldrich company (Germany) were used. Biologically synthesized gold nanoparticles were produced at Fermentia Microbiological Ltd. (Hungary) as detailed below.

B. Preparation, Functionalization and Purification of Bio Gold Nanoparticles

Bio gold nanoparticles were produced by using a bio synthetization method according to Vágó *et al.* [11]. For synthesis, gold salt (tetrachloroauric acid trihydrate - $HAuCl_4 \times 3H_2O$) precursor was used in a fungal cell-free extract (shake flask fermentation supernatant) of thermophilic fungi in an acetate buffer environment (pH 4.6). The fermentation was made in an orbital shaker at 28 ℃ for 3 days. The supernatant was prepared by centrifugation and the biosynthesis was made at 45.0 ℃ ± 0.5 ℃.

Biologically synthetized solution of AuNPs (8.0 mL, ~10^{-3} M in terms of gold atoms) and tetramethylammonium hydroxide (0.8 mL, 1.0 M) was mixed and put in a vessel with a dichloromethane solution of 11-mercaptoundecanoic acid (8.0 ml, 10^{-3} M). The two-phase system was stirred for 20 hours at room temperature, and then the organic phase was removed, and in order to remove the excess of 11-mercaptoundecanoic acid, the water phase was washed three times with 4.0 mL of dichloromethane. Upon addition of hydrochloric acid solution (4.0 ml, 0.1 M) the AuNPs precipitated. After centrifuging the precipitate, the liquid phase was removed, then the solid phase was washed three times with hydrochloric acid solution (8.0 mL, 0.1 M) in order to prevent the dissolution of the AuNPs. After the purification steps, the aggregated AuNPs were redispersed in water with addition of a few drops of 1.0 M tetramethylammonium hydroxide solution.

Biologically produced gold nanoparticles were originated from several different subspecies of fungi. Table I contains the origin and the median particle size of the applied gold nanoparticles.

TABLE I. The Origin and the Median Particle Size of the Applied Gold Nanoparticles

	Origin of AuNP	Median size
A	Without AuNP	-
B	Sigma 765430	5 nm
C	Sigma 765538	15 nm
D	Sigma 765473	30 nm
E	*Humicola insolens* CBS 147.64	76.5 nm
F	*Rhizopus pusillus* WFPL 267A (ATCC 16458)	60.1 nm
G	*Thermoascus aurantiacus* TUB F-43 (ATCC 58156)	54.2 nm
H	*Thermomucor indicae-seudaticae* NRRL 6429 (ATCC 28404)	69.5 nm
I	*Thielavia terrestris* NRRL 8126 (ATCC 38088)	124.0 nm

C. Instrument and Sensors

For the measurements a Quartz Crystal Microbalance with Impedance (QCM-I) instrument (MicroVacuum Ltd., Hungary) was used. The instrument was operated by the BioSense 3 software (MicroVacuum Ltd., Hungary). The gold coated quartz crystal sensors (5 MHz fundamental frequency, AT-cut) were purchased also from Micro Vacuum Ltd., (Hungary).

The flow rate was generated by an NE-1002X syringe pump (New Era Pump Systems Inc., Farmingdale, USA), for sample injection a Rheodyne no. 7125 injector (Cotati, USA) with 500 µL loop was inserted into the system.

D. Surface Modification

Before surface modification, the sensor surface was cleaned with: 1 M NaOH for 20 min, 1 M HCl for 5 min and 12 M HCl for 5 minutes twice. In each case, 100 µL of the proper solution was pipetted onto the surface of the sensor. After each step, the sensor surface was rinsed with distilled water and the surface was dried at room temperature.

At first, the surface modification was carried out inside the FIA system. 500 µL of proper solutions were injected into the system one after another: L-cysteine (0.1 M), EDC/NHS (4:1 ratio, 0.5 M – 0.5 M), AuNPs, EDC/NHS (4:1 ratio, 0.5 M – 0.5 M) and BSA solution in proper concentration.

The next surface modification method was carried out outside. The sensor surface was modified with the

following method: L-cysteine solution (0.1 M in distilled water) for 30 min, EDC/NHS solution (4:1 ratio, 0.5 M – 0.5 M) for 30 min, the applied AuNPs solution in distilled water (if AuNPs were used) for 30 min, EDC/NHS solution (4:1 ratio, 0.5 M – 0.5 M) (if AuNPs were used), BSA solution in distilled water. In each case, 100 μL of the proper solution was pipetted onto the sensor surface and between each step the sensor surface was rinsed with distilled water and was dried at room temperature.

E. Measurement Setup

TRIS buffer (pH 6.5, 42 mM) served as the eluent in the fluid injection analysis (FIA) system. Before the measurement, ethanolamine (10 mM) was injected to block the active binding places in the EDC/NHS complex where the applied antigens could not bound. HCl (50 mM) solution was used as a regeneration material after ethanolamine and after each sample. The HCl solution cleaves the immunobinding reaction between the antigen and antibody. As samples, proper concentrations of anti-BSA solutions were used.

