Supplementary Information (SI) for Sensors & Diagnostics.

Ultrasensitive electrochemical detection of methotrexate in biological fluids using $NiMn_2O_4$/CNT nanocomposite-modified electrode

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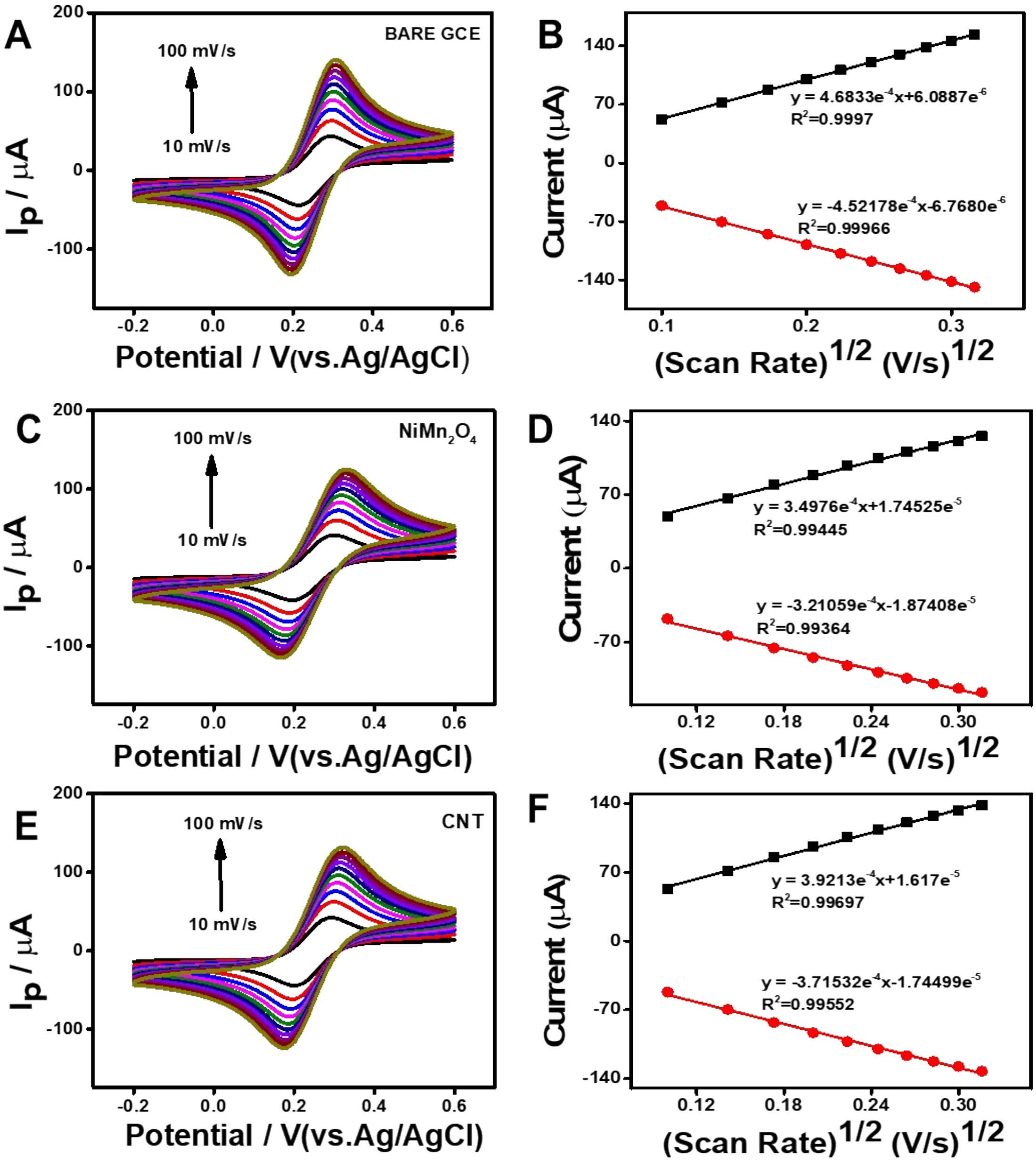

Fig. S1. (A, C, E) CV curves recorded at various scan rates ranging from 0.01 to 0.1 V s^{-1} for **(A)** Bare GCE, **(C)** $NiMn_2O_4$-GCE, and **(E)** CNT-GCE in 5 mM $[Fe(CN)_6]^{3-}$ solution containing 0.1 M KCl. **(B, D, F)** Corresponding linear plots of $\upsilon^{1/2}$ versus redox peak currents (I_{pa}/I_{pc}) for **(B)** Bare GCE, **(D)** $NiMn_2O_4$-GCE, and **(F)** CNT-GCE.

