Molecularly imprinted sensor for lipoic acid quantification in serum: A proof-of-concept for diagnosis of NELL-1 membranous nephropathy and kidney failure

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Contents

EXPERIMENTAL SECTION

Characterization

Surface morphology and microstructure of both unmodified and modified electrodes were examined using a field-emission scanning electron microscope (FE-SEM; FEI Inspect S50). Structural characterization of the polymer was performed using Fourier-transform infrared spectroscopy (FTIR) with a Cary 630 FTIR spectrometer (Agilent Technologies), recording spectra over the range of 4000–650 cm^{-1}. SEM micrographs were further processed using WS×M 4.0 Beta 9.3 software.[1]

Electrochemical Sensor Measurements

All electrochemical measurements were conducted using a PalmSens 4 potentiostat interfaced with PSTrace software (PalmSens BV, The Netherlands). The redox solution consisted of 2.5 mM equimolar $K_4[Fe(CN)_6]\cdot 3H_2O$ and $K_3[Fe(CN)_6]$ with 0.05 M KCl, prepared in phosphate-buffered saline (PBS, pH 7.4). Lipoic acid (LA) stock and calibration solutions (1–500 μM) were prepared in the same redox medium, supplemented with 0.1 M NaOH to enhance solubility. Selectivity studies were performed using 500 μM solutions of potential interferents (urea, glucose, spermine, glutamine, guanine, uric acid, cholic acid, and cholesterol) in the same redox background.

Electrochemical impedance spectroscopy (EIS) was conducted using an AC amplitude of 6 mV at a DC bias of 0.1 V, with 50 points per decade. Cyclic voltammetry (CV) was performed over a potential range of −0.4 V to +0.4 V at a scan rate of 100 mV/s, using a potential step of 0.01 V and recording three consecutive cycles per measurement. Scan rate-dependent CVs were also obtained (10–200 mV/s) to analyze redox behavior. Differential pulse voltammetry (DPV) was performed from −0.5 V to +0.5 V using the following parameters: equilibrium time 10 s, step potential 0.005 V, pulse amplitude 0.1 V, and pulse width 0.02 s.

Changes in peak currents corresponding to the redox couple $[Fe(CN)_6]^{3-/4-}$ were used as analytical signals for LA detection. Sensitivity, limit of detection (LOD), and limit of quantification (LOQ) were calculated using the standard equations: $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, where σ is the standard deviation of the blank signal (y-intercept of the calibration curve) and S is the sensitivity (slope of the calibration curve).[2] All measurements were conducted in triplicate ($n = 3$) using three independently fabricated sensors.

Pre-Treatment and Analysis of Human Serum

To evaluate the practical applicability of the MIP sensor for LA detection, electrochemical measurements were conducted using human serum samples. Serum samples (2 mL each) were obtained from five healthy volunteers by the Health Centre, University of the Punjab, after obtaining informed consent from all participants. The collection and handling of human samples were conducted with the approval of the Chief Medical Officer of the Health Centre and the Head of the School of Chemistry. Ethical clearance for the study was granted by the Institutional Ethics Review Board (IERB) of the University of the Punjab. All experimental procedures complied with institutional ethical standards and relevant national regulations, including the principles outlined in the Declaration of Helsinki.

Each 2 mL serum sample was first diluted with phosphate-buffered deionized water to a total volume of 6 mL. The diluted samples were centrifuged at 4000 rpm for 10 minutes at room temperature to remove cellular debris and suspended particles. The resulting supernatants were carefully transferred to clean vials and passed through 0.22 μm syringe filters to eliminate large proteins and other interfering macromolecules.

