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Comparative study of thermal stability of magnetostrictive biosensor between two kinds of biorecognition elements

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ABSTRACT

Magnetostrictive biosensors specific to *Salmonella typhimurium* were prepared by immobilizing antibody or phage as biorecognition elements onto the magnetostrictive sensor platform. The sensors were stored at temperatures of 25 °C (room temperature), 45 °C and 65 °C, respectively, and the ability to bind *S. typhimurium* was detected by testing the resonant frequency shift using a HP network analyzer after exposure to 1 mL of 1×10^9 cfu/mL of *S. typhimurium* at a predetermined schedule. The binding of *S. typhimurium* to biosensors was confirmed by Scanning Electron Microscopy (SEM). The results showed that there existed an initial sudden drop in the average density of *S. typhimurium* bound to the biosensor surface versus duration at different temperatures for the two kinds of recognition elements, and the binding ability to *S. typhimurium* of phage-immobilized biosensors was much better than that of antibody-immobilized biosensors, with longevity longer than 30 days at all tested temperatures, though decreasing gradually over the testing period. While the longevity of antibody-immobilized biosensors was only about 30, 8 and 5 days at room temperature (25 °C), 45 °C and 65 °C, respectively. Meanwhile, the activation energy of the two kinds of biosensors was investigated, and it was found that phage immobilized sensors showed much higher activation energy than antibody immobilized sensors, which resulted in less dependency on temperature and thus having much better thermal stability than antibody immobilized sensors.

1. Introduction

Salmonella typhimurium, a kind of food-borne pathogen, is one of the greatest concerns related to fresh fruits and vegetables [1]. The symptoms of *Salmonella* infection are diarrhea (88%), fever (80%), abdominal cramps (65%), nausea (42%), occasional vomiting (35%), and headache (29%) [2]. The contamination of fresh food with *Salmonella* can occur at any point from the farm to the table, and the amount of consumption of poultry products, milk and vegetables is getting more and more for recent years, therefore it would be desirable and urgent for fresh food to be systematically detected using biosensor during production and distribution [3–7].

A biosensor essentially consists of two main components viz., a physical transducer and a biorecognition element. In this study, a magnetostrictive platform served as the transducer, since it offers wireless or remote detecting, which is a unique advantage over conventional sensor platforms, and polyclonal antibody or filamentous phage was used as the biorecognition element.

Antibodies are immune system-related proteins called immunoglobulins, which can selectively bind target antigen because of their special structure and different amino acid sequence. However antibodies are generally fragile and filamentous phage has emerged recently as a substitute to antibodies [8–11] as bio-recognition elements for biosensors owing to their environmental robustness [12].

For all practical applications, it is very essential for both major components to be robust enough to withstand the rigors of the field conditions. However, in most cases, the biorecognition element is quite susceptible to suffer from the changes of the field environment. Hence it is of utmost importance to test the stability of the bio-recognition element. Though some literatures found about the thermal stability of free biorecognition element [3,13,14], no report found about those immobilized on a sensor platform. Since the immobilization may alter their stability, it is necessary to investigate the stability of the two kinds of bio-recognition elements at three different temperatures after immobilizing on a magnetostrictive sensor platform, and make a comparison analysis of them.

2. Materials and methods

2.1. Materials and cultures

METGLAS® 2826 MB alloy (Conway, SC) in the form of a long roll of ribbon obtained from Honeywell International was used as the sensor platform; the composition is $Fe_{40}Ni_{38}Mo_4B_{18}$. Magnetostrictive sensors

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with the size of 5 mm × 1 mm were cut using an auto-controlled micro dicing saw. The diced sensors were ultrasonically cleaned in methanol solution to remove grease and debris left by the dicing process followed by a thermal anneal in a vacuum oven at 200 °C for 2 h. To improve the environmental stability as well as the bioactivity of the biosensors, thin layers of chromium and gold were coated on both sides of the sensors using a Denton™ (Moorestown, NJ) high vacuum RF sputtering system.

