# A low-cost and facile electrochemical sensor for trace level recognition of flutamide in biofluids using large area bimetallic NiCo₂O₄ micro flowers

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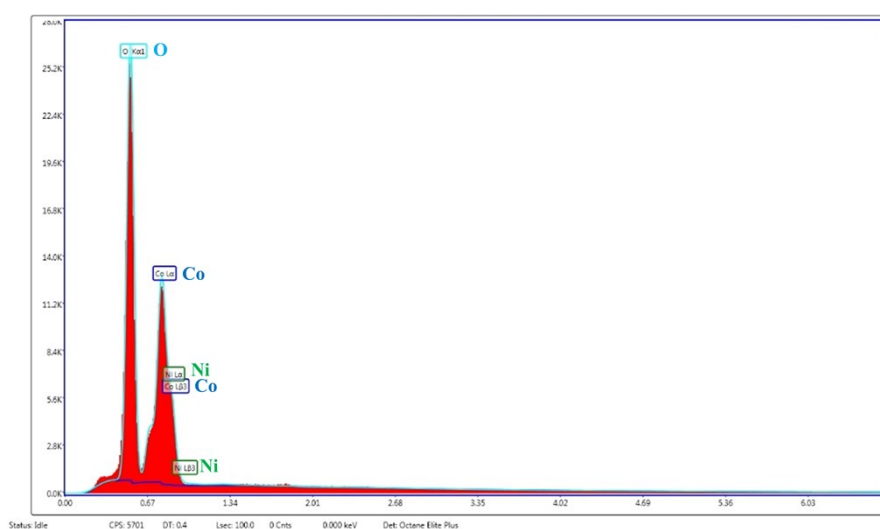
## Supporting Information



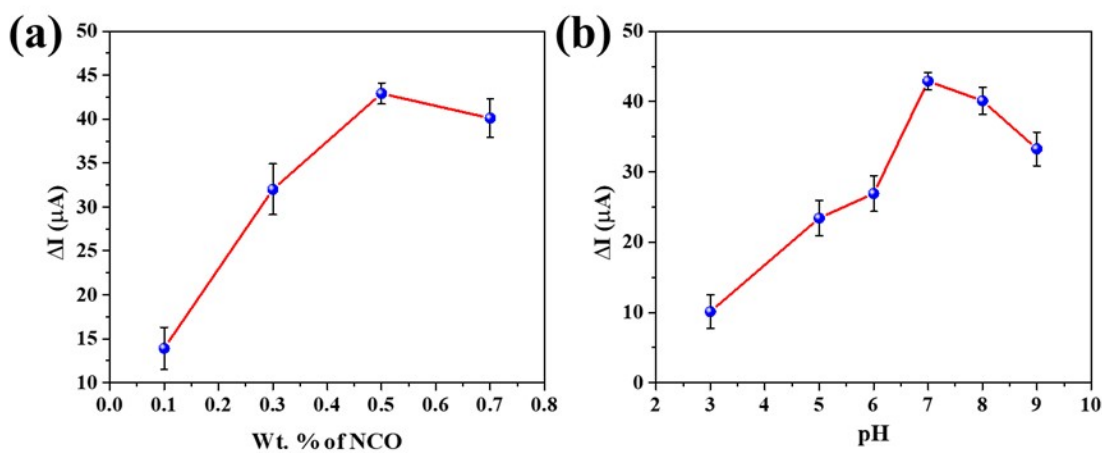**Figure S1 EDS spectra of NCO micro flowers**

**Figure S2. Performance evaluation plot of NCO/GCE at different (a) wt.% of NCO and (b) pH of the supporting electrolytic solution.**

## Electrochemical Analysis

The electrochemical studies of the NCO/GCE sensor were performed using 5 mM Fe $(CN)_6^{3-/4-}$ containing 1 M KCl electrolytic solution as the redox probe. The cyclic voltammetry (CV) analysis technique was employed to estimate the electrochemical active surface area (ECSA) of NCO/GCE. The Randle-Sevcik equation, shown in the Eqn. 1 was used to calculate the ECSA of NCO/GCE and GCE.

