ANALYTICAL SCIENCES JUNE 2016, VOL. 32

A Simple DNA-based Electrochemical Biosensor for Highly Sensitive Detection of Ciprofloxacin Using Disposable Graphene

Syazana A LIM* and Minhaz U AHMED†**

**Environmental and Life Sciences Programme, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE 1410, Brunei Darussalam*
***Biosensors and Biotechnology Laboratory, Chemical Science Programme, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE 1410, Brunei Darussalam*

In this work we exploited the electrostatic interaction of double stranded DNA (dsDNA) with drug components to construct a simple, but highly sensitive, DNA-electrochemical sensor for detecting ciprofloxacin. The following straightforward three-step procedure was performed to determine ciprofloxacin: (i) dsDNA-layer immobilization on the surface of the working graphene-modified screen-printed carbon electrode; (ii) dsDNA-ciprofloxacin interaction for 2 min; and (iii) electrochemical measurement using square-wave voltammetry. An increased oxidation of the guanine component was observed, at +1.0 V, as a result of the electrostatic interaction of positively charged ciprofloxacin with the negatively charged nucleic acid sugar phosphate. Based on the International Conference on Harmonization Guidelines, a linear relationship between the guanine oxidation peak and ciprofloxacin concentration (0.1 to 100 µM) was obtained with a detection limit of 0.1 µM. Our developed sensor is straightforward to construct and use, requiring no multi-step time-consuming preconditioning of electrodes. It is highly sensitive and selective in the detection of ciprofloxacin, and has the potential to be useful in the future fabrication of rapid and portable on-site food safety analysis devices.

Keywords Ciprofloxacin, DNA electrochemical biosensor, graphene, screen-printed electrodes

(Received January 11, 2016; Accepted February 23, 2016; Published June 10, 2016)

Introduction

Ciprofloxacin is an antibiotic in the fluoroquinolones group, which is effective in the treatment of a number of infections, including urinary-tract infections, Enterobacteriaceae-induced osteomyelitis, Ear-Nose-Throat infections, gonococcal infections and chronic bacterial prostatitis.[1] As a stable antibiotic, ciprofloxacin does not undergo complete metabolism in the body, with 30 – 90% of excreted ciprofloxacin remaining unaltered. Particularly, patients taking medicinal drugs that are primarily metabolized by cytochrome P450 1A2 (*e.g.*, theophylline and tizanidine), and other common drugs, such as terbinafine, can experience adverse effects from the co-administration of ciprofloxacin.[2] In addition, the widespread administration of this antibiotic to livestock has led to rising public concern about drug residues entering the food supply chain. Since ciprofloxacin is highly stable in soil and wastewater systems, this has the potential to cause environmental pollution in agricultural and aquacultural practices.[3] Conventional analytical procedures of drug residues have heavily depended on traditional methods, such as high-performance liquid chromatography and liquid chromatography–mass spectrometry, which are laborious and time-consuming and unsuitable for screening large number of samples.[4,5] Thus, there is an urgent need for a highly sensitive and rapid screening ciprofloxacin device to measure environmental contamination. Lately, recent developments in DNA-based biosensors have focused on the interaction of drugs and DNA, providing a convenient method for detecting drugs, possible miniaturization as well as portability.[6] Additionally, the construction of DNA sensors has gained considerable attention owing to the excellent ability of DNA to attach molecules covalently (by chemically modifying various DNA components) and/or non-covalently (by outer electrostatic interaction, groove binding, and intercalation) with high affinity and specificity.[7-14] In DNA-electrochemical biosensors, DNA is used as a biomolecular recognition element, where specific binding of molecules with DNA can be measured electrochemically and quantified.[15]

Ciprofloxacin (Fig. 1) has two functional groups, which can

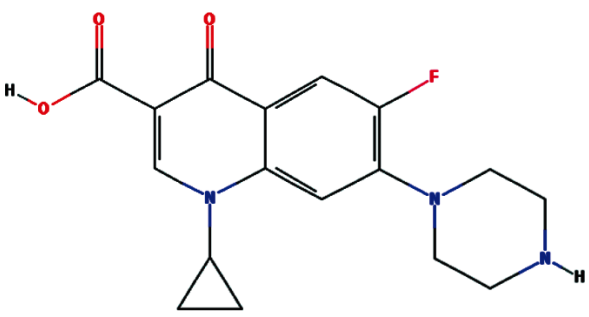

Fig. 1 Chemical structure of ciprofloxacin (Source: PubChem).

