Supplementary Material

Environmentally Friendly Electrochemical and Spectrophotometric Strategies for Sensitive Determination of Nintedanib in Complex Matrices

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Materials and Reagents

In this study, Glucose (99.5 %), L-arginine (98.0 %), L-methionine (99.5 %), sodium hydroxide (98%), potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$ (99.5 %), hydrochloric acid (37%), sodium Acetate (99%), ascorbic acid (99%), uric acid (99.0 %), acetic acid (99%), potassium chloride, sodium phosphate, sodium sulfate were purchased from Sigma Aldrich Co. (https://www.sigmaaldrich.com, Germany). Phosphate-buffered saline was made of sodium dihydrogen phosphate and potassium dihydrogen phosphate. Britton-Robinson (B-R) Buffer was made of Boric acid, phosphoric acid, potassium chloride, and acetic acid. All chemical compounds were analytical grade and used without additional refinement.

Apparatus

Voltammetric experiments were carried out using an AUTO LAB system with PGSTAT204 electrochemical workstation (Metrohm Inc., Switzerland) with a glassy carbon electrode system in a one-compartment of 10 mL electrochemical cell. All electrochemical measurements were performed at 25 °C unless otherwise specified.

The pH of the supporting electrolyte was monitored with a pH meter (Hanna Instruments, Woonsocket, Rhode Island, USA). Spectroscopic device, the UV–vis absorption spectra were recorded using a Perkin Elmer UV-Lambda 35 spectrophotometer. Measurements were performed at room temperature using quartz cuvettes with a path length of 1 cm. Appropriate blank solutions were used to correct for background absorption, and all spectra were recorded in the wavelength range of 276 nm. This ensures reproducibility and clarity of the experimental procedure.

All electrochemical measurements were carried out in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl as the supporting electrolyte (ionic strength ≈ 0.1 M). A conventional three-electrode system was employed, consisting of MWCNTs-modified carbon paste electrode (CPE, 0.071 cm^2) as the working electrode, an Ag/AgCl (3 M KCl) reference electrode, and a platinum wire counter electrode. All experiments were performed at room temperature (~25 °C) under unstirred conditions without degassing.

Preparation of real samples

The fabricated MWCNTs/ CPE was effectively used for the utilization of NIN in human plasma, tablet, and human urine samples. Commercially available NIN tablets were finely ground and dissolved in a 1:1 (v/v) mixture of water and methanol. The solution was filtered using a 0.45 μm PTFE syringe filter, followed by appropriate dilution with 0.1 M BR to obtain working concentrations suitable for electrochemical analysis. For urine sample preparation, NIN at known concentrations within the linear detection range (0.02–2.0 μM) was spiked into untreated human urine. To prepare protein-free human serum, 1.0 mL of human serum albumin was mixed with 1.0 mL of acetonitrile, serving as a protein precipitant. The mixture was vortexed for 2 minutes, then centrifuged at 8000 rpm for 20 minutes to separate the precipitated proteins. The resulting supernatant, containing the protein-depleted serum, was carefully collected and diluted to the desired concentration using a 1×10^{-3} M NIN solution in a calibrated volumetric flask. Quantitative analysis and recovery studies were then performed on these spiked samples under optimized electrochemical conditions to assess the sensor's accuracy and applicability in real biological matrices.

Human plasma serum and synthetic urine used in this study were commercially obtained from Sigma-Aldrich. No human participants or patient samples were directly involved in this research; therefore, ethical approval and informed consent were not required.

$$K^0 = \frac{RT}{F^2 CA R_{ct}} \qquad (S1)$$

$$I_0 = \frac{RT}{n F R_{ct}} \qquad (S2)$$

k^0 represents the standard heterogeneous electron transfer rate constant (cm s^{-1}), I_0 is the standard exchange current density (A cm^{-2}), R stands for the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the temperature (298.15 K), F is the Faraday constant (96485 C mol^{-1}), R_{ct} is the electron transfer resistance (Ω), A is the electrode surface area (cm^2), n is the number of electrons transferred, and C is the concentration of the $[Fe(CN)^6]^{3-/4-}$ solution (5×10^{-6} mol cm^{-3}).

