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Magnetically-assisted impedimetric detection of bacteria using phage-modified carbon microarrays

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ABSTRACT

This study presents an investigation on the possibility of improving the detection limit of bacteria with an inexpensive electrochemical, impedimetric sensor platform, by integrating the sensor with magnetic manipulation. The approach uses T4 bacteriophage coated Dynabeads to selectively capture and concentrate *E. coli K*12 cells from samples, to increase the sensitivity of detection at the surface of functionalized screen-printed carbon microarrays. Fluorescence and flow cytometry measurements indicate that the surface modification of the magnetic beads, with phages, and binding with the bacteria, were successful. Integration of the screen-printed carbon-based impedimetric sensor, with a magnetic manipulation system, was found to improve the sensitivity of the device, decreasing the limit of detection of *E. coli K*12 from 10^4 to 10^3 cfu/mL. We have also demonstrated that this approach provides for more specific detection of bacteria, enabling the operator to account for non-specific adsorption, and detection of bacteria in more complex (real) samples (milk).

1. Introduction

Bacterial contaminations in different environments, especially in the food industry, are a major source of many of the health problems encountered in this era. Environmental, clinical, and industrial analysts are looking for inexpensive, rapid and easy-to-use methodologies to monitor the contamination caused by microorganisms and prevent potential widespread outbreaks [1–4].

Various techniques including biochemical tests or immunological techniques, such as enzyme-linked immunosorbent assay (ELISA), have been widely studied [5–9]. However, depending on what procedure is used (pre-enrichment of cultures or use of an optically active reagent conjugated with a secondary antibody, for example), the detection limits are known to vary, and in many cases to be too high. An additional setback common to all of these techniques is long detection times.

Biosensors were introduced as alternative analytical devices to enable a more rapid, and on-site, detection of these microorganisms. A variety of biosensors for the detection of bacteria are based on the use of labeled-secondary antibodies. The quartz crystal microbalance (QMC) and surface plasmon resonance (SPR), as label-free (direct) biosensing devices, offer the advantage of rapidity of detection and simplicity of operation. Impedance-based measurements, as an alternate inexpensive electrochemical method, have also been used for the development of label-free biosensors. Generally, impedance measurements require a three electrode set-up containing a metal foil or a wire immersed in the sample solution. In recent years, several electrode geometries and designs, such as interdigitated microarray electrodes or screen-printed electrodes, were used to improve the sensitivity of impedimetric sensors. But the problem with the majority of sensors remains the low efficiency in capturing the intended bacterial target, especially when antibodies are used as the immobilized bioreceptor, resulting in high detection limits.

In order to overcome this problem, other interesting strategies were used to increase the sensitivity of the detection system, including the use of magnetic particles. In these approaches, instead of immobilizing antibodies onto the electrode surface, they were immobilized onto magnetic particles. An example of this method has been reported by Varshney and Li [10], where magnetic beads were used for the detection of *E. coli* O157:H7 in samples of ground beef. The magnetic beads were immobilized with anti-*E. coli* through biotin–streptavidin interaction, and then they were mixed with bacteria containing solution and impedance variations were

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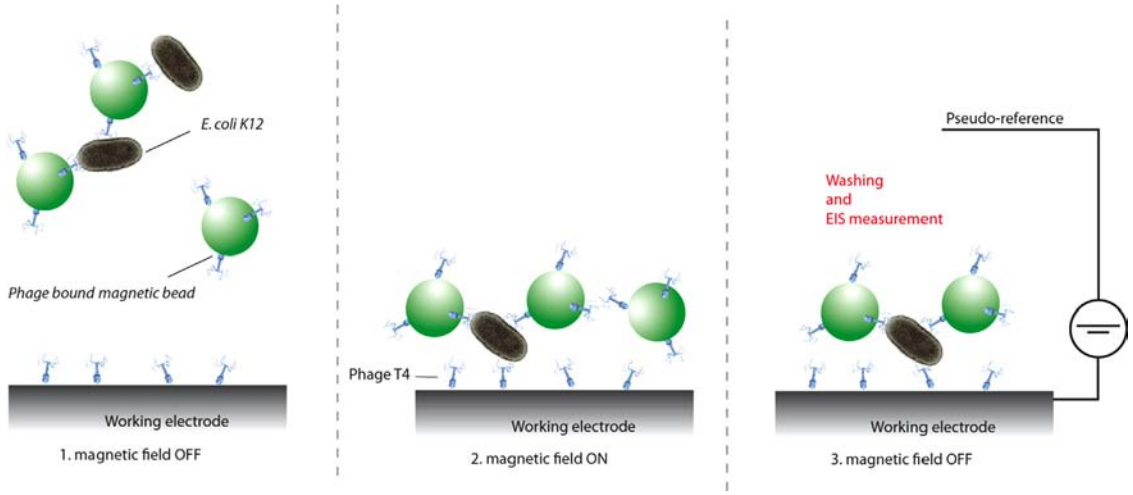

Fig. 1. Illustration of the screen-printed carbon electrode microarrays sensor and its use for the EIS detection of magnetically separated *E. coli K*12.

measured. The magnetic particle–bacteria complex was attracted onto the surface of the sensing electrode using a magnetic field and the detection limits of this system were found to be 10^4 cfu/mL and 10^5 cfu/mL in pure culture and ground beef, respectively.

