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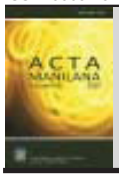

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Electrochemical Impedance Spectroscopy (EIS)-based measurement of the binding and lysis activity of immobilized bacteriophage UP87 on *Aeromonas hydrophila*

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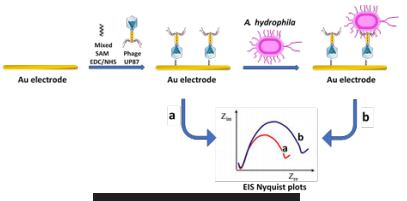
Graphical Abstract



Abstract

In this study, a measurement system based on Electrochemical Impedance Spectroscopy (EIS) was assembled to determine whether bacteriophage UP87 immobilized on a gold electrode surface would exhibit binding and lytic activity towards *Aeromonas hydrophila*. Bacteriophage UP87, a lytic phage for *A. hydrophila*, was immobilized on gold screen-printed electrodes (Au-SPEs), which contained a self-assembled monolayer (SAM) of 11-mercaptoundecanoic acid (MUA) and dithiothreitol (DTT), via carbodiimide cross-linker chemistry. The phage solution incubation time on the Au SPE was optimized to ensure maximum phage coverage of the electrode. The optimum time for phage UP87 immobilization was 60 minutes. To confirm the successful immobilization of the phage on the electrode surface, the Au-SPEs were characterized using Fourier Transform Infrared Spectroscopy (FTIR). The study of the binding and lysis of *A. hydrophila* was done through bacterial solution incubation on the treated Au-SPEs for different durations. Binding of the bacterial cells was shown by the general increase in the charge transfer resistance (R_{ct}) up to a maximum value obtained after 60 minutes of incubation. Lysis of the captured bacterial cells was indicated through the decrease in R_{ct} value after 80 minutes of incubation. In addition, the phage-modified Au-SPE exhibited higher R_{ct} values at 60 minutes of incubation period for *A. hydrophila* compared with the non-target bacteria Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, thus showing satisfactory selectivity. This high selectivity was attributed to the pre-treatment of the Au-SPE which blocked nonspecific interactions and highly favored the binding of *A. hydrophila*. The results of this study indicate that surface immobilization of phages can be used to concentrate them for binding and lysing bacteria in solution. The concentration-dependence of the bacteria's binding to the phage-modified surface hints at the possibility of using the phage UP87 as a biorecognition element in a selective biosensor for *A. hydrophila* using the EIS technique.

Keywords: *Aeromonas hydrophila*; Bacteriophage; Electrochemical Impedance Spectroscopy; Gold Screen-Printed Electrode

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Introduction

Bacteriophages, or simply phages, are viruses that kill and control the host's cellular mechanisms [1]. These viruses can interact with target sites on the bacterial surface and implant their genetic material inside the infected bacteria. The phage uses this process to control the bacteria for the replication of new virions that cause lysis of the bacteria. These phages range in size from 20-200 nm and can be wild-type or engineered [2]. In 2014, the group of the third author isolated a phage, designated as Bacteriophage UP87, from sewage samples that is lytic towards *A. hydrophila*. The phage is classified under the Family Myoviridae possessing an icosahedral head, a contractile tail, and linear, double-stranded DNA genome. The UP87 phage decreased the total amount of *A. hydrophila* in the blood of the Nile Tilapia (*Oreochromis niloticus*) and did not cause mortality among the infected fish similar to the results obtained using oxytetracycline, a commonly used antibiotic in aquaculture [3]. In comparison to antibiotics, which have a broad effect on microorganisms, phages can be used as targeted killers of pathogenic bacteria due to their host-specificity [4].

It has been shown that phage therapy can be used to prevent diseases caused by *A. hydrophila* in the laboratory by simply adding phages to the water at a high concentration and immersing the fish for 30 minutes [5]. However, in contrast to the controlled environment of laboratory setups, the water volumes in fish cages in lakes or in fishponds can be exceedingly high in a typical fish farm environment and this could have the effect of diluting or removing the injected phages through the water flow. In response to this need, immobilizing phages on a surface through physisorption or covalent bonding to impart a long-term action against water-borne bacteria could be the best answer [4]. Immobilized phages have been shown to have an antimicrobial impact in its investigations on food packaging [6] and medical equipment [7].