$$i_{pa} \text{ or } i_{pc} = 2.69 \times 10^5 \times A_e \times D^{1/2} \times n^{3/2} \times v^{1/2} \times c \qquad \rightarrow (1)$$

where n is the number of electrons, $A_e$ is the effective surface area or ECSA, $i_{pa}$ or $i_{pc}$ is the anodic/ cathodic peak current, v is the rate of voltage sweep (mV/s), C is the concentration of potassium ferricyanide (mol/cm$^3$), D - diffusion coefficient [1]. Figure S3 (a) shows the characteristic CV curve of GCE and NCO/GCE at 10 mV/s scan rate. The significant increase in the peak currents of NCO/GCE was ascribed to the electrocatalytic active sites of NCO micro flowers with $Ni^{2+}/Ni^{3+}$ and $Co^{2+}/Co^{3+}$ redox couples. The ECSA estimated by considering anodic peak current ($i_{pa}$) was quantified as 0.285 cm$^2$ which is ~123 % larger than the GCE. This shows that the surface modification process of the GCE has a significant impact on the electrocatalytic activity of the electrode which can be attributed to the electrocatalytic active sties of NCO micro flowers. Figure S3 (b) illustrates the CV curve of NCO/GCE at a varied scan rate from 10 mV/s to 200 mV/s. The linear increase in the anodic and cathodic peak currents was observed with the linear increase in the scan rate of CV. This augmentation in the peak current can be attributed to the upsurge in the concentration gradient of the redox species at the electrode-electrolyte interface which was induced by the faster voltage sweep. Besides, significant shifts in the redox potential (i.e., shift towards the higher potential for oxidation peak and lower potential for reduction peak) with the increase in the scan rate can

be attributed to the polarization of the NCO/GCE electrode [2]. Figure S3 (c) shows the linear calibration curve between the peak currents ($I_{pa}$ and $I_{pc}$) of NCO/GCE versus the log of scan rate (mV/s). The linear regression equations of $I_{pa}$ and $I_{pc}$ has been evaluated with a regression coefficient ($R^2$) of 0.996 and 0.995, respectively. The slope obtained for $I_{pa}$ and $I_{pc}$ were greater than 0.5 which confirms the existence of an adsorption-controlled process at the electrode-electrolytic interface [3]. Likewise, the linear calibration curve between the peak voltage ($E_{pa}$ and $E_{pc}$) and log (scan rates (mV/s)) was estimated as illustrated in figure S3 (d). The linear regression equation of Epa and $E_{pc}$ has been derived with a regression coefficient $R^2$ of 0.996 and 0.998, respectively. Using the regression equation and slope values various other kinetic parameters of the electrode such as charge transfer coefficient ($\alpha$) and apparent charge transfer rate constant ($K_s$) was quantified using Laviron's model [1].

$$E_{pa} = E^o + \frac{RT}{\alpha_a nF} ln\frac{\alpha_a}{m}$$
→ (2)

$$E_{pc} = E^o + \frac{RT}{\alpha_c nF} ln\frac{\alpha_c}{m}$$
→ (3)

$$m = 0.025(\frac{k_s}{n_\alpha})$$
→ (4)

where $k_s$ is the apparent charge transfer rate constant, $E^o$ is the reduction potential, n is the number of electrons transferred and $\alpha$ is the charge transfer coefficient.

$$log\ K_s = \alpha log\ (1-\alpha) + (1-\alpha)\ log\ \alpha - log\frac{RT}{nF\theta} - \alpha\ (1-\alpha)\frac{nF\Delta E_p}{2.3\ RT}$$
→ (5)

The slope value (m) estimated from the regression equations of $E_{pa}$ and $E_{pc}$ was equated to the following Eqn. 6 and 7, respectively.

$$0.079 = \frac{2.303\ RT}{\alpha_a nF}$$
→ (6)

$$0.091 = -\frac{2.303\ RT}{\alpha_c nF}$$
→ (7)

The charge transfer coefficients $\alpha_a$ and $\alpha_c$ was estimated as ~0.74 and ~0.64, respectively which are close to the unity confirming the reactant like transition state for an oxidative reaction [4]. The average $k_s$ value of NCO/GCE was evaluated as 0.831 cm s$^{-1}$.

Finally, the chronocoulometric analysis of NCO/GCE was performed to obtain the active surface coverage area ($\Gamma$) of reactant ions from the electrolyte. The $\Gamma$ was estimated employing Eqn. 8,

$$Q = nFA\Gamma$$
→ (8)

