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Voltammetric investigation of complex growth media at a bare glassy carbon electrode: A case study of oxytetracycline

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ABSTRACT

Reports regarding the voltammetric properties of microbiological growth media are scarce in the literature and limited focus has been placed towards the application of electroanalysis for analyte monitoring in these complex media. This work aims to investigate the viability of voltammetry as a quantification method for analytes in microbiological growth media, using oxytetracycline (OTC) as a model analyte. Analysis of both commercially available and laboratory prepared growth media indicated the presence of interfering media components which produced anodic peaks at potentials ranging from ~+0.85 V to ~+1.30 V (vs. Ag/AgCl) under acidic conditions and ~+0.62 V to ~+1.35 V (vs. Ag/AgCl) under neutral pH. These peaks were identified as originating from proteinaceous components of growth media and correlated to the presence of peptone, malt extract and yeast extract. Electrochemical impedance spectroscopy demonstrated significant increases in the charge transfer resistance for $Fe(CN)_6^{3-/4-}$ redox probes at glassy carbon electrodes in the presence of peptone-comprised media (130.3 Ω) compared to media-free buffer (50.4 Ω). Adsorption of the aforementioned media components to the electrode surface thus contributes to analytical interference through faradaic and non-faradaic processes. By adapting the growth media for analyte detection purposes, this study proves the feasibility of detecting OTC, as well as the use of dilution of the media to further decrease the interferent effects of growth media. A 50-fold dilution of the media provided a 96.7% recovery of the OTC peak current at 20 μM concentration. The empirical detection limit of OTC in 50-fold diluted media was determined to be 0.5 μM, which makes it applicable to current industrial OTC fermentation processes.

1. Introduction

The bioprocess industry directed at the production of high value primary and secondary metabolites of microorganisms, such as anticancer agents, amino acids and antibiotics [1–3] is ever more reliant on sensor technology. These include sensors for monitoring of factors vital to the regulation of optimal microorganism growth such as pH, temperature, dissolved oxygen and nutrient levels as well as monitoring of the production of the drug or product. Whether through off-line sampling, or on-line analysis, the ability of the diagnostic measures [4,5] to permit analysis with limited to no pre-treatment is a key determinant for rapid decision making.

The turbidity of fermentation media [6] in which microorganisms are grown necessitates separate pretreatment steps prior to sample analysis where traditional chromatographic or colourimetric methods are employed. One of the distinct advantages of voltammetric methods of analysis is that it is not limited by solution turbidity [7,8]. Surprisingly few studies have explored the application of voltammetric methods as a viable alternative for bioprocess control measurement.

A wide range of different microbiological growth media is used in the bioprocess industry; either purchased in a preformulated mixture, adapted or custom made. The selection of the media is often guided by the particular components (including salts, amino acids, vitamins) required by the microorganism for optimal growth.

In particular, scant information exists in the literature examining the voltammetric profile of such different growth media used in biological processes. A reference to an assessment of tissue culture media (Dulbecco's modified Eagle's medium; DMEM) by Lawrence et al. [9] indicated the potential for interference from electroactive components in certain media, with anodic peaks at +0.8 V (vs. SCE), attributed by the author to the oxidation of a tyrosine phenolic group (a common component of growth media).

Through impedimetric and voltammetric methods, this study aimed to probe the feasibility and limitations associated with utilising electrochemical methods for analytical purposes in a range of commonly used complex microbiological growth media. The study

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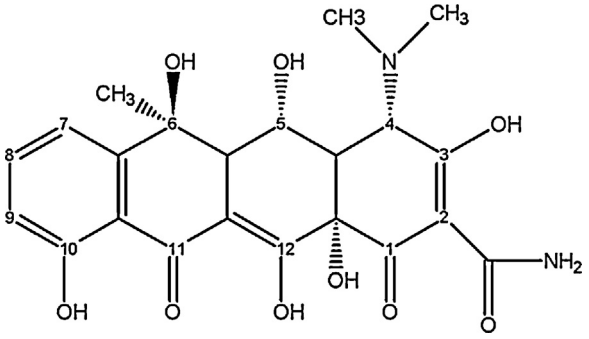

Fig. 1. Molecular structure of oxytetracycline [18].

also sought to identify particular media components which may hinder voltammetric analysis.

The antibiotic oxytetracycline (OTC, Fig. 1) is widely used as both a human and veterinary drug in the treatment of various diseases caused by gram positive and gram negative bacteria and pathogenic Rickettsia [3,10,11]. OTC has also shown promise as a low-dose/long term growth promoter for livestock [11,12]. Production of OTC by *Streptomyces rimosus* in fermentation vessels in the bioprocess industry has been explored in a wide range of different microbiological growth media for optimal generation of OTC [13–17] which, coupled with its wide-spread use, provides a relevant case study for this work.