A number of studies have immobilized biorecognition elements such as bacteriophages through the entrapment in conducting polymer matrices [8], magnetic particles for the capture of bacterial cells [9], adsorption through reagentless and physisorption methods [10], modification with sugars, amino acids to facilitate attachment of wild-type bacteriophages [11], amino acid or lipid tagging mechanisms using phage receptor binding proteins (RBPs) [12], genetic biotinylation of the capsid heads of bacteriophages and the natural affinity of the biotin/streptavidin recognition [13], attachment through plasma treatment [14], and covalent binding through the use of self-assembled monolayers or surface-imprinted polymers [4, 8, 14, 15]. The formation of self-assembled monolayer (SAM) has been a widely used immobilization method that is advantageous due to its simplicity, high selectivity, specificity, terminal functionality, blocking of non-specific interactions, and excellent stability over other immobilization methods [8, 15]. Immobilization of lytic bacteriophages with the use of SAM and other methods have been utilized for the detection of various bacteria such as *Staphylococcus arlettae* [1], *Mycobacterium smegmatis* [8], *S. aureus* & *Bacillus anthracis* [9], *E. coli* [2, 8, 10, 16], *Bacillus cereus* & *Salmonella typhimurium* [17].

In biosensor applications, phages that are covalently immobilized to a graphene or gold surface have been found to enhance the phage density and its lytic activity [1, 2]. The long chains in MUA provide stability for phage immobilization while its carboxylic moiety allows the phage to connect to the gold electrode. DTT acts as an interfacial layer that blocks nonspecific interactions such as physical adsorption and spaces out the MUA. Its hydroxyl groups provide a hydrophilic microenvironment which is favorable for binding reactions and enhance the non-fouling properties of the layer. The advantages of using mixed SAM for the phage immobilization is that the long carbon chain molecules act as a support for the phage while the short chain molecules favor the electron transfer process [15]. The NHS reagent is often included in EDC coupling protocols to improve efficiency or create dry-stable (amine-reactive) intermediates. EDC couples NHS to carboxyls, forming an NHS ester that is considerably more stable than the O-acylisourea intermediate while allowing for efficient conjugation to primary amines at physiological pH. An increase in resistance was observed in the MUA+DTT/Au electrode incubated with bacteria. This phenomenon is due to the formation of a barrier of captured cells which hinders the probes close to the electrode surface [8, 15].

This study therefore aimed to investigate if bacteriophage UP87 that are immobilized on a gold electrode surface will exhibit binding and lytic activity towards *A. hydrophila*. The binding and lysis events were measured using Electrochemical Impedance Spectroscopy (EIS), which is a powerful electrochemical technique capable of detecting small changes occurring at the solution-electrode interface.

Materials and Methods

Materials. The host bacteria, *A. hydrophila* (IFO 3820), was obtained from the Philippine National Collection of Microorganisms (PNCM) at the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños. Bacteriophage UP87 (vB_AehM_UP87) was acquired from the collection of the third author [3]. The gold screen-printed electrode (SPE) was purchased from Metrohm Dropsens (Spain). Tryptic soy agar (TSA), Tryptic soy broth (TSB), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), phosphate buffer saline (PBS) tablet, N-hydroxysuccinimide (NHS), 11-mercaptoundecanoic acid (MUA), and dithiothreitol (DTT) were purchased from Sigma-Aldrich® (Singapore). The washing buffer used was composed of the phosphate buffer saline tablet in solution (pH 7.4 at 25°C). Alumina slurry with 0.3 and 0.5 µm-sized particles were purchased from Buehler (USA). Analytical grade reagents and solvents were used all throughout this study. Characterization of the treated gold screen-printed electrodes were utilized through the use of Fourier-Transform Infrared Spectrophotometer IRPrestige-21 (Shimadzu, Japan) and absorbance of the McFarland standards were measured using SmartSpecTM 3000 UV-Vis Spectrophotometer (Bio-Rad Laboratories, USA). All glassware were cleaned with freshly prepared 3:1 HCl/HNO_3 (aqua regia), rinsed thoroughly with ultrapure water and oven-dried prior to use. All solutions were prepared using ultrapure water (TOC <5.00 pbb; resistivity: 18.2 MΩ cm^{-1}).

