Sensors and Actuators B 190 (2014) 295–304

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Automated flow-injection immunosensor based on current pulse capacitive measurements

Dag Erlandsson[a], Kosin Teeparuksapun[a,b], Bo Mattiasson[a,b], Martin Hedström[a,b,*]

[a] Capsenze HB, Annebergs gård 5520, SE-26021 Billeberga, Sweden
[b] Department of Biotechnology, Lund University, Box 124, SE-22100 Sweden

ARTICLE INFO

Article history:
Received 6 March 2013
Received in revised form 23 August 2013
Accepted 24 August 2013
Available online xxx

Keywords:
Current pulse
Capacitive
Automated flow-injection
HIV-1 p24

ABSTRACT

This document describes a new concept for assessing capacitance based on a constant current pulse to the biosensor transducer. The biosensor has a working electrode that is coated with an insulating molecular layer including a ligand which forms an affinity surface. A sensor electrode is brought into contact with electrolyte solution, and the new measuring principle then involves steps where three different constant currents (I_1, I_2 and I_3) are serially pulsed on the sensor surface during pre-determined time periods. The potential that is built up (rising) across the sensor surface is sampled every 6.8 μs. The inclination of the registered potential profile corresponding to the current pulsed was utilized to calculate both capacitance and resistance. The new current-based measurement method shows a 10-fold increase in stability for the capacitive measurement as compared to the potential pulse technique. Quantitation of HIV-1 p24 using monoclonal anti-HIV-1 p24 antibodies was used as a model system for the evaluation of the technique. The binding of HIV-1 p24 antigens to the immobilized antibodies causes the capacitance to decrease. The change in capacitance was proportional to the concentration of HIV-1 p24. The capacitance measurement using the current pulse method offers a stable sensing technique with a broad range of potential applications.

1. Introduction

When selecting an analytical technique for quantitative determination of trace amount of biomolecules, a sensitive and precise analytical method is needed [1–3]. Conventional quantitative methods e.g. mass spectrometry [4,5], HPLC [6,7], NMR [8,9] and chromatography [10,11] are available. For certain applications where the target is present at trace levels, more sensitive methods are needed. One such group of assays is represented by sensitive affinity-based biosensors. Among these, electrochemical sensors have achieved great interest due to their high sensitivity when compared to other sensors like gravimetric and optical techniques [1,12–14]. The electrochemical biosensors are based on a combination of biological molecules and electrodes.

During the past decade, capacitive measurement has attracted increasing interest for the analysis of biomolecules using the affinity based approach [1,3,15–22]. The capacitive biosensor is constructed by arranging the ligand molecules (i.e. antibody, DNA, lectin etc.) on a pre-modified working electrode surface. The concentration of the target analyte in solution can be quantified by measuring the change in dielectric properties upon interaction between analyte and ligand occurring on the sensor surface. The affinity interaction will contribute to the decrease in measured capacitance and the amplitude of capacitance change is correlated to the concentration of the analyte [15–22]. There are different approaches that can be theoretically applied to measure capacitance at the electrode/solution interface with the ligands attached to the surface of the working electrode. The most employed techniques are impedance spectroscopy (IS) [21,22] and potential pulse [15–20].

Evaluation of capacitance through impedance measurements has been widely described [21,22]. Impedance is an effective method to exhibit the electron-transfer resistance features of surface-modified electrodes and evaluate capacitance. However, recording a full impedance spectrum within a wide range of frequencies is time consuming [22] and interpretation of data is complex [16].

Capacitive biosensors based on a potential pulse utilize the transient current response when a small potential pulse (e.g. +50 mV) is applied to the working electrode. This method has been used in various different applications [1,3,16–22]. However, a capacitive biosensor that operates according to the potentiometric pulse concept is sensitive to external electronic disturbances which can lead to inaccurate measurement and poor baseline stability [23]. The sharp potential pulse that is applied to the working electrode surface may affect the affinity layer with the bound ligands in such a way that they may partly be destroyed. The working electrode would then need to be replaced by a new one, resulting

* Corresponding author. Tel.: +46 705585311
E-mail address: martin.hedstrom@biotek.lu.se (M. Hedström).