This biosensor was further improved by the same group [11] using a microfluidic cell. A microchamber with a volume of 60 nL was made by bonding a poly-(dimethysiloxane) (PDMS) microchannel to the gold interdigitated microelectrode array. This microfluidic cell was used to detect bacteria in the active layer above the microelectrodes. The active layer is a sensitive region of a few micrometers above the electrode surface, where variations in impedance can be detected efficiently. The complex sample containing antibody-coated magnetic beads and bacteria was injected into the chamber, and this enabled the detection limits to be reduced to 10^2 cfu/mL and 10^3 cfu/mL, with a measurement time of 35 min, for the pure culture and ground beef samples, respectively. Using a thin microfluidic chamber to increase analyte proximity to the electrode surface decreases the time for the diffusion of conductive ions to the active layer, resulting in a more rapid reaction [11].

Another impedimetric approach to detect *Salmonella* cells using interdigitated microelectrodes and magnetic particles has been demonstrated by Yang et al. [12]. Magnetic beads modified with anti-*Salmonella* were used to capture bacteria cells in the media and then impedance measurements were performed. The variation in impedance in this case was found to be related to changes of the double layer capacitance. The decrease in the capacitance was observed to be due to an increase in bacterial growth. A linear relationship for capacitance variation was found with the logarithm of bacteria concentrations ranging from 10 cfu/mL to 10^6 cfu/mL. For the detection of 10 cfu/mL, 8 h was required, while the detection time for 10^6 cfu/mL of bacteria was found to be 1.5 h.

Magnetic separation is a simple and rapid method to capture and concentrate bacteria present in complex (real) samples. Techniques based on magnetic bead separation do not require centrifugation, filtration or expensive columns [13]. The magnetic separation approach has been coupled with various detection methods such as chemiluminescence [14], flow cytometry [2], immunoassays [15], and electrochemical methods [16,17].

Here, we investigated the feasibility of coupling magnetic separation/manipulation, with our impedimetric detection system, to further reduce the detection limits and illustrate the method's potential to detect specific bacteria in complex (real) samples. This method was adapted from previous work on the detection of *E. coli K*12, where T4 phage was directly immobilized onto screen-printed carbon electrode microarrays, to act as a specific probe [18] (Fig. 1). To the best of our knowledge, this is the first time that inexpensive, rapid, and specific impedimetric detection of bacteria using a combination of carbon microarrays and phage-modified magnetic beads, has been demonstrated.

2. Materials and methods

2.1. Chemicals and reagents

2-[N-morpholino] ethane sulfonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochlroride (EDC), sodium hydroxide (NaOH), fluorescein isothiocyanate (FITC), Tris–HCl buffer, N-dimethylformamide (DMF), bovine serum albumin (BSA), were purchased from Sigma-Aldrich and used as received. Carboxylic acid functionalized Dynabeads (1 μm) were purchased from Invitrogen Inc. Bacteria and phage cultures were obtained from Biophage-pharma Inc. SM buffer was prepared by mixing 5.8 g of NaCl, 2.0 g of $MgSO_4 \cdot 7H_2O$, 50 mL of 1 M Tris–HCl (pH 7.5), and 1 mL of 10% (w/v) of gelatin in MilliQ water.

2.2. Bacteriophage and bacteria preparation

T4 bacteriophage (wild type) was amplified by pipetting 100 μL 10^6 cfu/mL of *E. coli K*12 and 100 μL 10^6 pfu/mL of T4 phage in a test tube and using a vortex. The mixture was incubated at room temperature for 15 min and was then added to a 20 mL tube containing LB media. The mixture was incubated for 6 h at 37 °C in a shaking incubator. The solution was then centrifuged at 2500 g for 20 min, followed by filtering the supernatant with 0.22 μm Millex filter (Millipore) to remove any remaining bacteria. After that, the supernatant was centrifuged at high speed (12000*g*) for 1 h followed by removing the supernatant and resuspending the phage pellet in 1 mL of SM buffer. Phage counting was performed using soft agar plate and expressed in pfu/mL [19]. *E. coli K*12 cells were grown at 37 °C in 4 mL LB media using an incubator-shaker for 3 h, followed by 3 centrifugations at 2500*g* for 20 min, in order to exchange the media with SM buffer. Enumeration of bacteria was performed by the plate count technique and expressed in cfu/mL.