Preparation of Bacteriophage. A 24-hour old culture of the host bacteria, *A. hydrophila*, was prepared in TSB. A 2 x 10 mL volume of TSB was prepared followed by the addition of 300 µL bacteriophage solution and 100 µL of host bacteria suspension. The mixture was incubated for 24 hours at 30-36°C. Removal of residual bacteria was done through filtration of the solution using a 10 mL syringe with 0.45 µm Acrodisc© filter into sterile test tubes. Double agar overlay method was used to determine the titer of the prepared bacteriophage solution. The bacteriophage filtrates were serially diluted 9 times (10^{-1}-10^{-9}) in PBS. About 0.5 mL of *A. hydrophila* broth culture and 0.5 mL of the diluted bacteriophage UP87 filtrate were added to 3 mL of molten 0.7% soft TSB and poured on a TSA plate. The plates were incubated overnight at 30-36°C and examined for plaques the following day. A plate with an ample number of colonies (10^6 x sample) was analyzed, and the titer of the bacteriophage (measured in plaque forming units per mL) in the stock and other diluted samples were estimated using the equation:

$$pfu\ mL^{-1} = \frac{1000\ x\ number\ of\ plaques\ x\ reciprocal\ of\ dilution\ factor}{volume\ of\ bacteriophage\ sample\ (\mu L)}$$

The effectivity of the bacteriophage was evaluated through standard spot test method. A plate containing *A. hydrophila* was prepared according to the aforementioned procedure, and divided into four test zones. About 10 µL of the bacteriophage sample was distributed at the center of each test zone. Visual analysis was performed after the plate was incubated at room temperature for 24 hours. The presence of a spot indicated that the bacteriophage culture was still functional and could still be used.

Bacterial 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 (10) µL of each of the diluted samples was pipetted into a sterile TSA plate and incubated at 37°C overnight to allow the colony formation. The bacteria colonies were counted the day after to give the exact concentration of bacteria present at the time of detection. Plates with only 30-300 colonies were counted [18].

Pre-treatment of the Gold Substrate. The Au-SPEs were of a DS 220BT type with a 4 mm diameter working electrode and with total dimensions of 33 x 10 x 0.5 mm (length x width x height). It is a three-electrode system wherein the working and counter electrodes are made of gold, while the reference electrode and electric contacts are made of silver. The working electrode area of the Au-SPE was pre-treated as reported in related literature [15, 19]. Gold disk electrodes were polished with 0.3 and 0.5 µm alumina slurry, rinsed with water, and dried under flowing nitrogen gas. A clean, gold electrode was immersed into a 100 µL solution of 1 µM MUA and 10 µM DTT, which is the mixed self-assembled monolayer (SAM) reagent. The setup was then incubated in a humidified chamber (refrigerator) at 4°C for 14 hours to yield the MUA+DTT/Au electrode [15]. The resulting electrode was washed with PBS and activated in 100 µL freshly prepared solution composed of 2 mg/mL EDC and 5 mg/mL NHS for 30 minutes and rinsed with 10 mM PBS. The activated electrode was then immersed in 100 µL of 1 mg/mL Phage UP87 for 1hr at 25°C which were the parameters obtained from the optimization studies and rinsed with washing buffer.

The surface-active sites of the MUA+DTT/Au electrode were blocked using 100 µL of 1% bovine serum albumin (BSA) for 30 minutes to prevent unnecessary interactions with nontarget analytes. The electrode was rinsed with washing buffer after each step to remove adsorption components. The phage-modified electrode was then stored in a 4°C humidified chamber.

Electrochemical Measurement of Bacterial Binding. An amount of 2 mL of a 24-hr *A. hydrophila* culture was added and mixed to a test tube containing 9 mL PBS. The absorbance of the mixture at a wavelength of 420 nm was measured using a SmartSpecTM 3000 UV-Vis spectrophotometer. A set of McFarland standards with corresponding nephelometric values (0.5 to 6) was used to determine the volume (in µL) of bacterial culture and PBS needed to obtain a sample with a concentration of 10^8 CFU/mL. Serial dilution was performed to obtain samples with the following concentrations: 10^1, 10^2, 10^4, and 10^6 CFU/mL. All samples were placed in sterilized Eppendorf tubes. The McFarland standard solutions were composed of barium chloride ($BaCl_2$) and sulfuric acid (H_2SO_4) which produced a $BaSO_4$ fine particle precipitate. This was used to approximate the number of bacteria or the colony-forming units (CFU/mL) in a liquid suspension through the comparison of the turbidity of the standard solutions with that of the sample. Measurement of the samples at wavelengths 420-660 nm using the spectrophotometer prevents killing the bacteria, the maximum absorbance wavelength of *A. hydrophila* is near the standardized wavelength of 420 nm. *A. hydrophila* can be detected for as low as 352 nm [15].