† To whom correspondence should be addressed.
E-mail: minhaz.ahmed@ubd.edu.bn; minhazua@gmail.com

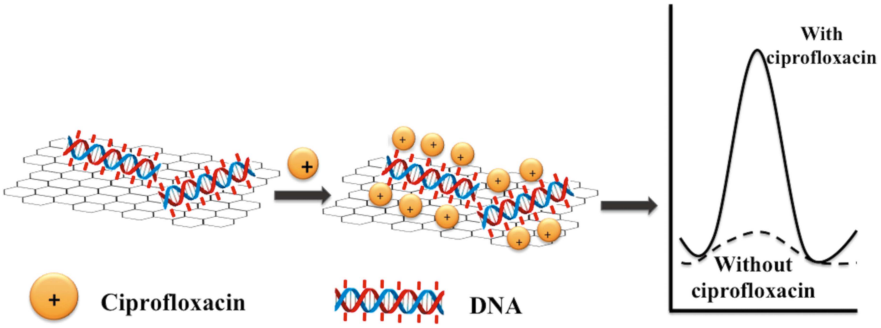

Fig. 2 Schematic representation of the detection principle used in this study.

be ionized, and depending on the pH of solution used, ciprofloxacin can be present in the forms of cation, neutral (unionized form), zwitterion and anion.[16] In an acidic medium, ciprofloxacin acquires a positive charge and binds to DNA through electrostatic interactions with the negatively charged nucleic acid sugar-phosphate backbone of DNA.[17] This becomes the principle of our detection method. Other reported DNA-based biosensors using binding affinities of analytes to DNA have been employed for various studies, such as in the detection of pathogenic microorganisms, other types of drugs, toxic compounds, carcinogens, and pollutants, such as Sudan II,[6] thalidomide,[18] leuprolide[19] and taxol.[20]

In recent years, the inclusion of nanomaterials in biosensors to improve the sensitivities and detection limits has shown great progress and advancement.[20] Carbon nanomaterials are often incorporated in DNA-based biosensors owing to their high surface area, excellent electrical conductivity and biocompatibility. In comparison with bare glassy carbon, platinum and gold, the surface area for the electrochemical activity of carbon nanomaterials is much larger, as confirmed by the relatively enhanced background current. This, in effect, provides a larger surface area for DNA immobilization on nanomaterials. Another advantage of using nanomaterials as electrochemical transducers in DNA-based biosensors is that the surface pretreatment step, which is needed to improve the electrochemical response, is unnecessary and omitted.[21] In this work, a graphene-modified screen printed carbon electrode (SPCE) was chosen on the basis of its high surface area and enhanced electron transfer, qualities that render it suitable for use as a sensing material in electrochemical analyses.[9,22] To the best of our knowledge, no report on the voltammetric detection of ciprofloxacin using a DNA-graphene SPCE has been published. The prime objective of this study is to provide a simple, rapid, efficient and sensitive procedure to detect ciprofloxacin by exploiting the excellent and superior attributes of graphene. Herein, a simple methodology was developed to electrochemically detect ciprofloxacin using a disposable graphene-modified SPCE as the detection platform. The electrostatic interaction of double stranded DNA (dsDNA) with drug components was utilized to construct a simple yet highly sensitive DNA-electrochemical sensor to detect ciprofloxacin, as illustrated in Fig. 2.

Materials and Methods

Reagents and materials

Ciprofloxacin, salmon sperm dsDNA, sodium acetate, acetic acid, uric acid, ascorbic acid, dopamine hydrochloride, NaOH, HCl, Na_2HPO_4, $NaH_2PO_4{\cdot}2H_2O$, KH_2PO_4, potassium ferrocyanide and potassium ferricyanide were purchased from Sigma-Aldrich (MO, USA). Graphene nanopowder was obtained from Graphene Supermarket (USA). All solutions were prepared and diluted using double-distilled water.

Instrumentation

Cyclic voltammetry (CV), square-wave voltammetry (SWV) and differential pulse-voltammetry (DPV) measurements were carried out using an Autolab PGSTAT101 III (Metrohm, Netherlands) that works in conjunction with Nova 1.10 software. The screen-printed electrodes were acquired from DropSens (Oviedo, Spain) and consisted of (i) graphene-modified and (ii) carbon as working electrodes (area of working electrodes 12.6 mm^2), a carbon counter-electrode and a silver reference electrode. All measurements were made at room temperature (21 ± 1°C).

Preparation of graphene-modified electrode

The graphene-modified glassy carbon electrode (GCE) was prepared, using a protocol, as described by Zhang *et al.*[23] *via* electrodeposition. Firstly, the bare GCE was polished with alumina slurry and rinsed with dubious amount of distilled water. Next, ultrasonication of GCE was carried out in ethanol for 5 min. Graphene was then electrodeposited in 10 mL of 0.1 M KCl solution containing 200 µL of 1 mg/mL graphene by applying a potential of +1.7 V. The electrode was rinsed gently using distilled water to remove any unbound graphene.

Sensor fabrication

A stock solution of 100 ng/mL salmon sperm dsDNA was prepared with 0.2 M acetate buffer (pH 4). A layer of DNA was immobilized on the surface of the working graphene-modified SPCE by casting 10 µL of a DNA solution, and allowing it to dry at room temperature for 2 h, or until becoming dry.

Characterization of electrode materials

The bare GCE, bare SPCE, graphene-modified SPCE, electrodeposited graphene on bare GCE and DNA immobilized graphene SPCE were electrochemically characterized using the redox probe $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ (5 mM, PBS 10 mM, pH 7.4). The CV parameters were set as the following: initial potential, –0.2 V, end potential, +0.6 V and scan rate, 100 mV/s.

