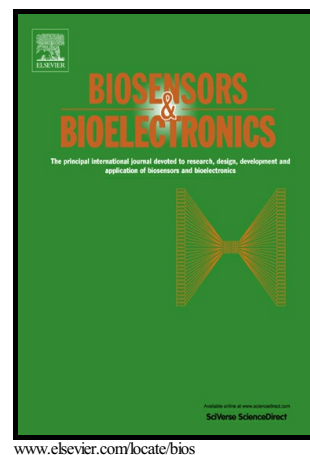


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PII: S0956-5663(17)30621-8
DOI: <http://dx.doi.org/10.1016/j.bios.2017.09.014>
Reference: BIOS9988

To appear in: *Biosensors and Bioelectronics*

Received date: 6 June 2017
Revised date: 2 September 2017
Accepted date: 11 September 2017

Cite this article as: Adriano Santos, Paulo R. Bueno and Jason J. Davis, A dual marker label free electrochemical assay for *Flavivirus* dengue diagnosis, *Biosensors and Bioelectronics*, <http://dx.doi.org/10.1016/j.bios.2017.09.014>

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A dual marker label free electrochemical assay for *Flavivirus* dengue diagnosis

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Abstract

Dengue is a RNA viral illness of the genus *Flavivirus* which can cause, depending on the pervasiveness of the infection, hemorrhagic dengue fever, known as dengue shock syndrome. Herein we present an electrochemical label free approach enabling the rapid sensitive quantification of NS1 and IgG (a tool supporting an ability to distinguish primary and secondary infections). Using a bifunctional SAM containing PEG moieties and tethered redox thiol, both markers are detectable across clinically relevant levels by label free impedance spectroscopy derived redox capacitance. A subsequent frequency specific immittance function approach enables even more rapid assaying (within seconds) with no impairment of analytical parameters (linearity, sensitivity and variance).

Keywords: molecular diagnostics, biosensor, electrochemical capacitance spectroscopy, electrochemical immittance analysis, dengue, NS1, IgG.

1. Introduction

Dengue is an infection caused by RNA virus (DENV), a member of *Flavivirus* genus. [1] DENV virus has four different serotypes (DENV1, DENV2, DENV3 and DENV4) which can cause, depending on the incidence (first or secondary infection), [2, 3] dengue fever (DF) or dengue hemorrhagic fever (DHF) alternatively known as dengue shock syndrome (DSS) [2]. The symptoms vary from little physical prognosis to a severe disease status as characterized by bleeding and circulatory failure. Dengue is transmitted from one person to another by the bites [2] of a female contaminated *Aedes* mosquito, [4] which propagates in urban areas in subtropical and tropical regions. [2] Frequent outbreaks are reported in Asia-Pacific region, both central and south America and southeast of the Gulf of Mexico. [2] In Brazil alone, over a million cases of dengue were registered from January to May of 2015 and, in 2016, an increase of about 50% was registered in the first two months of the year, over 2015 levels. Epidemics have additionally been reported in 2010 in Philippines, Caribbean, Central America and Sri Lanka. [2] It is important to highlight that, due to climate change [5] and an increase in global travelling, [1] dengue re-emerged in 2009 in Florida [2] and an outbreak in 2012 was reported in Portugal, the first since the 1920s. [6] It is now considered a global public health issue [7] where 3.6 billion people over 124 countries are estimated to be at-risk of infection. [8] In view of this scale, and in the absence of efficient vaccines or therapeutics, [4] rapid, accurate and practical diagnosis enabling immediate patient management and a control of spread is of considerable value. [3]

Virus isolation and cultivation in vitro followed by an immunofluorescence assay is generally considered the “gold standard” approach for dengue detection. [1, 2] Though highly specific, the methodology requires a long incubation time (7-12 days) and lab facilities with skilled personnel. [1] In addition, the appropriate window for virus culturing is limited to up seven days following the onset of symptoms, making this methodology impractical for routine screening. [1] Diagnosis based on RNA detection by reverse transcription polymerase chain reaction (RT-PCR) is used for detecting the viral RNA and, again, though specificity is high, this is a costly, laborious, and time intensive process [1, 2, 4]. The latter consists of an approximately three-hour-procedure [9] involving RNA isolation, the production of a single-strand complementary DNA copy, PCR amplification, separation, detection and quantification by optical [10] or electrochemical [11] measurements. PCR methods are, additionally, only effective shortly after disease onset [2] and suffer from false-positives through cross-contamination with dengue virus PCR products in the laboratory. Serological tests such as MAC-ELISA (monoclonal antibody capture-enzyme linked immunosorbent assay), [2]

are not accessible in resource-limited healthcare settings nor readily portable (enzyme labeled secondary antibodies, repetitive incubation steps and UV-VIS readout are required). [2, 4] Electroanalytical methods offer much to deal with challenges involving sensitivity, portability and additionally can be both rapid and label free. [12, 13]