Characterization of the binding of *A. hydrophila* on the modified electrode surface was done using EIS measured through the use of an electrochemical impedance analyzer (eDAQ ERZ100) coupled to a potentiostat (eDAQ EA163). The block diagram of the EIS instrumentation system is shown in Figure 1. The EIS parameters were recorded in the presence of $Fe^{3+/2+}$ redox probe (5 mM $K_3Fe(CN)_6$ + $K_4Fe(CN)_6$ • $3H_2O$, prepared in 0.1 M PBS). The frequency range used was from 0.1 Hz to 1 MHz with a fixed amplitude of 5 mV. After activation, the bacteriophage-immobilized SPEs were incubated with 25 µL aliquots of a stock bacterial solution (1 x 10^8 CFU/mL, concentration standardized with standard colony counting method) and its serial dilutions (10^1, 10^2, 10^4, 10^6) at 37°C for an hour and rinsed with the washing buffer. The surface-active sites of the MUA+DTT/Au electrode were blocked using 100 µL of 1% bovine serum albumin (BSA) for 30 minutes to prevent unnecessary interactions with nontarget analytes. The treated Au-SPEs were stored at 4°C in a humidified chamber.

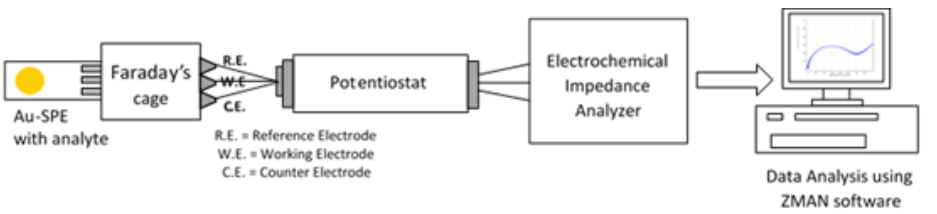

Figure 1. Block diagram of the electrochemical impedance spectroscopy instrumentation system.

Characterization of Bacteriophage-immobilized Au-SPE. Fourier Transform Infrared Spectroscopy (FTIR) analysis using an attenuated total reflectance (ATR) accessory was also employed to verify bacteriophage immobilization on the 0.5 mm thick Au-SPE. The phage-modified gold electrode of the Au-SPE was placed on top of the ATR crystal towards the IR source which created an evanescent wave that is absorbed by the sample and reflected internally. A comparative study between the FTIR spectra of the bare SPEs and the phage-modified SPEs was done to verify the successful immobilization of the phage by observing characteristic peaks at ~3300 cm^{-1} (-NH group) and 1650 cm^{-1} (amide group). The structure of the modified gold screen-printed electrodes involves long carbon chains, hydroxyl (–OH), carbonyl (C=O), and amide (–NH) groups.

Assessment of Selectivity. Selectivity tests were utilized against two ubiquitous bacteria, *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). The bacteria solutions were prepared and subjected to EIS similarly with the method of *A. hydrophila*. The same amount (25 μL) was dispensed onto the Au SPE and incubated for 60 minutes. The electrode was then placed into the Faraday's cage and the redox probe was added dropwise until the whole surface was covered.

Results and Discussion

Bacteriophage-modified Surface. The preparation of the bacteriophage-modified surface was based on the method reported in a previous literature, but revised by using bacteriophage UP87 as the biorecognition element and *A. hydrophila* as the target bacteria. An Au-SPE was used because of the ability of gold to interact with thiol groups which formed the self-assembled monolayer (SAM). The formation of the mixed SAM by co-assembling MUA and DTT on gold surface is a widely used immobilization method due to its several advantages, such as simplicity, terminal functionality, prevention of non-specific adsorption, and good stability [8, 15].

