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One step preparation and electrochemical analysis of IQS, a cell–cell communication signal in the nosocomial pathogen *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa uses a hierarchical cell–cell communication system consisting of a number of regulatory elements to coordinate the expression of bacterial virulence genes. Sensitive detection of quorum sensing (QS) molecules has the potential for early identification of *P. aeruginosa* facilitating early medical intervention. A recently isolated cell–cell communication molecule, a thiazole termed IQS, can bypass the *las* QS system of *P. aeruginosa* under times of stress, activating a subset of QS-controlled genes. This compound offers a new target for pathogen detection and has been prepared in a one step protocol. A simple electrochemical strategy was employed for its sensitive detection using boron-doped diamond and glassy carbon electrodes by cyclic voltammetry and amperometry.

Bacterial communication allows microorganisms to coordinate behaviour and respond co-operatively and quickly to potentially harmful changes in their surrounding environment. Pathogenic bacteria act as a population, rather than as an individual cell, to bypass the immune response of the host in order to survive and persist.[1,2] Quorum sensing (QS), an important chemical cell-to-cell communication process used by bacteria, is regulated by small extracellular signalling molecules, which allows bacterial populations to collectively control gene expression and synchronise group behaviour. QS is generally associated with attaining a high population density.[3] QS signals are low molecular weight, diffusible molecules which act as a means of intercellular communication to co-ordinate bacterial behaviour such as secondary metabolite production, virulence, biofilm development and swimming and swarming motility.[4,5]

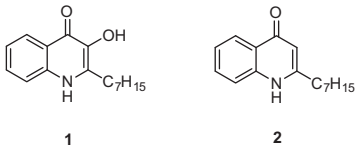

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Figure 1. Structure of *P. aeruginosa* AHQ signals.

As a ubiquitous Gram-negative bacterium, *Pseudomonas aeruginosa* is an opportunistic human pathogen. It is of great

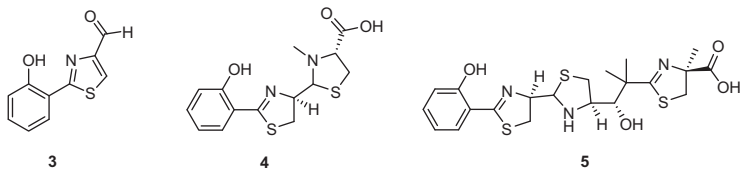

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Figure 2. IQS and 2-(2-hydroxyphenyl)-thiazoline containing siderophores.

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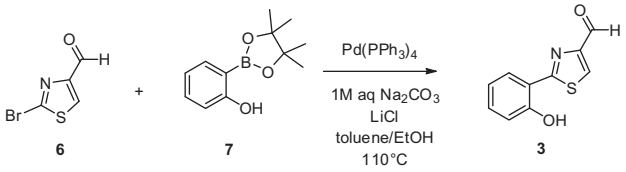

Scheme 1. Synthesis of 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS).

clinical importance due to its prevalence as a hospital-acquired infection, particularly in immunocompromised patients, and is a common cause of morbidity and death in people with cystic fibrosis (CF).[6,7] Such overwhelming infections stem from bacterial chemical interaction with its environment. Perhaps the most important self-defence mechanism attributed to the severity of *P. aeruginosa* infections is the formation of biofilms, that is, microcolonies surrounded by an exopolysaccharide alginate. The film acts