Recent studies have shown that non-structural protein 1 (NS1) can be used as a biomarker for detection of dengue. [14-16] NS1 is a ~45 KDa glycoprotein [17] expressed in infected cells and can be detected within a week of symptom onset in a concentration range of 0.04-2.00 $\mu\text{g/mL}$ in serum of primary infected patients and 0.01-2.00 $\mu\text{g/mL}$ in secondary. [15] There are additional reports that a combined detection of NS1 antigen and its antibodies (IgM and/or IgG) improves diagnostic sensitivity and aids the identification of secondary infection, critical in detecting likely severe forms of this disease. [18, 19] NS1 represents an early stage biomarker, [15] with IgM detection possible from the third day after contamination, and persisting for approximately three months. [20] IgG levels spike 5-14 days after contamination. [20, 21] In secondary infections, however, the IgG titer increases within 1-2 days to levels that are several orders of magnitude higher than observed prior to infection. [21] IgG analysis thus can be used as confirmatory of secondary infection. [19, 20] (see supplementary material-section S1, SM-S1).

To date a number of low cost diagnostic platforms for NS1 have been reported, including those based on impedimetric analyses. [1, 4, 22-28] For example, Cecchetto et al. [26] have reported an impedimetric biosensor to detect NS1 both in PBS and serum using a surface tethered anti-NS1 antibody. Similarly, Darwish et al. [27] have reported an impedance immunosensor for NS1 detection through the multistep construction of an immobilized gold nanoparticle-anti-NS1 antibody film. Traditional impedimetric (faradaic or non-faradaic) approaches are inherently spectroscopic, with data acquisition typically across a broad range of applied AC frequencies (1 MHz to 10 mHz) prior to subsequent fitting of experimental data to a convenient electric circuit. [13] In the dominant faradaic approach, it is additionally necessary to pre-dope with an amplifying excess of redox probe [29] prior using the resolved charge transfer resistance (R_{ct}) as signal transduction. As an alternative, we have introduced the use of redox tagged receptor interfaces and redox capacitance (C_r) (from electrochemical impedance-derived capacitance spectroscopy) as underpinning a fully label free sensory approach. [30-32] Although this approach brings significant practical advantages, by default, it also requires a full spectrum analysis. We have also introduced an immittance function methodology that enables a more facile analysis without any data fitting.[32, 33] The concept is based on the relationship between potential perturbation and

measured current being described by mathematically exchangeable complex immittance functions (e.g. impedance Z^* , capacitance C^* , admittance Y^* and modulus M^*). It is possible, within this, to obtain different transduction signals using only one data set without any reference to equivalent circuits. [32-34] The benefit of using immittance function approach is that it is, in essence, a “plug and play” concept wherein a sensor “chip” is coupled to the read out device, where an algorithm can be set up to search for the most sensitive function, simultaneously mapping its frequency response. In previous work we have demonstrated this application to a number of markers. [32-34]

Herein we expand this approach to Dengue diagnostics using a three self-assembly monolayer (SAM) functionalities, that is passivation of the electrode, redox tagging and finally non-fouling characteristics. Using these SAM characteristics we compare the analytical potency of the obtained redox capacitance signal and electrochemical immittance methods. These methodologies illustrate on the first dual electrochemical dengue marker assay reported to date, quantifying both markers across their full clinically relevant ranges, and ultimately enabling the future development of a useful mobile diagnostic platform.

2. Experimental

2.1 Chemicals and biochemical reagents

The chemicals described in this section were purchased from Sigma-Aldrich, except the PEG-thiol (HS-C11-EG3-OCH₂-COOH, EG = ethylene glycol) and 11-(ferrocenyl)-undecanethiol (11Fc) which were obtained from Prochimia [purity > 95%]. Non-structural protein 1 (NS1) and IgG antibody (anti-NS1, reactive against four different dengue viruses [I-IV]) were purchased from Abcam. Solutions of anti-NS1, NS1 and bovine serum albumin (BSA) 1 mg/mL were prepared in phosphate buffered saline (PBS) pH 7.4 containing NaCl (137 mmol/L), KCl, 2.7 mmol/L and freshly prepared sodium phosphate (10 mmol/L). Solutions of PBS, NaOH 500 mmol/L, H₂SO₄ 500 mmol/L, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) 400 mmol/L, *N*-Hydroxysuccinimide (NHS) 100 mmol/L and sodium perchlorate (NaClO₄) 100 mmol/L were prepared in Milli-Q water (Millipore system with 18.2 MΩ cm at 25 °C). Mixed solutions of PEG-thiol (0.02 mmol/L) and 11Fc (2 mmol/L) were prepared in anhydrous ethanol. Commercial human serum was obtained from Sigma and used diluted in PBS at 20% (v/v).