Filamentous phage clone E2 used in this research was derived from a landscape f8/8 phage library [10], which has been studied and verified to be highly specific and selective towards *S. typhimurium* [15]. Polyclonal antibody to *S. typhimurium* which was immune system-related proteins called immunoglobulins was purchased from Abcam Inc (Cambridge, MA) with the concentration of 1 mg/mL, which exhibited good specificity to *Salmonella* bacteria [16]. Subsequent dilution of the suspensions was performed using TBS solution. *S. typhimurium* cultures (1×10^9 cfu/mL) were prepared in the Department of Life Science at Auburn University. All test solutions were maintained at 4 °C before using.

2.2. Principle of detection

Due to the magnetoelastic nature of the amorphous magnetostrictive alloy, the sensor exhibits a physical resonance when it undergoes a time-varying magnetic field, thus emitting magnetic flux, and this can be monitored remotely without the use of direct physical connections. As Fig. 1 denotes, the frequency spectrum of the sensor can be obtained by sweeping an AC magnetic interrogation field over a predetermined frequency range while monitoring the response of the sensor using a pickup coil. If the frequency of the AC field is equal to the mechanical resonant frequency of the sensor, the conversion of the magnetic energy into elastic energy is maximal and the sensor undergoes a magnetostrictive resonance. For a thin, ribbon-shaped sensor of length L vibrating in its basal plane the fundamental resonant frequency of the longitudinal vibrations is described by [17],

$$f = \sqrt{E/\rho \cdot (1-\sigma^2)}/2L \tag{1}$$

where E denotes Young's modulus of elasticity, σ is the Poisson's ratio, ρ is the density of the sensor material, and L is the long dimension of the sensor. Due to the shape demagnetizing factors of the ribbon-like sensor the magnetic permeability is greatest along its length; hence an incident magnetic field will generate longitudinal vibrations in the sensor from almost any orientation except normal to the basal plane of the sensor.

When the testing temperature, humidity and other environmental parameters are constant, the resonant frequency change of the magnetostrictive sensor depends only on the mass change on its surface. An additional mass Δm to the mass of the sensor M corresponds to a change in the resonant frequency. If the mass increase is small compared to the mass of the sensor the shift in the resonant frequency is given by [18],

$$\Delta f = -f \cdot \Delta m/2 \cdot M \tag{2}$$

where f is the initial resonant frequency, M is the initial mass, Δm is the mass change, and Δf is the shift in the resonant frequency of the sensor.

Eq. (2) shows that the resonant frequency shifts linearly and decreases with increasing mass on the sensor surface. Therefore capturing of the target organism onto the surface of the biosensor causes a mass increase with a corresponding decrease in its resonant frequency. Magnetostrictive materials have been used to develop environmental as well as chemical sensors [19–23]. This study has shown that, by employing a suitable bioprobe for detecting pathogens, this material can be used as a biosensor.

2.3. Immobilization of biorecognition elements

The Langmuir–Blodgett (LB) technique was used for antibody immobilization on the magnetostrictive sensors. Seven monolayers containing antibodies are transferred onto the magnetostrictive sensor surface using a LB film balance KSV 2200 LB (KSV Chemicals, Finland). Phage was immobilized on sensors using physical adsorption by immersing the sensor platform in a phage suspension for 1 h. The ME resonator platforms were then washed with 1× TBS buffer and sterilized distilled water (DW) in order to remove any unbound bioprobe and debris. After immobilization, any unbound area of the ME resonator platform was blocked with 300 μL of 1% bovine serum albumin (BSA, Sigma-Aldrich Co., St. Louis, MO, USA) at 22 °C for 1 h. Finally, the ME resonator platform was washed three times with sterilized DW and then dried in air.

