Electrochemical Immunosensor based on Nanostructured Lanthanum oxide substituted reduced graphene oxide Interface for Ultralow Ciprofloxacin Detection in Spiked Milk Samples

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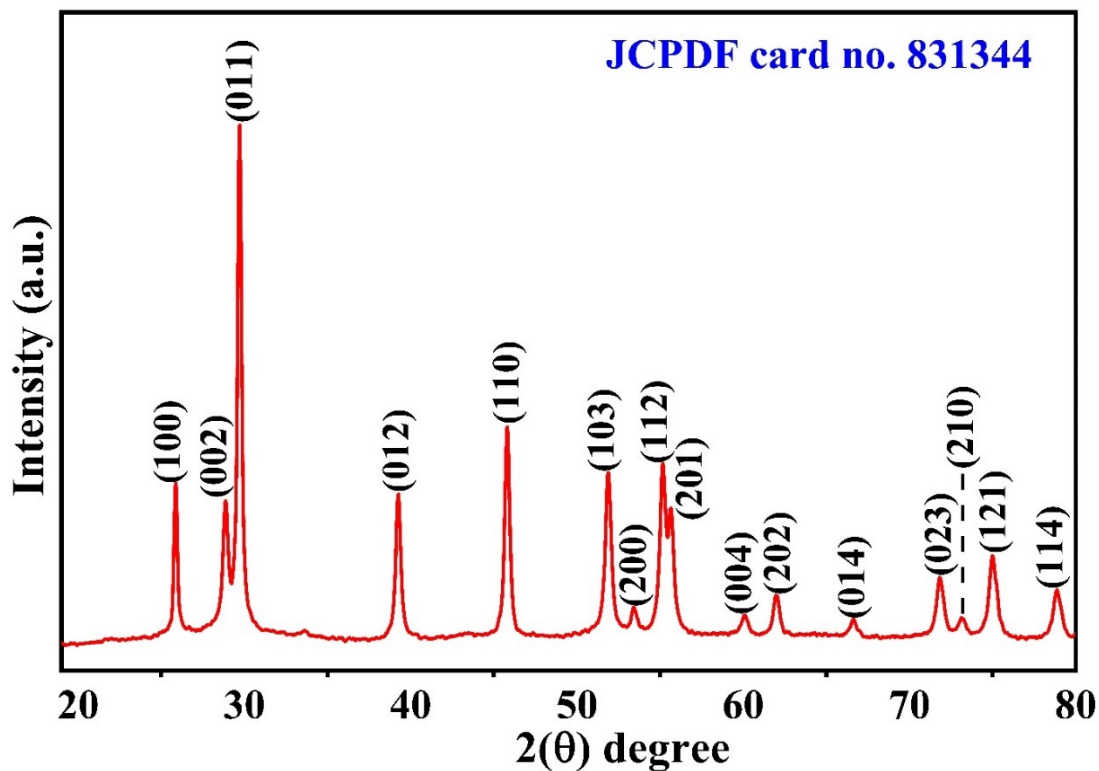**SUPPLEMENTARY INFORMATION**