2.2 Electrochemical measurements

All experiments were performed at room temperature (25 °C) using NaClO₄ electrolyte at 100 mmol/L. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements were performed using an AUTOLAB potentiostat equipped with a frequency response analysis (FRA) module and the NOVA software from Metrohm. A three-electrode system was used, including the gold electrode as working electrode (1.6 mm diameter from Basi), a platinum disc as counter electrode and Ag|AgCl (3 mol/L NaCl, from ALS Co., Ltd) as reference electrode. All potentials referred to in this work are relative to Ag|AgCl (3 mol/L NaCl). All solutions used were deaerated by N₂ purging for 25 min before use.

2.3. Surface preparation and characterization

Gold electrodes were mechanically polished using 0.1 and 0.05 µm grain-sized aluminum oxide aqueous suspensions (Buehler) followed by sonication in deionized water for 15 min to remove adhered particles. CVs for electrochemical desorption (see SM-S2) were performed in NaOH 500 mmol/L, from -1.7 to -0.5 V, 400 cycles, at the scan rate of 50 mV/s. Electrochemical cleaning CV's were performed in H₂SO₄ 500 mmol/L from 0.3 to 1.5 V, 500 cycles, at scan rate of 100 mV/s, followed by CV gold oxide stripping in H₂SO₄ 500 mmol/L from 0.3 to 0.6 V, 10 cycles, 100 mV/s. Electroactive areas were calculated on the basis of cyclic voltammograms from the electrochemical cleaning step by integrating the cathodic peak on the last scan using a value of 482 µC/cm². [35, 36] The surface roughness was determined by dividing the electro-active area by the geometric area. These determinations (0.036 ± 0.009 cm², with a corresponding surface roughness of 1.8 ± 0.4) were used to normalize subsequent electrochemical transduction signals.

The electro-active bifunctional SAMs were constructed by immersing cleaned electrodes for 20 h at 25°C in a mixed solution containing 0.02 mmol/L PEG-thiol (with low fouling features)[37-39] and 2.0 mmol/L 11Fc in anhydrous ethanol. The carboxyl group of the PEG-thiol was then activated with an aqueous solution containing EDC 200 mmol/L and NHS 50 mmol/L for 30 min. Subsequently, the electrode was washed using water, dried with nitrogen gas and immersed in either 97 µg/mL anti-NS1 solution or 10 µg/mL NS1 antigen in PBS for 1 h. Finally, the electrodes were immersed in a BSA solution (1mg/mL) for 30 min (Figure 1) and then washed with PBS and water.

After SAM formation, CV analysis was performed from 0.0 to 0.8 V (50 cycles) at a scan rate of 100 mV/s to resolve the redox-in potential $E_{in} = (E_{ox} + E_{red})/2$, where E_{ox} and E_{red} are the oxidation and reduction potentials peak potentials, respectively. Impedance analyses were carried

out at E_{in} in a frequency range of 100 kHz to 0.1 Hz (50 frequencies logarithmically arranged), amplitude of 10 mV, after each step of the interface construction.

Electrochemical capacitance analysis was performed by determining the capacitance from raw impedance data using the relationship $C^*(\omega) = 1/i\omega Z^*(\omega)$, where ω is the angular frequency and i is the complex number $i = \sqrt{-1}$. [40] In processing $Z^*(\omega)$, it is possible to obtain both the imaginary $C'' = \phi Z'$ and real $C' = \phi Z''$ portions of capacitance, noting that $\phi = 1/(\omega|Z|^2)$, where $|Z|$ is the modulus of impedance. The 11Fc coverage (Γ_{11Fc} , mol/cm²) was calculated from $\Gamma_{11Fc} = 4C_r k_B T / eF$, where C_r is the electrochemical or redox capacitance per cm² (estimated by the diameter semicircle of the capacitive Nyquist plot, as detailed in references [13, 30]), k_B is the Boltzmann constant, T is the absolute temperature (293 K), F is the Faraday constant, and e the elementary charge. [41] Γ_{11Fc} results obtained using redox capacitance were compared with those estimated from classical voltammetry, $\Gamma_{11Fc} = Q/FA$, assuming $Fc^+ + e^- \rightleftharpoons Fc$ and Q is the charge obtained by the integration peak of the reduction/oxidation process after compensating for non-faradaic contributions. [42] Γ_{11Fc} redox coverage was also calculated by analysis of the voltammetric current peaks resolved at different scan rates (100–700 mV/s). [43]