2.4. Thermal stability experimental

After immobilization, the sensors were divided into 3 sets and maintained in 3 constant temperature humidity chambers with temperatures of 25 °C, 45 °C and 65 °C, respectively. At the predetermined schedule, six sensors from each of the three sets were taken to undergo testing and sensors were allowed to attain room temperature before testing. They were immersed in *S. typhimurium* water solution with the volume of 1 mL and the concentration of 1×10^9 cfu/mL for 30 min to bind bacterial cells. The resonant frequency of the sensors was measured using a HP network analyzer 8751A with S-parameter test set at 87511A before and after binding of bacterial cells to the surface of the sensor. Then the sensors were removed and exposed to Osmium tetraoxide (OsO_4) vapor for 1 h to fix the bacterial cell wall to facilitate Scanning Electron Microscopy (SEM) observations. Finally, SEM images were examined

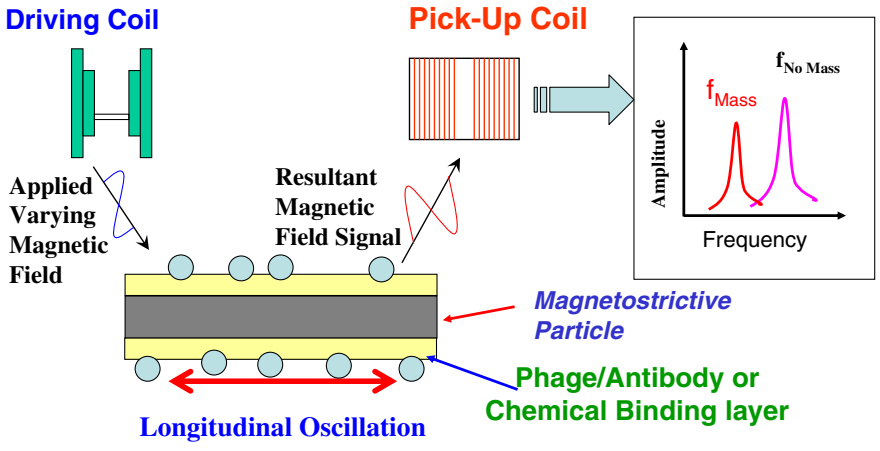

Fig. 1. Schematic illustration of the wireless nature of the magnetostrictive biosensors and its basic principle for bacteria detection.

using the Scanning Electron Microscope JEOL 7000F, operating at 5 kV. Each data point in all figures represents the average value obtained from six independent sensors subjected to treatment under identical experimental conditions.

3. Results and discussions

3.1. Response curves

The resonant frequency of magnetostrictive biosensors before and after exposure to *S. typhimurium* solutions was measured. Fig. 2 shows the real-time frequency spectrum of the antibody immobilized biosensor before and after exposure to *S. typhimurium* solution with a concentration of 1×10^9 cfu/mL. An obvious shift in resonant frequency due to binding of bacteria on the biosensor surface was observed before and after being exposed to bacteria.

3.2. Frequency shifts

Fig. 3 shows the resonant frequency shifts over the study period at different temperatures after exposure to a solution containing *S. typhimurium* with a concentration of 1×10^9 cfu/mL.

It indicates that all sensors at tested temperature exhibited duration dependence and a decline in sensitivity with duration was observed, and it was found that the higher the temperature, the more rapidly the resonant frequency shift decreases with duration. The degradation in the sensor activity can be attributed to the inactivity of the bio-recognition element at higher temperatures. And it can be seen that all phage-immobilized sensors had good thermal stability and showed obvious resonant frequency shift on day 35, though there existed gradual decrease in resonant frequency. However, antibody-immobilized sensors showed much worse thermal stability, for those kept at 25 °C, zero shifts in resonant frequency were seen on day 30. And those maintained at higher temperatures showed a more remarkable reduction in the frequency shift, and with no shift observed on days 8 and 5 for the sensors maintained at 45 °C and 65 °C. All the sensors showed an initial sudden drop in the resonant frequency shifts, which might result from the degradation rule of the biological activity versus duration [3,13,14].