Following pre-treatment, each serum sample was further diluted in a 1:2 ratio with standard phosphate-buffered saline (PBS) containing the redox probe $[Fe(CN)_6]^{3-/4-}$ and KCl. These samples were then analyzed using CV to determine the baseline concentration of LA. To assess recovery, each serum sample was spiked with 100 μM LA, and CV measurements were repeated under identical conditions. Percent recovery was calculated by comparing the observed concentration increase to the expected value, following standard methods.[3]

RESULTS

Figure S1

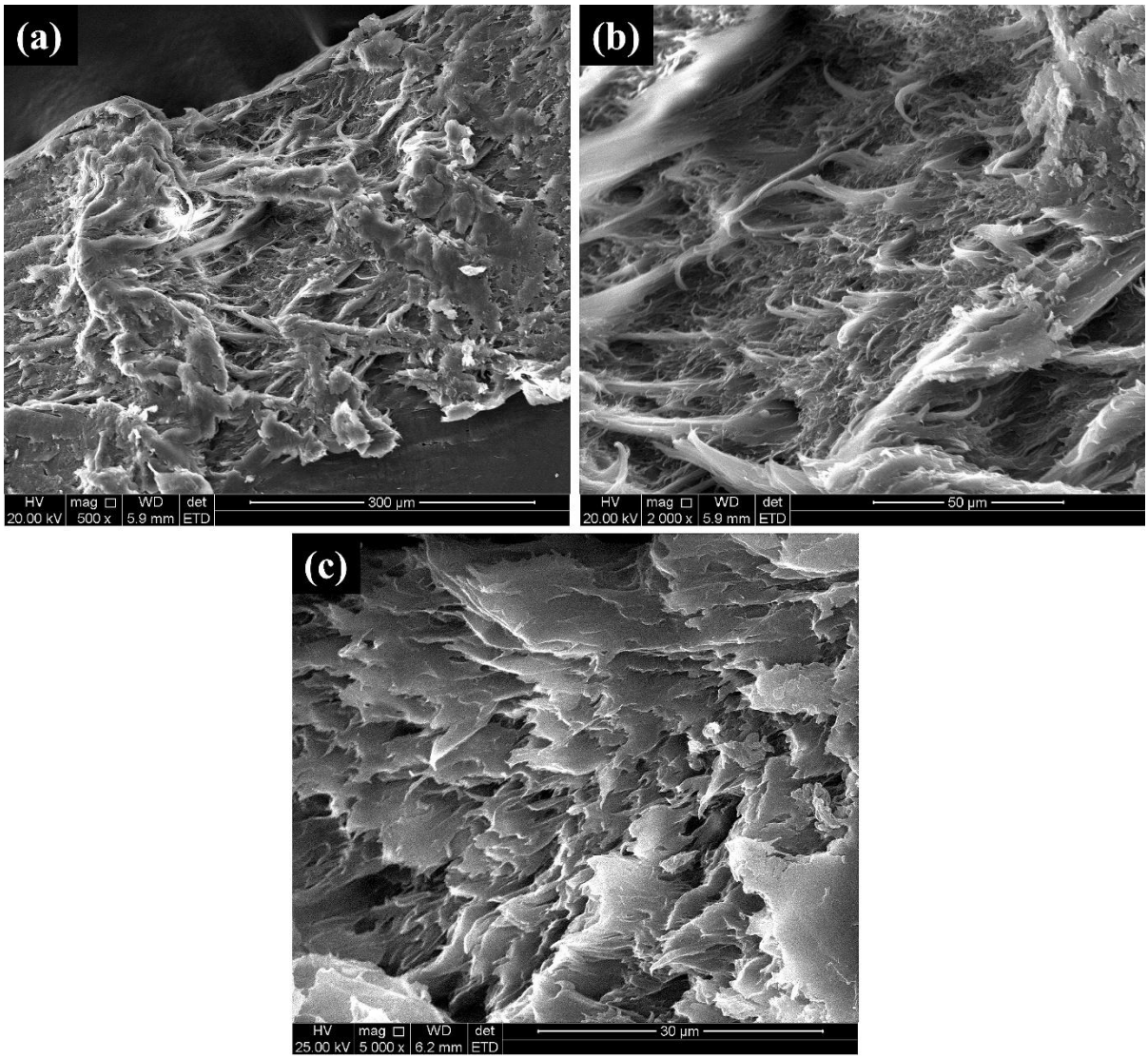

Figure S1. Surface morphology of PAni–PmAP copolymer. (a–c) SEM micrographs at progressively higher magnifications: (a) 500× (scale bar: 300 µm), (b) 2,000× (scale bar: 50 µm), and (c) 5,000× (scale bar: 30 µm).