F. Statistical Methods

To examine the differences between groups, two-sample t-tests were made at a 0.05 level of significance. For creating calibration curves, non-linear least square regression method was made. For statistical analysis, SPSS software (IBM Corp., USA) and R-Project (R Development core Team, Vienna, Austria) was utilized.

III. Results and Discussion

A. Measurement Parameters

At first, model measurements were performed to define and prove the possibility of the use of different type of AuNPs. The effects of the physicochemical and biochemical parameters were examined. The effect of the injected sample volume was investigated at two levels (200, 500 μL). Results showed that larger amounts of samples have increased the gained signal intensity significantly.

The effect of the applied flow rate was also investigated from 50 to 200 μL min^{-1}. Results showed that there is an optimum value at around 100 μL min^{-1}. Below this value, the obtained signal was too low and above this value the noise of the baseline was significantly higher.

Different layering methods were also examined throughout the preliminary phase. First, surface modification was made inside the FIA system by injecting the reagents into the loop one after another. In case of chemically produced AuNPs with a median diameter of 15 nm and an applied BSA concentration of 1000 μL min^{-1}, the sensor signal for 20 μL min^{-1} anti-BSA solution was 3.14 ± 0.67 Hz. Results showed that the obtained signals are better in case if the surface modification of the sensor was carried out outside from the instrument. In this case, with the same parameters the sensor signal was 4.96 ± 0.14 Hz. Since the sensor signals were significantly greater in case when the surface modification was carried out outside from the instrument ($p < 0.05$), this method was continued forward.

B. Without Gold Nanoparticles

After the preliminary phase, a model system was built to further investigate the effect of the concentrations of the reagents. The concentration of the applied BSA solution had a significant effect on the signals. Fig. 1 shows the obtained signal intensity at different BSA concentrations. Samples were 20 μL min^{-1} anti-BSA solutions.

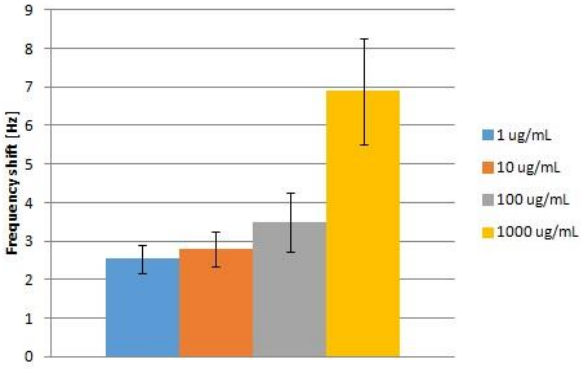

Figure 1. The QCM-I frequency shift at different immobilized BSA concentrations. (Samples were 20 μg/mL anti-BSA)

While results with 1, 10 and 100 μL min^{-1} BSA were not significantly different from each other (p>0.12 for each cases), the result with 1 μL min^{-1} BSA had significantly higher signal intensity (6.88 ± 1.36 Hz) (p<0.05). Fig. 1 also shows that as the signal intensity increases, the standard deviation also increases.

C. Results with Gold Nanoparticles

Chemically produced AuNPs were also examined to enhance the sensor sensitivity. There was only significant increase between the signal intensity of the sensors prepared without AuNPs and prepared with chemically produced AuNPs with a 15 nm median diameter (p = 0.04). The dilutions of the AuNPs were investigated at three levels: 100×, 20× and 4×. Results showed that the best effect can be achieved with more diluted AuNPs (100×: 5.95 ± 0.89 Hz, 20×: 4.92 ± 0.65 Hz, 4×: 9.48 ± 1.01 Hz). At the best case the mean value of the signals were increased by 38% (from 6.88 ± 1.36 Hz to 9.48 ± 1.01 Hz). Fig. 2 shows the sensor signals of the applied chemically produced AuNPs with different median particle size.

Results showed that, there were decreases in the sensor signals in case of the AuNP with 5 and 30 nm median diameter compared to the signals obtained in the absence of AuNPs (from 6.88 ± 1.36 to 3.92 ± 0.64 Hz and to 4.64 ± 0.55 Hz).

The application of bio-AuNPs also did not cause a significant change in the signal intensity. In case of (I) bio-AuNP (which has the highest median particle diameter: 124.0 nm), the sensor signals increased by 14% (from 6.88 ± 1.36 Hz to 7.84 ± 0.99 Hz) and the standard deviation decreased by 27%. In case of (F) bio-AuNP, there was just a slight increase in the signal intensity (~3%) but the standard deviation of the signal intensity

decreased to 41% (from 6.88 ± 1.36 Hz to 7.09 ± 0.55 Hz), therefore it seems that the application of bio-AuNPs can increase the signal intensity and the accuracy (by reducing the standard deviation) of the measurements. Fig. 3 illustrates the gained signal intensity for 20 µL min^{-1} anti-BSA sample solutions in case of the use of biologically produced AuNPs with different origin.

