**Supporting Information for**

# A Zinc Oxide-Silver Nanocomposites-based SERS Nanoplatform for Ultrasensitive Ofloxacin Determination in Beef and an Ophthalmic Solution: Effects of ZnO and ZnO content on Electron Transfer and SERS enhancements

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**Calculation of limit of detection (LOD)**

The standard curve of linear detecting range was given as:

$$Y = A + B \times Log(X) \quad (1)$$

where A and B are intercept and slope of regression equation obtained through the plot of the logarithmic SERS intensity (Y) – logarithmic concentration (X).

The LOD is calculated using the following equation [1]:

$$LOD = 10^{\left[(Y_{blank} + 3SD)/Y_{blank} - A\right]/B} \quad (2)$$

where $Y_{blank}$ and SD are the SERS signal and the standard deviation of blank sample, respectively.

SD is calculated via the well-known formula:

$$SD = \sqrt{\frac{1}{n-1} \times \sum_{i}^{n} (x_i - x_{average})^2} \quad (3)$$

where $x_i$ if the "i" sample of the series of measurements, $x_{average}$ is the average value of SERS signal obtained from the blank sample repeated n times.

**Calculation of relative standard deviation (RSD)**

The RSD value of repeatability and reproducibility is calculated via the well-known formula:

$$\text{RSD} = \frac{SD \times 100}{x_{average}} \quad (4)$$

where SD is the standard deviation that calculates using equation 4 and $x_{average}$ is the average value of SERS signal obtained from each measurement.

**Calculation of enhancement factor (EF)**

The EF value is calculated according to the well-established equation, which was employed in several published studies [2, 3]:

$$EF = \frac{I_{SERS}}{I_{Raman}} \times \frac{N_{bulk}}{N_{surface}} \quad (5)$$

where $I_{SERS}$ and $I_{Raman}$ are Raman signal intensity of the analyte with and without SERS from the substrate, respectively; and $N_{bulk}$ is the number of analyte molecules that are probed on the Raman spectrum, while $N_{surface}$ is the number of analyte molecules probed using SERS.

$N_{bulk}$ can be calculated following:

$$N_{bulk} = \frac{A_{laser} \times h \times \rho}{M} \times N_A \quad (6)$$

where $A_{laser}$, h, ρ and m are the laser spot area, the focal length, the density of the solid analyte and its molecular weight, respectively; and $N_A$ is the Avogadro number.

$N_{surface}$ can be expressed as:

$$N_{surface} = \frac{C \times V}{A_{substrate}} \times N_A \times A_{laser} \quad (7)$$

where C, V, $A_{substrate}$ are the concentration, the volume drop-casted of the analyte, and the area of the substrate, respectively; $N_A$ is the Avogadro number; and $A_{laser}$ is the laser spot area.

Thus, EF can be calculated as:

$$EF = \frac{I_{SERS}}{I_{Raman}} \times \frac{N_{bulk}}{N_{surface}} = \frac{I_{SERS}}{I_{Raman}} \times \frac{h \times \rho \times A_{substrate}}{M \times C \times V}$$

(8)

In our case, $I_{SERS}$ and $I_{Raman}$ is Raman signal intensity with and without SERS substrate of Ofloxacin (1407 $cm^{-1}$), h = 2 μm = $2 \times 10^{-4}$ cm, ρ = 1.2688 (estimate) $g/cm^3$, M = 361.37 g/mol, $A_{substrate} = 4\pi$ $mm^2 = 4\pi \times 10^{-2}$ $cm^2$, C = $10^{-6}$ mol/L, V = 5 μL = $5 \times 10^{-6}$ L.

$I_{SERS}$ and $I_{Raman}$ values of Ofloxacin based on AgNPs, ZnO/Ag, and Ofloxacin powder were estimated using the spectra in Figure S5.