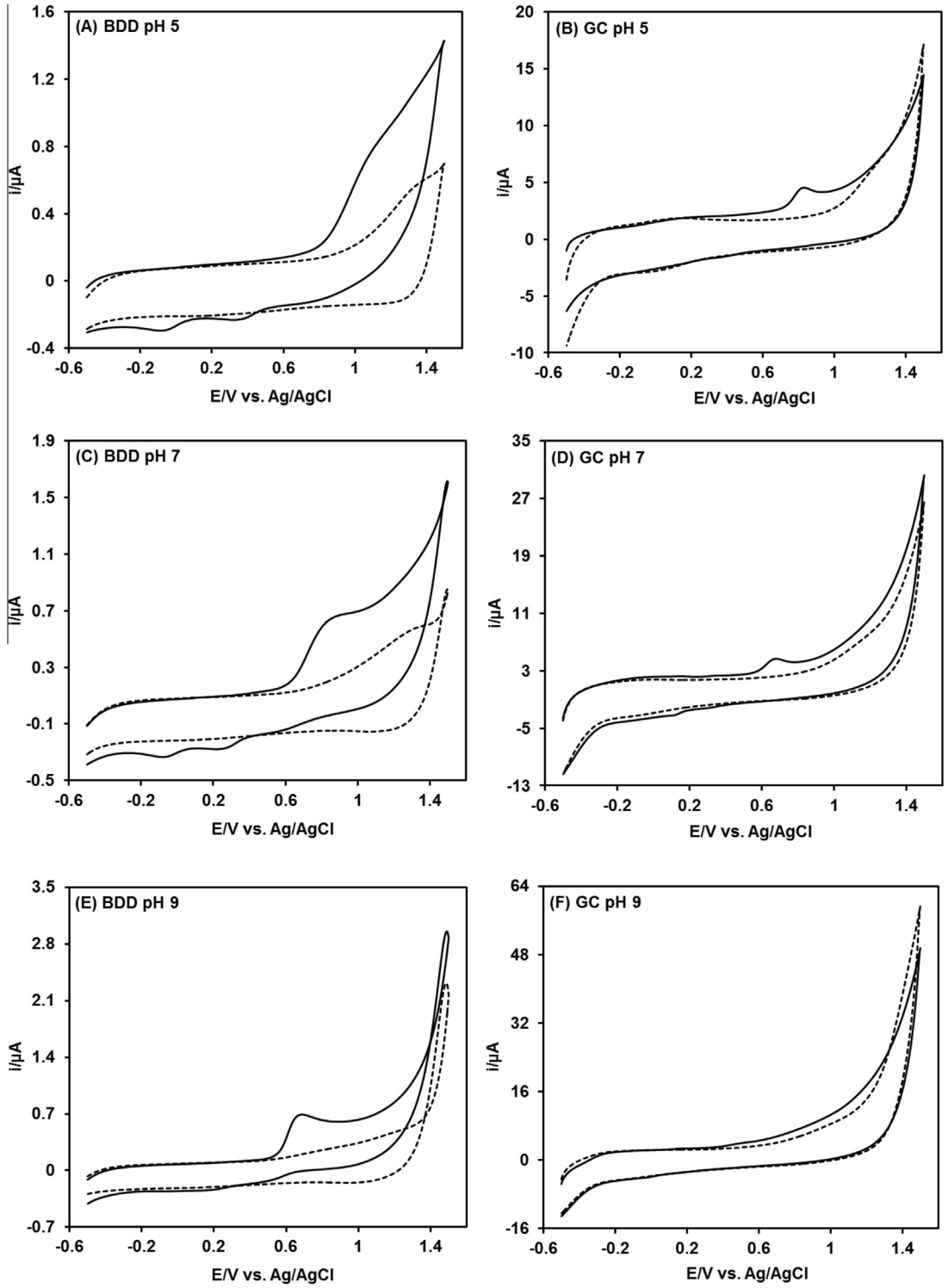

Figure 3. Cyclic voltammograms of the BDD and GC electrodes without (dashed line) and with (solid line) the presence of 100 μM IQS. (A–B) electrolyte: 50 mM acetate buffer (pH 5) containing 20% ACN; (C–D) electrolyte: 50 mM phosphate buffer (pH 7) containing 20% ACN; (E–F) electrolyte: 50 mM phosphate buffer (pH 9) containing 20% ACN.

as a direct barrier to phagocytic cells and offers innate resistance to antibiotics.[8] The QS framework of *P. aeruginosa* consists of two N-acylhomoserine lactone (AHL) regulatory circuits (LasIR and RhlIR) linked to the 2-alkyl-4(1H)-quinolone (AHQ) system, forming a sophisticated hierarchical network controlling global gene expression.[9,10] The primary components of the AHQ signalling pathway are 2-heptyl-3-hydroxy-4(1*H*)-quinolone **1** (referred to as the *Pseudomonas* Quinolone Signal, or PQS) and its biosynthetic precursor 2-heptyl-4(1H)-quinolone **2** (HHQ) as depicted in Figure 1.[11,12]