Preparation of real samples

The human urine sample was centrifuged at 1800 rpm for 5 min so as to remove precipitates, and then diluted 100-fold with acetate buffer (pH 4, 0.2 M). Human serum from a volunteer was used directly without centrifugation, and diluted

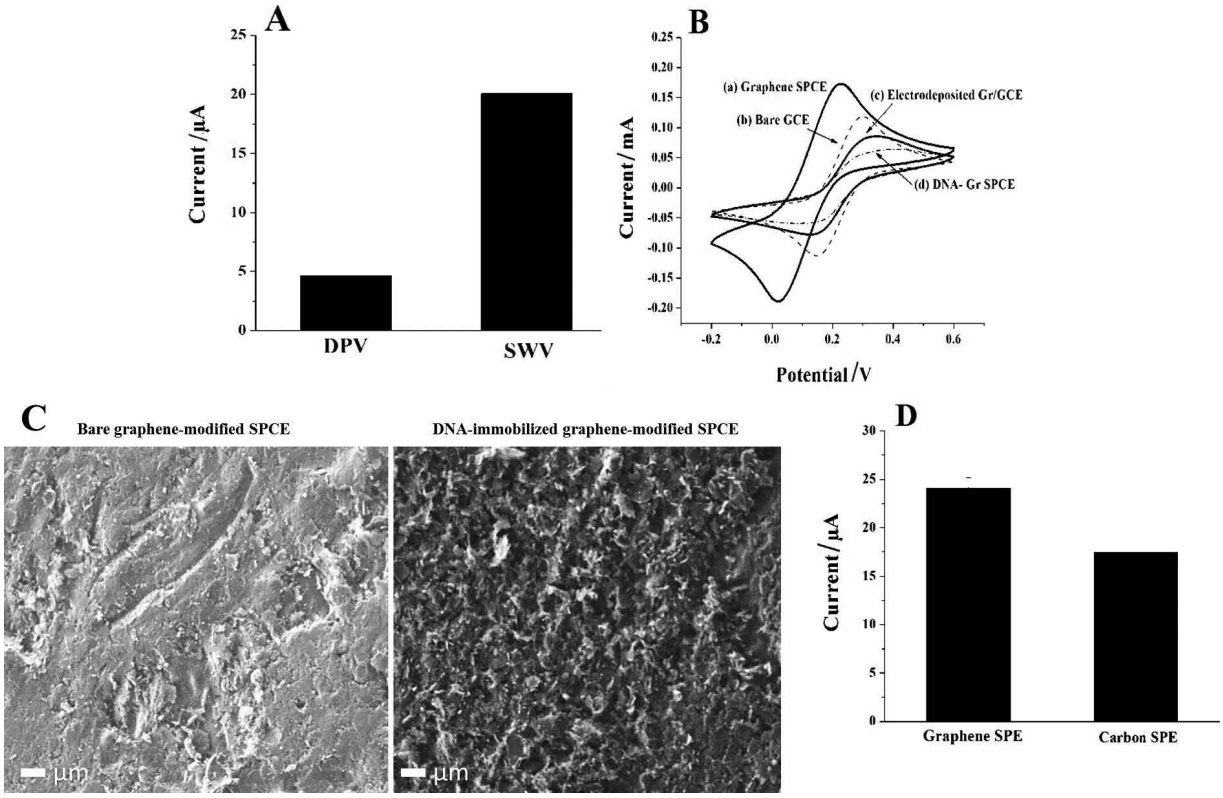

Fig. 3 (A) Graph illustrating the current responses for the detection of 100 μM of ciprofloxacin using DPV and SWV (n = 3). The interaction time was 120 s. DPV parameters: initial potential, 0 V; end potential, 1.4 V; step potential, 0.005 V; modulation amplitude, 0.025; modulation time, 0.05 s; and scan rate 100 mV/s. SWV parameters: initial potential 0 V, end potential +1.6 V, step 0.015 V, amplitude 0.04 V and frequency 25 Hz. (B) CV of different electrode materials: (a) graphene SPCE, (b) bare GCE, (c) electrodeposited graphene on bare GCE, and (d) DNA-immobilized graphene SPCE. All measurements were taken in 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ (PBS 10 mM, pH 7.4). CV parameters: initial potential –0.2 V, end potential of +0.6 V and a scan rate of 100 mV/s. (B) SEM images of (a) bare graphene-modified SPCE and (b) DNA-immobilized graphene-modified SPCE. (D) Comparison between carbon and graphene-modified SPE for ciprofloxacin detection at 100 μM using SWV (n = 3).

100-fold with acetate buffer (pH 4, 0.2 M). After spiking the diluted samples with different concentrations of ciprofloxacin, 50 μL of the spiked sample solution was dropped onto the DNA-modified electrode for electrochemical analysis. The recovery of spiked samples was then calculated.

