Journal of Colloid and Interface Science 362 (2011) 517–523

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

# Diagnosis of dengue infection using a modified gold electrode with hybrid organic–inorganic nanocomposite and *Bauhinia monandra* lectin

Cesar A.S. Andrade [a,*], Maria D.L. Oliveira [b], Celso P. de Melo [c], Luana C.B.B. Coelho [b], Maria T.S. Correia [b], Maurício L. Nogueira [d], Pankaj R. Singh [e], Xiangqun Zeng [e]

[a] *Centro Acadêmico de Vitória, UFPE, 55608-680, Vitória de Santo Antão, PE, Brazil*
[b] *Departamento de Bioquímica, UFPE, 50670-901 Recife, PE, Brazil*
[c] *Departamento de Física, UFPE, 50670-901 Recife, PE, Brazil*
[d] *Departamento de Doenças Infecciosas e Parasitárias, FAMERP, 15090-000 São Jose do Rio Preto, SP, Brazil*
[e] *Department of Chemistry, Oakland University, 2200 Squirrel Road, Rochester, MI 48309, USA*

ARTICLE INFO

*Article history:*
Received 30 May 2011
Accepted 2 July 2011
Available online 3 August 2011

*Keywords:*
Impedance spectroscopy
Cyclic voltammetry
Dengue
Lectin
*Bauhinia monandra*

ABSTRACT

A sensitive and selective biosensor for dengue serotyping was successfully developed. The biosensor uses a novel gold nanoparticles-polyaniline hybrid composite (AuNpPANI) for the immobilization of *Bauhinia monandra* lectin (BmoLL). The nanocomposite was applied to a bare gold electrode surface by chemical adsorption, and BmoLL was subsequently electrostatically adsorbed to the nanocomposite-modified surface. Atomic force microscopy (AFM), cyclic voltammetry (CV) and electrochemical impedance (EI) techniques were applied to evaluate the immobilization of BmoLL on AuNpPANI. The AFM images for AuNpPANI-BmoLL-DEN systems indicate a homogenous, compact and dense film of the conjugate. In the EI analyses, an obvious difference of the electron transfer resistance between the AuNpPANI-modified electrode and the bare gold electrode was observed. Among three dengue serotypes studied, dengue serotype 2 (DEN2) has higher values for $R_{CT}$, and lower values for both $n$ and $Q$. These are indications of a larger blocking effect and smaller capacitive dispersion, resulting from the higher agglutination of glycoproteins from the DEN2 sera. The selective BmoLL recognition for various dengue serotypes may be attributed to different patterns of glycoproteins in the sera produced by the glycoprotein immunoresponse from patients infected by the dengue virus.

© 2011 Elsevier Inc. Open access under the Elsevier OA license.

## 1. Introduction

Dengue viral diagnostics are typically performed by bioassays such as the growth of the virus in a cell culture from a specimen taken from the patient, detection of virus-specific antibodies in the blood, detection of virus antigens, detection of virus nucleic acids, examination of virus particles by electron microscopy and hemagglutination assay. However, these methods have been either too complex, time-consuming, lacking the necessary sensitivity and specificity or too expensive to be widely deployed. Thus, diagnostic methods that can identify the disease reliably and rapidly, and subsequently treat dengue virus infection at an early stage are urgently needed [1].

The dengue virus nonstructural glycoprotein, NS1, may present with different molecular mass, depending on the glycosilation. Some data suggest conflicting results for the use of this protein as a potential dengue hemorrhagic fever (DHF) predictor [2]. On the other hand, the level of some cytokines and the soluble vascular cell adhesion molecule 1 (VCAM-1) in blood samples may indicate a pattern for dengue fever (DF) and DHF recognition [3]. VCAM-1 is expressed on the surfaces of endothelial cells following stimulation with endotoxin and cytokines such as IL-1, TNF, IL-4, and IL-13 [3], as well as in dengue infections. Recent studies [3] have shown that the VCAM-1, a highly conserved glycoprotein produced in both membrane-associated and secreted forms and abundant in the serum of patients in the early stages of the immunoresponse of dengue virus infection may be an appropriate marker of acute and hemorrhagic dengue virus infection.

Lectins are carbohydrate-binding proteins of nonimmune origin and are involved in numerous cellular processes according to their characteristic structure and common interaction principles [4]. Previous studies of our group demonstrated the electrochemical response of the Concanavalin A (Con A) modified electrode for evaluation of a biosensor response for serum glycoproteins from patients infected by the dengue virus. This study revealed different responses for the DHF and DF sera [5]. Thus, we hypothesize that naturally occurring lectins can be used to detect serum glycoproteins immunoresponse from patients infected by the dengue virus. In the present work, the *Bauhinia monandra* (BmoLL),

* Corresponding author. Fax: +55 81 3523 3351.
*E-mail address:* csrandrade@gmail.com (C.A.S. Andrade).

0021-9797 © 2011 Elsevier Inc. Open access under the Elsevier OA license.
doi:10.1016/j.jcis.2011.07.013

a galactose-specific lectin, purified and characterized from leaves of *B. monandra Kurz* obtained from the northeast region of Brazil [6–8], was shown to be able to detect the dengue virus using electrochemical impedance readout that is sensitive, rapid and serotype-specific.

Studies by electrochemical impedance spectroscopy (EIS) demonstrate that proteins do not interact significantly with a gold electrode surface, not even through nonspecific interactions [9]. Thus, the introduction of accessible cysteine residue by genetic engineering techniques has served as a valuable method to immobilize proteins onto a metal electrode surface in a controlled manner [10]. Genetic engineering techniques frequently include expensive reagents and time-consuming procedures. New immobilization schemes and advanced sensing materials are therefore highly valued for improving the analytical capabilities of biosensing devices.

Because of the large specific surface area of nanoparticles, excellent conductivity and surface charge existing in the gold nanoparticles-polyaniline hybrid composites (AuNpPANI), BmoLL can be easily immobilized with high density and high activity. Polyaniline (PANI) reveals redox functions only in acid media ($pH < 4$) [11] a feature that limits its broad use, specifically in combination with biomaterials. However, BmoLL is stable in this pH range and has a negative surface charge [12].