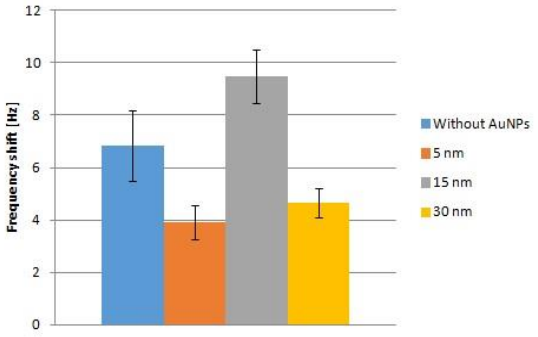

Figure 2. QCM-I sensor signals for 20 µg/mL anti-BSA samples. For surface modification 1000 µg/mL BSA solutions were applied with chemically produced AuNPs with different median particle sizes.

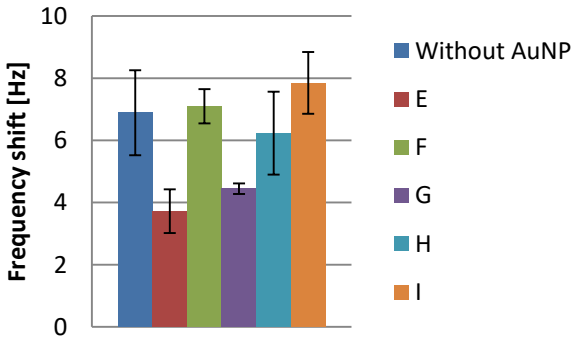

Figure 3. The QCM-I signal intensity with applying different bio AuNPs. (BSA concentration was 1000 µg/mL. Samples were 20 µg/mL anti-BSA)

Nanoparticles with the highest median particle size (I) resulted the highest signal intensity and nanoparticles with the lowest median particle size (G) resulted the lowest standard deviation, therefore the highest accuracy. Fig. 4 shows the gained signals in case of the (I) bio AuNP for different concentrations of anti-BSA samples.

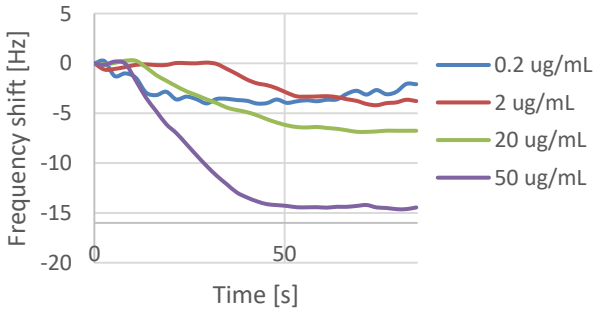

Figure 4. Obtained QCM-I signals for different anti-BSA samples with bio AuNPs (I). (BSA concentration was 1000 µg/mL, samples were: 0.2, 2, 20 and 50 µg/mL anti-BSA)

As Fig. 4 depicts, the sensor signals for different anti-BSA concentrations can be distinguished from each other. The sensor can detect and quantify from 200 µL min^{-1} (data not shown) down to 0.2 µL min^{-1}.

D. Calibration

The next step was to evaluate the interrelation between the concentration of the injected anti-BSA samples and the frequency shift of the signal. Fig. 5 illustrates a calibration curve in case of AuNPs (I) and applied BSA concentration of 1000 µL min^{-1}.

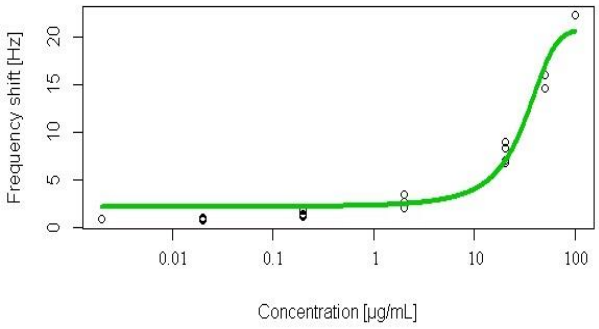

Figure 5. Calibration curve of anti-BSA measurements with AuNPs (I) and 1000 µg/mL BSA.

The samples were: 100, 50, 20, 2, 0.2, 0.02 and 0.002 µg/mL of anti-BSA solutions in TRIS buffer (42 mM, pH 6.5). The sigmoid models coefficient of determination is 0.96 and the models level of significance is below 0.01. The model parameters are also significant (p<0.01 in each cases). Fig. 5 shows, the dynamic measurement interval is between 100 and 2 µg/mL. Equation (1) describes the calibration curve.

$$f(x) = \frac{a}{1+e^{-b*(x-c)}} \quad (1)$$

where:

$f(x)$: is the frequency shift estimation
x: is the concentration of the anti-BSA
a: is the saturation parameter (a = 20.702)
b: is the derivative parameter (b = 0.073)
c: is the inflexion parameter (c = 29.275)

IV. Conclusion

To conclude, biologically produced AuNPs can enhance sensor sensitivity and also the accuracy of the measurement. The examination of the methods parameters and therefore the applied setup can be used in the future for aflatoxin detection and quantification in food samples.

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