2.3. Electrode microarray preparation

Screen-printed electrodes (SPEs) were fabricated using graphite ink (electrodag 423 SS, (Acheson, Erstein, France)) and a DEK 248 screen-printing machine (DEK, Erstein, France). The SPE platform was designed to provide multi-probe capability, easily

produced by screen-printing ink onto polyester sheets. The polyester sheets produced, each carrying 16 separate electrode microarrays (each microarray being 1 cm × 2.5 cm in size) were subsequently baked 10 min at 100 °C to dry the thermoplastic carbon ink (Supplementary material 1). This was followed by printing an insulating, hydrophobic, polymer (MINICO M 7000, (Acheson, Erstein, France)) onto the microarrays in order to define a window easily covered with a 40 μL drop of solution that does not spread. This window serves to isolate the active area composed of eight 0.2 mm^2, individually addressable, working electrodes, one ring-shaped reference electrode, and one central auxiliary electrode (see illustrations presented as appendix).

2.4. Activation of magnetic, carboxylic acid-coated, Dynabeads

In order to immobilize T4 phage onto the surface of the Dynabeads, they were first activated with the carbodiimide compound (EDC). 300 μL of Dynabeads (1 μm in diameter, 10 mg/mL) with a carboxylic acid coating was washed twice with the same amount (300 μL) of 0.01 M NaOH for 10 min with good mixing. This was followed by a threefold washing with 300 μL of de-ionized water. Two hundred microliters of 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide hydrochlroride (EDC) (20 mg/mL) were then added to the Dynabeads with mixing, and incubated for 30 min with slow tilt rotation. After incubation, the mixture was placed onto a magnet for 4 min and the supernatant was removed, then the beads were washed with cold de-ionized water and with 25 mM 2-[N-morpholino] ethane sulfonic acid (MES) (pH=6). At this point the Dynabeads are activated and are ready for the attachment of the phages.

2.5. Coating of Dynabeads with phage T4

After activation of the beads with EDC, the solution is removed and 100 μL of T4 phage (10^{10} pfu/mL) is added to coat the Dynabeads. Then, 100 μL of MES was added and incubated for 30 min with slow rotation at room temperature. The supernatant was removed by placing the tube on the magnet for 4 min. In order to quench non-reacted carboxylic groups, the beads were coated with phages incubated with 300 μL of 50 mM Tris–HCl buffer (pH=7.4) for 15 min at room temperature with slow rotation. Then, the solution was washed with 300 μL of 50 mM Tris–HCl buffer four times, and re-suspended into the Tris buffer and stored at 2–8 °C for further use. In order to bind the bacteria with the phage-coated magnetic beads, 20 μL of beads (10 mg/mL) was mixed with 1 mL of bacteria cells (at different concentrations) for 10 min with rotation at room temperature.

2.6. Preparation of fluorescence-labeled bacteriophage

The phage T4 was labeled with fluorescein isothiocyanate (FITC) according to a modified procedure [20]. Sixty microliters of phages at 10^{10} pfu /mL in SM buffer were mixed with 0.0105 g of FTIC and 2.5 mL of *N*, *N*-Dimethylformamide (DMF). The solution was stirred overnight at 4 °C and then purified by membrane dialysis.

2.7. Bacteria counting

Dilutions of an initial bacteria culture of 10^8 cfu/mL (approximate concentration) were prepared, to cover a range of 10^8 to 10^1 cfu/mL. Ten microliters of each of the diluted samples were pipetted into a checkered sterile Petri dish, previously covered with agar. The Petri dish was left for 15 min to let the samples properly absorb into the agar, and were then incubated at 37 °C overnight to allow colony formation. The next day the bacteria colonies were counted to give the exact concentration of bacteria present at the time of detection.

2.8. Flow cytometry measurements

Flow cytometry analysis for GFP expression was performed on a BD LSRII(tm) flow cytometry system (Becton-Dickinson Biosciences, CA) equipped with a 488 nm argon ion laser as an excitation source. The green fluorescence emission was detected using a 530/30 nm band pass filter set. The GFP expressing *E. coli K*12 was obtained from Dr. Roland Brousseau (Biotechnology Research Institute, Montreal).

2.9. Magnetic separation

Twenty microliters of phage-coated bead solution (10 mg/mL) were mixed with 1 mL of *E. coli K*12 (10^8 cfu/mL) in SM buffer, or a mixture of *Salmonella* (10^8 cfu/mL) and *E. coli K*12 (10^8 cfu/mL) in SM buffer, and rotated at room temperature for 10 min. After applying a magnetic field the supernatant was removed. The beads were washed 3 times with 1 mL of SM buffer, and re-suspended in the milk solution. Twenty microliters of this sample were deposited onto the phage T4-modified electrodes of the sensor for impedimetric detection.