In this work, a simple method of wet chemistry was developed to obtain a AuNpPANI with SH-terminal groups on its surface. The $H^+$ ion from $HAuCl_4$ leads to the formation of anilinium cations ($PhNH_3^+$) and this involves the generation of a radical cation accompanied by the release of an electron. $[AuCl_4]^-$ acts as the oxidizing agent and is capable of oxidizing $PhNH_3^+$ [13] (Fig. 1), resulting in polyaniline (oxidative product). Some authors have obtained similar AuNpPANI composites [13,14]. Up to now, no AuNpPANI systems with SH-terminal groups on the composite surface have been obtained. In our work, we stabilized the nanocomposite by 3-mercaptopropyl-trimethoxysilane (MPTS). MPTS was applied, due to the strong coordination of a mercapto group to Au to obtain thiol-capped gold nanoparticles. Subsequently, we promoted the hydrolysis of MPTS [15] to obtain thiol groups on the surface of AuNpPANI composites. AuNpPANI composites can thus be chemically absorbed onto metal electrodes.

Our biointerface design shown in Fig. 1 is especially suitable for electrochemical impedance based biosensors in which a semi-conductive biointerface is preferred to obtain a wide dynamic range and sensitivity [5,16]. In addition, EIS is an effective technique for the development of biosensors for real samples [17,18]. Atomic force microscopy (AFM), cyclic voltammetry (CV) and electrochemical

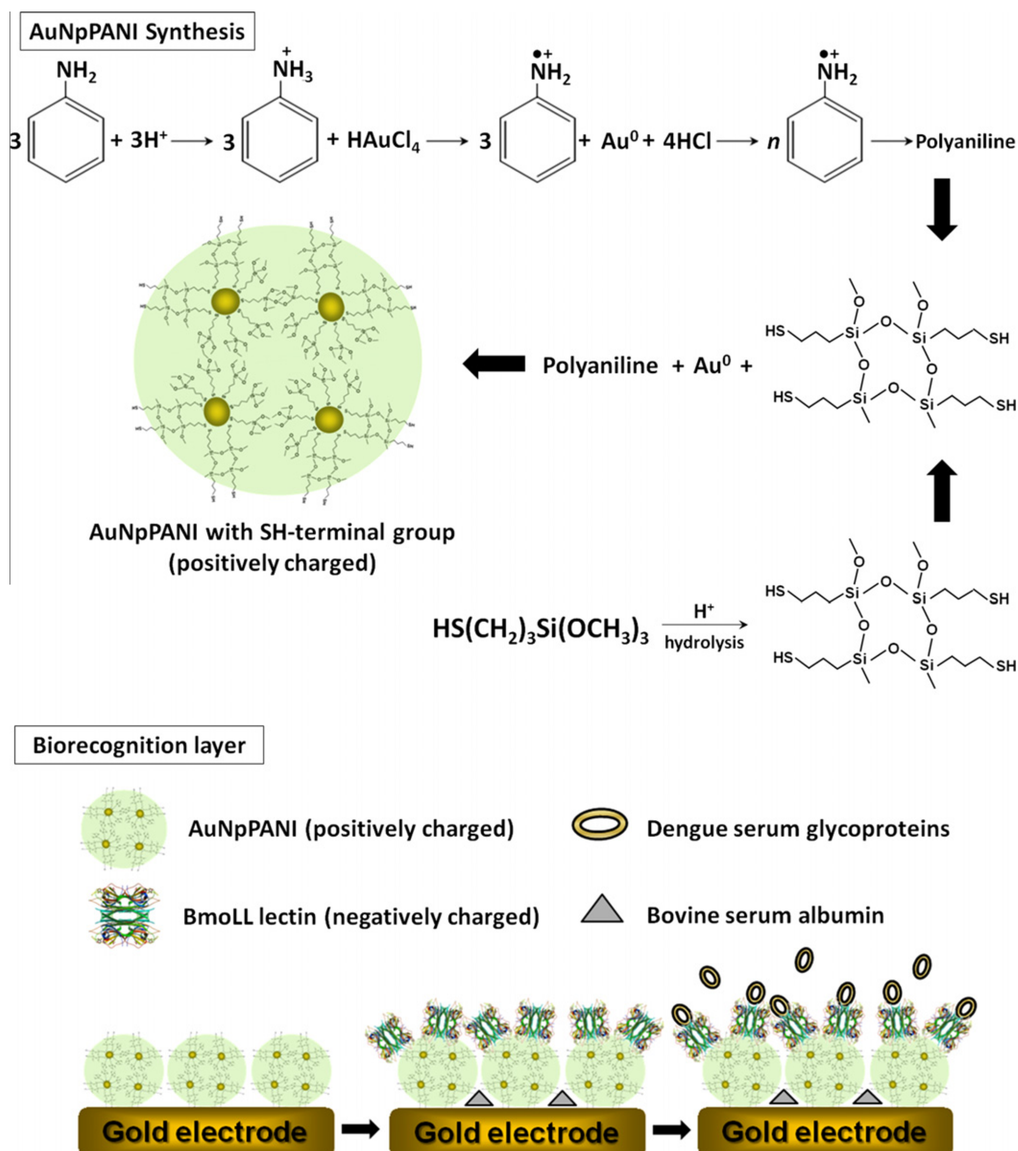


**Fig. 1.** Mechanism for the reaction of aniline with chloroauric acid trihydrate and schematic representation of the AuNpPANI-BmoLL-BSA-DEN biosensor system.

impedance spectroscopy (EIS) techniques were used to investigate the immobilization of BmoLL on AuNpPANI to evaluate its interaction with the patients' sera infected by dengue serotypes 1, 2, and 3 (DEN1, DEN2, and DEN3).

## 2. Materials and methods

### 2.1. Materials

BmoLL lectin was purified by one of us (Coelho, LCBB) [6] from the UFPE's Laboratory of Glycoproteins. The dengue sera were obtained from the Laboratory of Virology/FAMERP stocks. In this work we used the sera of patients contaminated with dengue serotypes 1, 2 and 3 (three patients for each serotype) and serum control (three patients). All sera were previously characterized as dengue negative (serum control) and dengue positive (serotypes 1, 2 and 3) using RT-PCR [19]. Aniline was purchased from Vetec (Brasil) and only used after distillation. $HAuCl_4{\cdot}3H_2O$, 3-mercaptopropyl-trimethoxysilane (MPTS), bovine serum albumin (BSA) and potassium ferricyanide were purchased from Sigma Chemical (St. Louis, MO, USA). Potassium ferrocyanide was obtained from Merck. Phosphate buffer saline (PBS) pH 7.2 was purchased from Gibco (Carlsbad, CA, USA).