Recently, Lee et al. reported the identification of a new cell-to-cell communication molecule, 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde **3** (Fig. 2), which they gave the name IQS.[13] Global gene profiling analysis shows that IQS controls the expression of many QS- and virulence-associated genes, indicating that IQS is a likely component of the QS mechanisms that govern *P. aeruginosa* physiology and virulence. IQS production is controlled by *las* under normal culture conditions but is also activated by phosphate limitation (a stress which bacteria commonly encounter while establishing an infection post major surgery or organ injury). The 2-(2-hydroxyphenyl)-thiazoline motif is commonly found in iron-chelating molecules or "siderophores" in pathogenic bacteria (e.g., pyochelin[14] **4**, yersiniabactin[15] **5**) which are released in response to low-iron conditions in the host. IQS may, potentially, be related to bacterial siderophores, perhaps as a precursor or a degradation product, being first identified in *P. aeruginosa* as a reduction product of aeruginoic acid, itself produced by the incomplete biosynthesis of pyochelin.[16–18]

To date, strategies used for the identification of *P. aeruginosa* are largely based on standard culture methods or costly and labour intensive real-time PCR.[19] Analytical methods for QS signalling molecules, such as LC/MS/MS,[20] capillary electrophoresis[21] and fluorometry, require sample pretreatment, lengthy analysis and high costs, or lack selectivity.[22] The demands for accurate and rapid point-of-care diagnosis of bacterial infection have stimulated efforts to develop simple and sensitive electrochemical strategies. Besides biosensor-based assays for PQS and HHQ,[23–25] cyclic voltammetry and amperometry using a boron-doped diamond (BDD) thin-film electrode could be an excellent method for the sensitive detection of HHQ, PQS and other 3-alkyl quinolones.[26] Electrochemical techniques exhibit high sensitivity, fast response, simplicity, low cost and potential for miniaturisation. This electrochemical procedure allows for the early diagnosis of *P. aeruginosa* infection prior to biofilm formation, facilitating early medical intervention and increasing life expectancy.

This work herein involved a one-step preparation of a potential cell-to-cell communication molecule, IQS. Its electrochemical properties are studied using BDD and glassy carbon (GC) electrodes. The BDD electrode displays high current density, wide potential window, low background current, extreme electrochemical stability and high resistance to fouling.[27] The GC electrode features include good electrical conductivity and positive potential range, low porosity and permeability to gases, high biocompatibility and hardness.[28] To our knowledge, no detection of IQS based on electrochemical strategies has been reported.

Thiazole **3** has previously been prepared as an intermediate towards **4** (and analogues thereof) by a number of alternate four-step procedures starting from 2-hydroxybenzonitrile and (R)-cysteine.[14,29] Here, we proposed a facile, one-step preparation of IQS via a Suzuki–Miyaura coupling[30,31] between the commercially available 2-bromothiazole-4-carboxaldehyde **6** and 2-hydroxyphenyl boronic acid pinacol ester **7** (Scheme 1).[32] This represents a cost and time-effective protocol furnishing IQS in an isolated yield of 26%. The product was confirmed by HRMS, ^{1}H NMR and ^{13}C NMR.

The redox behaviour of IQS on the BDD and GC electrodes was compared using cyclic voltammetry and amperometry, and optimisation of the supporting electrolyte pH and detecting potential was carried out. With 50 mM acetate buffer (pH 5) as the supporting electrolyte, IQS started to oxidise on the BDD electrode around +0.5 V with a broad peak in the potential range of +0.8 V to +1.5 V (Fig. 3A), compared to a well-defined peak at +0.82 V obtained by the GC electrode (Fig. 3B). The BDD electrode also detected two small reduction peaks (−0.09 V and 0.33 V), which were not detectable by the GC electrode. Both oxidation peaks of IQS on the BDD and GC electrodes shifted to +0.85 V and +0.68 V, respectively in a 50 mM phosphate buffer, pH 7 (Fig. 3C and D). The CV of IQS on the BDD electrode at pH 9 revealed a well-defined peak at +0.65 V while the GC electrode only showed a very weak response towards IQS electrooxidation (Fig. 3E and F). Consequently, the BDD and GC electrodes should be performed at pH 9 and pH 7, respectively, to provide the best detection sensitivity.