Electrochemical signal measurement

Stock solutions of ciprofloxacin (Sigma-Aldrich) were prepared daily in 0.2 M acetate buffer (pH 4) and kept in the dark before use. For the quantitative analysis of ciprofloxacin, 50 μL of ciprofloxacin (0 – 100 μM) was placed onto the electrode surface, covering all three zones (in-built working, counter and reference) of the electrode. Prior to any electrochemical measurement, dsDNA was allowed to interact with ciprofloxacin for 120 s on the electrode surface in an open circuit. Electrochemical quantification was conducted using SWV with the following parameter settings: initial potential, 0 V, final potential, +1.6 V, step, 0.015 V, amplitude, 0.04 V and frequency, 25 Hz. The oxidation response of the guanine component and ciprofloxacin was observed at +1.0 V (*vs.* Ag SPE) as a result of the electrostatic interaction of positively charged ciprofloxacin with the negatively charged nucleic acid sugar phosphate.

Results and Discussion

Choice of voltammetric technique

The choice of the voltammetric method used in analytical studies will greatly contribute to the performance of a biosensor. Pulse voltammetric techniques present an advantage that is capable to discriminate background noises from the amperometric current; this "discriminative capacity" is responsible for the attainment of lower detection limits.[24] Hence, to maximize the analytical current response and to enhance the capacity of this proposed sensor, an initial investigation was carried out by analyzing 100 μM of ciprofloxacin on 100 ng/mL DNA-immobilized graphene SPCE after 120 s interaction time. As illustrated in Fig. 3A, a four-fold difference in the current peak height was observed between the DPV and SWV techniques, consistent with previously reported by Dogan-Topal *et al.*,[19] and therefore SWV, being more sensitive than DPV by four-fold, was used throughout this work. Additionally, SWV offered the advantage of a faster analytical time, and therefore minimized any possible complications arising from a blockage of the electrode.

Characterization of DNA-graphene modified electrode

The main objective of this work was to fabricate a convenient

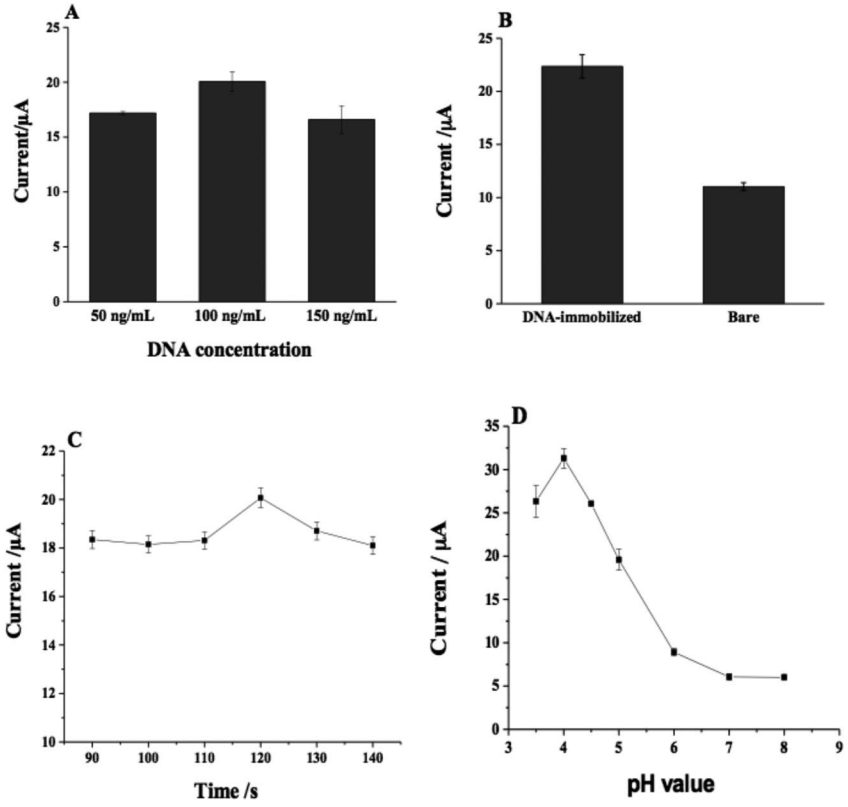

Fig. 4 (A) Optimization of the DNA concentration (n = 3) immobilized on graphene-modified SPCE with ciprofloxacin determined at 100 μM in 0.2 M acetate buffer (pH 4). (B) Comparison between (a) bare graphene-modified SPE and (b) DNA-immobilized graphene-modified SPE for ciprofloxacin detection at 100 μM using SWV (n = 3). (C) Effect of the interaction time between dsDNA on a graphene-modified electrode and 100 μM ciprofloxacin (in solution) in 0.2 M acetate buffer (pH 4). (D) Effect of the pH of an electrolyte on the interaction of dsDNA on a graphene-modified electrode and 100 μM ciprofloxacin (in solution) in 0.2 M acetate buffer at room temperature.