XRD spectra of nLa$_2$O$_3$ NPs

Figure S1: XRD spectra of nLa$_2$O$_3$ NPs

Raman spectra of nLa$_2$O$_3$ NPs
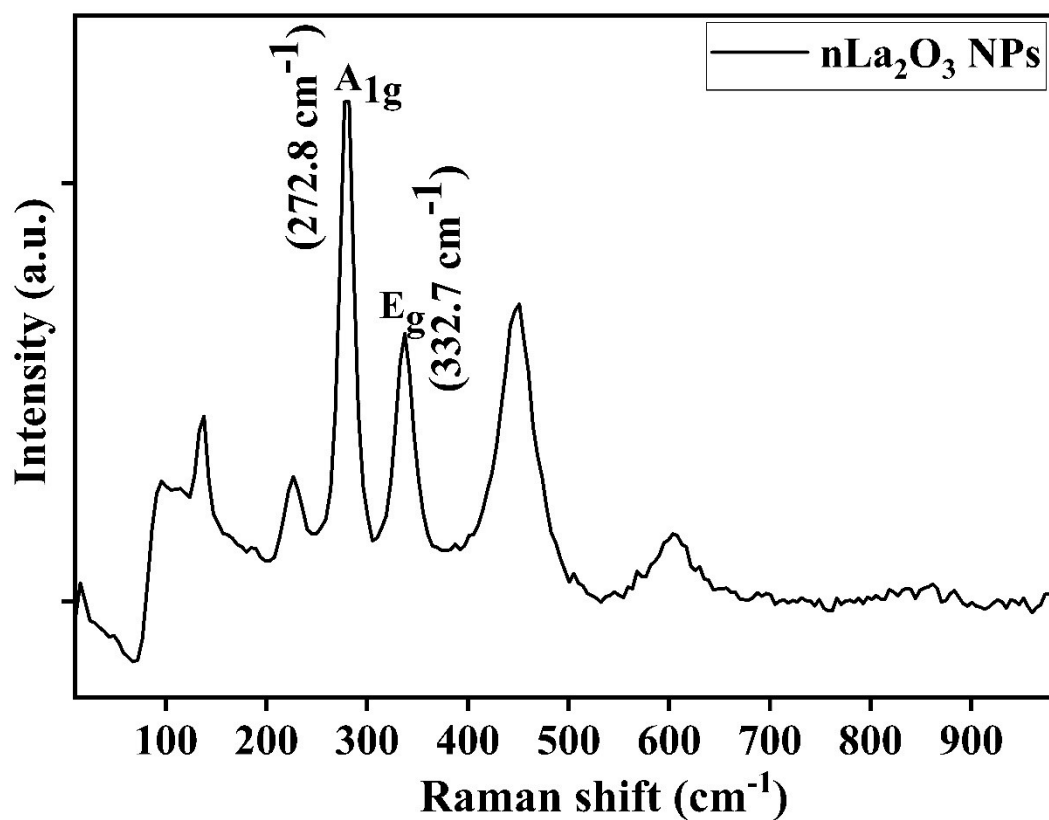
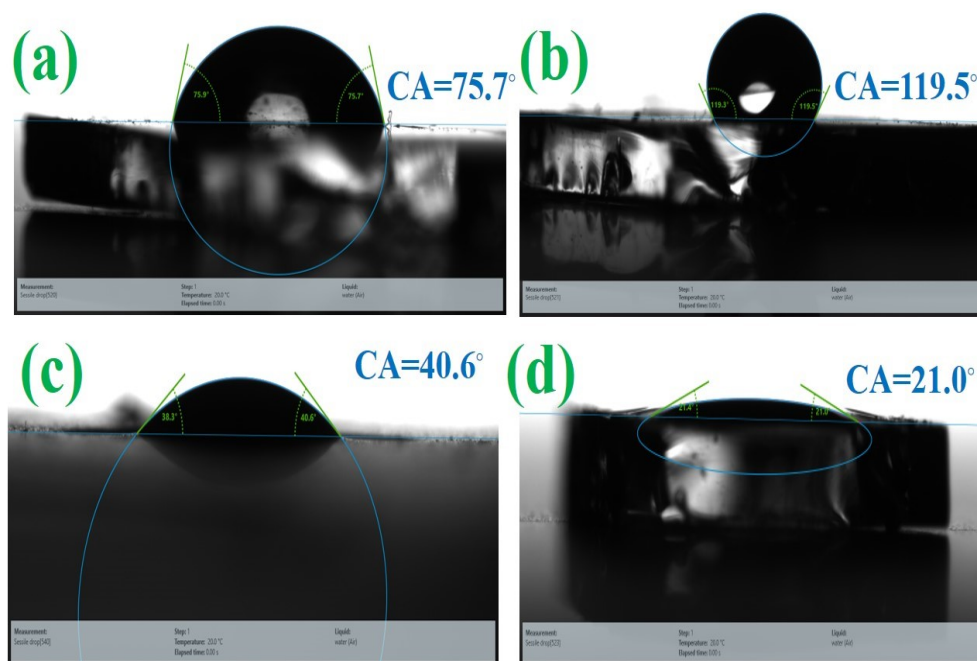**Figure S2:** Raman spectra of nLa$_2$O$_3$ NPs

Contact angle studies

Figure S3: Water contact angle of ITO (a), APTES/nLa$_2$O$_3$@rGO/ITO (b), anti-CPX/APTES/nLa$_2$O$_3$@rGO/ITO (c) and BSA/anti-CPX/APTES/nLa$_2$O$_3$@rGO/ITO (d).

