Electrochemical monitoring of LDH-A activity for anticancer inhibitor screening: overcoming UV–Vis interference limitations

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Highlights

- Electrochemical readout of LDH-A activity without optical bias.
- Sensitive NADH detection over extended concentration range.
- Inhibitor screening preserved after enzyme immobilization.
- Tool suitable for metabolic-target drug evaluation.

1. Introduction

Lactate dehydrogenase A (LDH-A) plays a central role in cancer metabolism and is strongly associated with tumor growth and proliferation[1]. For this reason, it has emerged as a promising pharmacological target for anticancer drug development, as its inhibition may enhance tumor sensitization to therapeutic treatments. However, the identification of effective inhibitors requires extensive screening campaigns, which remain time-consuming and economically demanding[2]. LDH-A activity is commonly monitored by following NADH oxidation through UV–Vis spectroscopy at 340 nm [3]. Although widely adopted, this approach may suffer from spectral interference when candidate inhibitors absorb in the same wavelength region as NADH, potentially compromising kinetic interpretation. In this context, an electrochemical approach is proposed as an alternative analytical platform for monitoring LDH-A activity and assessing inhibitor efficacy. The present study compares amperometric NADH detection at a Ti-modified glassy carbon electrode with conventional UV–Vis measurements, evaluating analytical performance and resistance to interference.

2. Methods

Human LDH-A (*h*LDH-A) performances were investigated both in soluble form and after the immobilization on functionalized mesoporous silica. Enzymatic reaction was carried out in 0.1 M pH 7.5 potassium phosphate buffer, at 37°C, in the presence of pyruvate (1.63 mM) and NADH (0.23 mM). Inhibition studies were conducted by NHI2 (20 μM) or galloflavin (5.46 μM). Activity was monitored through NADH oxidation. Electrochemical measurements were performed in a three-electrode configuration using a Ti-modified GCE as working electrode, Ag|AgCl reference electrode, and Pt counter electrode. Cyclic voltammetry (CV), linear sweep voltammetry (LSV), and electrochemical impedance spectroscopy (EIS) were employed for electrode characterization, while chronoamperometry (CA) at 0.66 V, identified as NADH oxidation peak, was used for NADH quantification and real-time monitoring of enzymatic reactions. Results were systematically compared with UV–Vis measurements at 340 nm.

3. Results and discussion

The Ti-modified GCE provided a stable and diffusion-controlled NADH oxidation, with a linear amperometric response in the 0.05–2 mM range. The calculated system sensitivity was 0.614, μA cm^{-2} mM^{-1}, as shown in Figure 1a, with a limit of detection (LOD) of 27.6 μM. High reproducibility and negligible current response to reaction substrates, products, solvents, and inhibitor molecules confirmed the selectivity of the electrochemical platform under screening conditions. In contrast, UV–Vis

spectroscopic analysis revealed significant spectral overlap between NADH and NHI2 at 340 nm, leading to unreliable kinetic interpretation under certain conditions. Moreover, optical detection was characterized by signal saturation for NADH concentrations above 0.4 mM, whereas the electrochemical approach maintained linearity across a broader range (Figure 1a). The proposed electrochemical platform successfully enables real-time monitoring of hLDH-A catalyzed reactions (Figure 1b). In the presence of inhibitors, the NADH oxidation rate decreased to 92% (NHI2) and 78% (Galloflavin) of the uninhibited value for soluble enzyme. Similar trends were observed for the immobilized form, confirming the ability of the system to discriminate inhibitory strength. The stronger inhibitory effect of Galloflavin is consistent with molecular docking results, which indicate stabilizing hydrogen-bond interactions within the enzyme structure. Overall, the electrochemical method not only matches the analytical capability of UV–Vis spectroscopy but effectively overcomes its key limitations.

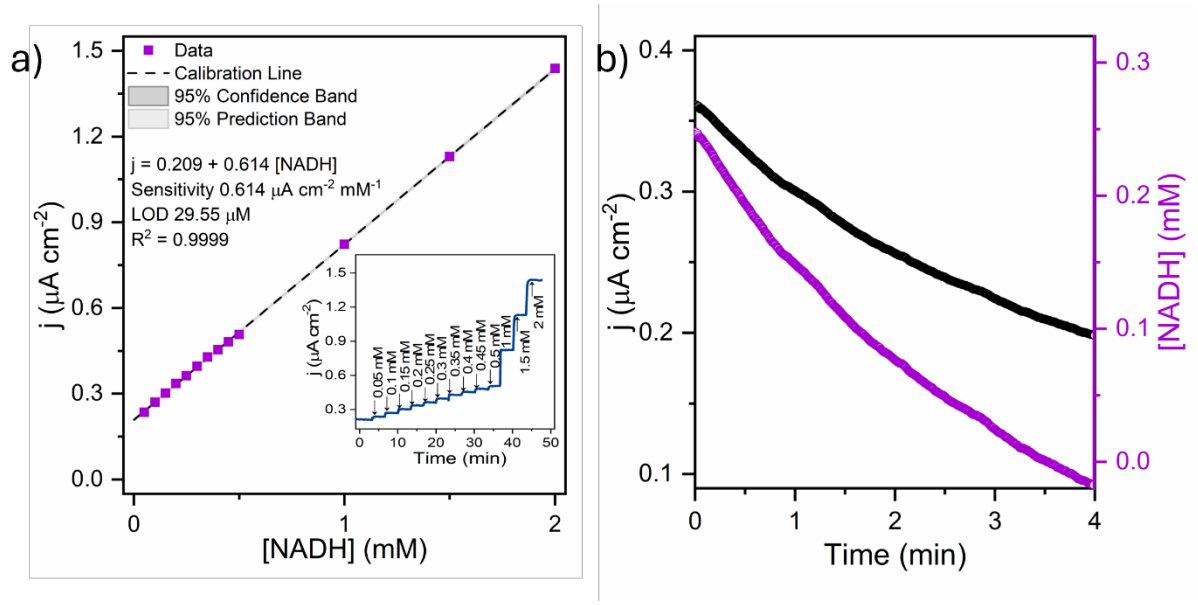

Figure 1. Electrochemical performance of the Ti-modified GCE. (a) calibration line for NADH quantification; inset current variation during amperometric test. (b) Real-time monitoring of LDH-A activity showing direct correlation between current decay and NADH consumption.

4. Conclusions

A Ti-modified glassy carbon electrode was demonstrated as an effective platform for amperometric monitoring of *h*LDH-A activity. The system combines high sensitivity, extended linear range, and resistance to interference from spectrally active inhibitor molecules. Compared to conventional UV–Vis spectroscopy, the electrochemical approach enables more accurate evaluation of inhibitory effects, particularly in the presence of spectrally active compounds. The proposed method represents a simple, cost-effective, and robust analytical tool for rapid screening of anticancer drug candidates targeting metabolic enzymes.

References

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Keywords

Enzyme inhibition; NADH detection; anticancer drug screening; electrochemical biosensing