### 2.2. Preparation of AuNpPANI composites

Hybrid organic–inorganic composites were obtained according to our early work [20]. The preparation of AuNpPANI was performed in a round bottom glass flask containing ethanol (20 mL) and the compounds aniline (0.030 mol/L), MPTS ($6.46 \times 10^{-2}$ mol/L), and $HAuCl_4{\cdot}3H_2O$ (0.81 mmol/L), which were subsequently added and submitted to agitation (1100 rpm) for 48 h. Thus, the samples were centrifuged for 10 min at 12,000 RPM (to remove large aggregates), and 500 μL of 0.1 M HCl was subsequently added to obtain positively-charged PANI and promote the hydrolysis of MPTS [15].

### 2.3. Electrode surface modification

The gold electrode surface was freshly polished prior to its use with 0.05 μm α-$Al_2O_3$ paste, and rigorously rinsed with doubly distilled water following each polish. Next, the electrode was immersed in freshly prepared Piranha solution (30% $H_2O_2$ and concentrated $H_2SO_4$, 3:1 v/v) for 5 min. The polished electrode was then cleaned ultrasonically sequentially in water for 5 min. After being sonicated in deionized water, the electrode was electrochemically pretreated by cyclic potential scanning between 1.4 and −0.2 V in 0.2 M $H_2SO_4$ until a cyclic voltammogram of clean gold electrode was obtained. BmoLL solution (0.3 mg/mL) was mixed with 300 μL of AuNpPANI composite in a beaker and kept under stirring for 10 min. The AuNpPANI-BmoLL-modified electrode was obtained by dropping AuNpPANI-BmoLL solution onto the bare gold electrode surface at 25 °C for 2 min. Finally, the AuNpPANI-BmoLL-modified electrode was rinsed with water to remove unbound lectin and incubated in a PBS solution containing 0.2% BSA for 20 min at 25 °C in order to block the remaining active sites. The AuNpPANI-BmoLL-BSA-modified electrode was exposed to negative serum control and serum from patients infected by dengue serotype 1, 2 and 3 diluted in 10 mM pH 7.4 PBS solution (dilutions of 1:25, 1:50, 1:75, 1:100 and 1:150) for 10 min at room temperature.

### 2.4. Atomic force microscopy measurements

Atomic force microscopy (AFM) measurements were performed with a commercial PicoPlus microscope (Molecular Imaging, USA). Cantilevers with a Cr–Au tip (NSC18, MikroMasch, $F_0$ = 90 KHz, nominal spring constant = 5.5 N m$^{-1}$) were used for the tapping mode AFM in air at room temperature (approximately 25 °C) [21]. Scan areas varying from 0.5 μm × 0.5 μm with a resolution of 512 × 512 pixels were obtained. The AFM Gwyddion software was used to analyze the recorded scans [22]. HOPG substrate was obtained from Advanced Ceramics Corp. (Grade ZYH, pyrolytic Graphite Monochromator). To eliminate artifacts, images were obtained from at least two macroscopically separated areas on each sample.

### 2.5. Electrochemical measurements

Electrochemical measurements were carried out on a PARSTAT 2263 (Princeton Applied Research, USA) potentiostat. The impedance spectra were recorded in the frequency range of 100 mHz to 100 kHz. All tests were conducted on an open circuit potential, and a single modulated AC potential of 10 mV was applied. Impedance measurement was performed in the presence of a 10 mM $K_4[Fe(CN)_6]^{4-}/K_3[Fe(CN)_6]^{3-}$ (1:1) mixture as a redox probe in 10 mM PBS solution containing 0.15 M NaCl [23]. CV measurements were performed in a conventional electrochemical cell containing a three-electrode setup with an Ag/AgCl (saturated KCl) reference electrode and swept the potential between −0.3 and +0.7 V with a scan rate of 80 mV s$^{-1}$. A platinum wire and a modified gold disk ($d$ = 2 mm) were used as auxiliary and working electrodes, respectively. All measurements were performed in triplicate.

## 3. Results and discussion

### 3.1. AFM characterization

Fig. 2 demonstrates AFM images of the AuNpPANI before (a) and after (b) BmoLL interaction, respectively. From Fig. 2a well-defined AuNpPANI particles are clearly seen on the surface and are well isolated from each other, owing to the electrostatic repulsion. The AuNpPANI particles formed were in the range of ~45 nm and height of 16 nm, resulting in a large surface area and easier attachment of BmoLL molecules. After BmoLL interaction, the diameter of the AuNpPANI slightly increased, indicating that BmoLL molecules were immobilized on the surface of AuNpPANI.

The AFM images for AuNpPANI-BmoLL-DEN systems indicate a homogenous, compact and dense film of the conjugate. Thus, BmoLL acts as a binding protein to the sensing glycoprotein, which anchors very well to the AuNpPANI sensor surface. Regardless of the dengue serotype, on adsorbing glycoproteins onto the AuNpPANI-BmoLL sensor layer, the AuNpPANI-BmoLL was covered by a fine structured film with a thickness ranging from 2 to 15 nm (Fig. 2c–e). However, DEN3 (Fig. 2e) and negative control (Fig. 2f) presented the weakest adhesion to AuNpPANI-BmoLL, with lower values for height than DEN1 (Fig. 2a) and DEN2 (Fig. 2b).

### 3.2. Electrochemical characteristics of AuNpPANI-BmoLL-DEN system

The stepwise modification on the gold electrode is accompanied by a decrease in the amperometric response and increase in the peak-to-peak separation between the cathodic and anodic waves of the redox probe. This is consistent with the hindering of the electron-transfer barriers introduced on assembly of these layers. In particular, after interactions with DEN2 an obvious disappearance

