423a Nonstationary Analysis of in Vivo Electrochemical Measurements Using Ac Voltammetry: from Patterns to Process Parameters

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In the last decades, electrochemical sensing techniques have been used to investigate a plethora of biological processes [1-4]. For instance amperometry, where the electrode voltage is kept constant, and voltammetry, where the electrode voltage is perturbed according to a predefined waveform, are the methods of choice for a wide range of measurements in neuroscience [5-7]. From the current response of the electrode, information about the characteristics, and therefore identity, of the analyte can be drawn. While amperometric methods offer good temporal resolution they cannot discriminate between different molecules undergoing an electrochemical reaction step at the electrode [8]. On the other hand, a large number of physiological functions in biology are regulated by dynamic multicomponent systems and thus, a technique that is capable of monitoring different analytes simultaneously is required in order to study them. Voltammetric techniques have been shown to offer improved selectivity as well as improved discrimination against non-faradaic contributions to the signal. Ultramicroelectrodes (voltammetric electrodes of typically less than 10 m) enable the accurate measurement of time-dependent currents and have proven to be a very useful tool for *in vivo* biological experiments offering unparalleled spatial and temporal resolution combined with good selectivity [9, 10].

The major difficulty of voltammetric methods lies in their interpretation. Electrochemical signals are intrinsically nonlinear and non-stationary. For a long time voltammetric measurements were confined in small-amplitude perturbations to avoid nonlinear effects of electron transfer. Lately, ac voltammetry, which uses a ‘slow' ramp superimposed on a ‘faster' large amplitude sinusoid as voltage perturbation, has drawn considerable attention due to its enhanced voltammetric detail as well as its ability to investigate phenomena on different timescales. Such large amplitude and frequency excitation waveforms though cause capacitance interference to the current to become substantial [11]. Hitherto there have been few alternatives to the FFT technique for frequency analysis, although fundamental assumptions of the theory, namely periodicity, continuity and linearity, are not satisfied [12]. As a result, ac voltammetric data-sets could either not be fully interpreted [13] or their interpretation involves very complicated computations [14].

We recently proposed a signal processing technique – the Hilbert transform (HT) – which considerably minimises the effect of capacitance without applying the often ambiguous background subtraction. The HT is suitable for nonstationary processes and has been used in the analysis of nonlinear dynamical systems [15, 16]. It results in the instantaneous attributes of the current response. Through time-domain pattern recognition, we have shown that this method can lead to high precision estimations of kinetic and thermodynamic parameters for surface confined processes [17].

In this contribution we will propose a methodology that enables the accurate estimation of a set of species- and process-specific parameters in order to monitor the dynamical behaviour of the environment under investigation as well as the sensor/environment interface when mass-transport is present. The spatiotemporal behaviour of the analyte will be investigated through theoretical models which will simulate the effect of electrochemical reaction- and mass transport-dynamics when the electrode voltage is perturbed. From the analysis of the electrode current response we will show how to deduce kinetic ($k0$,), thermodynamic (E_0), diffusion (D) and interface (C_{dl}) characteristic parameters as well as concentration changes in the bulk (C_{bulk}). We will illustrate that the proposed methodology proves very robust towards enhanced capacitance interference, mainly, and noise levels which normally preclude quantitative analysis with conventional tools. We will present experimental verification of our method through voltammetric measurements using serotonin, dopamine and noradrenalin [18]. These

molecules play a fundamental role in neurotransmission and cell signalling in general and are very difficult to discriminate *in vivo* with conventional electrochemical biosensing techniques. ‡

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