**Calculation of HOMO and LUMO energy levels**

The HOMO and LUMO energy levels of Ofloxacin can be estimated based on its onset oxidation and reduction potentials ($\phi_{ox}$ and $\phi_{red}$), respectively, using the equations:[4-6]

$$E_{HOMO} = -e\ (\phi_{ox} + 4.8 - \phi_{Fc/Fc+}) \quad (9)$$

$$E_{LUMO} = -e\ (\phi_{red} + 4.8 - \phi_{Fc/Fc+}) \quad (10)$$

in which $\phi_{Fc/Fc+}$ is the redox potential of ferrocene/ferrocenium couple ($Fc/Fc^+$) in the electrochemical system, assuming the energy level of $Fc/Fc^+$ to be -4.8 eV below vacuum level. Similar to the previous study, we set up an electrochemical system based on the description of Bin *et al.* with a Pt working electrode and a Ag/AgCl reference electrode.[4] Therefore, $\phi_{Fc/Fc+}$ was assumed to be 0.44 V versus Ag/AgCl.[4]

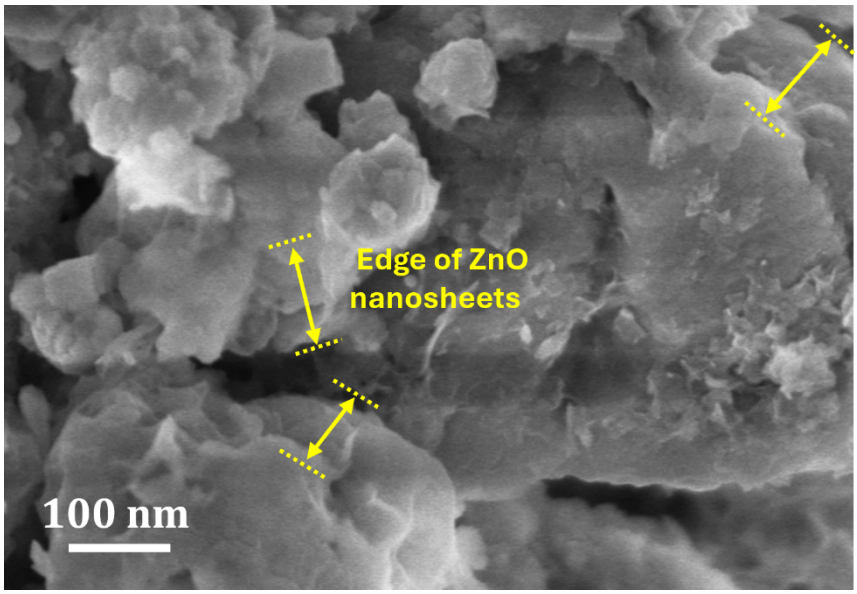


*Figure S1: SEM image of the ZnO nanosheets.*

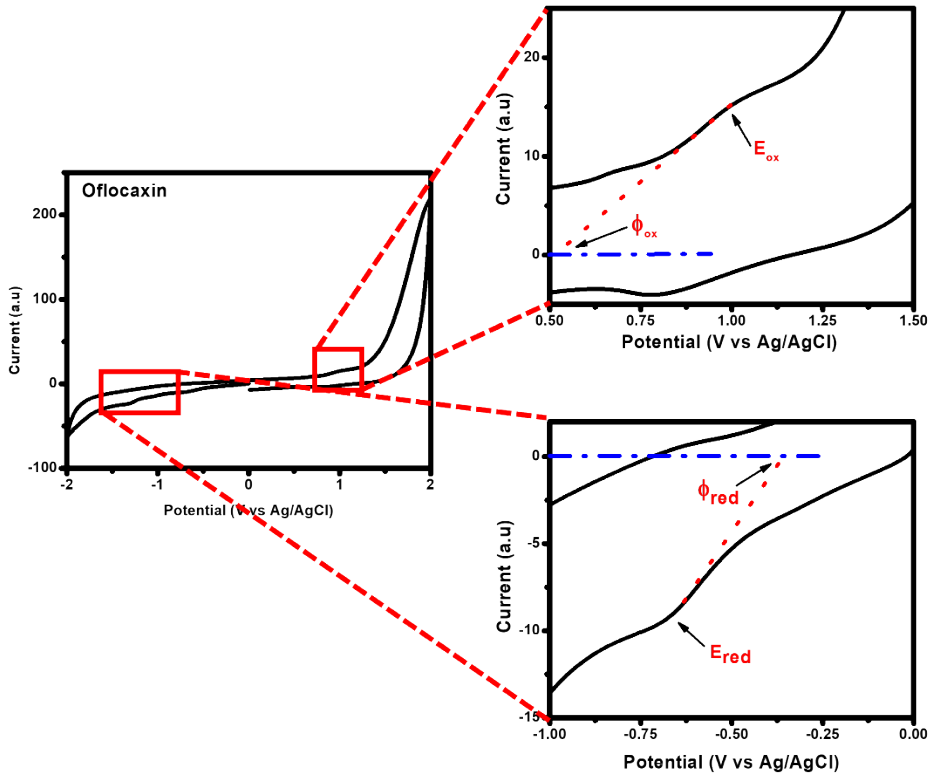


*Figure S2: Determination of the HOMO−LUMO energy levels of Ofloxacin analyte via cyclic voltammetry measurements.*

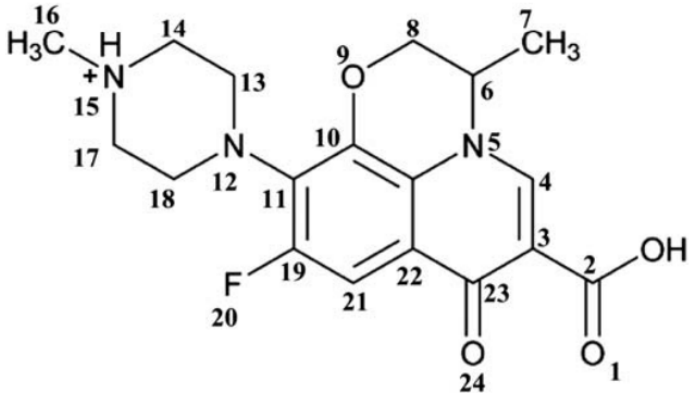


*Figure S3: Structure of Ofloxacin [7].*

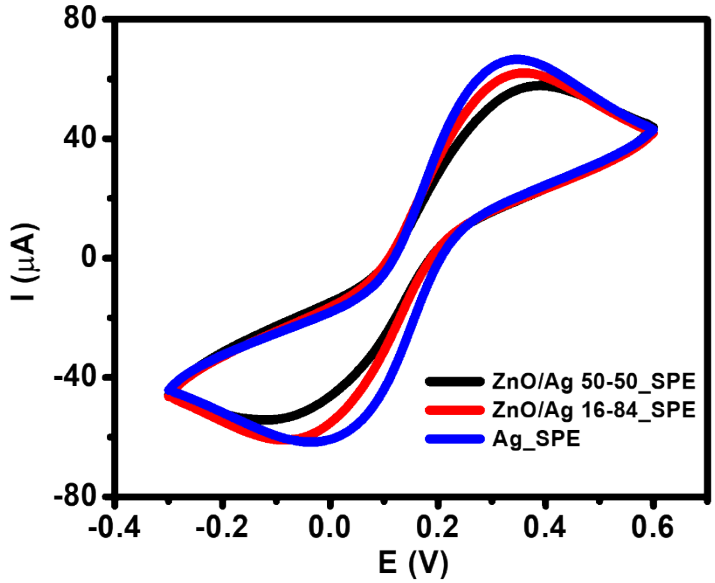


*Figure S4: CV curves (a) recorded on Ag_SPE, ZnO/Ag 16-84_SPE, ZnO/Ag 50-50_SPE in 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$.*