2.10. Magnetic separation from milk

Ten microliters of phage-coated bead solution (10 mg/mL) were mixed with 100 μL of *E. coli K*12 (10^8 cfu/mL) in 2% milk and rotated at room temperature for 10 min. After applying a magnetic field the supernatant was removed. The beads were washed 3 times with 1 mL of SM buffer, and re-suspended in the milk solution. 20 μL of this sample were deposited onto the phage T4-modified electrodes of the sensor for impedimetric detection.

2.11. Electrochemical measurements

For phage immobilization, the electrodes were functionalized with 50 μL of 0.1 M EDC in 0.12 N HCl, through chronoamperometry with applying a potential of +2.2 V for 10 min. They were then washed with deionized water and immersed in 2 mL of T4 bacteriophage solution (10^8 pfu/mL in SM buffer, pH 7.5) and left on a shaker for 2 h. Subsequently, they were rinsed with SM buffer and covered with BSA (1 mg/mL) for 1 h to block the surface.

All EIS measurements were performed in SM buffer (pH 7.5) using a Voltalab electrochemical workstation (model PGZ 301 by Radiometer, Copenhagen, Denmark). The Voltamaster software (version 4.0) was used to run the electrochemical experiments and collect the data. EIS measurements (Bode plots, impedance vs. frequency) were carried out over a frequency range varying from 100 Hz to 100 kHz with an applied dc potential of 400 mV, and a superimposed ac voltage of 20 mV amplitude. All tests were performed at room temperature.

3. Results and discussion

One of the important objectives of this work is to address/improve the specificity, sensitivity, and rapidity of an electrochemical biosensor for the detection of bacteria. The detection limit for *E. coli K*12 sensing, using the direct impedimetric approach previously developed by our group, was determined to be 10^4 cfu/mL.

This limited performance of the T4-based biosensor shall be associated with the impedimetric detection approach in which bacteria need to diffuse from the bulk solution to the electrode surface before being able to generate measurable signal. In these

conditions, and because the T4-bacteria interaction is a destructive process (taking 20–25 min to be complete), the first surface-captured bacteria may already have undergone lysis as the diffusing intact bacteria are gradually reaching the surface.

In order to solve this problem, an attempt was made to integrate the impedimetric sensor with a magnetic manipulation technique, to speed up the capture of bacteria by the immobilized phages acting as recognition receptors on the sensor surface. This integration is intended to significantly improve the detection limit, shorten the analysis time to a few minutes, and reduce the non-specific binding that cause false results. The detection setup that was developed to make use of magnetic manipulation is shown in Supplementary Material 2. In the setup, a commercially available rare-earth (neodymium–iron–boron) magnet was used to apply the magnetic field. As shown in Supplementary Material 2-A, a plastic base-plate was machined to incorporate piano wires, reproducing the pattern of 8 working electrodes of the screen-printed sensor array (Supplementary Material 2-B). The base-plate was used to effectively channel the magnetic field force lines and align/focus them right underneath the sensing electrodes.

3.1. Binding activity of T4 modified magnetic beads

In the present report, we are investigating the feasibility of coupling magnetic separation/manipulation, with an impedimetric detection system, to further reduce the detection limits and illustrate the method's potential to detect specific bacteria in complex (real) samples. This method was adapted from previous work on the detection of *E. coli K*12, where T4 phage was directly immobilized onto screen-printed carbon electrode microarrays, to act as specific probe. In an attempt to develop the analytic set up depicted in Fig. 1, 1 μm magnetic beads were modified with covalently bound T4 phages.

To confirm the successful attachment of the phage to the magnetic bead surfaces, magnetic beads were modified with FITC-labeled T4 phage and fluorescent images taken (Fig. 2). Single bright spots can be distinguished, clearly indicating that the beads are carrying immobilized FITC-labeled phage on the surface. Fig. 2 inset with higher magnification depicts a non-filtered, white-light, microscopic image of labeled phage-coated beads, clearly showing the beads as yellowish spots surrounded by green fluorescent phages.

In order to verify that the T4 phage-magnetic bead complex was still able to bind the target bacteria, a first row of experiments was performed, mixing various concentration of bacteria with the complex, incubate and then determining the remaining free bacteria using the plate method counting.

The binding efficiency was calculated as follows:

$$\%\ \text{Binding efficiency} = \frac{\text{Count before separation} - \text{Count in supernatant after separation}}{\text{Count before separation}} \times 100$$

The results are presented in Table 1.

The T4 phages bound to the magnetic beads were shown to still be able to bind the target bacteria, since the *E. coli K*12 concentrations after magnetic separation were found to be significantly lower than before. Binding efficiency of the beads was then found to be between 70% and 80% when in contact with bacteria cell concentrations ranging from 10^4 to 10^8 cfu/mL. The binding efficiency drops then significantly to 50% and 8% for the 10^3 and 10^2 cfu/mL concentration, respectively. These results are in good agreement with the classical behaviors of magnetic beads where binding efficiencies are generally expected/observed to be greater at higher bacteria concentrations.