and sensitive DNA-sensor that did not require multiple steps or a lengthy pretreatment of electrodes. The impressive attributes demonstrated by graphene, as a result of its sp^2 bonds and its electronic configuration,[25] led us to choose this material for our electrochemical sensor. To investigate the electrochemical performance of graphene, CV was carried out using a 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ probe (PBS 10 mM, pH 7.4). Figure 3B shows that graphene (Fig. 3B(a)) produced a wide and broad signal response in comparison with a screen-printed carbon electrode (Fig. 3B(b)), which demonstrates the remarkable charge-transfer ability of graphene. Additionally, for comparison, graphene was electrodeposited on the GCE; Fig. 3B(c) shows that the electron transfer for this electrode was suppressed as a result of the structural defects of a graphene and the drop in the electrical conductivity.[23] As shown in Fig. 3B(d), the redox peaks were reduced upon the immobilization of DNA on the graphene SPCE owing to the repulsion between DNA and the anions of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$.[26] Comparing Figs. 3B(a) and 3B(d), the difference between the cathodic and anodic peaks is about 200 mV, which must be acknowledged. The absence of a sufficient electrolyte, the resistive aspect of SPE, and the increased resistance due to the presence of DNA on the electrode surface are probable causes for this significant difference. Furthermore, Fig. 3C shows a change in the surface morphology upon the immobilization of DNA on the graphene SPCE. Figure 3C(b) shows a relatively rough surface after 100 ng/mL of DNA was drop-casted on the electrode, in comparison with the surface of untreated graphene SPCE. Based on these results, the electrochemical performance of commercial graphene SPCE was shown to be highly favorable for the construction of a simple and sensitive DNA-based electrochemical biosensor. A comparison study was conducted to detect 100 μM ciprofloxacin using the carbon SPE and graphene-modified SPCE. Also, as shown in Fig. 3D, the graphene-modified material provided a better current output as a result of enhanced electron transfer compared with that of carbon, as discussed earlier.

Parameters optimization

Optimization of DNA concentration. To attain the highest current response possible, optimization of different parameters was initially performed. One of the parameters that dictated the sensitivity, and thus ultimately the limit of detection in this sensor, was the concentration of DNA used for immobilization on the working electrode. Since applying a layer of film can decrease the signal response of an electrode, the concentration of immobilized DNA layer was studied. Figure 4A reveals that applying a 100 ng/mL concentration of DNA produced the highest current signal, and that this DNA concentration was used throughout this work. Using a lower DNA concentration produced weaker current response. This may be attributed to the lower concentration of DNA attracting less of the positively charged ciprofloxacin towards the electrodes, which would result in a lower signal output. Conversely, applying a higher

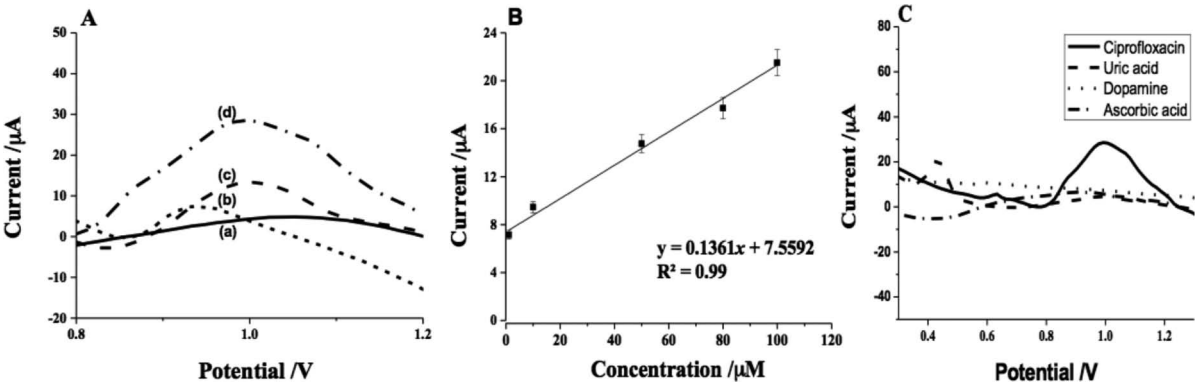

Fig. 5 (A) Typical voltammograms of different concentrations of ciprofloxacin: (a) acetate buffer only (b) 10 μM (c) 50 μM and (d) 100 μM ciprofloxacin. (B) Linear regression curve of the SWV current response *versus* the concentrations for the detection of ciprofloxacin ($n = 3$). (C) Current responses of 100 μM of ciprofloxacin, uric acid, ascorbic acid and dopamine in an acetate buffer (pH 4.0).

DNA concentration yielded a lower current response, because a thicker layer of DNA formed on the working electrodes, which hindered the transfer of electrons to the electrodes.

To test the role of DNA in the enhancement of ciprofloxacin detection, Fig. 4B illustrates the current responses of unmodified bare and DNA-modified (100 ng/mL) graphene SPCE for the detection of 100 μM of ciprofloxacin. The result indicated DNA-modified graphene produced a higher current yield than bare graphene under the same experimental procedure.

Optimization of interaction time. The time allowed for the interaction between dsDNA on the electrode surface and ciprofloxacin in solution will affect the amount of ciprofloxacin attracted to the negatively charged DNA. The effect of the interaction time was therefore studied to examine the optimum time needed for the DNA-ciprofloxacin interaction. For this experiment, interaction times from 90 to 140 s were investigated, using 100 ng/mL of DNA immobilized on the electrode surface to detect 100 μM of ciprofloxacin. It was observed that the SWV current response escalated at 120 s, then dropped and subsequently leveled off (Fig. 4C). This fall and leveling off in current was attributed to the saturation of the DNA-modified electrode with ciprofloxacin as the interaction time increased.[27] Based on this experimental data, 120 s was selected as the optimum interaction time between DNA and ciprofloxacin.

