Electrochemistry Communications 78 (2017) 43–46

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

Submicromolar quantification of pyocyanin in complex biological fluids using pad-printed carbon electrodes

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ARTICLE INFO

Keywords:
Pyocyanin
Human serum
Wounds
Voltammetry
Pseudomonas aeruginosa

ABSTRACT

Pyocyanin, a toxin produced by *Pseudomonas aeruginosa*, offers potential as a biomarker for the indirect detection of this bacterium of major importance for infections in burns, woundcare and cystic fibrosis.

Pad-printed carbon electrodes are herein explored using square wave voltammetry to detect pyocyanin in a range of buffered and biological media. Third-order polynomial baseline fitting was explored to enhance the analytical sensitivity and extend the linear range to submicromolar concentrations. These modelling baselines showed excellent correlation with the experimental data, confirmed by high Interclass Correlation Coefficients of 0.995–0.998, and enabled the quantification of pyocyanin – with linearity extended down to 0.18 μM in Human Serum and 0.336 μM in both Britton-Robinson buffer and Simulated Wound Fluid, and derived Limits of Detection of 0.17, 0.15 and 0.09 μM, respectively, in this proof-of-concept study.

Therefore, the use of very simple, cost-effective printed carbon materials enabled the detection of clinically relevant concentrations of this important biomarker through a new baseline fitting model and offers a novel approach for point-of-care diagnostics where *Pseudomonas aeruginosa* infections are critical.

1. Introduction

The ability to rapidly quantify biomarkers through point-of-care testing (PoCT) confers a huge advantage for the rapid diagnosis and controlled management of many diseases and conditions. New analytical methodologies and platforms are essential to drive these technologies forward thereby enabling PoCT to cover a wider range of diagnoses. An area where this is apparent is within woundcare and burns management; a recent eDEPLHI study found that clinician's top research priorities included the development of wound diagnostics and the management of wound infections [1]. Research towards PoCT to enhance woundcare have been targeted through standard in-vitro diagnostic approaches, but also the emerging concept of smart-bandages, capable of providing diagnostic information and detecting wound infection without the need for wound redressing using many electrochemical approaches [2–7].

One biomarker we have previously suggested is pyocyanin – a toxin produced by *Pseudomonas aeruginosa* infections – of particular importance in woundcare, burns and cystic fibrosis. The ability to reliably and sensitively measure pyocyanin has been approached through electrochemical means, such as the use of gold-electrode arrays to map pyocyanin production through agar-diffusion model [8], commercial carbon strip electrodes [9,10], and carbon fibre and glassy carbon [11]. Previous research has shown that such technologies have been capable of producing small calculated Limits of Detection, but often being unable to quantify pyocyanin below 1 μM [9,11]. The ability to develop sensitive analytical technologies to quantify pyocyanin, enabling rapid diagnosis or more specific management, would be of benefit to patients and healthcare providers. Physiologically and diagnostically relevant concentrations have yet to be fully determined; concentrations as high as 130 μM have been identified in the sputa of cystic fibrosis patients [12], but more generally (and in wounds), concentrations < 10 μM are more commonly found [13]. In terms of the physiological relevance of pyocyanin, a dose dependent formation of hydrogen peroxide in endothelial cells for 1–50 μM pyocyanin, concentrations of 5–10 μM have been found to arrest cell growth, and 25 μM to induce apoptosis [14,15].

The research detailed herein explores the proof-of-concept use of a non-modified printed carbon electrode using square wave voltammetry through simple peak-current analysis, and analysis following third-order polynomial baseline fitting. The use of baseline fitting or subtraction models have attracted some interest in electroanalytical chemistry –with asymmetric least squares and polynomial baselines shown to enhance detection, produce better calibration plots and enable lower Limits of Detection [16–20]. The research detailed here explores the potential use of a third-order polynomial baseline fitting to

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http://dx.doi.org/10.1016/j.elecom.2017.03.021
Received 19 January 2017; Received in revised form 24 March 2017; Accepted 31 March 2017
Available online 03 April 2017