Flow cytometry was then used as a complimentary method to verify the formation of complexes between the phage-coated beads and GFP-expressing *E. coli K*12 (10^8 cfu/mL). The flow cytometry analysis results are presented in Fig. 3.

In the presence of T4 coated magnetic beads and GFP-bacteria (Fig. 3-A), three distinct regions of interest (*Q*1–*Q*3) are observed due to three different populations. Region *Q*1 consists of the magnetic beads only whereas region *Q*3 is composed of non-fluorescent debris. Region *Q*2 is the most interesting, containing the particles with the largest size, indicating that phage-modified magnetic beads have formed large complexes through association with the bacteria cells. Formation of such large complexes between bead modified phage and bacteria has been reported previously [21].

Fig. 3-B shows the flow cytometry results obtained in the presence of non-fluorescent bacteria. These results reveal a significantly smaller number of events (population) closer to the *y*-axis, which may be associated to the presence of subcellular particles. Comparing Fig. 3-A and -B, the shift in forward and side scatter observed after mixing the bacteria with phage-modified beads indicates the formation of larger complexes, which have much larger forward and side scatter characteristics. As a control experiment, sterile filtered SM media (Fig. 3-D), and a mixture of beads and phage (Fig. 3-C) were analyzed in the same fashion. These yielded no more than 4 events for SM media, and 43 events for the bead and phage mixture.

Supplementary Material 3 shows single parameter histograms of the number of events observed as a function of side scattering. The different sizes of bacterial cells, and bacteria complexed with phage-coated beads, cause a shift in the peaks present in the single parameter histograms. This shift is most likely due to changes in the side scattering after incubation of the bacteria cells with the phage-coated beads, corresponding to a change in the size/

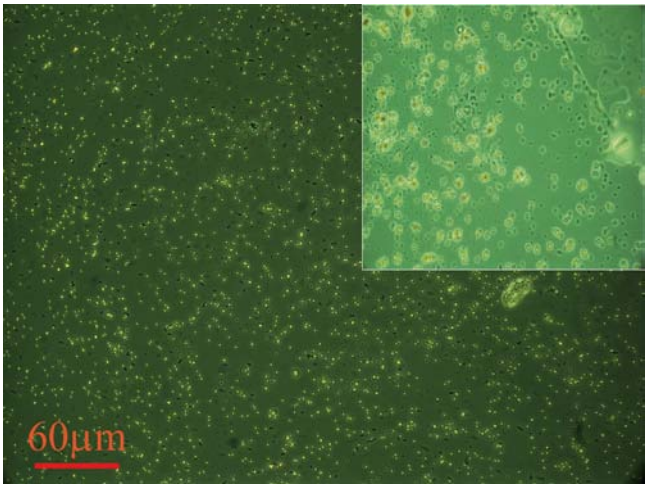

Fig. 2. Fluorescence images of FITC-labeled phage immobilized onto magnetic beads, magnification=400X;. Inset: non-filtered white-light microscopic image of labeled phage-coated magnetic beads, magnification=1000X.

Table 1
Binding efficiency of phage-coated magnetic beads with *E. coli K*12, in pure culture.

Bacteria concentration (cfu/mL)	Count before separation	Count after separation	% Binding efficiency
10^8	1.4×10^8	2.8×10^7	80
10^7	6.2×10^7	1.5×10^7	75
10^6	1.1×10^6	2.8×10^5	74
10^5	1.6×10^5	3.5×10^4	78
10^4	1.7×10^4	4.1×10^3	75
10^3	6.5×10^3	3.2×10^3	50
10^2	2.4×10^2	2.2×10^2	8