The typical current-time responses and calibration curves of the BDD electrode towards successive addition of 1 μM IQS are illustrated in Fig. 4. The BDD electrode was poised at +1.0 V, +0.9 V and +0.7 V for detection at pH 5, pH 7 and pH 9, respectively, due to the electrooxidation peak potentials observed on the CVs.

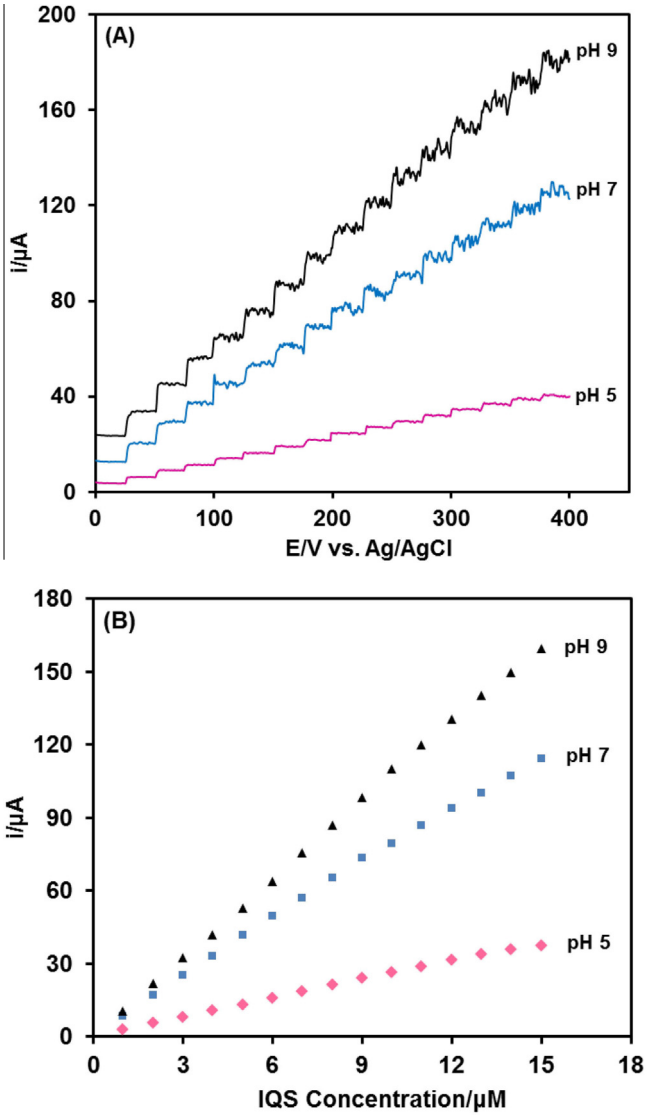

Figure 4. (A) Amperometric responses of the BDD electrode towards the addition of 1 μM IQS. (B) Calibration curves. Electrolytes: 50 mM acetate buffer (pH 5) containing 20% ACN and 50 mM phosphate buffer (pH 7 and 9) containing 20% ACN. Detection potentials: +1.0 V for pH 5, +0.9 V for pH 7 and +0.7 V for pH 9.