Where n is the number of transferred electrons, Q is the variance between reverse and forward intercepts, A is the active electrode area (0.0707 cm$^2$), $\Gamma$ is the active surface coverage area and F is the faraday constant. Figure S3 (e) illustrates the chronocoulometric study of GCE and NCO/GCE. The $\Gamma$ of NCO/GCE was calculated as 9.74 nmol cm$^{-2}$ which was higher than GCE. Herein, the electrocatalytic active sites of the NCO in the NCO/GCE were responsible for the augmentation of $\Gamma$.
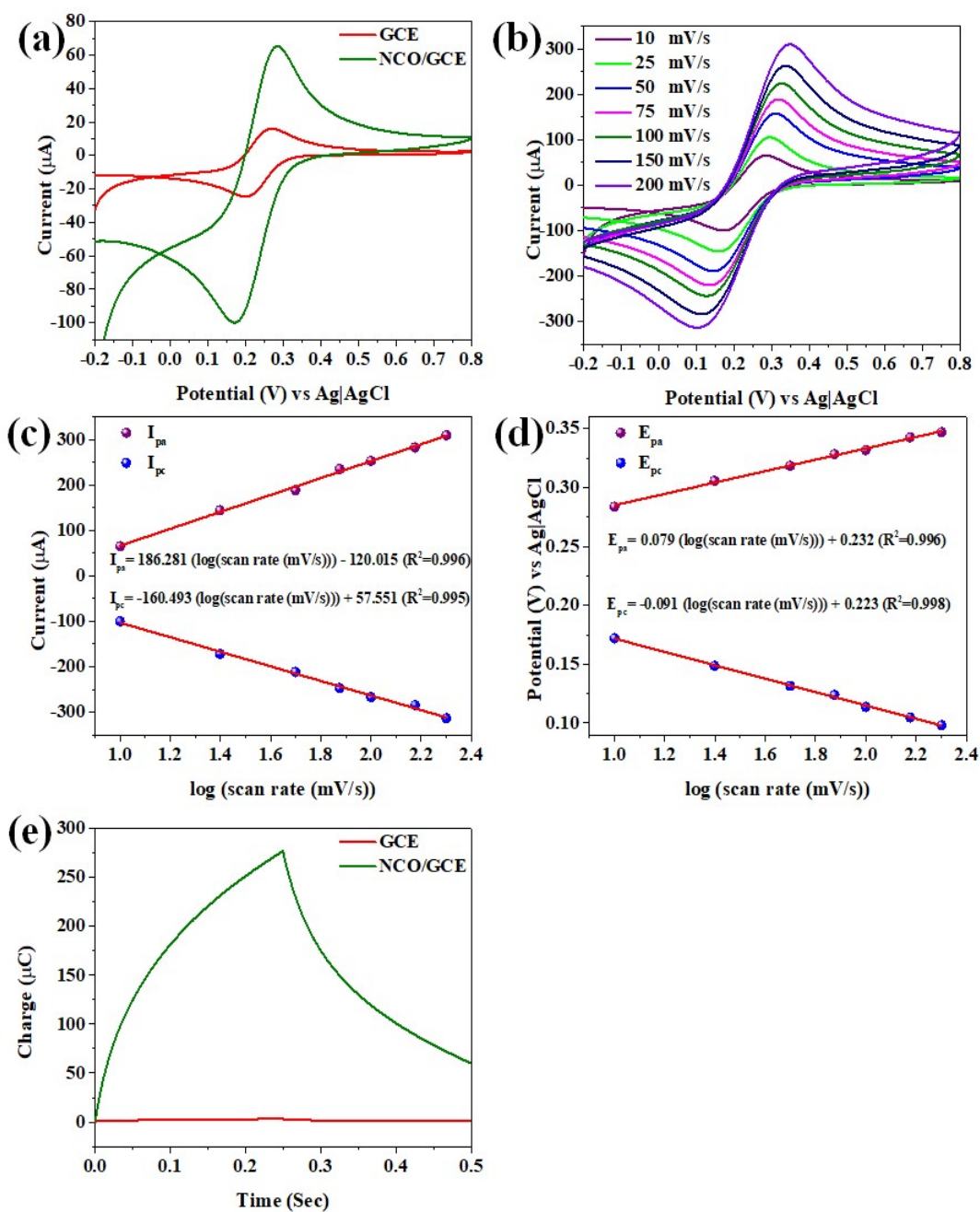
**Figure S3 (a)** CV response of NCO/GCE and GCE in 1M KCl comprising 5 mM of Fe $(CN)_6^{3-/4-}$ supporting the electrolyte at 10 mV/s **(b)** CV response at a varied scan rate, linear calibration curve between **(c)** Ip and log (scan rate (mV/s)) **(d)** Ep and log (scan rate (mV/s)) **(e)** chronocoulometric analysis of GCE and NCO/GCE.

**Table S1** Detection of flutamide in simulated blood serum samples via standard addition technique.

| Concentration of Flutamide in blood serum (μM) | Spiked Flutamide (μM) | Recovered concentration (μM) | Relative standard deviation (%) | Recovery (%) |
|---|---|---|---|---|
| 2 | 0.3 | 0.32 | 6.45 | 106.45 |
| 2 | 40 | 39.05 | 2.40 | 97.60 |

**References**

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