Table S1. EIS fitted parameters

Electrode	Parameter[a]	Unit	Fitted Value	Relative Std. Error (%)
Bare GCE	**R_s**	Ω	110.4	0.785
	C	F	5.256×10^{-7}	5.935
	R_{ct}	Ω	49.45	2.529
	W	$\Omega\cdot s^{-1/2}$	9.854×10^{-4}	1.039
CNT-GCE	**Rs**	Ω	100.3	1.421
	C	F	7.890×10^{-7}	8.98
	Rct	Ω	45.93	4.936
	W	$\Omega\cdot s^{-1/2}$	9.383×10^{-4}	1.939
$NiMn_2O_4$-GCE	**Rs**	Ω	95.77	1.171
	C	F	7.298×10^{-7}	2.476
	Rct	Ω	316.75	1.576
	W	$\Omega\cdot s^{-1/2}$	1.005×10^{-4}	3.508
$NiMn_2O_4$/CNT-GCE	**Rs**	Ω	82.23	1.144
	C	F	9.481×10^{-7}	4.366
	Rct	Ω	7.98	7.91
	W	$\Omega\cdot s^{-1/2}$	1.429×10^{-3}	1.163

[a] R_s: Solution Resistance, C: Capacitance, R_{ct}: Charge Transfer Resistance, W: Warburg Impedance

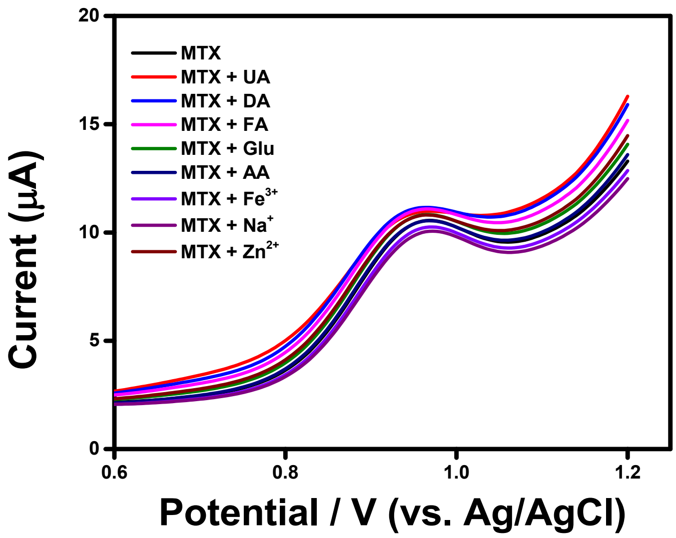

Fig. S2. DPV responses of $NiMn_2O_4$/CNT/GCE for the MTX (1 μM) detection in the presence of common interfering species (50-fold excess) in PBS (0.1 M, pH 7.0)

Calculation of n value

Using the Randles-Sevcik Equation

We use:

$$m_{MTX}= (2.69\times10^5) \times n^{3/2}_{MTX}\times A\times D^{1/2}_{MTX}\times C_{MTX}$$

Rearrange to solve for n:

$$n_{MTX}= (m_{MTX} / 2.69\times10^5\times A\times D^{1/2}_{MTX} \times C_{MTX})^{2/3}$$

Given Values

- mMTX=1.43×10^{-5} A (slope of I_p vs. $\nu^{1/2}$ for MTX)
- A= 0.098 cm^2 (electrochemically active surface area from ECSA)
- $D_{MTX} \approx 7.6\times10^{-6}$ cm^2/s (experimentally determined diffusion coefficient for methotrexate)[1,2]
- C_{MTX}=1.0×10^{-6} mol/cm^3 (1 μM MTX concentration)
- 2.69×10^5 (Randles-Sevcik constant at 25°C)

Step-by-Step Calculation

Compute $D^{1/2}_{MTX}$

$$D^{1/2}_{MTX} = (7.6\times10^{-6})^{1/2} = 2.67\times10^{-3}$$

Compute the denominator:

$$2.69\times10^5\times0.098\times(2.67\times10^{-3})\times(1.0\times10^{-6})$$

$$=2.69\times10^5\times0.098\times2.67\times10^{-9}$$

Compute the fraction:

$$m_{MTX}/\text{denominator} = 1.43\times10^{-5} / 7.05\times10^{-3}$$

$$= 2.03\times10^{-3}$$

Take the (2/3) power:

$$n_{MTX} = (2.03\times10^{-3})^{2/3}$$

Approximating:

$$n_{MTX} \approx 1.31$$

Final Answer:

$$n_{MTX} \approx 1.31$$

Since n must be an integer, we round to the closest whole number:

$$n_{MTX} \approx 1$$

References (For D value in calculation of n value)

1. H. Wan, X. Xie, H. Liu, S. Mahmud and H. Liu, *J Anal Chem*, 2024, **79**, 1640–1648.
2. Z. Chen, J. Liu, H. Wei, Y. Liang, X. Meng and D. Pan, *Ionics*, 2025, **31**, 2205–2214.