Figure S2

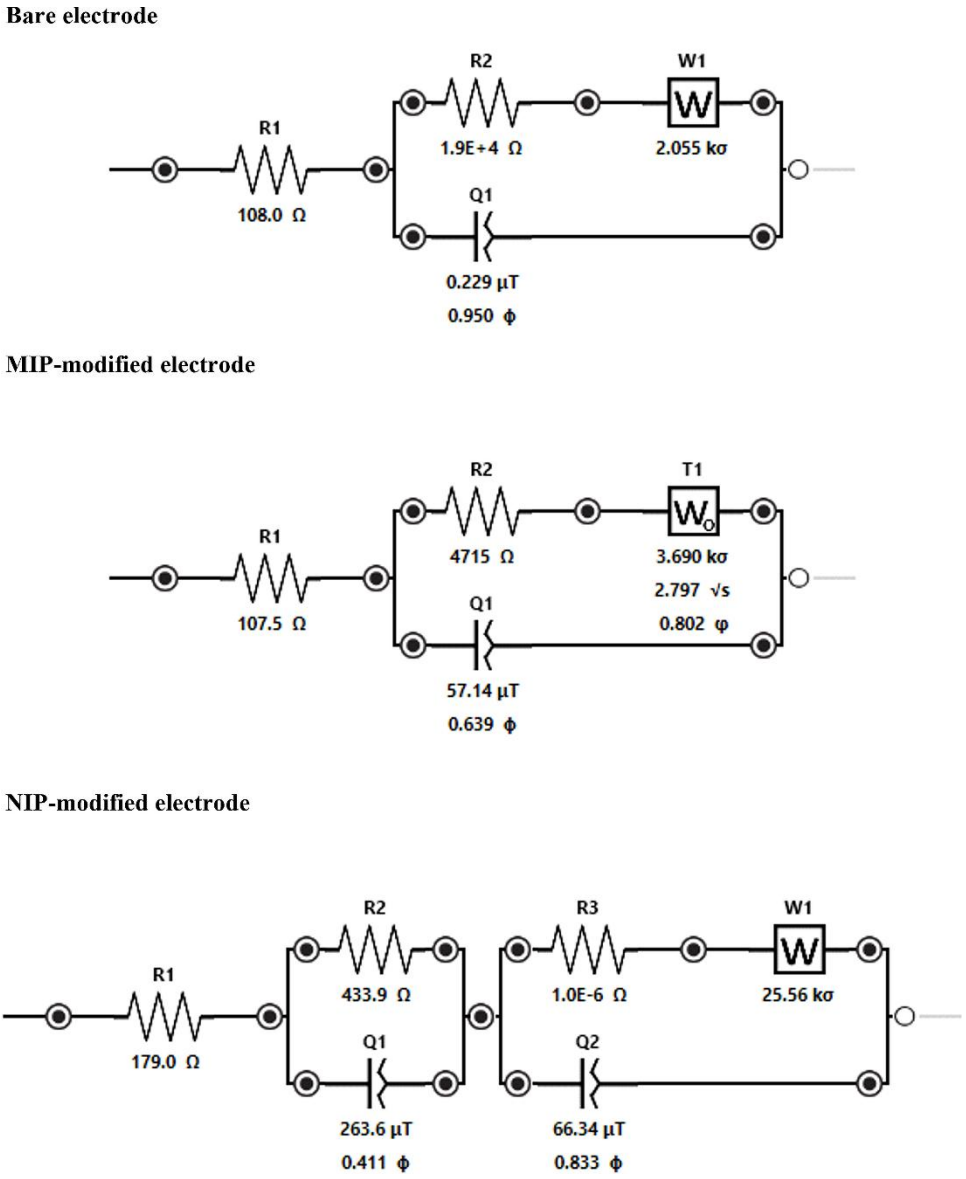

Figure S2. Equivalent circuit models and extracted fitting parameters for electrochemical impedance spectroscopy (EIS) analysis of bare, MIP-, and NIP-modified SPGEs.