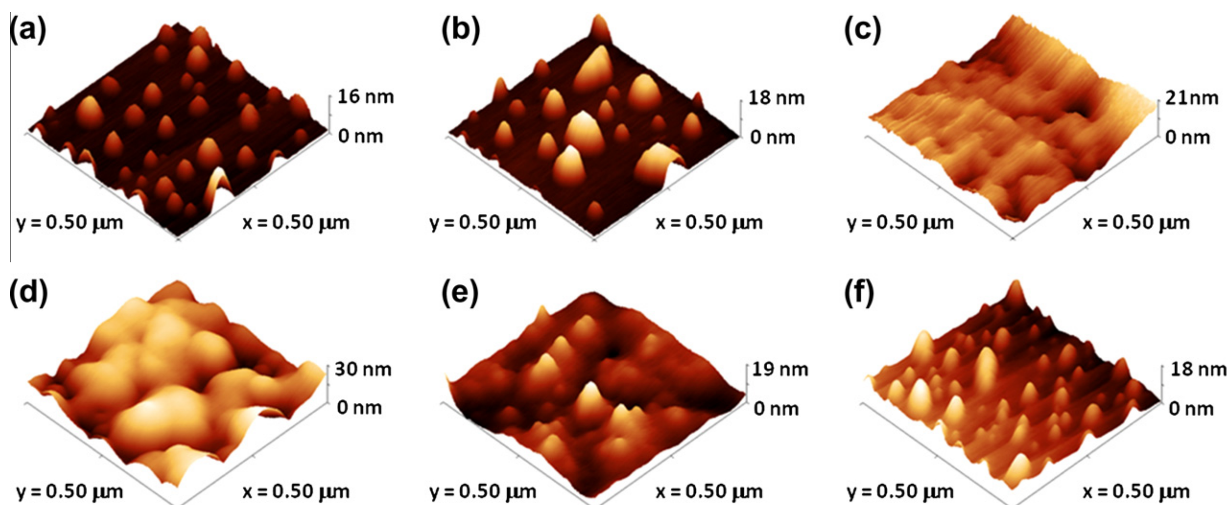


**Fig. 2.** An AFM topographic image of the HOPG surface modified with AuNpPANI (a), AuNpPANI-BmoLL (b), AuNpPANI-BmoLL-DEN1 (c), AuNpPANI-BmoLL-DEN2 (d), AuNpPANI-BmoLL-DEN3 (e) and AuNpPANI-BmoLL-DENnegative (f). The dilution used of dengue serotypes was 1:50. All scan areas were varied from 0.5 μm × 0.5 μm with a resolution of 512 × 512 pixels.

of the anodic and cathodic peaks was obtained, probably through a specific interaction between lectin-glycoproteins present in tested serum. The reason is that the lectin-glycoprotein complex acts as the inert electron and mass-transfer blocking layer and hinders the diffusion of ferricyanide toward the electron surface [16].

### 3.3. Characterization of surface assembly and recognition of glycoproteins from dengue serotypes by EIS

Faradaic impedance spectra were observed during the stepwise modification process (Fig. 3). The bare gold electrode reveals a very small semicircle domain, indicating a very low electron-transfer resistance $R_{CT}$. After the electrode was modified with AuNpPANI composite, the $R_{CT}$ was increased, due to the deposition of nanoparticles on the electrode surface with SH-terminal groups, which acts as an electrostatic barrier that restricts the ability of the redox probe to access the layer and retards the electron transfer kinetics between the redox probe and the electrode. BmoLL lectin molecules electrostatically combined with AuNpPANI again increased the $R_{CT}$, indicating that the AuNpPANI-BmoLL system obstructed the electron transfer of the electrochemical probe. To block remaining sites, the AuNpPANI-BmoLL-BSA system was obtained and a new increase in the $R_{CT}$ was observed (Fig. 3).

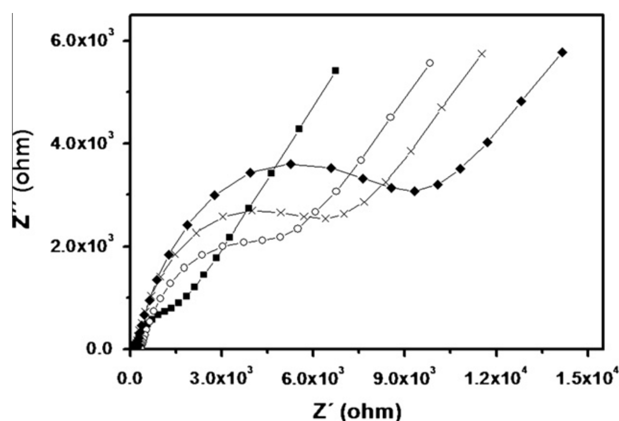


**Fig. 3.** Nyquist plots for the stepwise modification process: bare gold electrode (■), AuNpPANI (○), AuNpPANI-BmoLL (×) and AuNpPANI-BmoLL-BSA (♦). Supporting electrolyte 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ 1:1 containing 0.15 M NaCl in PBS solution pH 7.4.

Further optimized experimental factors include the dilution of dengue serum. Five different concentrations of dengue serotypes (1:25, 1:50, 1:75, 1:100 and 1:150) were tested (Fig. 4). We observed higher values for the dielectric response for DEN2 at different concentrations. However, significant differences were obtained for DEN2, suggesting a specific interaction between BmoLL lectin-glycoproteins at the modified electrode surface. The AuNpPANI-BmoLL system has a higher sensitivity for the detection of dengue glycoproteins, and the detection limit of dengue glycoproteins in serum reaches a dilution as low as 1:150. However, the low dengue serum dilution (1:25) might create a blockage of the AuNpPANI-BmoLL-modified electrode.

The impedance data were fitted with a Boukamp non-linear least square fitting program [24], using the modified Randles equivalent circuit (inset of Fig. 5), which includes the solution resistance ($R_{\Omega}$), electron transfer resistance ($R_{CT}$), constant phase element ($Q$) and Warburg impedance element ($W$). After the circuit description code has been specified and the starting values have been entered, the frequency range of the data is displayed. Weight factors were obtained based on the Nyquist graphical data, hence the frequency range selected for analysis by the NLLS-fit was 100 mHz to 100 kHz, while maintaining the parameters fixed in the fit procedure. We selected the value of 400 Ω for $R_{\Omega}$, and the value of $2.4 \times 10^3$ Ω for $R_{CT}$. The $Q$ value was $10^{-6}$ F and the value of $n$ was equal to 0.5, and finally the fit procedure was performed and for the Warburg element we adopted the value of $10^{-4}$. All analyses are used with high confidence data to start with a fast analytical fit procedure (lambda is then set to zero).

Ideally, $W$ and $R_{\Omega}$ represented the bulk properties of the electrolyte solution and diffusion features of the redox probe in solution and are thus not affected by modifications of the electrode surface. The $Q$ value depends on the dielectric constant of the layer separating the ionic charges and the electrode surface, surface area of the electrode, and the thickness of the separation layer. The $R_{CT}$, depends on the insulating feature at the electrode/electrolyte interface. The changes in $R_{CT}$ were much greater than those in the other impedance components. Thus, $R_{CT}$ proved to be a suitable signal for sensing the interfacial properties of the biosystem prepared during all the assembly procedures.