EIS Studies:

The electrochemical impedance spectroscopic studies of ITO (curve i), APTES/nLa$_2$O$_3$@rGO/ITO (curve ii), anti-CPX/APTES/nLa$_2$O$_3$@rGO/ITO (curve iii) and BSA/anti-CPX/APTES/nLa$_2$O$_3$@rGO/ITO (curve iv) have been done in PBS solution containing 5 mM [Fe(CN)$_6$]$^{3-/4-}$ in the frequency range of 100 kHz to 10 Hz [Fig. 5(d)]. The semicircle of Nyquist plot gives the charge transfer resistance (R$_{ct}$) of electrode that depends on the dielectric features of electrode surface and electrolyte interface. The value of R$_{ct}$ was obtained as 249.35 Ω for ITO glass substrate (curve i), which is the highest. However there is significant decrease in the value of R$_{ct}$ 205.2 Ω after the deposition of APTES/nLa$_2$O$_3$@rGO nanocomposite onto ITO surface (curve ii), indicates the high electrical conductivity of nLa$_2$O$_3$@rGO nanocomposite and fast diffusion of redox species [Fe(CN)$_6$]$^{3-/4-}$ to the electrode surface. However, after the covalent immobilization of anti-CPX antibodies to the free -NH$_2$ sites of APTES functionalized nLa$_2$O$_3$@rGO nanocomposite, the value of R$_{ct}$ drastically decreases to 165.58 Ω for anti-CPX/APTES/nLa$_2$O$_3$@rGO/ITO (curve iii). This decrease in the value of R$_{ct}$ can be assigned to the spatial orientation of anti-CPX molecule onto electrode surface that facilitate increased charge transfer process between redox species [Fe(CN)$_6$]$^{3-/4-}$ and free available functional group (NH$_2$) of anti-CPX molecule (Kumar et al. 2015). The value of R$_{ct}$ increases (176.42 Ω; curve iv) after the BSA immobilization on the anti-CPX/APTES/nLa$_2$O$_3$@rGO/ITO immunoelectrode due to the macromolecular structure and insulating structure of BSA that hinder the charge transfer between redox species [Fe(CN)$_6$] and electrode surface.

The heterogeneous electron transfer rate constant (K$_{ct}$) for all respective electrodes was calculated using Eq.

$$Kct = \frac{RT}{n^2F^2ARct[S]}$$

Where R is gas constant, T is the absolute temperature, n is the no. of transferred electrons per molecule of the redox probe, F is Faraday constant, A is the surface area of the electrode (cm^2) and [S] is the concentration of redox probe. The K$_{ct}$ value of ITO electrode was found as 2.006 × 10^{-6} while in the case of APTES/nLa$_2$O$_3$@rGO/ITO the value of K$_{ct}$ increases to 3.044 × 10^{-6}

exhibiting the faster electron transfer between the APTES/nLa$_2$O$_3$@rGO/ITO electrode and redox species. After immobilization of anti-CPX, the value of K$_{ct}$ again increases to 4.329 × 10^{-6} shows the fast electron exchange between immunoelectrode and redox species. However, after the BSA immobilization on anti-CPX/APTES/nLa$_2$O$_3$@rGO/ITO immunoelectrode, the value of K$_{ct}$ decreases to 2.160 × 10^{-6}, due to its insulating nature which resist the electron transfer.

The Time constant (τ) for each electrode has been calculated using the given equation as below

$$R_{ct}.C_{dl} = 1 / 2\pi\ f_{max} = \tau$$

After the EPD of APTES/nLa$_2$O$_3$@rGO on ITO substrate there is abrupt decrease in value of time constant was found as compared to ITO glass substrate (8.938 x 10^{-4} s). It reveals the fast diffusion of [Fe(CN)$_6$]$^{3-/4-}$ ions at the interface of electrolyte and APTES/nLa$_2$O$_3$@rGO/ITO electrode. The immobilization of anti-CPX on to the APTES/nLa$_2$O$_3$@rGO/ITO result in decrease of τ value 2.716 x 10^{-4} s due to the fast diffusion of [Fe(CN)$_6$]$^{3-/4-}$ ions. After the BSA immobilization the value of τ again increases (2.724 x 10^{-4} s) due the insulating behavior of BSA.

Table S1. EIS features of various electrodes

Electrode	Charge transfer Resistance (R$_{ct}$)	Heterogeneous electron transfer rate constant [K$_{ct}$ (cms^{-1})]	Time constant (τ) s
ITO	249.35 Ω	2.006 × 10^{-6}	8.938 × 10^{-4}
APTES/nLa$_2$O$_3$ NPs@rGO/ITO	205.2 Ω	3.044 × 10^{-6}	5.631 × 10^{-4}
anti-CPX/APTES/nLa$_2$O$_3$ NPs@rGO/ITO	165.58 Ω	4.329 × 10^{-6}	2.716 × 10^{-4}
BSA/anti-CPX/APTES/nLa$_2$O$_3$ NPs@rGO/ITO	176.42 Ω	2.160 × 10^{-6}	2.724 × 10^{-4}