CV electrochemical desorption was performed in NaOH 500 mmol/L with potential ranging from -0.2 to -1.4 V at scan rate of 50 mV/s to obtain the bi-functional SAM surface coverage (Γ_{SAM}), as described elsewhere. [35]

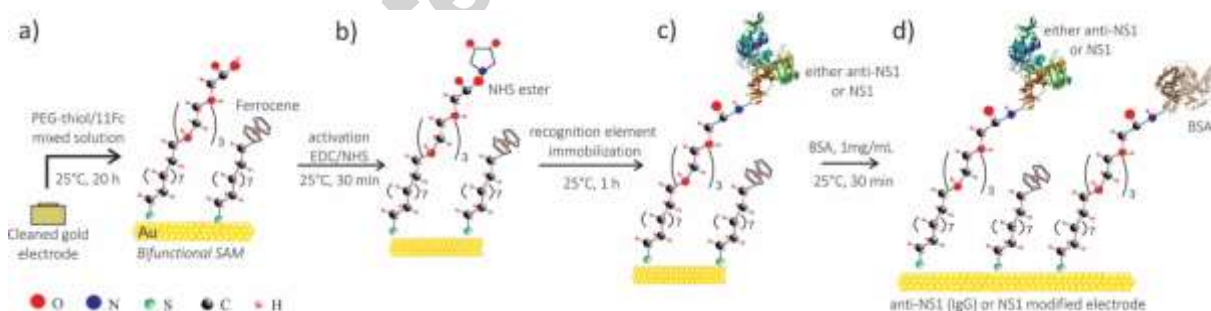


Figure 1. Schematic step by step preparative depiction of the anti-NS1 and NS1 modified electrode surfaces. (a) Functionalization with a mixed solution containing 11Fc and PEG-thiol. (b) Activation of carboxylic groups prior to coupling with amino groups present in the recognition element as shown in (c). (d) Blocking non-specific sites with BSA 1mg/mL (molecules not drawn into scale).

2.4 Detection of NS1 and IgG (anti-NS1)

After the surface construction and electrochemical characterization, the functionalized electrodes were exposed for 30 min in either NS1 or IgG mAb solutions at different concentrations, ranging from 1 to 5,000 ng/mL, prepared either in PBS or diluted serum at 20% (v/v) in PBS. The electrodes were then washed with water and impedance analyses carried-out at E_{in} using NaClO_4 supporting electrolyte. A typical acquisition time of impedance data over the full frequency range (50 frequency points, 100,000-0.1 Hz) is approximately 4 min. All assays were carried out in triplicate and results reported as average $\pm$ standard deviation ($\bar{x} \pm sd$).

As noted above, the signal transduction (S) used herein was initially the inverse of redox capacitance (i.e. $S = 1/C_r$, where C_r is obtained from the semicircle diameter of the complex capacitance plots) and then the inverse of the imaginary part of capacitance (i.e. $S = 1/C''$). The relative response (RR), defined as:

$$RR_{[target]}(\%) = [(S_{[target]} - S_0)/R_0] \times 100 \quad (1)$$

where S_0 represents the blank measurement and $S_{[target]}$ is the S value for a certain target (either NS1 or anti-NS1) concentration. Analysis of correlation coefficient (R), coefficient of determination (R^2), sensibility (slope of the linear regression in the analytical plot), limit of detection (LOD, defined as $3.3 \times SD$ where SD represents the standard deviation of the blank) [44] and relative standard deviation (RSD) were performed. In order to evaluate the non-specific interactions, serum at 20% (5 fold) dilution in PBS was used. In optimizing analytical frequency for $1/C''$ transduction analytical plots were resolved with a coefficient of determination $R^2 > 0.96$, with the highest sensitivity and the lowest limit of detection.

3. Results and discussion

3.1 Surface film characterization

An initial CV analysis of the 11Fc and PEG-thiol SAM (Figure 2a) resolves the expected reversible redox wave (peak separation 30 ± 5 mV and current peak ratio about unity (i.e. $i_{pa}/i_{pc} \approx 1$). The full width at half of the peak maximum height (FWHM), a representation of redox site homogeneity, [45] is ≈ 110 mV a little higher than the theoretical ideal for an electron process (90.6 mV), [45] but fully in line with prior reports. [43] A complete impedance characterization of the bifunctional SAM is shown in SM-S3.