Table 1 shows the equivalent circuit parameters of the fitting curves for the various steps of the dengue biosystem elaboration and the interaction between BmoLL and glycoproteins from dengue serotypes 1, 2 and 3. Our results revealed a different response

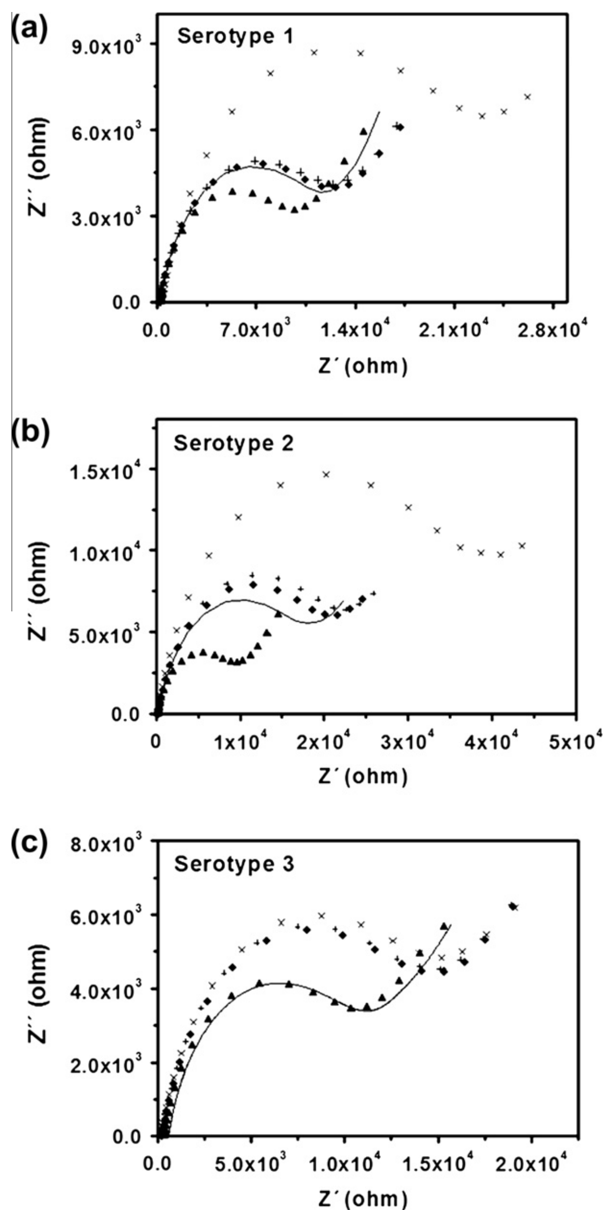


**Fig. 4.** Nyquist plots of AuNpPANI-BmoLL-BSA response after contact with DEN1 (a), DEN2 (b) and DEN3 (c) as follow: AuNpsPANI-BmoLL-BSA-$DEN_{(1:150)}$ (▲), AuNpsPANI-BmoLL-BSA-$DEN_{(1:100)}$ (-), AuNpsPANI-BmoLL-BSA-$DEN_{(1:75)}$ (♦), AuNpsPANI-BmoLL-BSA-$DEN_{(1:50)}$ (+), AuNpsPANI-BmoLL-BSA-$DEN_{(1:25)}$ (×). Supporting electrolyte 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ 1:1 containing 0.15 M NaCl in PBS solution pH 7.4.

for the each dengue serotype (1, 2 and 3) (Fig. 4). Optimum serum dilution was found to be 1:50 (v/v). Generalized observations of EIS spectra during the consecutive modification process for the AuNpPANI-BmoLL-BSA-DEN(1-3) system showed that after lectin recognition the values of $R_{CT}$ of equivalent circuit increased significantly, while reduction of $Q$ was observed simultaneously. Comments on the constant phase element parameters ($Q$ and $n$) would be very speculative. However, some remarks should be made concerning the variation of $n$. This parameter is close to 1 in most experiments, and close to 0.5 only for the systems with negative serum. This is an indication of some diffusional aspect of the charge transfer process at the interface. The EIS spectra demonstrate the higher $R_{CT}$ for serotypes 2, 1, 3, and negative serum.

The results presented in Fig. 3 clearly show that BmoLL was able to recognize glycoproteins in dengue serotypes with high specificity to dengue serotype 2, as can be seen in the increase in charge

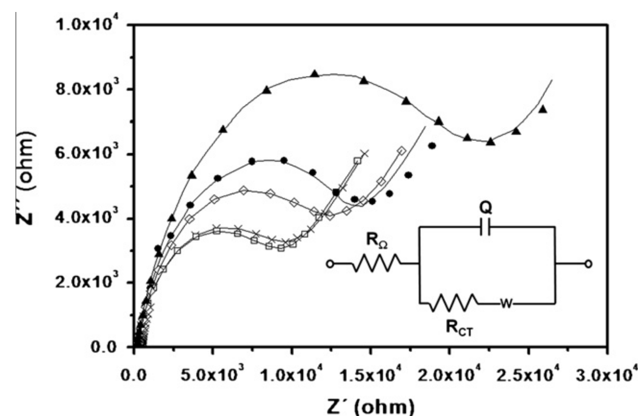


**Fig. 5.** Nyquist plot of the AuNpPANI-BmoLL-BSA (□), AuNpPANI-BmoLL-BSA-DENnegative (×), AuNpPANI-BmoLL-BSA-DEN3 (◇), AuNpPANI-BmoLL-BSA-DEN1 (●) and AuNpPANI-BmoLL-BSA-DEN2 (▲). Solid lines represent fitted data and scattered lines represent experimental data. Inset: modified Randles circuit. Supporting electrolyte 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ 1:1 containing 0.15 M NaCl in PBS solution pH 7.4. Serum dilution tested was 1:50 (v/v).