Table S1. Electrochemical features obtained from EIS and CV analyses for the bare CPE and MWCNTs –modified CPE.

Electrode	**ΔE_p (mV)**	**R_{ct} (Ω)**	**ESA (cm^2)**	**K^0 ($cm\ s^{-1}$)**	**I_0 ($A\ cm^{-2}$)**
Bare CPE	146	2800	0.09	1.8×10^{-4}	0.81×10^{-5}
MWCNTs/CPE	80	1000	0.17	2.8×10^{-4}	0.23×10^{-5}

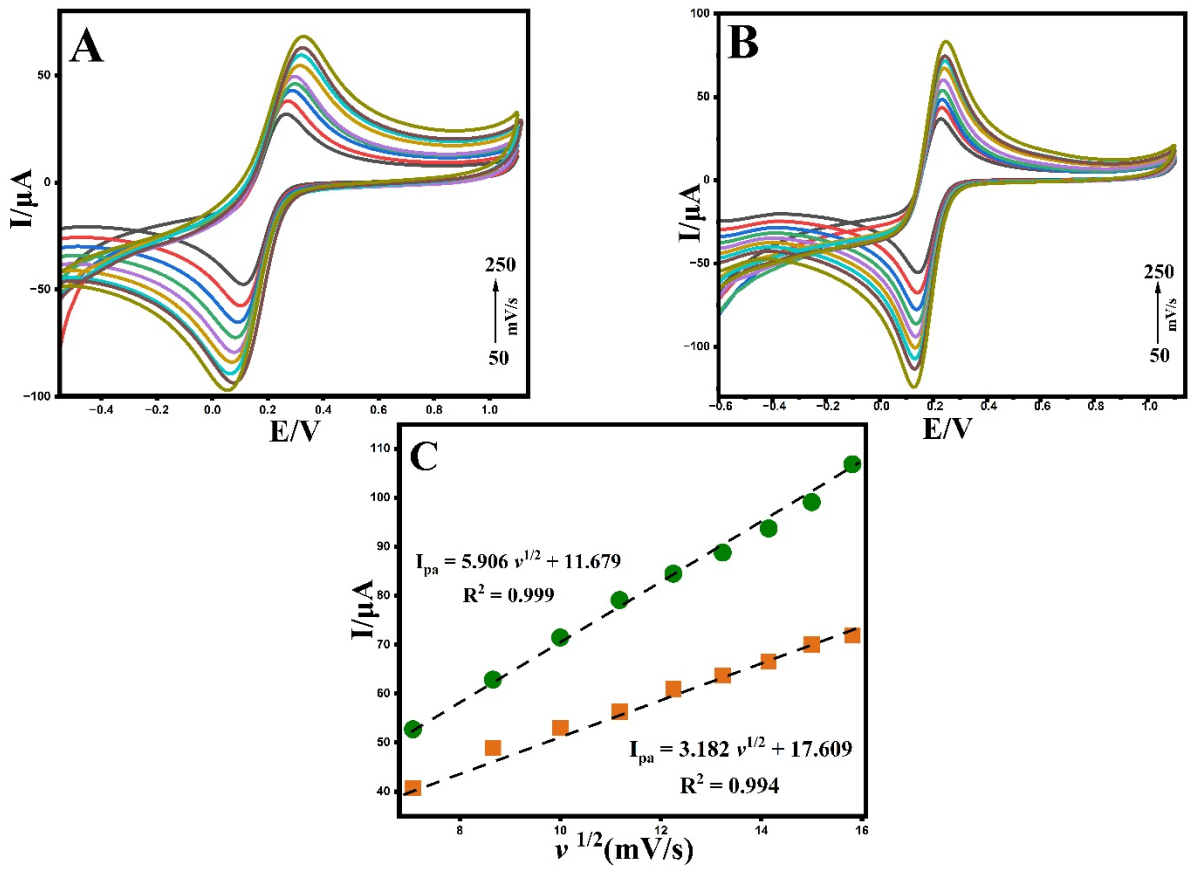

Figure S1. The recorded CV curves of bare CPE (A), MWCNTs-modified CPE (B), and the relationship between I_{pa} vs. $v^{1/2}$ (C) on the bare CPE and MWCNTs–modified CPE at various scan rates in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl.