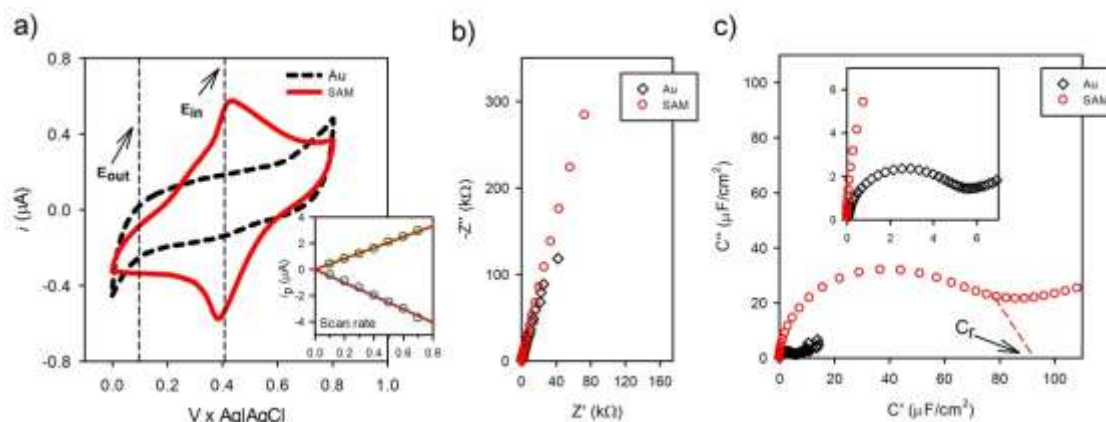


Figure 2. (a) CV obtained before (dashed black line) and after (red line) SAM formation. Conditions: 100 mM NaClO₄, potential range 0 to 0.8 V (x Ag|AgCl), scan rate 100 mV/s, 50 cycles. The 50th cycle is shown. The inset illustrates the linear dependence of current peak (anodic, i_{pa} ; and cathodic, i_{pc}) in function of scan rate, from 100 to 700 mV/s. (b) Nyquist impedance plot obtained at E_{in} potential for bare gold (black diamond) and SAM (red circles). The impedance raw data can be converted to capacitance complex function, as shown in (c). The redox capacitance (C_r) can be estimated by semicircle diameter.[30, 40, 46] Conditions: measurements performed at E_{in} potential (≈ 400 mV x Ag|AgCl). AC perturbation amplitude of 10 mV. Frequency range from 100 KHz to 0.1 Hz (50 frequencies). Supporting electrolyte: 100 mM NaClO₄ aqueous solution.

The 11Fc surface coverage (Γ_{11Fc}) determined by integration of CV peaks (Figure 2a) is $(1.8 \pm 0.5) \times 10^{-10}$ mol/cm². A similar value can be obtained using the linear dependence of the anodic or cathodic current peaks in function of scan rate (inset in Figure 2a), namely $(1.4 \pm 0.3) \times 10^{-10}$ mol/cm². For comparison, an approximation based in the redox capacitance (using an approximation of the number of states at E_{in} energy level)[47] is $(1.0 \pm 0.1) \times 10^{-10}$ mol/cm². The total SAM surface coverage (Γ_{SAM}) was evaluated by integrating the anodic peak (at ≈ -1.1 V) [48] (details in SM-S4) with a value of $\Gamma_{SAM} = (5 \pm 2) \times 10^{-10}$ mol/cm², in line with expectations for films of this type. [49]

The impedance behavior of the mixed SAM was studied at two different potentials, *redox in* ($E_{in} = 406 \pm 4$ mV) and *redox out* ($E_{out} = 100$ mV) as previously specified. *Redox in* potential corresponds to formal potential of the ferrocene couple, while *redox out* is only a charging potential, as illustrated in the vertical lines in Figure 2a (details of SAM impedance behavior can be found in SM-S3). From the Figure 2b, it is not possible to distinguish the differences in the impedance Nyquist plot (i.e. complex domain) from the data collected at E_{in} for bare gold and SAM modified surface. However, this problem can be overcome by converting the complex impedance raw data to capacitance. As can be seen, at the *redox in* potential for bare gold, only the double layer capacitance can be noted, 5 ± 1 μ F/cm². After SAM modification, the capacitance increases

considerably ($96 \pm 14 \mu\text{F}/\text{cm}^2$, associated with C_r from tethered redox SAM), with a C_r magnitude consistent with previous work ($\approx 100 \mu\text{F}/\text{cm}^2$). [50]

After SAM formation and electrochemical assessment, the carboxylic groups present in the PEG-thiol were activated with EDC/NHS standard chemistry [51] for IgG antibody (anti-NS1) or antigen (NS1) receptor coupling. To block non-specific sites to avoid unspecific interaction, BSA at 1mg/mL PBS was used. During both steps it was possible to observe a specific decrease in C_r signal (Figure S5).[52] For more details, please refer to SM-S5.

After initial electrochemical characterization, the redox active receptive films were applied to target detection. Control receptive films are unresponsive to successive incubations in PBS or serum (demonstrating specificity and signal stability – see SM-S6 and SM-S7).