3.3. SEM observations

In order to confirm that the resonant frequency shifts were caused by the binding of *S. typhimurium* to the biosensor, SEM images were taken for all the sensors after exposure to a solution containing *S. typhimurium* with a concentration of 1×10^9 cfu/mL. Fig. 4 shows some typical SEM images of the biosensors at different holding conditions for different

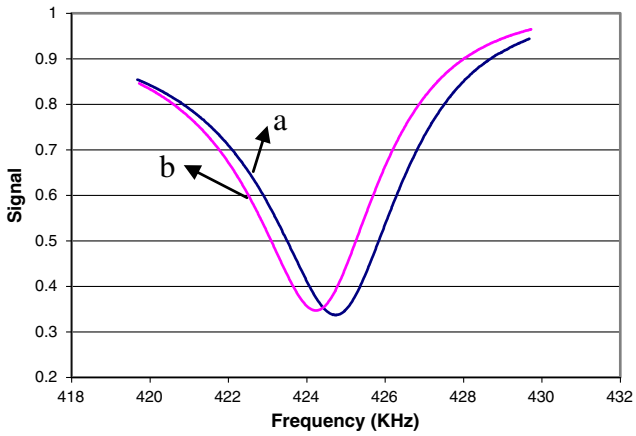

Fig. 2. Real-time frequency spectrum of the antibody immobilized biosensor (a) before and (b) after exposure to a solution containing *S. typhimurium* with a concentration of 1×10^9 cfu/mL.

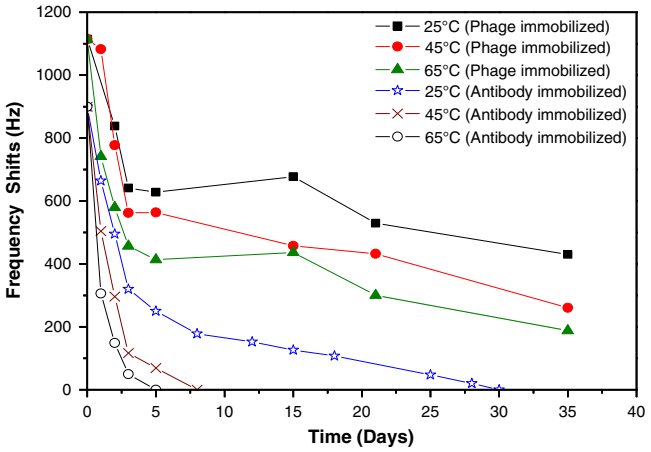

Fig. 3. The resonant frequency shifts with duration for both kinds of biosensors at different temperatures after exposure to a solution containing *S. typhimurium* with a concentration of 1×10^9 cfu/mL.

kinds of biorecognition elements after being exposed to the same concentration of *S. typhimurium*, which showed the big difference of bacterial density on the sensor surfaces for different cases.

3.4. Density of bacteria binding to the biosensors

Ten different regions of each sensor surface were examined and photographed using SEM. The number of cells bound to the sensor surface was then directly counted from SEM images and statistically convert this to an area density of bacteria attached to the sensor surface, calculation shown as below,

$$D = n/A \tag{3}$$

where D is the bacterial density, A is the area of the sensor surface, and n is the number of the bacteria bound to the sensor surface counted using SEM images.

The density of bacteria bound to the biosensor surface vs. duration (in days) for sensors maintained at the three different temperatures was generated as shown in Fig. 5.

It can be seen that Fig. 5 correlates quite well with the resonant frequency shifts in Fig. 3 at all cases, and all phage-immobilized sensors had much higher density of bacteria than antibody-immobilized sensors. They kept high density of bacteria even after day 30, though there existed an initial sudden drop. However, antibody-immobilized sensors showed zero density of bacteria at days 30, 8 and 5 for the sensors maintained at 25 °C, 45 °C and 65 °C, respectively.