Optimization of Phage Immobilization Time. The optimum immobilization time of phage UP87 was determined by obtaining impedance readings of MUA+DTT/Au electrodes wherein the phage was incubated at various times: 30 minutes, 1 hour and 2 hours. During the optimization step, the bacterial incubation time was set to a constant time of 1 hour ($1x10^8$ CFU/mL *A. hydrophila*). Nyquist plots of impedance spectra obtained for these electrodes are shown in Figure 2. The highest R_{ct} value (7356 Ω) was obtained from the electrode in which the phage was incubated for 1 hour. A comparison of R_{ct} values is shown in Figure 3. The error bars in the graph represent the standard deviations of the mean of three replicate measurements. These results are consistent with the reference procedure, which allotted the same amount of time to facilitate immobilization of the capture reagent [15]. Therefore, 60 minutes was selected as the optimum phage immobilization time. At 30 minutes, a lower charge transfer resistance is observed due to a lower electrode surface coverage by the phages and at about 2 hours, a plateau-like R_{ct} profile was reached which indicated the maximum possible number of phages linked to MUA+DTT/Au-SPE [15]. When the phage linkages with the SAM/EDC+NHS has reached its peak at 1 hour, the R_{ct} value after that immobilization time decreased by a small amount only. [10]

Optimization of Bacterial Solution Incubation Time on the Au-SPE. To determine the optimum time of maximum binding through the incubation of *A. hydrophila*, impedance readings of MUA+DTT/Au electrodes with bacteria that were incubated at various intervals, namely 10, 20, 30, 40, 60 and 80 minutes, were conducted. Nyquist plots of impedance spectra obtained for these electrodes are shown in Figure 4. The decrease in impedance at 80 minutes was inferred to be due to phage-induced lysis of the bacteria. The release of ionic cellular components following lysis causes the impedance of the solution to decrease [15].

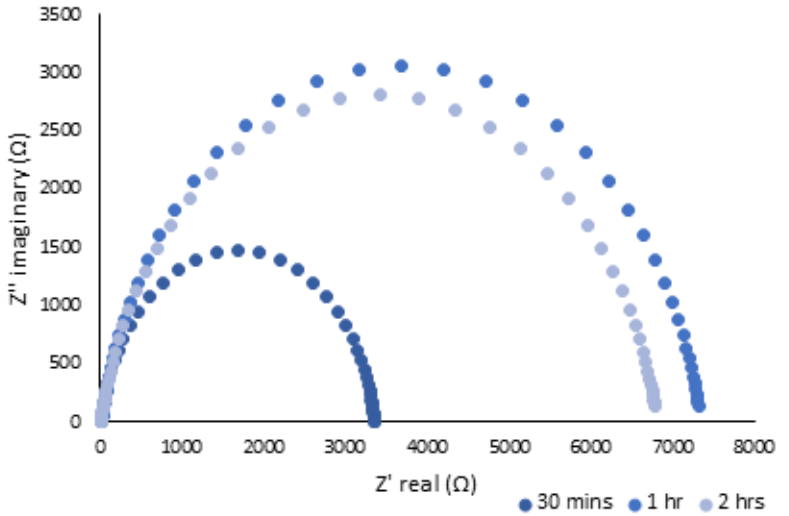

Figure 2. Fitted Nyquist plots of phage-immobilized Au-SPEs prepared using different immobilization times (30 min, 1 h, and 2h).

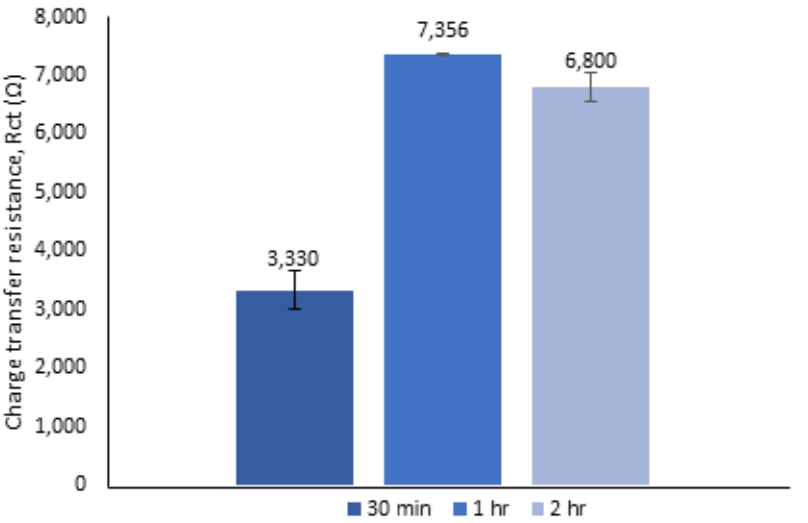

Figure 3. R_{ct} values of phage-immobilized Au-SPEs prepared using different immobilization times (30 min, 1 h, and 2h).