3.2 NS1 detection in PBS and diluted serum

For the NS1 assay, the target protein was diluted in both PBS (pH 7.4) and diluted serum (1:4, sera:PBS) across a clinically relevant concentration range 1 to 5,000 ng/mL. [15] Aliquots of 50 μL of NS1 solution were used with an incubation time of 30 min. Figure 3 shows the capacitance Nyquist and Bode plots subsequently obtained. All measurements were performed at the E_{in} potential (note that at E_{out} the capacitance is unresponsive to target recognition). A decrease in redox capacitance signal (semicircle diameter) was observed, as expected. [30, 31, 53] It is worth noting that the relative response RR in function of target concentration (i.e. $\text{RR}(\%) = 100 \cdot (1/C_{r, [\text{NS1}]} - 1/C_{r, \text{blank}}) / (1/C_{r, \text{blank}})$, where $C_{r, [\text{NS1}]}$ is the redox capacitance signal for a specified NS1 concentration and $C_{r, \text{blank}}$ the signal of the blank) is Langmuir in form (details in SM-S8). The derived association affinity, $K_a = (6 \pm 2) \times 10^8 \text{ L/mol}$ ($K_D = (1.8 \pm 0.6) \text{ nmol/L}$) is in the expected range. [30]

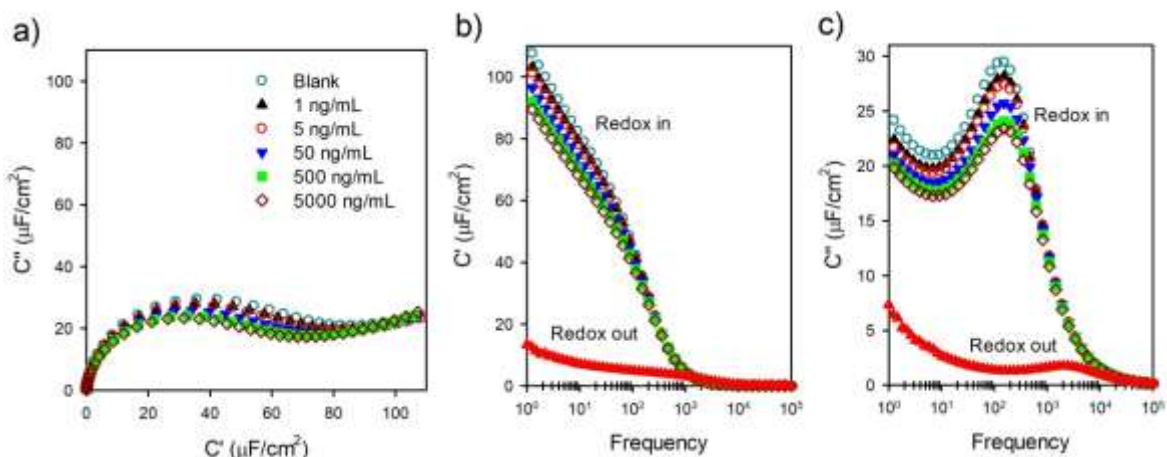


Figure 3. (a) Capacitive Nyquist plot obtained using different NS1 concentrations in PBS at E_{in} , which is possible to observe changes in C_r (semicircle diameter) in function of NS1 concentration. The real (b) and imaginary part of capacitance (c) are unresponsiveness at the *redox out* potential E_{out} but at potential E_{in} . Conditions: measurements performed at E_{in} potential (≈ 400 mV x Ag|AgCl). AC perturbation amplitude of 10 mV. Frequency range from 100 KHz to 0.1 Hz (50 frequencies). Supporting electrolyte: 100 mmol/L NaClO_4 aqueous solution.

From a constructed analytical curve (Figure 4) and derived analytical parameters (Table 1), it is demonstrable that NS1 across a 1-5,000 ng/mL linear range is detectable with a sensitivity of 4.5% per decade. The associated LOD is 340 pg/mL, thus lower than reported by others using carbon nanotube-screen printed electrodes (12 ng/mL) [54] or by means of conventional faradaic impedance using a redox probe in solution (3 ng/mL). [26] The average of the relative standard deviation (RSD) for all data points in the calibration curve ($RSD = sd/\overline{RR}$, where $\overline{RR}$ is the relative signal average for a certain target concentration, and sd the respective standard deviation) was 8.7%. As shown in Figure S8 (SM-S8), nonspecific responses using diluted serum at 20% were typically < 14% of the specific response.