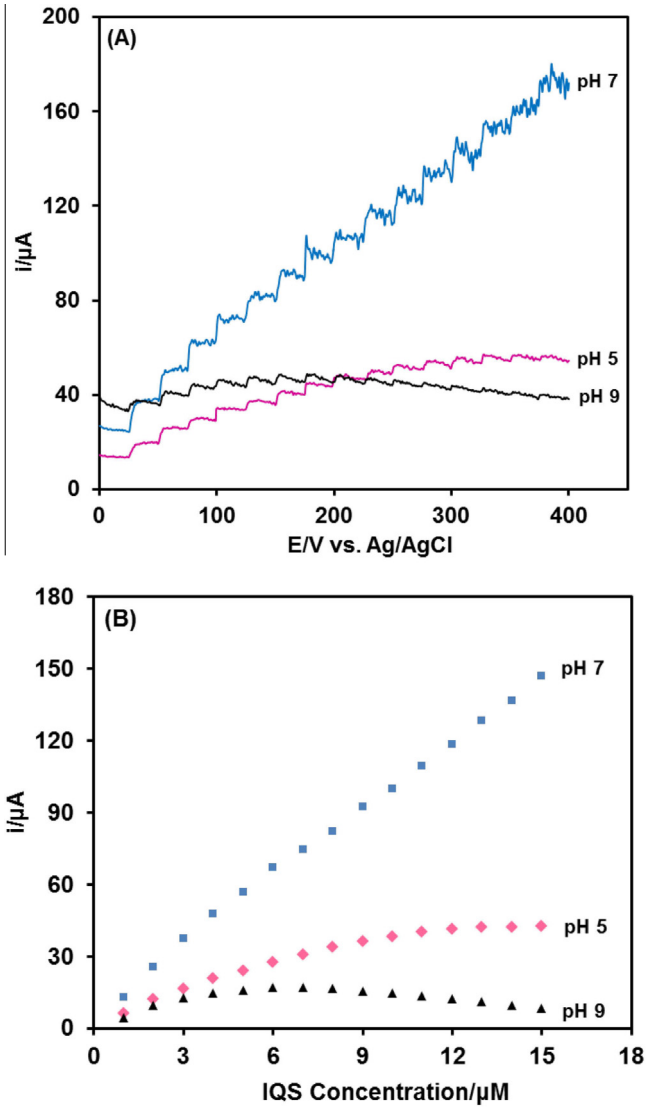

Figure 5. (A) Amperometric responses of the GC electrode towards the addition of 1 μM IQS. (B) Calibration curves. Electrolytes: 50 mM acetate buffer (pH 5) containing 20% ACN and 50 mM phosphate buffer (pH 7 and 9) containing 20% ACN. Detection potentials: +0.8 V for pH 5, +0.7 V for pH 7 and +0.5 V for pH 9.

As expected, the BDD electrode exhibited the highest sensitivity for IQS at pH 9, a 4-fold increase on that obtained at pH 5. However, the signal responses became less distinct upon increasing the buffer pH. The response time was only 2 s with linearity up to 14, 12 and 15 μM IQS and detection limits of 46, 20 and 12 nM (S/N = 3) for the BDD electrode at pH 5, 7 and 9, respectively.

The detection potentials of the GC electrode changed to +0.8 V, +0.7 V and +0.5 V for pH 5, pH 7 and pH 9, respectively, lower than those using the BDD electrode (Fig. 5), indicating that IQS needs lower oxidation energy on the GC electrode. Differing from the performances of the BDD electrode, the optimal detection condition for IQS using the GC electrode was at pH 7. Similar to the CV features, the signal response of IQS on the GC electrode at pH 9 was very low with significant background noise and a very narrow linear range. The response time was also 2 s with linearity up to 4, 7 and 3 μM IQS and detection limits of 152, 89 and 197 nM (S/N = 3) for GC at pH 5, 7 and 9, respectively. Overall, the BDD electrode at pH 9 and the GC electrode at pH 7 showed comparable amperometric responses towards IQS. However, the BDD electrode with extremely low background current, exhibited wider linear ranges and much lower detection limits compared to the GC electrode.

In conclusion, a recently reported thiazole was prepared using an efficient one step protocol using the Suzuki–Miyaura coupling reaction. Analysis by various electrochemical techniques allows for the sensitive detection of IQS using the BDD and GC electrodes. Future endeavour should focus on the validation of the optimised protocol by clinical samples.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.08.023.

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