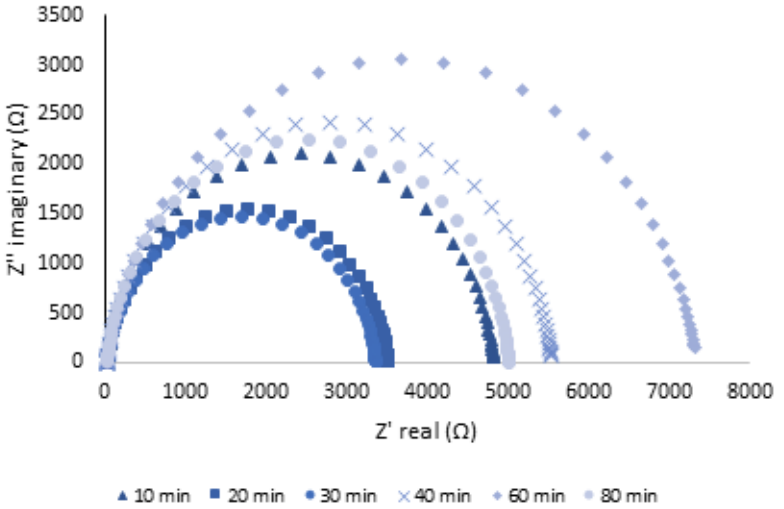

Figure 4. Fitted Nyquist plots of phage-immobilized Au-SPE incubated with 1x10^8 CFU/mL *A. hydrophila* solution at different time durations (10, 20, 30, 40, 60, 80 mins).

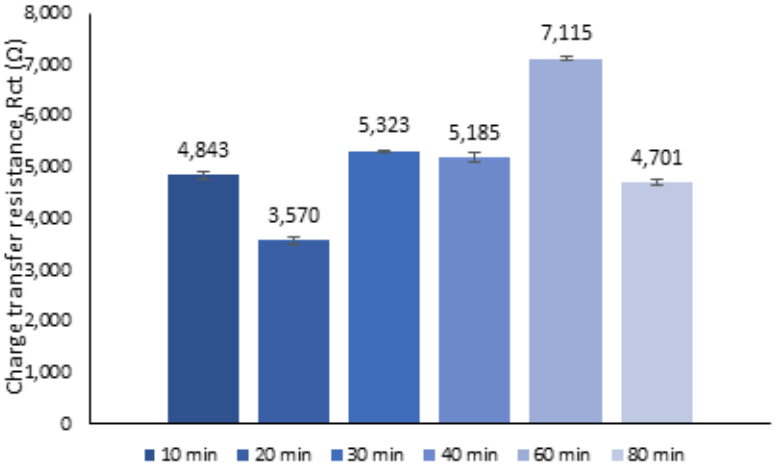

Figure 5. R_{ct} values of phage-immobilized Au-SPE incubated with 1x10^8 CFU/mL *A. hydrophila* solution at different time durations (10, 20, 30, 40, 60, 80 mins).

The plot with the highest R_{ct} (7115 Ω) was obtained from the electrode with a bacterial incubation time of 60 minutes. A comparison of R_{ct} values is shown in Figure 5 which suggested that the binding reaction was completed in about 60 minutes, i.e. optimum incubation time. After the optimum time, at 80 min the R_{ct} value decreased since at this time release of bacterial cellular components could have occurred due to cell lysis releasing newly made phages together with ionic cellular components into the solution.

Characterization of Au-SPE. The IR spectra of the MUA+DTT/Au electrode with bacteriophage UP87 shown in Figure 5 was obtained through ATR technique. The inset in Figure 6 shows a diagram of the MUA+DTT/Au electrode. Encircled regions indicate peaks of interest. The peak observed at around 3700 cm^{-1} corresponds to the O-H stretching of the hydroxyl groups of DTT, while the peak around 3600 cm^{-1} corresponds to N-H stretching coming from the amide part of MUA. Peaks located at ~2300 cm^{-1} are due to the alkyl C-H stretching in the long carbon chains of MUA molecules and hydrogen bonding vibrations. Meanwhile, a medium peak observed at around 1550 cm^{-1} corresponds to the C=O stretching of the carbonyl moiety of MUA (symmetric and antisymmetric bending). The peaks between 500 and 950 cm^{-1} corresponds to C-S and C-C stretching. The presence of these peaks therefore confirms the composition of the mixed SAM which connects the phage to the electrode surface. A number of studies have confirmed the successful immobilization of the bacteriophage through FTIR which showed characteristic peaks at 3280 cm^{-1} (broad signal of proteins), 1650 cm^{-1} (amide I absorption), 1400 (carbohydrates) and 1250 cm^{-1} ($-PO_2$) [1]. Immobilization of a T4-phage was confirmed by distinctive peaks between 500 and 950 cm^{-1} (C-S and C-C stretching), 1250 and 1500 cm^{-1} (methyl symmetric and antisymmetric bending), 1700 and 1750 cm^{-1} (C=O stretching), 2750 and 2000 cm^{-1} (NH_2 hydrogen bond symmetric and antisymmetric vibrations), and 2800-2950 cm^{-1} (alkyl chains vibrations) [10].