0925-4005/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.snb.2013.08.076

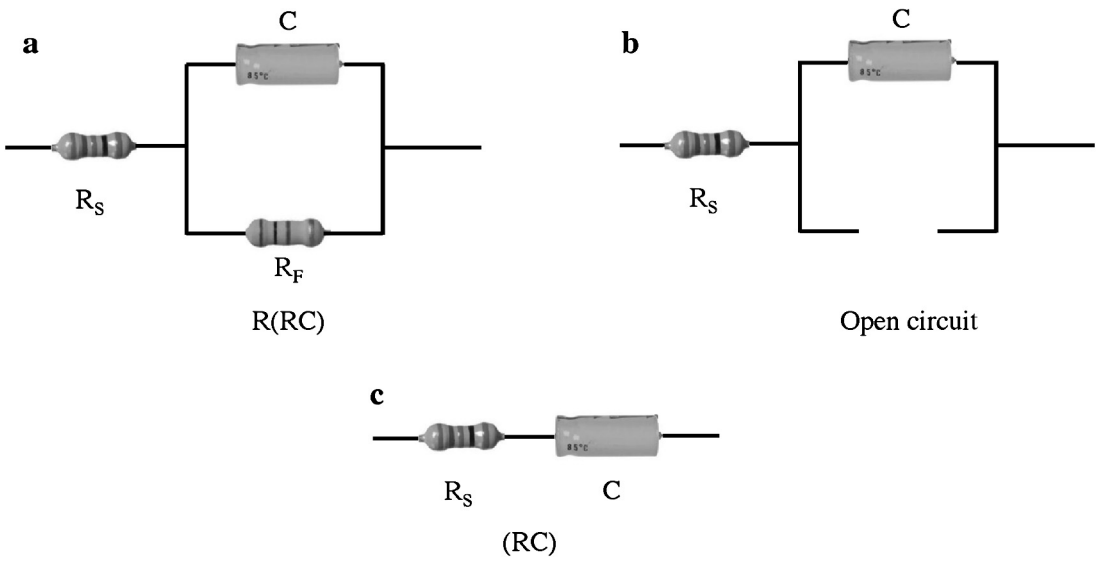

Fig. 1. Equivalent circuit for the measurement of capacitance (a) R(RC) circuit consists ohmic resistance of the insulation layer, R_F, the electrical resistance of elements serially connected to the layer, R_S and a capacitor, C (b) R_F is very much higher than R_S making it act like an open circuit, (c) open circuit is simplified as (RC) circuit having resistor serially connect to the capacitor.

in a time-consuming operation before baseline stability is re-established. Another critical step in designing capacitive biosensors is the immobilization of the biorecognition elements on the electrode. If the electrode is not sufficiently insulated, ions can move through the layer, causing a system short-circuit, which leads to a distorted or omitted signal. Interferences from redox couples in the electrolyte solution can also cause high Faradaic background currents, which might increase the resistance current and decrease the capacitance response [23].

The development of real-time capacitive-based assays has been proposed and further developed with some additional features, e.g. the ability to investigate the electrode properties during the measurement [24]. However, it is desirable to have a stable analytical system with improved capacitive measurements both with regard to increased sensitivity and accuracy of the biosensor.

Another alternative way to measure capacitance at the electrode/solution interface is to use a current pulse method. This technique has been proposed for the measurement of electrochemical capacitance in molten salt [25,26]. The theory for the current pulse technique is, similarly as for the potentiometric capacitive assays, based on the principle of an electrical double layer and the electrode solution interface is based on an assumption that the system could be described as a simple resistor–capacitor (RC) circuit model [16,23–26]. Generally for the capacitive biosensor assay, the electrode is modified with an insulating layer. Hence, an accurate description of the electrode interface would be an R(RC) circuit as shown in Fig. 1a [24]. As the electrode surface is insulated, the ohmic resistance (R_F) of the insulating layer is much higher than the electrical resistance of the element serially connected to the layer (R_S). This high R_F simulates an open circuit (Fig. 1b) and the system could thus be simplified as RC circuit having an extra resistor (R) serially connected to a capacitor (C) (Fig. 1c). When the RC circuit is charged by a constant current, I, as shown in Fig. 2a, the potential (U) increases linearly with time (t) as illustrated in Fig. 2b.

The potential response can be simultaneously sampled from the potential curve that is built up across the sensor when it is charged with a constant current. The electrical resistance (R) is calculated by using Ohms law (1).

$$U = I \times R \tag{1}$$

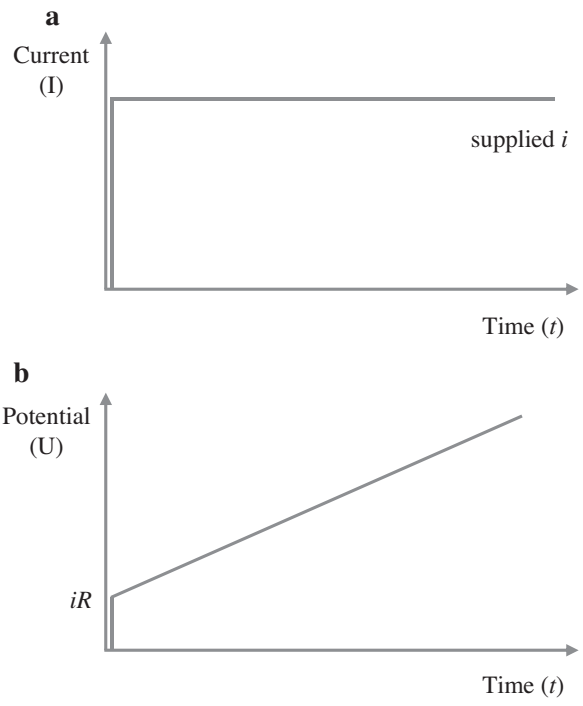

Fig. 2. The method for measuring capacitance using current step (a) supplied of constant current to the (RC) circuit, (b) the potential response rising up on the (RC) circuit resulting from the supplied of constant current.

where U is the voltage when time is zero and I is the applied current. The capacitance is then calculated from the slope of the potential curve through the following formula [23,25,26].

$$C = (I \times t)/U \tag{2}$$

where I is the current supplied to the sensor, t is the current pulse period and U is the slope of the voltage built up across the capacitor of the RC circuit multiplied with time [26,27]. The evaluated capacitances (nF) are then plotted against time (min).

There are a large number of reports where this method is used for capacitance measurement but to our knowledge this is the first