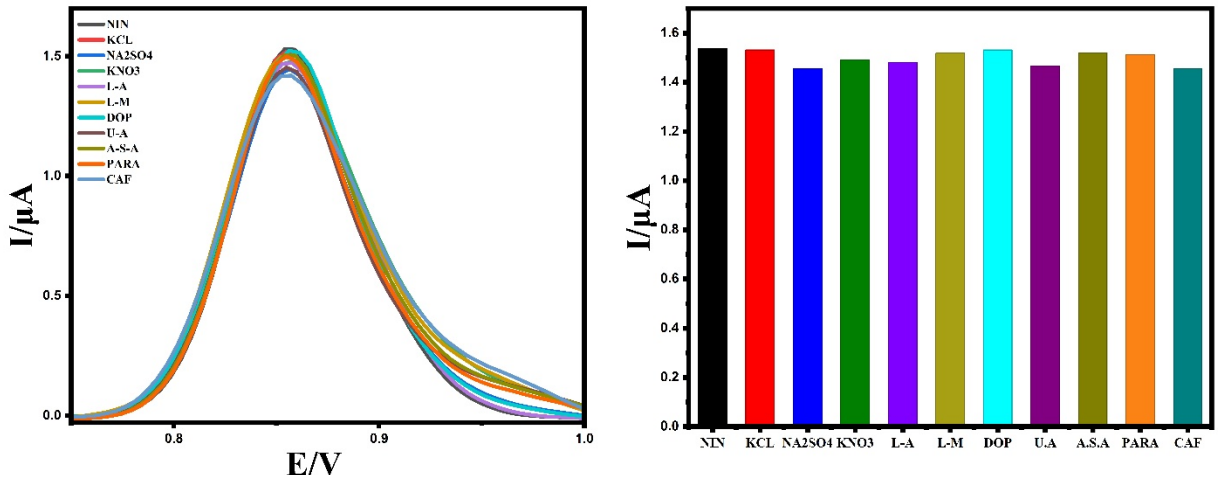

Figure S2. DPV curves and histogram of selectivity MWCNTs/CPE in 1.0 µM NIN (BR buffer, pH 2.0).

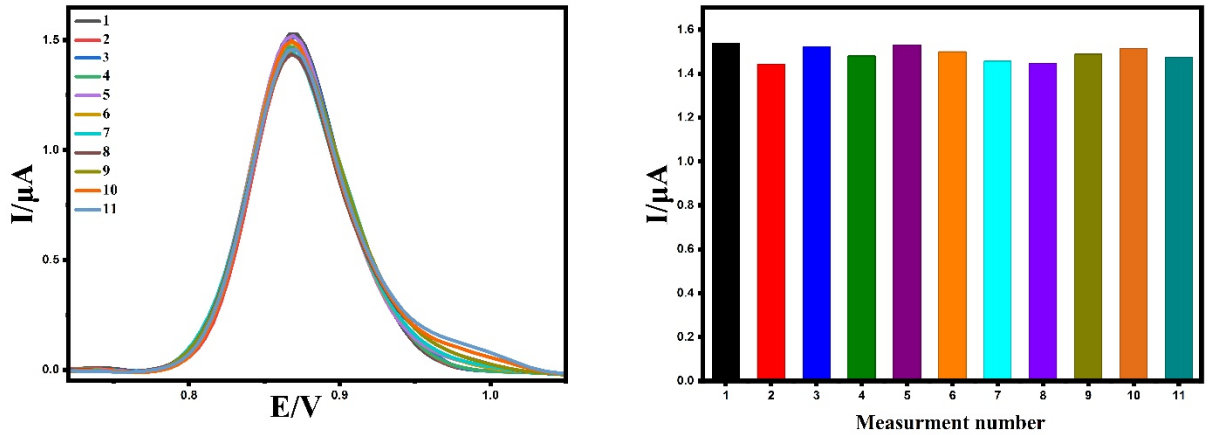

Figure S3. DPV curves and histogram of repeatability of MWCNTs/CPE in 1.0 µM NIN (BR buffer, pH 2.0).