**Table 1**
Values of the equivalent circuit elements from fitted impedance result.

| Sample | $Q$ (μF) | $n$ | $R_{CT}$ (kΩ) | W × $10^{-4}$ |
|---|---|---|---|---|
| Gold bare electrode | 9.61 ± 0.04 | 0.77 ± 0.04 | 1.80 ± 0.11 | 1.69 ± 0.12 |
| AuNpPANI (2 min) | 2.48 ± 0.09 | 0.83 ± 0.06 | 1.99 ± 0.18 | 1.19 ± 0.11 |
| AuNpPANI-BmoLL | 3.10 ± 0.08 | 0.86 ± 0.07 | 4.36 ± 0.18 | 1.36 ± 0.10 |
| AuNpPANI-BmoLL-BSA | 2.98 ± 0.04 | 0.84 ± 0.09 | 8.02 ± 0.16 | 1.48 ± 0.13 |
| *AuNpPANI-BmoLL-BSA-Dengue serotype 1* | | | | |
| Dilution 1:25 | 2.14 ± 0.14 | 0.81 ± 0.10 | 20.64 ± 0.45 | 1.40 ± 0.09 |
| Dilution 1:50 | 3.14 ± 0.10 | 0.84 ± 0.09 | 12.60 ± 0.40 | 1.41 ± 0.09 |
| Dilution 1:75 | 3.24 ± 0.19 | 0.82 ± 0.13 | 10.80 ± 0.32 | 1.42 ± 0.07 |
| Dilution 1:100 | 3.70 ± 0.10 | 0.87 ± 0.10 | 9.75 ± 0.34 | 1.33 ± 0.08 |
| Dilution 1:150 | 4.57 ± 0.16 | 0.83 ± 0.11 | 9.07 ± 0.32 | 2.15 ± 0.06 |
| *AuNpPANI-BmoLL-BSA-Dengue serotype 2* | | | | |
| Dilution 1:25 | 1.81 ± 0.13 | 0.84 ± 0.11 | 34.70 ± 0.28 | 1.15 ± 0.10 |
| Dilution 1:50 | 2.61 ± 0.11 | 0.81 ± 0.11 | 19.70 ± 0.29 | 1.29 ± 0.13 |
| Dilution 1:75 | 3.45 ± 0.13 | 0.81 ± 0.10 | 18.40 ± 0.15 | 1.30 ± 0.12 |
| Dilution 1:100 | 3.57 ± 0.15 | 0.81 ± 0.08 | 16.10 ± 0.12 | 1.32 ± 0.12 |
| Dilution 1:150 | 3.63 ± 0.14 | 0.85 ± 0.09 | 8.20 ± 0.17 | 1.43 ± 0.09 |
| *AuNpPANI-BmoLL-BSA-Dengue serotype 3* | | | | |
| Dilution 1:25 | 2.59 ± 0.13 | 0.81 ± 0.09 | 13.60 ± 0.27 | 1.52 ± 0.15 |
| Dilution 1:50 | 3.15 ± 0.15 | 0.83 ± 0.11 | 10.90 ± 0.20 | 1.43 ± 0.14 |
| Dilution 1:75 | 3.34 ± 0.16 | 0.83 ± 0.10 | 12.50 ± 0.25 | 1.38 ± 0.11 |
| Dilution 1:100 | 3.49 ± 0.13 | 0.86 ± 0.07 | 10.00 ± 0.10 | 1.45 ± 0.10 |
| Dilution 1:150 | 4.02 ± 0.14 | 0.83 ± 0.13 | 8.61 ± 0.12 | 1.49 ± 0.12 |
| *AuNpPANI-BmoLL-BSA-Dengue negative* | | | | |
| Dilution 1:25 | 4.50 ± 0.21 | 0.76 ± 0.11 | 8.48 ± 0.31 | 1.55 ± 0.14 |
| Dilution 1:50 | 3.19 ± 0.24 | 0.82 ± 0.10 | 8.43 ± 0.32 | 1.44 ± 0.13 |
| Dilution 1:75 | 4.71 ± 0.20 | 0.74 ± 0.14 | 7.68 ± 0.33 | 1.62 ± 0.14 |
| Dilution 1:100 | 4.12 ± 0.21 | 0.78 ± 0.09 | 7.62 ± 0.30 | 1.45 ± 0.15 |
| Dilution 1:150 | 2.81 ± 0.20 | 0.86 ± 0.07 | 6.99 ± 0.31 | 1.53 ± 0.13 |

transfer, $R_{CT}$. The performance of the modified electrode for detection of dengue glycoproteins was evaluated through the relative variation of this parameter ($\Delta R_{CT}$). This variation can be calculated according to the following equation:

$$\Delta R_{CT}(\%) = \left(\frac{R_{CT}(\text{BmoLL} - \text{DENserotype}) - R_{CT}(\text{BmoLL})}{R_{CT}(\text{BmoLL})}\right) \times 100$$

where $R_{CT}$(BmoLL) is the value of the electron-transfer resistance of the AuNpPANI-BmoLL-BSA-modified electrode. $R_{CT}$(BmoLL-DENserotype) is the value of the electron-transfer resistance of the AuNpPANI-BmoLL-BSA-modified electrode after exposure to the patients' sera containing dengue serotypes 1, 2 and 3.

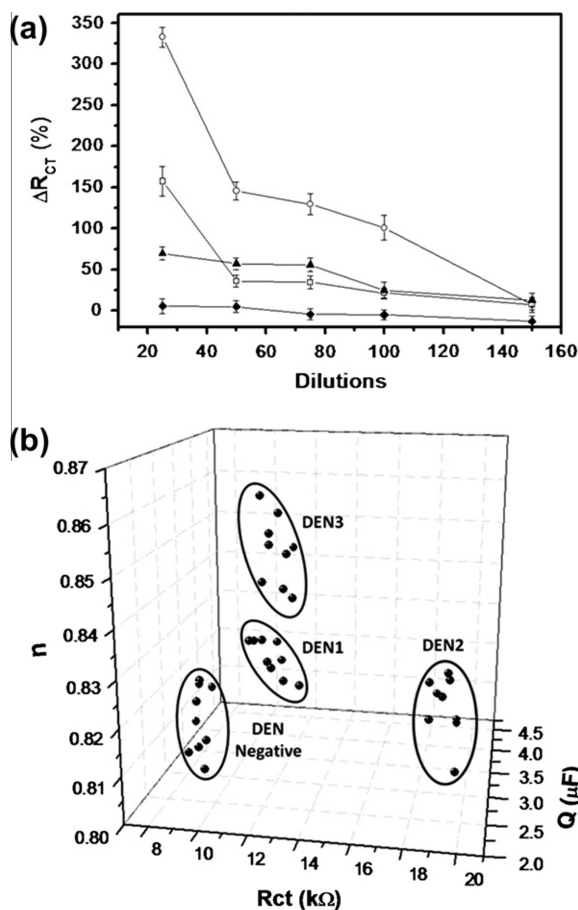


**Fig. 6.** $\Delta R_{CT}$% for the systems that corresponded to the AuNpPANI-BmoLL-BSA-DEN1 (□), AuNpPANI-BmoLL-BSA-DEN2 (○), AuNpPANI-BmoLL-BSA-DEN3 (▲) and AuNpPANI-BmoLL-BSA-DENnegative (♦) at different concentrations (1:25, 1:50, 1:75, 1:100 and 1:150) (a); and three-dimensional plot for values of $R_{CT}$, $Q$ and $n$ (b). The analyses of the sera of patients contaminated with dengue (three patients for each serotype) and serum control (three patients) were performed in triplicate.