3.5. Activation energy calculation

Activation energy can be calculated using Arrhenius equation [24] as below,

$$K = A \cdot \exp(-E_a/RT) \tag{4}$$

where K is the rate coefficient, A is a constant, E_a is the activation energy, R is the universal gas constant ($R = 1.987$ cal mol^{-1} K^{-1}), and T is the absolute temperature. Then K can be expressed using the following formula,

$$\ln K = \ln A - E_a/RT. \tag{5}$$

The Arrhenius plot showing the effect of temperature on the sensitivity for the two kinds of magnetostrictive biosensors over a temperature range of 25 °C to 65 °C was shown in Fig. 6. For the convenience, here we showed the plot of ln$K$ versus 1000/T.

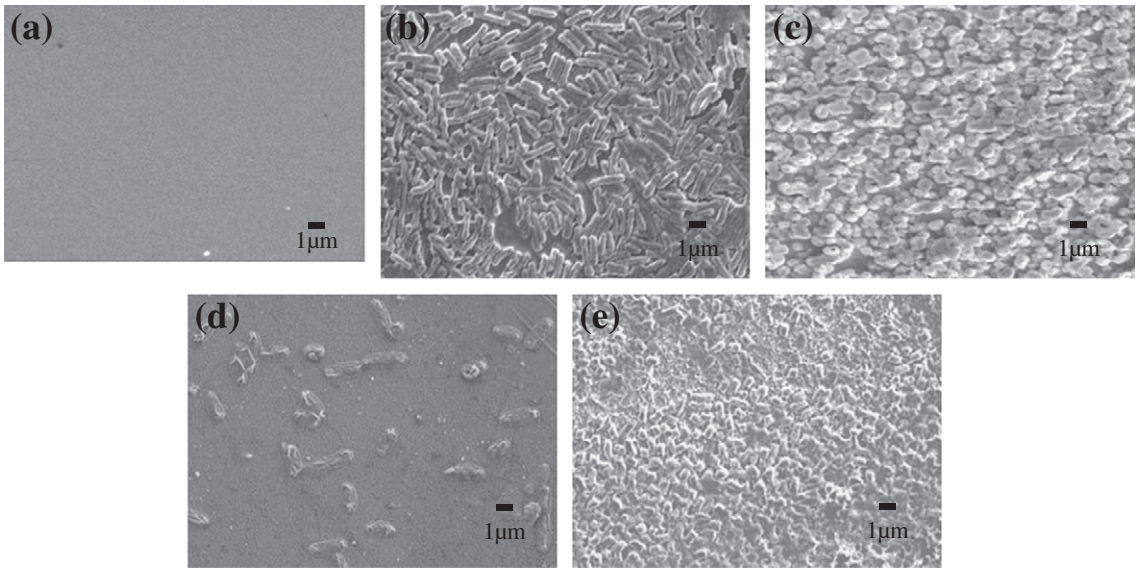

Fig. 4. Typical SEM images of the biosensors' surface before and after binding to *S. typhimurium* bacterium with different durations at 25 °C (a) before the binding, (b) antibody, 0 day (c) phage, 0 day (d) antibody, 15 days (e) phage, 15 days.