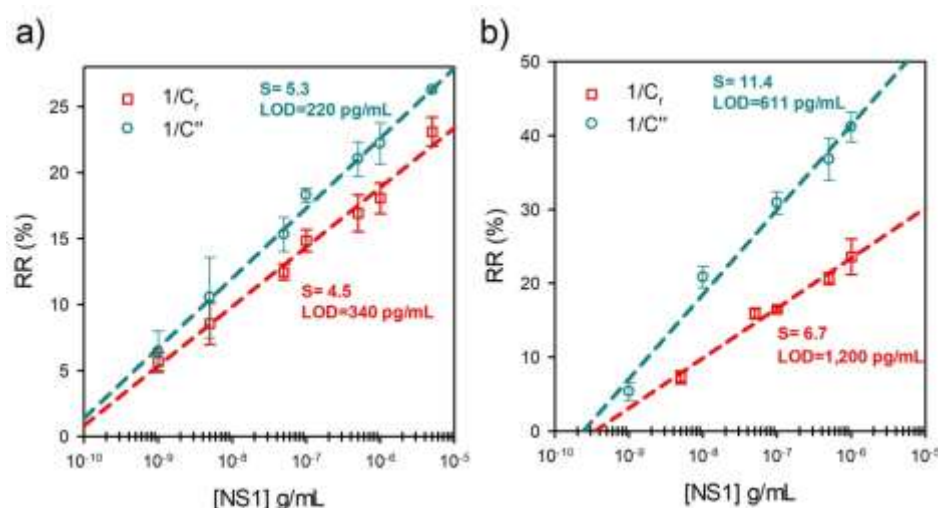


Figure 4. a) Analytical plot obtained for NS1 detection in PBS and (b) 20% serum. In red square, data obtained using $1/C_r$ as signal transduction, while in dark cyan circles for $1/C''$. The sensibilities (S) and limits of detection (LOD) are mentioned in the plots. The correlation coefficient (i.e. Pearson, R) shown a strong positive linear dependence of the transduction signal in function of logarithm of NS1 concentration ($R > 0.98$), with correspondent coefficient of determination $R^2 > 0.98$.

The same approach was used to detect NS1 in diluted human serum at 20%. The standard curve is shown in Figure 4b (red squares) and the analytical parameters are summarised in Table 1. The methodology reported here presents a clinical relevant linear range of target concentration from 5-1,000 ng/mL; NS1 is present in patients in concentrations from 40-2,000 ng/mL in primary and 10-2,000 ng/mL in secondary infections, respectively. In addition, the associated LOD of 1.2 ng/mL is a lower value than previously reported by traditional impedimetric approaches.[26]

Table 1. Analytical parameters obtained for NS1 and IgG (anti-NS1) assays in PBS and diluted serum (1:4) by ECS ($1/C_r$) and $1/C''$ (at 100 Hz). All measurements were carried out in triplicate.

Target	Approach	Assay condition	Linearity (ng/mL)	Sensitivity (% per decade)	LOD (pg/mL)	RSD* (%)	R^2
NS1	$1/C_r$	PBS	1-5,000	4.5	340	8.7	0.987
		Diluted serum	5-1,000	6.7	1,200	7.0	0.983
	$1/C''$	PBS	1-5,000	5.3	220	11.3	0.995
		Diluted serum	1-1,000	11.4	611	9.4	0.987
IgG	$1/C_r$	PBS	1-1,000	6.3	231	7.0	0.975
		Diluted serum	10-1,000	16.9	6,100	5.0	0.965
	$1/C''$	PBS	1-1,000	6.3	393	3.4	0.995
		Diluted serum	10-1,000	15.7	9,500	4.2	0.961

*Average of standard deviation calculated for data points obtained for the plot of the analytical curve.

3.3 IgG detection in PBS and diluted serum

In an extrapolation of this approach to surfaces constraining the NS1 antigen, anti-NS1 (IgG) assays can be developed. The resulting (Bode and Nyquist plots) are shown in SM-S9. Robust detection was possible across a log linear range of 1-1,000 ng/mL with a sensitivity of 6.3% per decade (Figure 5a) and LOD of 231 pg/mL. As shown in Figure S7 (SM-S7), the nonspecific responses are < 14% of specific response. In spiked dilute serum the relevant assay range is 10-1,000 ng/mL, with a LOD of 6,100 pg/mL.