The $\Delta R_{CT}$ clearly increases with serum concentration, indicating that the interactions between lectin and glycoproteins can be sensed by the modified electrode (Fig. 6a). This increase in $\Delta R_{CT}$ is different for the three serotypes, since the highest and most sensitive response was to DEN2. We observed very low changes in the electron-transfer resistance with experiments using negative dengue serum.

These results demonstrate that the BmoLL retains its capacity to recognize complex carbohydrates after adsorption and are a clear indication that this system could be applied to the detection for DEN2. It can be seen that $\Delta R_{CT}$ increased with the presence of glycoproteins in the blood serum, indicating lectin recognition. BmoLL lectin is specific for galactose sugar [6] and can be used as an affinity matrix to purify glycoproteins from human colostrums [25]. The values of $\Delta R_{CT}$, for fixed dilution of dengue serotypes (1:50), are highest for sera contaminated with dengue serotype 2 (145.60%), 1 (57.10%) and 3 (35.91%), respectively, indicating the capacity of BmoLL to recognize the glycoprotein pattern of these samples (Table 2). However, the higher $\Delta R_{CT}$ values obtained for DEN2, compared with the other serotypes, are statistically different at a 90% confidence level.

A three-dimensional plot with three variables, $R_{CT}$, $Q$ and $n$, is shown in Fig. 6b. It is clear that the data are distributed in the plot according to the dengue serum type to which they belong. The DENnegative sera results are located in the region of lower $R_{CT}$, $Q$ values, and n values, which corresponds to a smaller blocking effect of the biosensor surface associated with a smaller capacitive dispersion. The DEN1 and DEN3 sera results are distributed at intermediate values of $R_{CT}$ and higher values of $Q$ and n, indicating the intermediate dispersion and blocking of the biosensor surface. Finally, the data for DEN2 sera are located at higher values of $R_{CT}$, and at lower values of both $n$ and $Q$. This is an indication of a larger blocking effect and smaller capacitive dispersion, resulting from higher agglutination of glycoproteins from the DEN2 sera. Reproducibility of the biosensor system was evaluated from the $\Delta R_{CT}$ response for each dengue serotype at different biosensors and acceptable R.S.D. values of 5.70%, 2.33%, 5.28% and 1.99% for DEN1, DEN2, DEN3 and DENnegative, respectively, were observed. These results indicate the suitability of the AuNpPANI-BmoLL-BSA-DEN-modified biosensor to practical applications.

The BmoLL recognition for dengue serotypes may be attributed to different patterns of glycoproteins in the sera [16]. Patients with secondary DEN2 infection have significantly higher plasmatic levels of glycoproteins and interleukins [3]. The frequency of secondary DEN2 infection was higher in patients with DHF than in those with DF [26]. It is known that DHF-contaminated serum demonstrated a higher expression of plasmatic glycoproteins such as cytokines, NS1, ICAM-1, IFN$\alpha$ and IFN$\gamma$, and VCAM-1 involved in hemorrhagic events [3]. The higher values of charge transfer resistances and capacitances shown by DEN2 are involved in the higher levels of glycoproteins. To understand the higher blocking effect indicated by the DEN2, compared with DEN1 and DEN3, This effect may be associated with the high level of VCAM-1.

The presence of sialic acid in a terminal position of this protein plays an important role in its interaction, which is involved in the BmoLL interaction. Sialic acids are 9-carbon monosaccharide that link to the terminal galactose, N-acetylgalactosamine, or other sialic acids in carbohydrate chains that are attracted to glycoproteins or glycolipids [27,28]. VCAM-1 molecules are decorated with $\alpha$2-6-linked sialic acids [29]. In N-linked glycoproteins, sialic acids are attached to Gal$\beta$1-4GlcNAc or Gal$\beta$1-3GlcNAc with $\alpha$2-6 and $\alpha$2-3-linkages [30]. These aspects are important roles that account for the higher BmoLL interaction with sera contaminated with DEN2, owing to the BmoLL binding specificity. Studies with Con A lectin showed a different behavior with respect to processes for interaction with the studied glycoproteins present in the serum of patients with DF and DHF [16]. The results for Con A revealed a greater interaction with sera from patients with DF, who had lower levels of glycoproteins containing sialic acids residues rich in galactose. This process was involved in the ability of Con A to interact specifically with mannose and glucose residues.

Thus, the variable reaction of the modified electrode producing a different response among dengue serotypes 1, 2 and 3 is probably

**Table 2**
Relative charge transfer variation from impedance data.

| Condition | Before[a] (AuNpPANI-BmoLL-BSA) | After[1] DEN1 | After[2] DEN2 | After[3] DEN3 | After[4] DENneg |
|---|---|---|---|---|---|
| $R_{CT}$ (kΩ) | 8.02 | 12.60 | 19.70 | 10.09 | 8.43 |
| $\Delta R_{CT}$ (%) | – | 57.10 | 145.60 | 35.91 | 5.11 |

[a] Before contact with dengue serotypes and 1-after contact with dengue serotype 1, 2-after contact with dengue serotype 2, 3-after contact with dengue serotype 3 and 4-after contact with dengue negative serum.

be due to the ability of BmoLL detect distinct glycosylation patterns. As previously reported [16], the changes in metabolism and diseases reveal different glycosylation in secreted glycoproteins by infected cells [31,32], and our modified electrode may be useful as a biosensing system for DEN2 diagnosis. Nonetheless, the proposed system could also be useful for predicting and monitoring the course of the dengue disease, and hence help guide the prescription of preventive therapy in dengue patients in endemic areas of the world by detecting unusual carbohydrate structures by means of lectin [33].