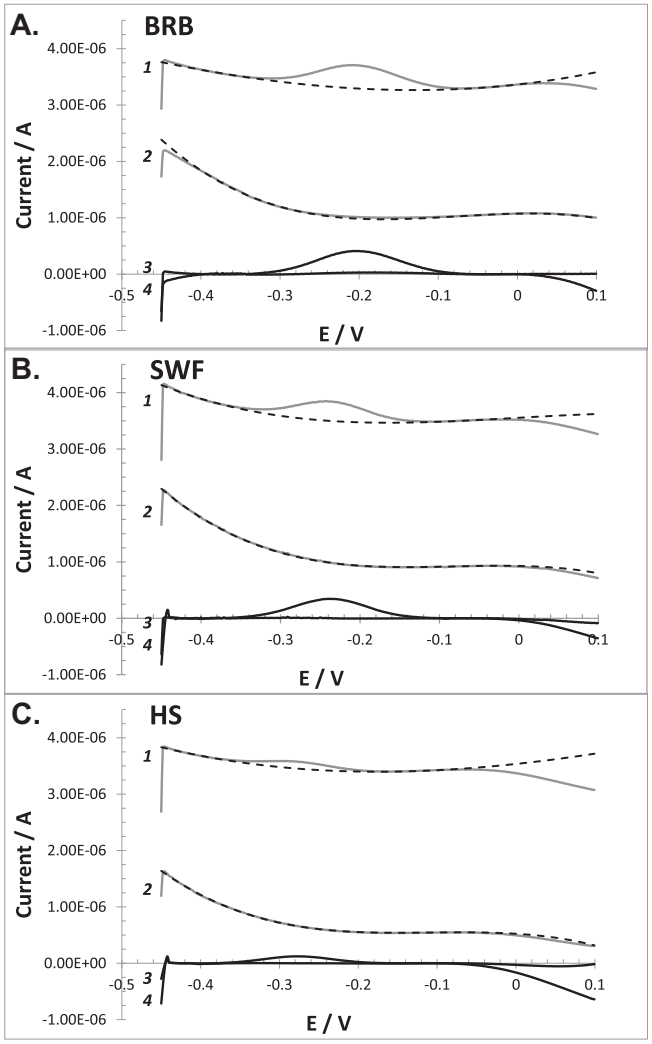

Fig. 1. Square wave voltammograms for pad-printed carbon electrodes for in 5 μM pyocyanin [*1*], pyocyanin free media [*2*], third-order polynomial baseline fitting lines [dashed lines], and residual plots for 5 μM pyocyanin [*3*], pyocyanin free media [*4*]. A. Britton-Robinson buffer (pH 7.0), B. Simulated wound fluid (pH 7.3), C. Human serum (pH 8.35).

enhance pyocyanin detection (Limits of Detection, Quantification and Linearity) in buffered and authentic biological media.

2. Material and methods

2.1. Materials and chemicals

All chemicals used were of reagent grade and used without further purification. Britton Robinson buffer (BR) was prepared from 40 mM of acetic, boric and phosphoric acids and adjusted to pH 7.0 prior to use. Simulated Wound Fluid (SWF) was prepared from 50% Equine Serum (Sigma Aldrich H1270) and 50% maximum recovery diluent (4.25 g/L NaCl (BDH) and 0.5 g/L MC-19 Beef Extract (Lab M)). Human Serum (H6914) and pyocyanin (P0046) (from *Pseudomonas aeruginosa* (≥ 98%)), were purchased from Sigma-Aldrich, and kept frozen (− 20 °C) until use.

2.2. Preparation of pad printed electrodes

The pad printing process of electrodes was performed as previously described [4]. Carbon Graphite Ink (Gwent Group-C2000802P2) was thinned with isophorone to produce an ink of consistent viscosity. After printing two carbon layers (~5 μm each), isophorone was evaporated through 18 h in a fume hood and then heated at 90 °C for 30 min. Dielectric paste (Gwent Group) was diluted with Diluent (Gwent Group-S70204D5) and printed followed by the same evaporation and thermal treatment step, defining a working electrode of an exposed area of ~5.6 mm^2. Electrical connection was made through conductive adhesive copper tape (Farnell, UK).

2.3. Electrochemical experiments

Electrochemical detection of pyocyanin was carried out in a three-electrode set-up using printed carbon working and counter electrodes, and a 3 M NaCl Ag/AgCl reference electrode (BASi). All electrochemical measurements were performed using Square Wave Voltammetry (SqWV) [a = 100 mV, f = 2.2 Hz.] in 3 mL of electrolyte, using a μAutolabIII (Eco Chemie) potentiostat and analysed through NOVA 1.10 software, at room temperature (20 °C). Prior to use, each electrode was electrochemically cleaned with SqWV in the corresponding electrolyte, for five scans − 0.45–+ 0.1 V prior to addition of pyocyanin. The anodic limit was set (E < 0.1 V) to avoid electropolymerisation of

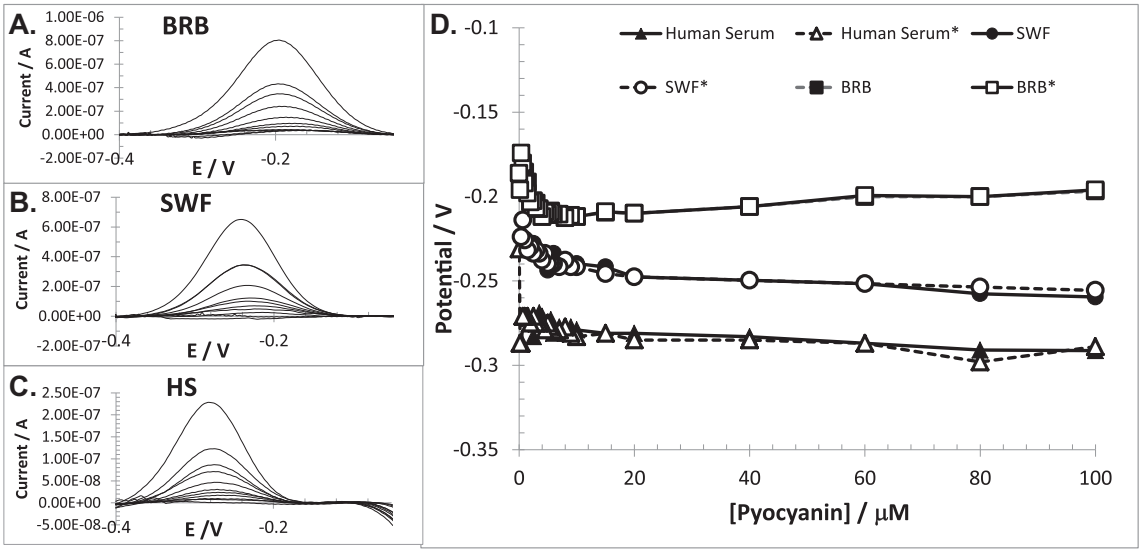

Fig. 2. Residual plots from third-order baseline fitted square wave voltammograms for the calibration of pyocyanin (0, 0.183, 0.334, 0.667, 1, 1.5, 2, 3, 4, 5, 10 μM). For air saturated: A. Britton-Robinson Buffer (pH 7.0), B. Simulated wound fluid (pH 7.3) and C. Human Serum (pH 8.35). D. *Epa* for increasing pyocyanin concentration (0–100 μM) for the three electrolytes studied (raw and baseline-fitted data).