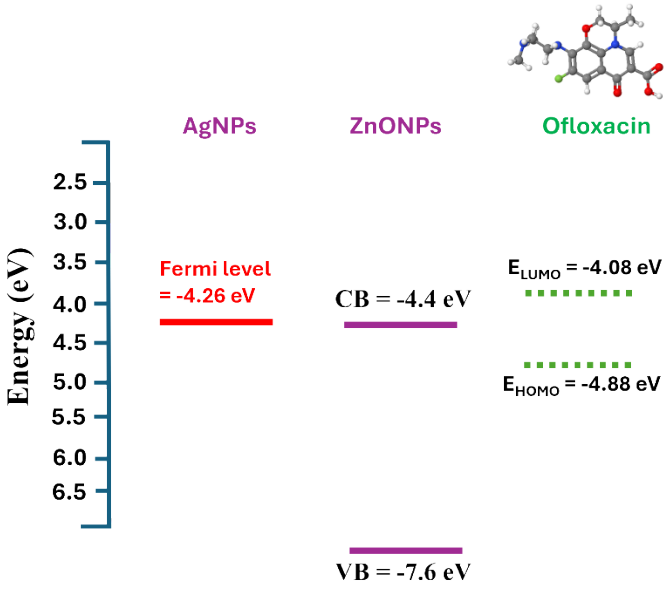


*Figure S5: The energy level of AgNPs, ZnO nanosheets, and Ofloxacin analyte.*

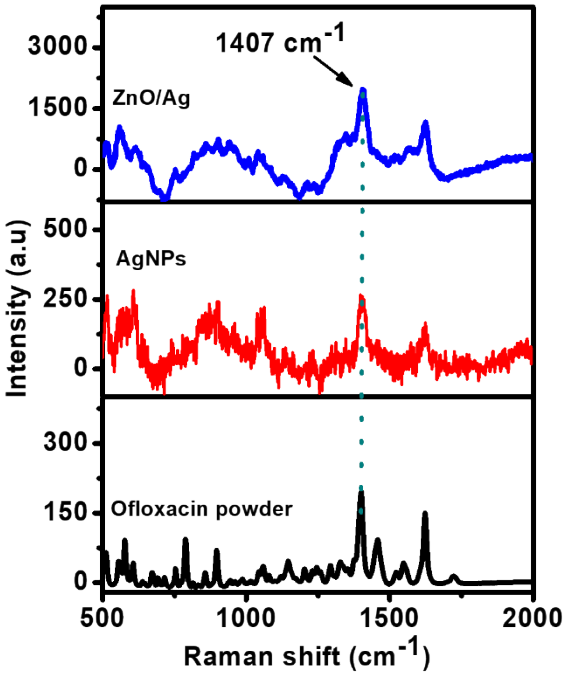


*Figure S6: Raman of TCZ; and SERS spectrum of AgNPs and ZnO/Ag for Ofloxacin ($10^{-6}$ M).*

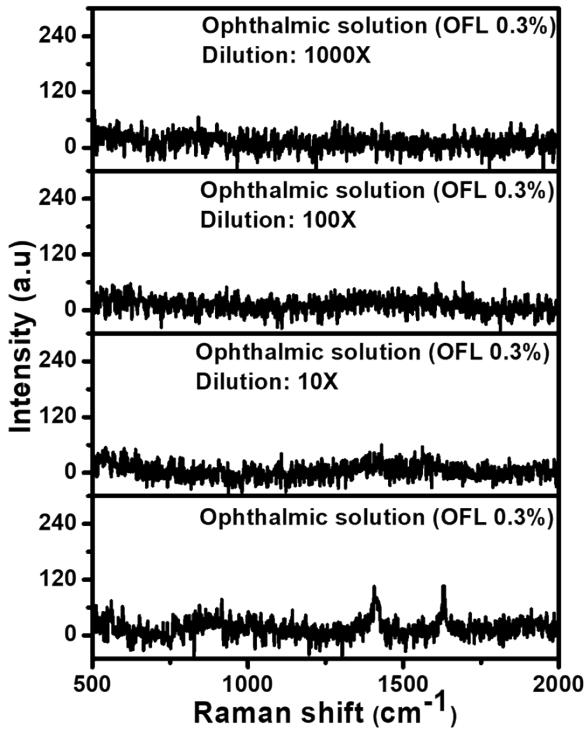


***Figure S 7: Raman spectra of original ophthalmic solution (OFL 0.3%) and several diluted ones.***

*Table S1: The recovery values for five concentrations of OFL in the beef sample.*

| Real sample | Analyte | Concentration of TCZ (M) | Recovery (%) |
|---|---|---|---|
| Beef | OFL | $10^{-5}$ | 91.41 |
| | | $10^{-6}$ | 89.86 |
| | | $10^{-7}$ | 89.37 |
| | | $10^{-8}$ | 86.57 |
| | | $10^{-9}$ | 87.95 |

## References


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