## 4. Conclusions

The large specific surface area, excellent conductivity and surface charge of the AuNpPANI enable it to be used as a preeminent matrix for protein immobilization. BmoLL lectin adsorption onto AuNpPANI surfaces was mainly driven by the electrostatic interaction between the positively charged AuNpPANI and negatively-charged lectin residues. The AuNpPANI-BmoLL was able to detect glycoproteins from each of the three dengue virus serotypes with a high degree of specificity. Our results demonstrate that AuNpPANI-BmoLL interacts with glycoproteins of dengue serotype 2. AuNpPANI-BmoLL was used to manufacture a novel dengue glycoprotein biosensor. The biosensor exhibits excellent reproducibility and shows good selectivity for the detection of dengue glycoproteins in the sera samples. We believe that this study provides strong evidence that naturally occurring BmoLL lectin recognition for dengue serotypes may be attributed to different patterns of glycoproteins in the sera produced by the glycoprotein immunoresponse from patients infected by the dengue virus.

## Acknowledgments

The authors are grateful for the support provided by the Rede ELINOR de Nanobiotecnologia/CAPES and FACEPE. CASA would like to thank CAPES for a postdoctoral grant (BEX 3561/10-0) and XZ likes to thank NIH support 1R21EB006495.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcis.2011.07.013.

## References

[1] G. Bock, J. Goode, New Treatment Strategies for Dengue and other Flaviviral Diseases, Novartis Foundation, John Wiley & Sons Ltd., Chichester, UK., 2006.
[2] E.M.B. Lemes, M.P. Miagostovicsh, A.M.B. Alves, S.M. Costa, A.M.B. Fillipis, G.R.G. Armoa, M.A.V. Araujo, J. Clin. Virol. 32 (2005) 305.
[3] R.F. Chen, K.D. Yang, L. Wang, J.W. Liu, C.C. Chiu, J.T. Cheng, Trans. R. Soc. Trop. Med. Hyg. 101 (2007) 1106.
[4] N. Sharon, Trends Biochem. Sci. 18 (1993) 221.
[5] M.D.L. Oliveira, M.T.S. Correia, F.B. Diniz, Synth. Metals 159 (2009) 2162.
[6] L.C.B.B. Coelho, M.B.R. da Silva, Phytochem. Anal. 11 (2000) 295.
[7] M.L.R. Macedo, M.G.M. Freire, M.B.R. da Silva, L.C.B.B. Coelho, Comp. Biochem. Physiol., Part A 146 (2007) 486.
[8] V. Rosilio, M.M. Boissonnade, L.C.B.B. Coelho, N.S. Santos-Magalhaes, C.A.S. Andrade, A. Baszkin, Colloids Surf., A 250 (2004) 491.
[9] J. Wang, L.A. Luck, I.I. Suni, Electrochem. Solid-State Lett. 10 (2007) J33.
[10] F. Mizutani, Sens. Actuators, B 130 (2008) 14.
[11] C. Sheng-Yun, P. Su-Moon, Synth. Met. 105 (1999) 91.
[12] C.A.S. Andrade, A. Baszkin, N.S. Santos-Magalhães, L.C.B.B. Coelho, C.P. de Melo, J. Colloids Interface Sci. 289 (2005) 371.
[13] K. Mallick, M. Witcomb, M. Scurrell, A. Strydom, J. Phys. D 42 (2009) 095409.
[14] W. Qiu, H. Huang, S. Zeng, T. Xue, J. Liu, J. Polym. Res. 18 (2011) 19.
[15] H. Zhang, R. Liu, Q. Sheng, J. Zheng, Colloids Surf. B 82 (2011) 532.
[16] M.D.L. Oliveira, M.T.S. Correia, F.B. Diniz, Biosens. Bioelectr. 25 (2009) 728.
[17] A.G. Mantzila, C. Strongylis, V. Tsikaris, M.I. Prodromidis, Biosens. Bioelectr. 23 (2007) 362.
[18] M.I. Prodromidis, Electrochim. Acta 55 (2010) 4227.
[19] A. Mondini, R.V.M. Bronzoni, I.L.S. Cardeal, T.M.I.L. Santos, E. Lázaro, S.H.P. Nunes, G.C.D. Silva, M.C.F.S. Madrid, P. Rahal, L.T. Figueiredo, F. Chiaravalloti Neto, M.L. Nogueira, J. Clin. Virol. 40 (2007) 84.
[20] C.P. de Melo, C.G. dos Santos, C.A.S. Andrade, Fluorescent nanoparticle composites themselves, process for the preparation of such composites, and use in rapid diagnosis systems with affinity to biological molecules, Patent PCT/BR2009/000117, 2009.
[21] D.M.N. Luna, E.P.S. Falcao, S.J. Melo, C.A.S. Andrade, Colloids Surf. A 373 (2011) 22.
[22] D. Necas, P. Klapetek, C. Anderson, Gwyddion, <http://www.gwyddion.net> 2008.
[23] M.D.L. Oliveira, C.P. de Melo, O. Glaucius, C.A.S. Andrade, Colloids Surf., B 82 (2011) 365.
[24] B.A. Boukamp, Solid State Ionics 18–19 (1986) 136.
[25] E.G. Santana, Lectinas de sementes de Cratylia mollis (cramoll) e de folhas de *Bauhinia monandra* (Bmoll): imobilizações e aplicações biotecnológicas, PhD Thesis, Federal University of Pernambuco, Brazil, 2004.
[26] R.F. Chen, J.W. Liu, W.T. Yeh, L. Wang, J.C. Chang, H.R. Yu, J.T. Cheng, K.D. Yang, FEMS Immunol. Med. Microbiol. 44 (2005) 43.
[27] Y. Abe, C.W. Smith, J.P. Katkin, L.M. Thurmon, X. Xu, L.H. Mendoza, C.M. Ballantyne, J. Immunol. 1 (1999) 2867.
[28] A. Varki, FASEB J. 11 (1997) 248.
[29] K. Hanasaki, A. Varki, L.D. Powell, J. Biol. Chem. 31 (1995) 7533.
[30] S. Basu, M. Basu, S.S. Basu, in: A. Rosenberg (Ed.), Biological Specificity of Sialyltransferases, in Biology of the Sialic Acids, Plenum Press, New York, 1995.
[31] A. Azizan, J. Sweat, C. Espino, J. Gemmer, L. Stark, D. Kazanis, J. Virol. Methods 138 (2006) 211.
[32] T.S. Raju, J.B. Briggs, S.M. Borge, A.J.S. Jones, Glycobiology 10 (2000) 477.
[33] S. Sell, Human Pathol. 21 (1990) 1003.