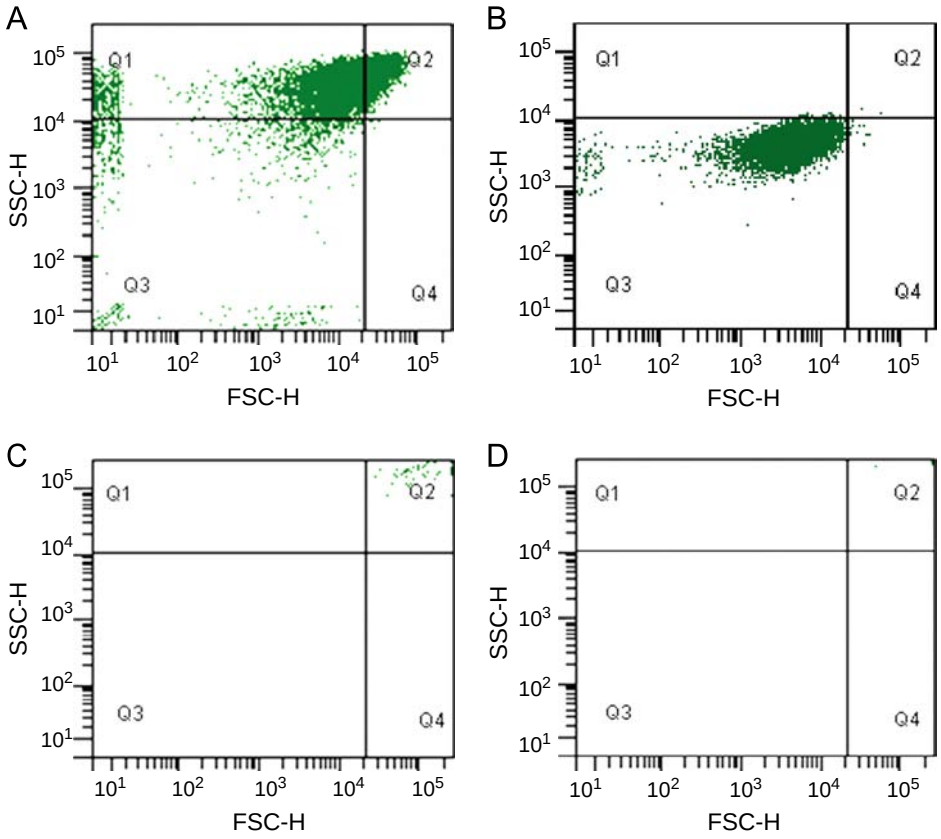

Fig. 3. Dual parameter contour plot of side scattering (vertical axis) and forward scattering (horizontal axis) for the complex of phage T4 immobilized onto beads, mixed with: (A) GFP-expressing bacteria. (B) Non-fluorescent bacteria. (C) A mixture of bead with phage only, as control. (D) SM media only.

granularity of the particles, further confirming the formation of the larger bacteria/bead complexes.

3.2. *Integration of magnetic field manipulation with the impedimetric sensor system*

The binding assay process was performed as illustrated in Fig. 1. The phages were immobilized onto the magnetic beads. The beads were then mixed with the bacteria sample for 10 min, and the mixture was deposited onto to the phage-modified screen-printed carbon electrode microarray surface. The magnet was then placed under the chip for 2 min to attract the magnetic beads, along with the captured bacteria, to the sensor surface. As a result, the response of the sensor (impedance) changes due to the added bacteria captured by the phage. Then, the magnet was removed and the surface washed to get rid of the unbound magnetic beads from the sensor.

Fig. 4-A presents the Bode impedance plots (impedance versus frequency) observed for different concentrations of *E. coli K*12 ranging from 10^3 to10^8 cfu/mL. In the low frequency domain (10^2–10^4 Hz), the impedance spectra of the bacteria suspensions vary significantly with concentration, whereas the impedance spectra show no significant difference in the high frequency domain (10^4–10^5 Hz).

At frequencies ranging from 10^2 Hz to 10^4 Hz the impedance spectra are sensitive primarily to changes in electrical resistance [22], and reflect the contribution to the impedance coming from the resistive components of the electrochemical detection system (see the corresponding Randles equivalent circuit, given as Supplementary Material 4), which includes Zw (Warburg impedance, resistance due to mass transfer), Rct (charge transfer resistance), and Rs (resistance of the electrolyte) [23]. The observation of increasing impedance with increasing bacterial cell concentration is in good agreement with the expected increase in resistance caused by the greater amount of bacteria being captured at the electrode surface. The bacteria cell membrane is highly insulating, having a conductivity of approximately 10^{-7} S/m [24]. In addition, the combination of bacteria with phage-coated beads, produce large bead-bacteria complexes which also contribute to increase the resistive behavior of the system.

As a control, the same series of experiments were performed with lysed bacteria, incubated with the T4-beads for 2 h (ensuring complete lysis of the bacteria). Fig. 4-B shows the Bode plots of lysed cells obtained from different bacteria concentrations, ranging from 10^3 to 10^8 cfu/mL. An increase in impedance with increasing concentrations is also observed with the lysed cells but, in this case, the variation is much less pronounced compared to that of intact cells, and can be attributed to the adsorption of lysed material at the electrode surface.

In order to demonstrate the impact of the application of the magnetic field on the detection performances, a control experiment was performed that involved acquiring the Bode plots for the detection of *E. coli K*12 without application of the magnetic field. These results are shown in Fig. 4-A and shows that very little shift in impedance occurs, even in the presence of the highest concentrations of bacteria, indicating the important impact and direct necessity of applying the magnetic field in order to get an improved detection response.

Finally, a specificity control was performed which involved testing the detection system in the presence of non-target bacteria. Fig. 4-B presents the Bode plots for the detection of *Salmonella* which gives much less significant impedance shifts compared to those observed for target *E. coli K*12 bacteria (Fig. 4-A), even at the higher concentrations. These small shifts can again be attributed to adsorption of bacteria onto the electrode surface.