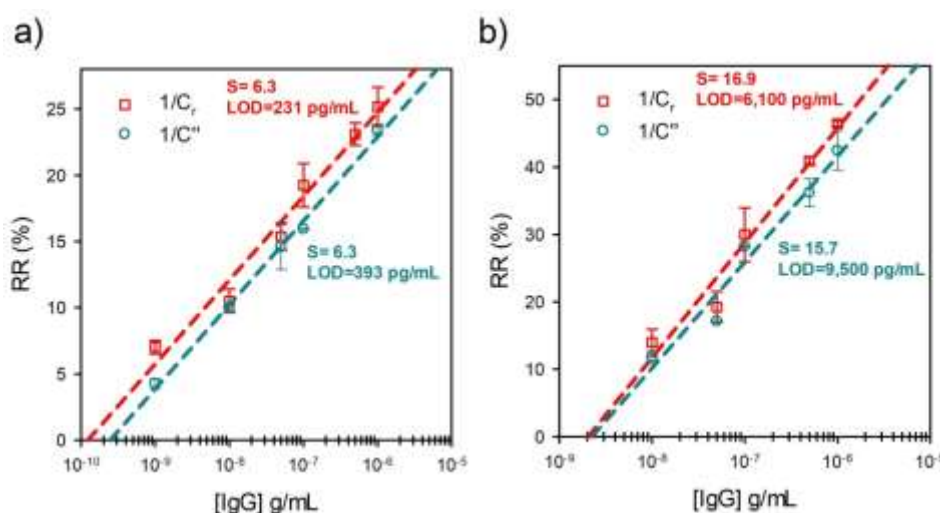


Figure 5. a) Representative analytical plots for IgG detection in PBS and (b) diluted serum at 20%. Data obtained using $1/C_r$ as signal transduction (red squares) and for $1/C''$ (dark cyan circles). The sensitivities (S) and limits of detection (LOD) are mentioned in the plots. The correlation coefficient (i.e. Pearson, R) shown a strong positive linear dependence of the transduction signal in function of logarithm of NS1 concentration ($R > 0.97$), with correspondent coefficient of determination $R^2 > 0.96$.

3.4 An alternative signal transduction for dengue detection: using $1/C''$ immittance function

Although the label free EIS-derived redox capacitance approach is, then, both sensitive and analytically well-behaved in clinically-relevant immunorecognition, assay times can be dramatically reduced by moving to an immittance platform (with no change in the interface or the experiment otherwise). In previous work, we have identified the inverse of imaginary capacitance as an effective transducer signal of molecular recognition at higher analytical frequencies of a redox tagged interface (~ 7 -100 Hz) when compared with purely capacitance analysis as described in the previous section. [32, 33] Accordingly, the optimized frequency found is of 100 Hz (Figure 6). The target-responsive data shown in Figure 6, for example, is acquired in <4 seconds at this frequency. The derived analytical data shown in Figures 4 and 5, and described in Table 1 (resolving likewise a

sensible association constant [$K_a = (8 \pm 2) \times 10^8$ L/mol, details in SM-S8] confirms the validity of the approach.

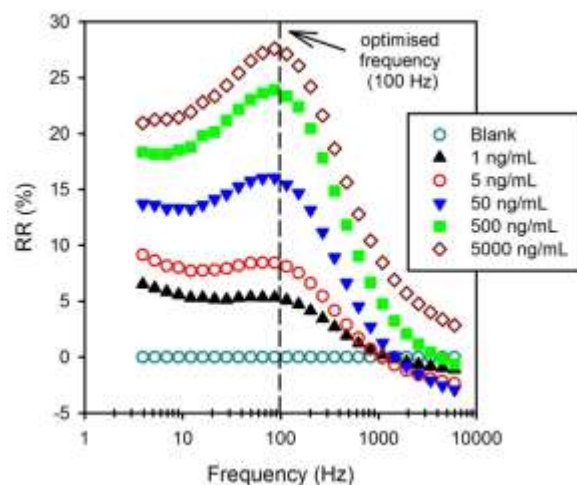


Figure 6. Example of $1/C''$ immittance function obtained for NS1 detection in PBS. The optimised frequency, 100 Hz, corresponds to the sensitivest condition with $R^2 > 0.96$.

4. Conclusions

New methodologies for dengue detection are required since the standard methods typically suffer from long data acquisition time and the need for skilled personnel. Standard impedimetric approaches, although label free and sensitive, require an amplifying pre-doping of each analytical solution with redox probe and the acquisition of data across a broad range of frequencies prior fitting to an equivalent circuit representing the electrical response of the interface. Redox capacitive methods are cleaner, equivalently sensitive and, as presently shown, can be additionally improved in such a way that acquisition times are significantly reduced. Accordingly, here we demonstrated that both dengue primary antigen and antibodies are reliably assayed in serum across clinically relevant ranges in *a few seconds*. This study is readily extrapolated to include the real-time assessment of additional markers and represents, we believe, a practically relevant contribution to dengue diagnostics.

Acknowledgement

The authors thank Royal Society of Chemistry, FAPESP (Sao Paulo State Research Foundation) and CAPES.

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Highlights

Redox tethered was used to generate transducer signal.

The redox capacitive transducer signal concept was applied.

NS1 and IgG proteins were successfully detected in low concentrations.

The immittance function concept was used to optimize signal analysis within seconds.