Figure S3

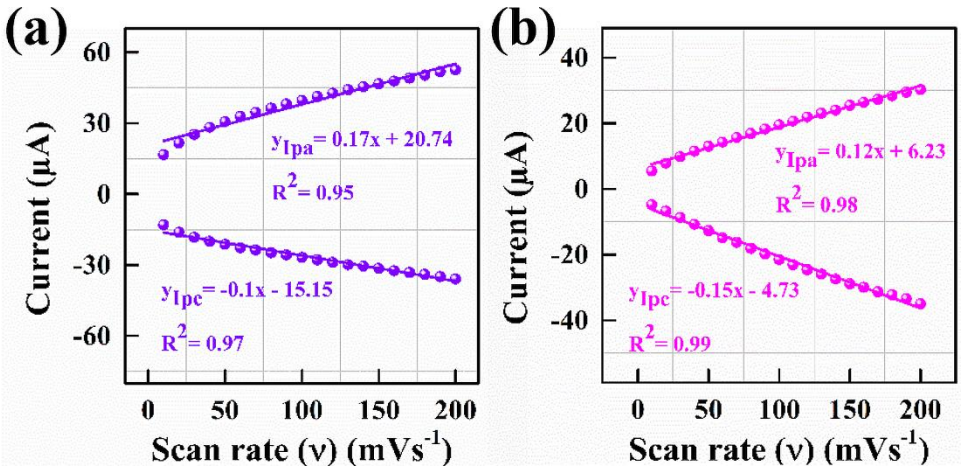

Figure S3. The linear regression plots of anodic (I_{pa}) and cathodic (I_{pc}) peak currents vs. the scan rate for (a) MIP and (b) NIP sensors, respectively.