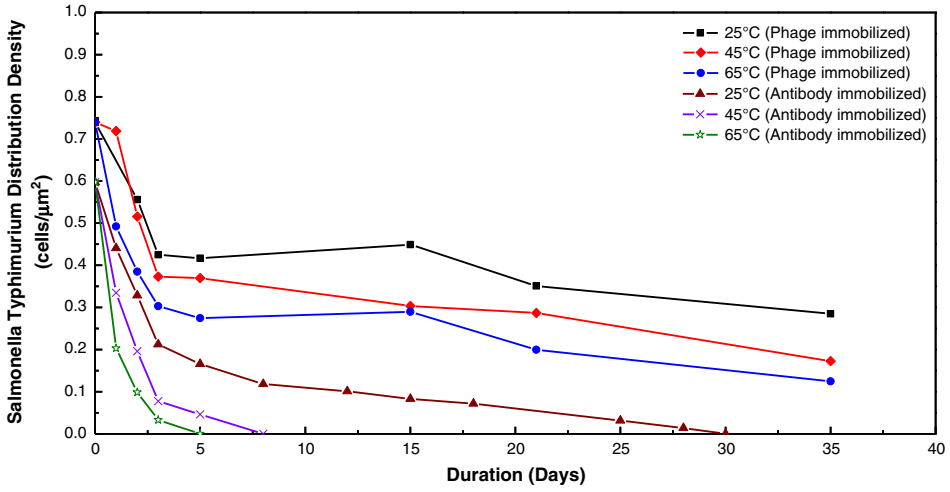

Fig. 5. The average density of *S. typhimurium* bound to the biosensor surface versus duration at different temperatures for the two kinds of recognition elements.

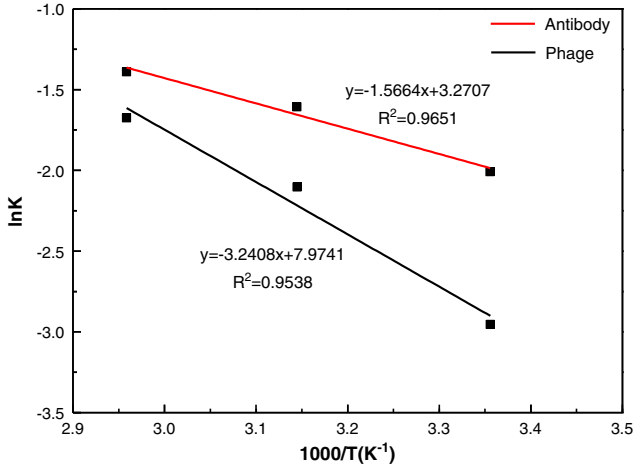

Fig. 6. ln*K* versus 1000/*T* showing the effect of temperature on the longevity of the antibodies. Activation energy of the antibodies was calculated from the slope of the plots.

The slope of Arrhenius plots is commonly used to determine the activation energy of the rate-limiting step in reacting systems. From Fig. 6, the activation energy determined for antibody immobilized sensors was 3112.4 cal/mol and the activation energy for phage immobilized sensors was 6439.5 cal/mol. The result indicates that antibody immobilized sensors show much lower activation energy than that of phage immobilized sensors. Lower activation energy suggests that the antibody used as a biorecognition element shows strong dependency on the temperature than phage, i.e. antibody tends to degrade more rapidly at higher temperatures. Therefore, phage immobilized biosensors possess much better thermal stability than antibody immobilized sensors.

4. Conclusions

Magnetostrictive biosensors specific to *S. typhimurium* were prepared by immobilizing different kinds of biorecognition elements onto the sensor. Antibodies and phage were immobilized using the Langmuir–Blodgett (LB) technique and physical adsorption, respectively.

And the ability to bind *S. typhimurium* was detected by testing the resonance frequency shift using a magnetostrictive platform after exposure to *S. typhimurium* solution. The binding of *S. typhimurium* to biosensors was confirmed by Scanning Electron Microscopy (SEM) micrographs. The results showed that at each temperature, an initial sudden drop existed in the average density of *S. typhimurium* bound to the biosensor surface versus duration for the two kinds of recognition elements due to the degradation of the biological activity versus duration, and the binding ability to *S. typhimurium* of phage-immobilized biosensors was much better than that of antibody-immobilized biosensors, the longevity of phage on the magnetostrictive sensor platform was longer than 30 days at all tested temperatures, while the longevity of antibody was about 30, 8 and 5 days at room temperature (25 °C), 45 °C and 65 °C, respectively. And the reason why phage immobilized biosensors possess much better thermal stability than antibody immobilized sensors is attributed to the higher activation energy. Therefore, it is reasonable to get the conclusion that phage immobilized sensors will be quite suitable to use in field assay at high temperatures due to their good thermal stability.

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