Assessment of Selectivity. In assessing the selectivity of the binding of the immobilized phages towards *A. hydrophila*, the binding interaction of Gram-positive *S. aureus* and Gram-negative *E. coli* with the phage-modified Au SPE was characterized using EIS. The same concentration ($1x10^8$ CFU/mL) for the three bacteria was used. Figure 7 shows the results of the R_{ct} for *E. coli* and *S. aureus* were 16% and 12% of the R_{ct} for *A. hydrophila*, respectively. These results suggest that the bacteriophage in the immobilized state is highly selective to the target bacteria. A bare unmodified gold electrode was also subjected to EIS measurements after incubation with $1x10^8$ CFU/mL *A. hydrophila* and rinsing, and a mean R_{ct} value of 1,038 Ω was obtained. From these results, it can be inferred that bacterial cells could indeed non-specifically adsorb on the gold electrode surface [20]. The R_{ct} values of *A. hydrophila* was significantly different ($p<0.05$) from that of the other two bacteria. This proves that the treatment applied to the Au-SPE was successful in the binding and lysis of the target bacteria. Targeting a specific bacterium, *A. hydrophila*, could be employed in the detection of the bacterium through a biosensor.

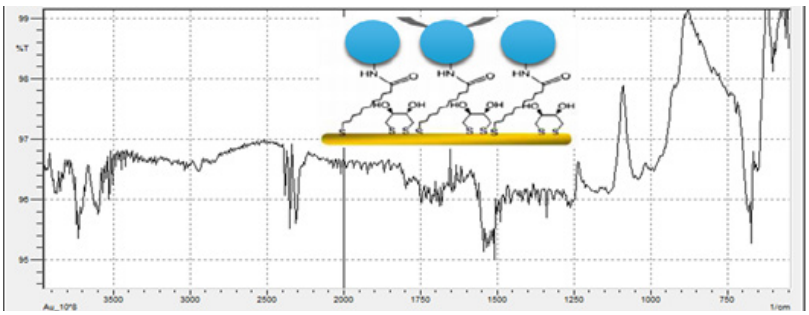

Figure 6. Infrared spectra of phage-immobilized Au-SPE (with MUA+DTT self-assembled monolayer).

Concentration Effects on Binding. The dependence of R_{ct} on the concentration of *A. hydrophila* was investigated to determine if the binding of the bacteria to the bacteriophage-modified surface is concentration-dependent. Figure 8 shows the logarithmic relationship between R_{ct} and the logarithm of *A. hydrophila* concentration: 10^1, 10^2, 10^4, 10^6, and 10^8 CFU/mL. The error bars in the graph represent the standard deviations of three replicate measurements. The logarithm of the charge transfer resistance (R_{ct}), in ohms, was plotted against the logarithm of concentration, measured in CFU/mL to obtain a linear plot.

The results obtained from the study suggest that binding and lysing the bacteria in solution could be achieved through the immobilization of phage. Furthermore, the concentration-dependence of the binding point to the potential of the phage UP87 to be used as a biorecognition element in a selective biosensor for *A. hydrophila* based on the EIS technique. Bacteriophages as recognition elements for a desired host which is made possible through immobilization on an electrode surface to achieve a bacteriophage-based biosensor should fulfill the following conditions: (a) the immobilized bacteriophages should have the ability to infect and link to the desired analyte, (b) inactivation of the bacteriophages by rapid changes of conditions should be prevented, (c) equal distribution of phages is needed to increase the sensor's sensitivity, and (d) no desorption process from the surface of the electrode should happen within the analyte solution [9].

Previous researches have dealt with bacteriophage-based studies for pathogen detection which include, but not limited to, *S. arlettae* [1], *M. smegmatis* [8], *S. aureus* & *B.anthracis* [9], *E. coli* [2, 8, 10, 16], *B. cereus* & S. *typhirium* [17]. These detections were achieved through the use of different transduction principles such as, EIS, colorimetry, fluorescence, electrogenerated chemiluminescence (ECL), Faradic impedance, fiber optic microchannel, fast fourier transform square wave voltammetry, and DNA electrochemistry.