Optimization of pH. Since the ciprofloxacin ionized state in solution relied to a great extent on the solution pH, the effect of the pH was examined. Acetate buffers of different pH values were made at room temperature and used to determine the current output of 100 μM of ciprofloxacin. Figure 4D explicates that pH 4 produced the highest current response. In addition, experiments to observe the effect of the current responses in buffer solutions of pH above the pK_a value of ciprofloxacin were performed. It can be observed in Fig. 4D that increasing the pH of buffer solution caused the current to decrease further, and to level off at pH 8. This may be explained by saying that in a buffer solution higher than pK_a of ciprofloxacin, the molecules acquired negative charges, and therefore less ciprofloxacin species were attracted to the surface of DNA-modified electrode.

Effect of NaCl

In this study, the addition of NaCl to the supporting electrolyte (sodium acetate buffer, pH 4) was also investigated. It was found that the oxidation peak at +1.0 V immediately disappeared upon the addition of a relatively low concentration of NaCl (20 mM) for the detection of 100 μM of ciprofloxacin. The addition of NaCl to an aqueous solution will dissociate the salt to positive ions (Na^+) and negative ions (Cl^-). The positive Na^+ will surround the sites of the negatively charges of the DNA, and the negative Cl^- will be attracted to the positively charged ciprofloxacin, leading to the suppression and shielding of electrostatic interaction between DNA and ciprofloxacin molecules. This fact suggests an interaction mechanism that at this pH value, the positively charged ciprofloxacin bound *via* an electrostatic interaction to the negatively charged phosphate backbone of dsDNA at low ionic-strength surroundings.[16]

Analytical performance of DNA based biosensor

Our method of detection was based on the electrostatic interaction between negatively charged DNA and positively charged ciprofloxacin. The pH of buffer solution (pH 4) rendered ciprofloxacin (pK_a of ciprofloxacin = 6.09) positively charged in solution. The oxidation peak of the guanine residue was discerned at +1.0 V, and increasing the ciprofloxacin concentration also increased its corresponding cathodic peak, as depicted in Fig. 5A. Although it has been reported that ciprofloxacin interacts with dsDNA both electrostatically and *via* intercalation,[17,28] this study demonstrated that it is the electrostatic interaction between dsDNA and ciprofloxacin that plays a more significant role than the non-electrostatic binding. This can be observed from the disappearance of the current peak when 0.2 mM of NaCl was added into the acetate buffer solution. Here, NaCl acted as an electrostatic screening of DNA that shielded the negative charge sites of DNA molecules thus significantly reducing the electrostatic interaction between DNA and ciprofloxacin. Furthermore, in cases where intercalation of drug with DNA is dominant increasing the concentration of drug caused an analogous decrease in the electrochemical oxidation signal.[17,28] It has also been reported that the relatively short interaction time between DNA and ciprofloxacin contributed to the electrostatic binding of DNA and ciprofloxacin. Longer incubation times would cause the oxidation peak to reduce where intercalation of the drug with DNA will take over as the main interaction mechanism.[29-31] Figure 5B shows a linear regression curve obtained ($r^2 = 0.99$) for the detection of ciprofloxacin in acetate buffer (background measurement was subtracted from each reading) with a wide linear range of

0.1 – 100 μM and a detection limit of 0.1 μM . The limit of detection in this investigation, which corresponds to the amount of ciprofloxacin that can be truly detected in a sample solution,[32] was acquired by visual evaluation. The low limit of detection achieved by employing this straightforward protocol opens up the possibility of the commercialization of our sensor, because it eliminates the laborious and time-consuming electrode preconditioning processes detailed by Brett *et al.*[33] that are typically required to improve the efficiency of DNA immobilization. The high sensitivity and wide dynamic range obtained for this sensor can be explained as follows. (1) The high affinity of ciprofloxacin towards DNA.[16] (2) The excellent transfer of electrons between graphene and DNA, which is a consequence of the stacking arrangement of DNA nucleobases and graphene. Overlap of their π-orbitals is minimal thus repulsion effects for charge transfer are reduced.[34] (3) The enhanced analytical sensitivity of the SWV technique in detecting minute amounts of ciprofloxacin[19] due to its excellent discrimination of capacitance current, low concentrations of analyte and broad linear range can be detected by SWV in comparison to other electrochemical techniques. In addition, for practical on-site purposes, SWV was an efficient technique for the analysis of ciprofloxacin by virtue of its excellent analytical speed, owing to its high effective scan rate, which significantly reduced the time needed for scanning.