Currently, quantification of OTC production during fermentation occurs mainly through two off-line processes: high performance liquid chromatography (HPLC) of pre-treated samples [19,20], or variations of the disc-diffusion method which, based on the required incubation period of 16–24 h, appears an impractical method for the OTC production process which itself spans only 5–10 days [13,17,21]. A rapid monitoring system for OTC during production in bioprocesses would greatly improve on current process monitoring for this industry [22].

2. Experimental

2.1. Instrumentation

Cyclic voltammetry (CV), square-wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) studies were performed using a PGSTAT 302 potentiostat (Metrohm Autolab B.V., Netherlands) coupled to a Voltammetric Analytical stand (VA 663, Metrohm Autolab B.V). A three electrode electrochemical cell was used throughout the study, consisting of a glassy carbon working electrode (GCE, 3 mm diameter) from BioAnalytical Systems, a platinum wire and a Ag|AgCl electrode (BioAnalysital Systems, 3 M KCl, NaCl saturated) serving as the counter- and reference electrode respectively. Electrodes were equidistantly spaced for each analysis at RT. All CV analyses were performed at 100 mV s^{-1}, and SWV was performed with a step potential of 5 mV and a frequency of 20 Hz. The GCE was cleaned between analyses, using a sequence of rinsing with MilliQ water (18 MΩ cm^{-1}), absolute ethanol, MilliQ water, polishing on a Buëhler pad (BAS) with aluminium oxide slurry (<10 μm, Sigma–Aldrich) and finally, repeating the rinsing procedure above with absolute ethanol and MilliQ water. The electrode was scanned cyclically (10 times) in the absence of oxytetracycline and growth media prior to each analysis in order to establish a stable baseline.

2.2. Analysis of data and statistical treatment

Data analysis was performed using General Purpose Electrochemical Systems (GPES version 4.7.9, Eco Chemie B.V.), Frequency Response analyser (FRA, version 4.9.007, Metrohm Autolab B.V.) and Microsoft® Excel 2007 software packages. Data was analysed using Student's *t*-test, with statistical significance assigned at $p < 0.05$. CV and SWV data was background subtracted [23] prior to analysis and is displayed as such in this work.

2.3. EIS analysis

EIS was performed in the presence of the selected growth media. The electrode was scanned cyclically (10 scans) followed by a single scan after the addition of 1 mM $Fe(CN)_6^{3-/4-}$ to confirm the open-circuit potential (OCP). A frequency range of 100 mHz–15 kHz was scanned logarithmically at an applied potential equal to the observed OCP for the respective sample, with an alternating voltage of 5 mV rms.

2.4. Buffer and analyte preparation

For analysis of oxytetracycline and growth media, Britton–Robinson (BR) buffer (0.2 M) was prepared using equimolar solutions of boric acid, phosphoric acid, and acetic acid, and titrated to pH 2 using NaOH. Reference standards for $Fe(CN)_6^{3-/4-}$ were analysed in 0.1 M KCl. Oxytetracycline (OTC) hydrochloride (VETRANAL® grade, ≥98% pure) stocks were prepared in 0.2 M BR buffer (pH 2). $Fe(CN)_6^{3-/4-}$ (1 mM) was prepared in MilliQ prior to each experiment using equimolar solutions of $K_4[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$ sourced from Fluka and Merck, respectively. For voltammetric studies in growth media, BR buffer was used in a volumetric ratio of 49:1 (v/v) BR:growth media.

2.5. Preparation of growth media

For this work, three commercially available growth media i.e. clostridium nutrient medium (CNM, Fluka), Luria Bertani broth (LB, Biolab) and nutrient broth (NB, Biolab), and three laboratory made media i.e. minimal salts media (MSM), a growth media optimised for OTC production (referred to here as AZ media, modified from Abou-Zeid et al. [24]) and finally laboratory derived media (LDM) (formulated in this study), were analysed. All growth media were prepared in MilliQ water. The composition of the growth media tested can be found in Table 1. Growth media concentrations were standardised for each experiment, and are provided within the relevant sections.

3. Results and discussion

3.1. Anodic profile of growth media and selected media components

Fig. 2(a) and (b) illustrates the potential for anodic interference of commercially available (CNM, LB and NB) and laboratory prepared growth media (MSM, LDM and AZ), respectively at low pH.

The anodic profile of the growth media (at different pH values) typically displayed the presence of two distinct groups of peaks, denoted Group A which starts at ~+0.85 V and Group B which starts at ~+1.15 V (vs. Ag|AgCl) as seen in Fig. 2(a) and (b) at pH 2. Analysis of MSM revealed no observable anodic peaks in the potential window studied. The peak groups (A and B) shifted consistently cathodically with increasing pH as observed in Fig. 2(c) and (d) with a third group, Group C, becoming visible at potentials more positive than Group B at pH 7. For the purposes of this study voltammetric data was collected at pH 2 to permit comparison with studies performed at this pH with OTC analysis (Section 3.3).

As observed the anodic wave for AZ media in Group A (Fig. 2(b)) is larger than that observed for LDM at the same potential. As the