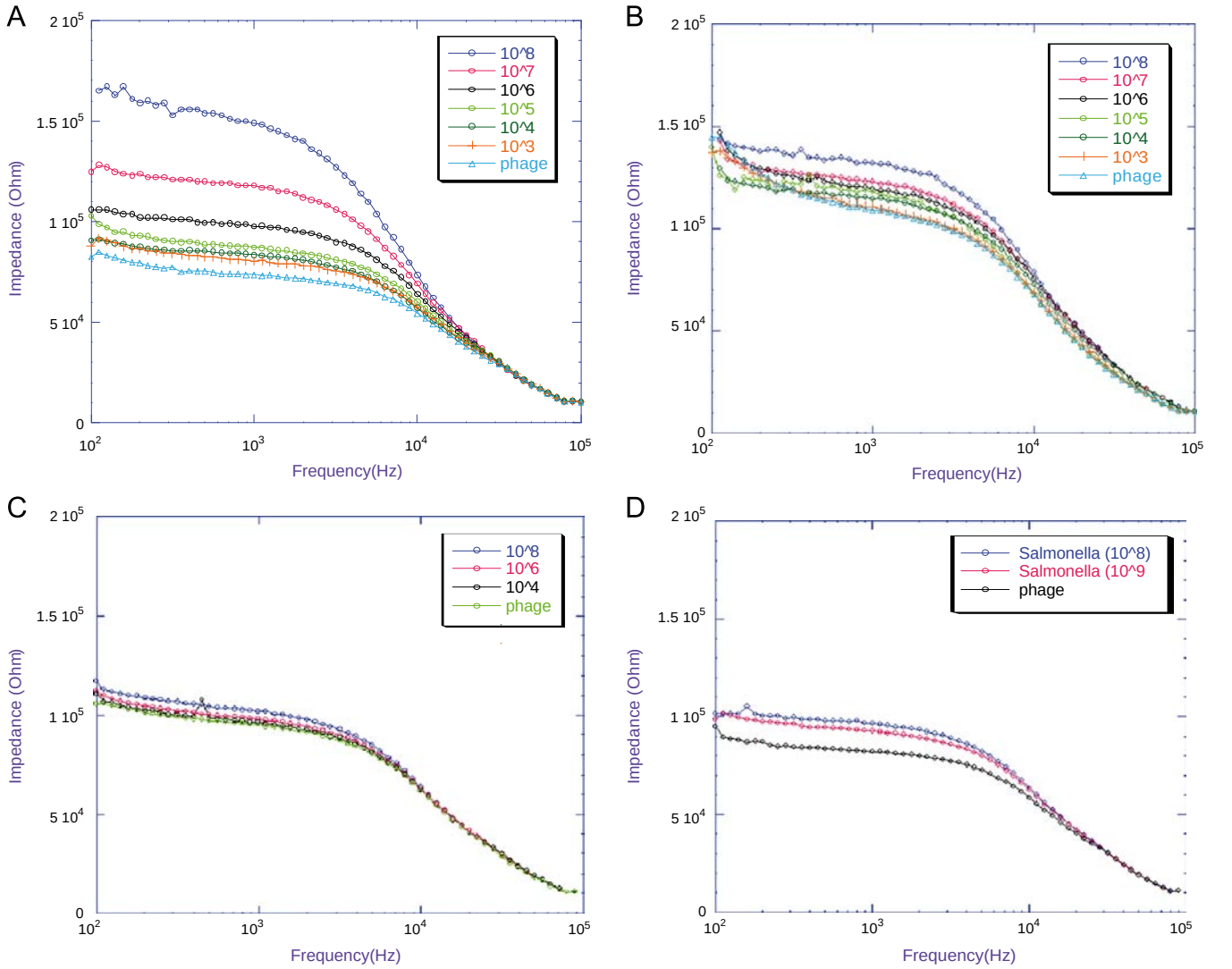

Fig. 4. (A) Bode impedance plots for live bacteria (10 min beads binding) at different concentrations. (B) Bode impedance plots for lysed bacteria (120 min beads binding) at different initial (intact) bacteria concentrations. (C) Bode plots for the control experiment performed without applying the magnetic field. (D) Bode plots for the control experiment performed with non-target bacteria.

Fig. 5-A presents the variation in impedance values (at 10^3 Hz) as a function of bacteria concentration. The standard deviations were obtained from 3 measurements performed at each of the concentrations. The detection limit, (calculated by dividing 3 times the noise of the blank, by the slope of the calibration curve) was determined to be 10^3 cfu/mL, which correspond to a one order of magnitude improvement over what was obtained for the detection *E. coli K*12 without the use of magnetic manipulation [18].

3.3. *Separation of bacteria from milk using magnetic manipulation and detection*

In order to demonstrate the potential of the technique for the capture, enrichment and detection of bacteria present in more complex media, milk samples (2% milk) were inoculated with *E. coli K*12. Then, T4 phage-coated beads were used to specifically separate *E. coli K*12 cells from the mixture.