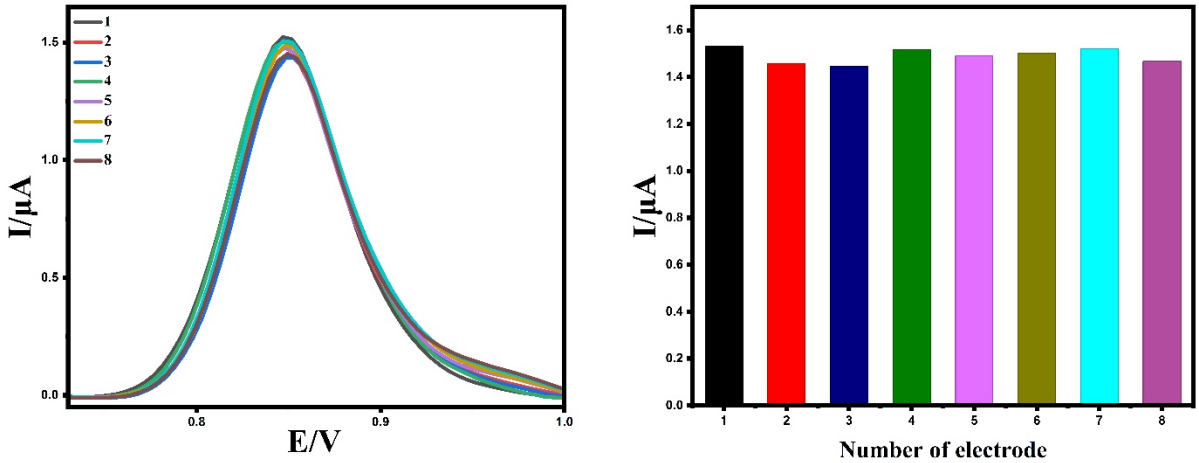

Figure S4. DPV curves and histogram of reproducibility of MWCNTs/CPE in 1.0 µM NIN (BR buffer, pH 2.0).

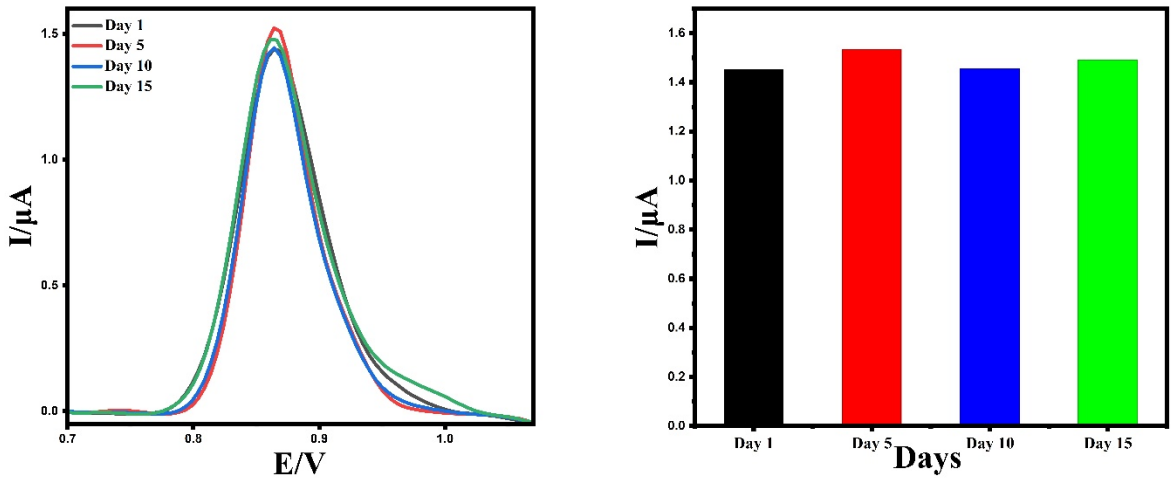

Figure S5. DPV curves and histogram of stability of MWCNTs/CPE in 1.0 µM NIN (BR buffer, pH 2.0).

Table S2. Recovery studies were conducted to evaluate the accuracy of the spectrophotometric method for NIN determination.

Added RIT (µM)	Found RIT (µM) [a]	Recovery %	RSD % (n = 3)
27.5	27.43	99.5	0.71
37.5	37.66	100.4	0.53

[a] Average of three replicate measurements.