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Designing a Colorimetric Sensor for NADH Assay Using Silver Nanoprisms

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ABSTRACT

Introduction: Nicotinamide adenine dinucleotide (NAD) and its reduced form (NADH) play a critical role in various cellular reactions, particularly in energy production. Thus, any NADH or NAD+ level shift can harm cell function. Additionally, the concentration of NADH in cancer cells, which is influenced by their metabolic activity, can be impacted by specific anticancer agents. Therefore, monitoring NADH levels in cancer cells is necessary to discover new anticancer drugs. Several methods, including electrochemical, chromatographic, and spectroscopic techniques, are often complicated, time-consuming, and costly. Given the importance of NADH assay in biological samples, we have used silver nanoprisms (AgNPRs) as a platform to develop a simple, sensitive, and cost-effective colorimetric sensor to analyze NADH.

Methods and Materials: AgNPRs were synthesized using a seed-mediated synthesis method by adding NaBH4 into a solution containing $AgNO_3$, sodium citrate, and PVP in ultra-pure water. The formation of AgNPRs resulted in a blue color appearing in the solution. For the NADH colorimetric assay, varying amounts of NADH standard solution or actual serum samples were added to a test tube, containing 500 µL of AgNPRs and 50 µL of Britton–Robinson buffer (0.04 M), and then incubated for 5 min. Afterward, 10 µL of H2O2 (0.01 M) was added to the tube, and the final volume was adjusted to 1.0 mL with distilled water. Following 10 min incubation at the ambient temperature, the mixture absorption spectrum was measured in a 1.0 cm quartz cuvette using a UV–vis spectrophotometer.

Results: T*ransmission electron microscopy* images revealed the triangular shape of AgNPRs. Also, three characteristic peaks of surface plasmon resonance for AgNPRs were observed in the absorption spectrum at wavelengths 330, 490, and 752 nm. The color of the experimental solution containing AgNPRs changed from blue to purple upon adding H_2O_2, indicating a shape transformation of the AgNPRs. In the presence of NADH, the solution color returned to blueish. The alteration in color from purple to blue was proportional to the NADH concentration. These phenomena were utilized to develop a colorimetric method for determining NADH.

Conclusion and Discussion: Our colorimetric sensor can measure NADH concentrations ranging from 15 to 300 nM, with a detection limit of 5.0 nM and a relative standard deviation of 2.0% at 200 nM. Therefore, a simple and cost-effective colorimetric method has been developed to analyze NADH in pharmaceutical and biological samples.

Keywords: Colorimetry, Nicotinamide-adenine dinucleotide, Silver