Fig. 5-B depicts the Bode plots (impedimetric detection with and without magnetic field) observed for *E. Coli K*12 (10^8 cfu/mL) suspended in 2% milk. It should be emphasized here that the separation step consists in the removal of the beads from the milk sample using a magnet, which is separate from the impedance measurements performed with the sensor in the presence or absence of a magnetic field. In Fig. 5-B, the *E. coli K*12-magnetic bead complexes extracted from milk samples inoculated with *E. coli K*12 only, show the trend expected when impedance measurements with the sensor are performed before and after applied magnetic field (i.e. significant increase in signal when the field is applied). This is also indicative of the effectiveness of the method in separating the target analyte from a complex sample (milk).

In an attempt to correlate the impedance shift induced by a concentration of 10^8 cfu/mL extracted from milk to the impedance shift observed in pure buffer, the shift obtained from Fig. 5-B was reported to the Fig. 5-A calibration curve. As a matter of fact, a strong matrix effect was observed inducing an underestimation of the calculated bacteria concentration (10^6 vs. 10^8 cfu/mL). Notwithstanding this contribution to impedance shifts caused by non-specific adsorption, these results still clearly indicate the effectiveness of the protocol in providing bacterial detection in complex sample.

4. Conclusion

In conclusion, we have demonstrated the feasibility of integrating an inexpensive, screen-printed carbon-based impedimetric

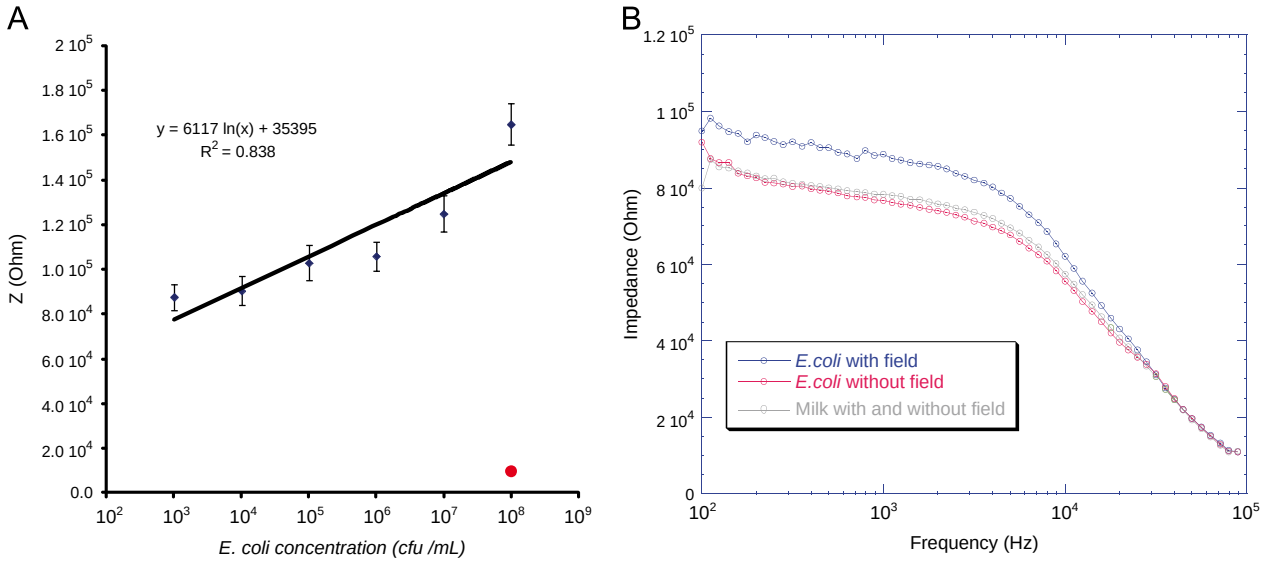

Fig. 5. (A) Impedance variation as a function of the bacteria concentration. The red dot represents the non-specific adsorption taken from Fig. 4-D plot. Error bars are the standard deviation of data extracted from 3 replicate Bode plots. Frequency = 10^3 Hz. (B) Bode impedance plots with or without magnetic separation from milk samples containing *E. coli K*12 (10^8 cfu/mL).

sensor, with a magnetic manipulation system for improving the sensitivity of the device. We have been able to show through fluorescence and flow cytometry measurements that the surface modification of the magnetic beads, with phages, and binding with the bacteria, were successful. We have also demonstrated that the detection of bacteria was specific, enabling the operator to account for non-specific adsorption, and can be used to detect bacteria in more complex (real) samples. Finally, we have demonstrated that the sensitivity is improved (from 10^4 to 10^3 cfu/mL) through the use of a simple magnetic manipulation system.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.07.078.

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