Selectivity of the biosensor

The selectivity of the proposed biosensor towards other compounds was studied using 100 μM of ascorbic acid, uric acid and dopamine and compared with 100 μM of ciprofloxacin. From Fig. 5C, 100 μM of uric acid, dopamine and ascorbic acid demonstrated minimum peak at +1.0 V in comparison with 100 μM of ciprofloxacin under the experimental conditions described above. Since different compounds exhibit redox peaks at distinct potentials in different experimental parameters (such as pH, applied voltage); these compounds can be distinguished from each other. Here, uric acid produced oxidation peaks at 0.4 V when scanned from 0.1 to 1.4 V. Zou *et al.* also reported the electro-oxidation of ascorbic acid, dopamine and uric acid at various potentials of 0, 0.2 and 0.4 V, respectively.[36]

Application of biosensor to real samples

A determination of ciprofloxacin in urine samples was performed to do as evaluate the feasibility of the proposed sensor in real samples; 100 μM ciprofloxacin was spiked into samples of urine and serum. The measurements were carried out in triplicate for each ciprofloxacin concentration, and the results are listed in Table 1. The recoveries in urine and serum samples obtained were found to be 107.8 and 84.3% with RSDs of 9.2 and 8%, respectively.

Comparison with DNA-based electrochemical sensors

Table 2 presents the DNA-based electrochemical sensors for the detection of ciprofloxacin reported by other research groups. Our DNA-based sensor achieved almost the same sensitivity as the device of Diab *et al.*,[16] but our protocol produced a wider linear range. The work by Diab *et al.*[16] produced a small linear working range, possibly due to a lack of electrode pre-conditioning steps. This is in contrast to a study by Nawaz *et al.*,[17] where the group included pre-conditioning of electrode, and thus better linear range was obtained. Our sensor did not require any cumbersome pre-conditioning steps of the electrode to enhance its electrochemical response, which could facilitate the straightforward construction of a DNA-based sensor for the detection of ciprofloxacin for on-site screening applications.

Inspired by our previous reported work on carbon nanofibers,[37] we believed that the adsorption of DNA on the transducer surface could be further enhanced, and hence better sensitivity could be achieved. This provides a great opportunity for further nanomaterial-based sensor studies that use this strategy in the detection of other analytes that have high affinity for DNA.

Table 1 Determination of ciprofloxacin in spiked urine and serum (n = 3)

Real sample	Ciprofloxacin concentration/μM	Ciprofloxacin actual detected/μM	Recovery, %	RSD, %
Urine	100	107.8	107.8	9.2
Serum	100	84.3	84.3	8.0

Conclusion

In this work, a simple method of fabrication for the electrochemical detection of ciprofloxacin in a buffer solution and real samples was described. We have exploited the basic electrostatic interaction between dsDNA and ciprofloxacin to develop a simple and sensitive DNA-based electrochemical biosensor. In addition to the DNA attracting ciprofloxacin—bringing the analyte closer to the working electrode—the use of SWV as the mode of detection assisted in the sensitive detection of the drug. Graphene played a significant role in the construction of our sensor because it eliminated the steps needed to precondition electrodes to give an enhanced electrochemical signal. This procedure was applied to urine and serum samples

Table 2 Comparison of DNA-based electrochemical sensors for the detection of ciprofloxacin

Electrode	Electrochemical technique	Portability	Assay type	Pre-conditioning of electrode	Limit of detection/μM	Linear range/ μM	Ref.
GCE	Cyclic voltammetry	Bulk electrode, laboratory based	Quantitative	No	0.117	1.0 – 10	16
GCE	Constant current potentiometry	Bulk electrode, laboratory based	Quantitative	Yes	24	40 – 80	17
	Differential pulse voltammetry				9		
Multiwalled CNT/GCE	Cyclic voltammetry	Bulk electrode, laboratory based	Quantitative	No	0.99	0.24 – 67.3	38
Graphene SPCE	Square wave voltammetry	Disposable electrode, on-site application	Quantitative	No	0.1	0.1 – 100	This work

with good recovery, increasing the potential for the development of an on-site and portable ciprofloxacin sensor in the future.

Acknowledgements

S. A L. would like to thank Graduate Research Office of Brunei Darussalam for financial assistance and express gratitude to Ministry of Education, Brunei Darussalam for the opportunity to undertake a Ph.D course at Universiti Brunei Darussalam.

Conflict of Interest

The authors declare no financial or commercial conflict of interest.