Figure S4

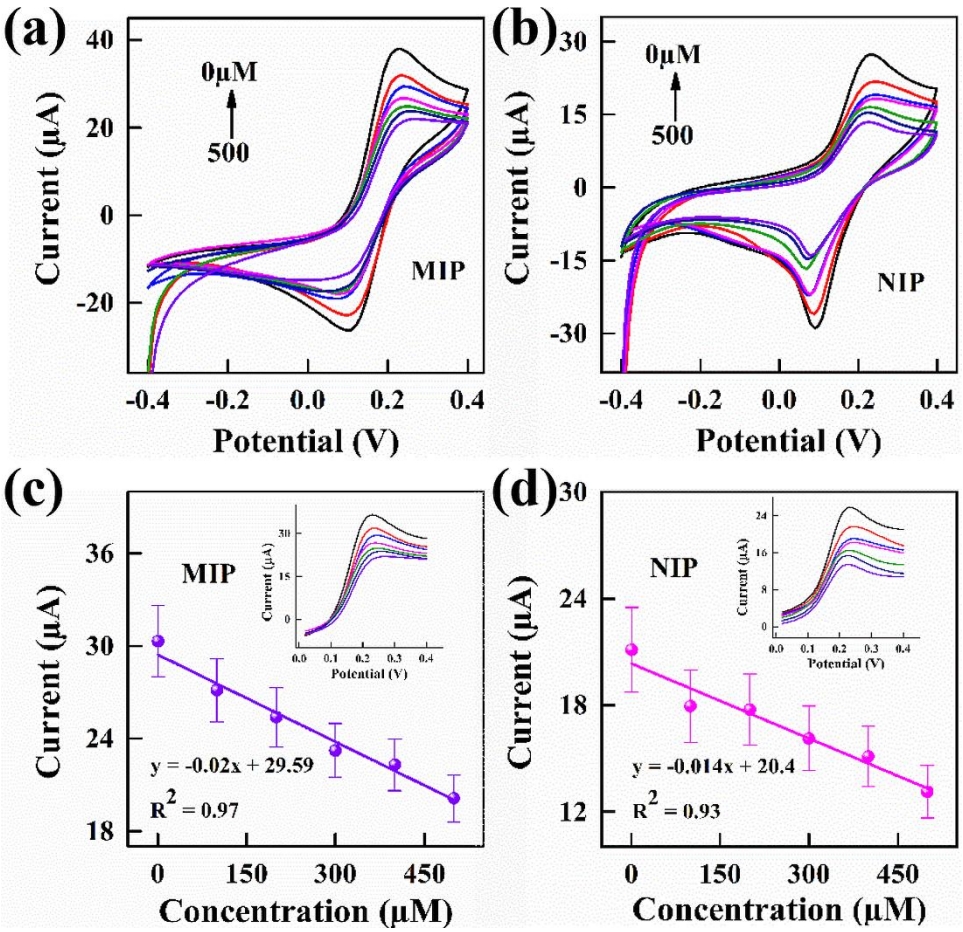

Figure S4. CV scans of (a) MIP and (b) NIP sensors at varying LA concentrations (0–500 µM) in a standard redox solution containing 2.5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.05 M KCl in phosphate buffer (pH 7.4) at 25 °C with a scan rate of 100 mV/s. The corresponding calibration plots of current response vs. LA concentration for (c) MIP and (d) NIP sensors.

Figure S5

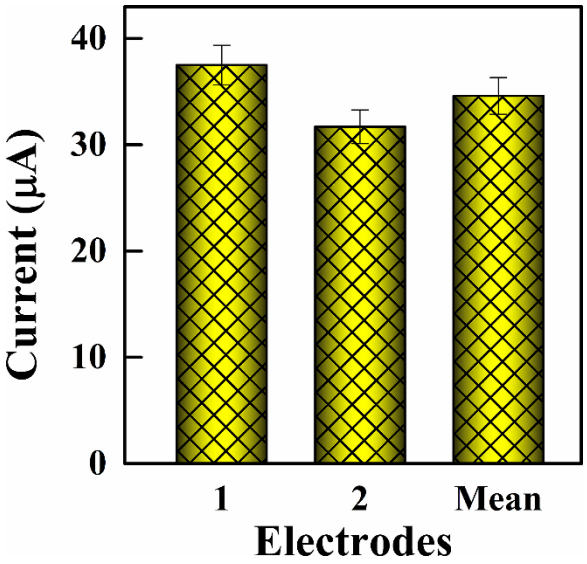

Figure S5. Batch-to-batch reproducibility. Anodic peak current (I_{pa}) of independently fabricated MIP sensors measured in a standard redox solution containing 2.5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.05 M KCl in phosphate buffer (pH 7.4) at 25 °C with a scan rate of 100 mV/s. The mean current was 35.0 µA with a relative standard deviation (RSD) of 11.8% (n = 3).

Table S1

Table S1. Comparison of the PAni-PmAP sensors' performance with previously reported electrochemical sensors and other analytical methods for lipoic acid quantification.

Materials / Reagents	Method	Detection range (µM)	Sensitivity	LOD (µM)	Ref.
1-benzyl-2-chloropyridinium bromide	HPLC-UV	0.2–50	–	0.1	[4]
GCE	HPLC-ECD	0.0024–4.86	–	0.0024	[5]
Pt-electrode	DPV	50–800	–	4.07	[6]
PV-CS/f-MWCNTs/GCE	DPV	0–3000	0.0466 µA/µM	0.012	[7]
Fluorine-doped tin oxide electrodes	SWV	5–200		3.68	[8]
Boron-doped diamond electrode	DPV	0.3–105	0.0236 µA/µM	0.088	[9]
Cobalt phthalocyanine/PG	CV	7.3–260	–	0.25	[10]
GCE	DPV	1–150	–	1.8	[11]
MWCNTs/GCE	LSV	26–180	17.7 µA/µM	19	[12]
NIP/SPGE	CV	1–500	0.014 µA/µM	–	This work
	DPV	1–500	1.15 µA/cm^{2}µM	0.352	
MIP/SPGE	CV	1–500	0.02 µA/µM	–	This work
	DPV	1–500	2.04 µA/cm^{2}µM	0.189	

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