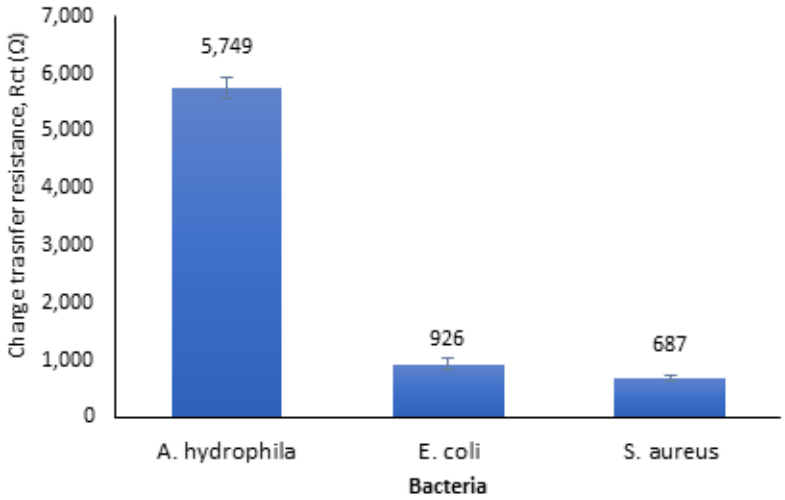

Figure 7. R_{ct} values of phage-immobilized Au-SPE incubated with $1x10^8$ CFU/mL solutions of *A. hydrophila, E. coli*, and *S. aureus* for 60 mins.

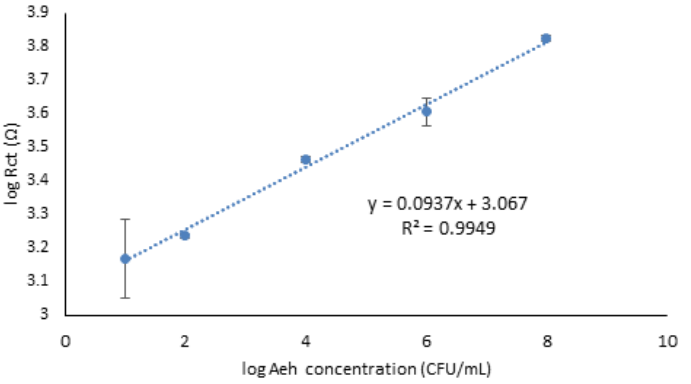

Figure 8. Log-log plot of R_{ct} values of phage-immobilized Au-SPE incubated with *A. hydrophila* solutions at different concentrations.

Conclusions

The phage UP87 was successfully immobilized on the gold screen-printed electrode (Au-SPE) surface. The optimized bacteriophage immobilization and the time need for the binding of the *A. hydrophila* was determined to be at 60 minutes. EIS measurements have demonstrated that the phage-modified Au-SPE surface was able to bind selectively *A. hydrophila* and indications of lysis was exhibited after 80 minutes of incubation of the bacterial solution. The immobilized phage was able to distinguish between Gram negative *A. hydrophila* from Gram-positive *S. aureus* and showed a much higher response compared with Gram-negative *E. coli*. The findings of this study suggest that immobilizing phages can be used to concentrate them for the purpose of binding and lysing bacteria in solutions. The results of this study indicate that surface immobilization of phages can be used to concentrate them for binding and lysing bacteria in solution. The concentration-dependence of the bacteria's binding to the phage-modified surface hints at the possibility of using the phage UP87 as a biorecognition element in a selective biosensor for *A. hydrophila* using the EIS technique. Compared with conventional detection methods for the target bacteria, such a biosensor could enable a more rapid means of detection while providing ample sensitivity and selectivity.

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Conflict of interest

The authors declare no conflict of interest.

Author Contributions

MNDD, DVB, DMDCP, and JHB all contributed significantly to the conduct of the study and drafting of the paper. JHB conceived of the work, DMDCP supplied the phage UP87 and bacteria, DVB supplied the electrodes and reagents and directly supervised MNDD, and MNDD performed the laboratory work. The final version of the manuscript has been read and approved by all authors.

Institutional Review Board Statement

The study did not involve any human participants or animal subjects.

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