References

1. A. M. Emmerson and A. M Jones, *J. Antimicrob. Chemother.*, **2003**, *51(suppl. 1)*, 13.
2. G. J. Khan, R. A. Khan, I. Majeed, F. A. Siddiqui, and S. Khan, *Professional Med. J.*, **2015**, *2*, 001.
3. Y. Zhuang, F. Yu, J. Ma, and J. Chen, *New J. Chem.*, **2015**, *39*, 3333.
4. J. De Smet, K. Boussery, K. Colpaert, P. De Sutter, P. De Paepe, J. Decruyenaere, and J. Van Bocxlaer, *J. Chromatogr. B*, **2009**, *877*, 961.
5. H. T. Zhang, J. Q. Jiang, Z. L. Wang, X. Y. Chang, X. Y. Liu, S. H. Wang, K. Zhao, and J. S. Chen, *J. Zhejiang Univ. Sci. B*, **2011**, *12*, 884.
6. B. Rezaei, M. K Boroujeni, and A. A Ensafi, *Bios. Bioelectron.*, **2015**, *66*, 490.
7. M. U. Ahmed, Y. Yoshimura, M. Hossain, E. Tamiya, and K. Fujimoto, *BioChip J.*, **2011**, *5(3)*, 206.
8. M. Safavieh, M. U Ahmed, E. Esen Sokullu, A. Ng, L. Braescu, and M. Zourob, *Analyst*, **2014**, *139*, 482.
9. M. U. Ahmed, I. Saaem, P. C. Wu, and A. Brown, *Crit. Rev. Biotechnol.*, **2014**, *34(2)*, 180.
10. M. U. Ahmed, M. M. Hossain, M. Safavieh, Y. L. Wong, I. A. Rahman, M. Zourob, and E. Tamiya, *Crit. Rev. Biotechnol.*, **2016**, *36*, 495.
11. M. Safavieh, M. U. Ahmed, A. Ng, and M. Zourob, *Biosens. Bioelectron.*, **2014**, *58*, 101.
12. M. U. Ahmed, S. Nahar, M. Safavieh, and M. Zourob, *Analyst*, **2013**, *138*, 907.
13. C. Tlili, E. Sokullu, M. Safavieh, M. Tolba, M. U. Ahmed, and M. Zourob, *Anal. Chem.*, **2013**, *85*, 4893.
14. S. Nahar, M. U. Ahmed, M. Safavieh, A. Rochette, C. Toro, and M. Zourob, *Analyst*, **2014**, *140*, 931.
15. A. D. R Pontinha, S. Sparapani, S. Neidle, and A. M. Oliveira-Bretts, *Bioelectrochemistry*, **2013**, *89*, 50.
16. N. Diab, I. Shqair, R. Salim, and M. Al-Subu, *Int. J. Electrochem. Sci.*, **2014**, *9*, 1771.
17. H. Nawaz, S. Rauf, K. Akhtar, and A. M. Khalid, *Anal. Biochem.*, **2006**, *354*, 28.
18. C. B. Oliveira, A. M. Chiorcea-Paquim, S. M. Ribeiro, A. T. P. Melo, M. Vivan, and A. M. Oliveira-Brett, *Bioelectrochemistry*, **2009**, *76*, 201.
19. B. Dogan-Topal, S. A. Ozkan, and B. Uslu, *The Open Chemical and Biomedical Methods Journal*, **2010**, *3*, 56.
20. A. Chen and S. Chatterjee, *Chem. Soc. Rev.*, **2013**, *42*, 5425.
21. M. I. Pividori and S. Alegret, *Top Curr. Chem.*, **2005**, *260*, 1.
22. S. A. Lim, H. Yoshikawa, E. Tamiya, and M. U. Ahmed, *RSC Adv.*, **2014**, *4*, 58460.
23. X. Zhang, Y. Wei, and Y. Ding, *Anal. Chim. Acta*, **2014**, *835*, 29.
24. B. Uslu, B. Topal, and S. A. Ozkan, *Talanta*, **2008**, *74*, 1191.
25. D. Chen, L. H. Tang, and J. H. Li, *Chem. Soc. Rev.*, **2010**, *39*, 3157.
26. K. Maruyama, J. Motonaka, Y. Mishima, Y. Matsuzaki, I. Nakabayashi, and Y. Nakabayashi, *Sens. Actuators, B*, **2001**, *76*, 215.
27. P. K. Brahman, R. A. Dar, and K. S. Pitre, *Sens. Actuators, B*, **2013**, *177*, 807.
28. S. Tajik, M. A. Taher, H. Beitollahi, and M. Torkzadeh-Mahani, *Talanta*, **2015**, *134*, 60.
29. V. C. Diculescu and A. M. Oliveira-Brett, *Bioelectrochemistry*, **2016**, *107*, 50.
30. O. M. Popaa and V. C. Diculescu, *Electrochim. Acta*, **2013**, *112*, 486.
31. M. Hasanzadeh and N. Shadjou, *Mater. Sci. Eng., C*, **2016**, *61*, 1002.
32. A. Shrivastava and V. B. Gupta, *Chron Young Sci.*, **2011**, *2*, 21.
33. C. M. A. Brett, A. M. O. Brett, H. P. Silvia, and S. H. P. Serrano, *Electrochim. Acta*, **1999**, *44*, 4233.
34. S. Gowtham, R. H. Scheicher, R. Ahuja, R. Pandey, and S. P. Karna, *Phys. Rev. B*, **2007**, *76*, 33401.
35. M. F. Barroso, N. de-los-Santos-Álvarez, M. J. Lobo-Castañón, A. J. Miranda-Ordieres, C. Delerue-Matos, M. B. P. P. Oliveira, and P. Tuñón-Blanco, *Biosens. Bioelectron.*, **2011**, *26*, 2396.
36. H. L. Zou, B. L. Li, H. Q. Luo, and N. B. Li, *Sens. Actuators, B*, **2015**, *207*, 535.
37. S. A. Lim and M. U. Ahmed, *Biosens. Bioelectron.*, **2015**, *70*, 48.
38. L. Fotouhi and M. Alahyari, *Colloids Surf., B*, **2010**, *81*, 110.