

ELECTROCHEMICAL SENSOR BASED ON ZnONPs WITH MWCNTs FOR DETERMINATION OF MALIC ACID

¹Eliška BIRGUSOVÁ, ¹Zuzana BYTEŠNÍKOVÁ, ¹Amirmansoor ASHRAFI, ¹Pavel ŠVEC,
¹Vojtěch ADAM, ¹Lukáš RICHTERA

¹*Department of Chemistry and Biochemistry, Mendel University in Brno, Brno, Czech Republic, EU,*
xbirguso@mendelu.cz, richtera@mendelu.cz

<https://doi.org/10.37904/nanocon.2023.4798>

Abstract

Malic acid is one of the most important acids in the food industry and in fermentation processes. Especially in the wine fermentation process, its decreasing concentration plays a significant role. During the process of malolactic fermentation of wine, it is converted into lactic acid, so accurate monitoring of malic acid could lead to improvements in winemaking technology. An electrochemical sensor based on nanomaterials could be used as a suitable tool. Traditionally sensors used enzymes for the determination of malic acid, but they are usually hard to store and expensive. One possibility to replace them is nanomaterials with enzymatic activity (nanozymes). Nanozymes, on the other hand, demonstrate robustness in various environments, making them invaluable tools in both industrial and biomedical applications. One key advantage of nanozymes is their tailorability. By manipulating their size, composition, and structure, researchers can design nanozymes with specific catalytic activities, mimicking natural enzymes or even surpassing their efficiency. This experiment used a combination of ZnONPs (zinc oxide nanoparticles) with a combination of MWCNTs (multi-walled carbon nanotubes) as a tool for the determination of malic acid.

Keywords: Electrochemistry, sensors, carbon nanomaterials, zinc oxide, nanoparticles, nanozymes

1. INTRODUCTION

Organic acids play a crucial role in the composition of wine, imparting essential aromas and serving as indicators of various biochemical processes that occur during wine production. Notably, malic acid undergoes a transformation into lactic acid during a fermentation phase known as malolactic fermentation. This secondary fermentation is highly desirable, particularly in the production of red wines. However, if a winemaker wishes to prevent malolactic fermentation, they must vigilantly monitor the levels of both acids in the wine and halt the process in a timely manner by adding sulfur. Interrupting malolactic fermentation during its progression can potentially compromise the quality of the wine [1]. Currently, there are several methods available for detecting malic acid. One method involves titration, which measures the overall acid content. Alternatively, instrumental methods such as liquid chromatography [2] or electrophoresis [3] can be employed. These techniques, however, can be financially prohibitive for smaller-scale winemakers, often necessitating sample analysis at centralized laboratories, which can be both time-consuming and costly [1]. As a viable alternative, biosensors, especially those utilizing electrochemical principles, show great promise. Electrochemistry offers a low detection limit, ease of operation, and cost-effective instrument procurement. Enzymatic sensors, in particular, have gained popularity [4]. While enzyme-based sensors provide selective responses, their production and storage requirements can be demanding [5]. Nanomaterials present a promising alternative, given their generally straightforward storage conditions and more cost-effective production methods [6]. Nanozymes have

demonstrated their ability to deliver selective and sensitive detection of specific analytes, such as malic and lactic acids in wine production. Their low detection limits and robust performance make them suitable candidates for applications where precision and accuracy are paramount, such as monitoring and controlling fermentation processes [6] [7]. In summary, the integration of nanozymes into electrochemical detection systems holds significant promise for enhancing the efficiency, cost-effectiveness, and accuracy of acid detection processes. This contribution extends to advancements in quality control and production optimization across diverse fields. Continued research and development in this domain are likely to unveil further opportunities for harnessing the potential of nanozymes in analytical chemistry and beyond [8] [9]. In the work by Smutok et al. [6], a biosensor modified with nanomaterials mimicking peroxidase activity was described for the detection of lactic acid in beverages, achieving a limit of detection (LOD) of 2 μM . This study underscores the potential of using nanozymes even in complex analytes like beverages. Additionally, the study utilized mixtures of zinc oxide nanoparticles (ZnONPs) and multi-walled carbon nanotubes (MWCNTs) as materials simulating enzyme-like activity for the detection of malic acid.

2. MATERIALS AND METHODS

2.1 Reagents

Chemicals used for solution and buffer preparation: sodium dihydrogen phosphate, sodium hydrogen phosphate, potassium nitrate, potassium hexacyanoferrate, potassium hexacyanoferrate, potassium hydroxide, sodium hydroxide (VWR international, MA, USA), potassium chloride and malic acid. For cleaning the surface of the electrode, following chemicals were used: ethanol absolute 99.8% (Lachner, Neratovice, Czech Republic), polishing suspension containing Al_2O_3 particles with a size of 1 μm , polishing suspension containing Al_2O_3 particles with a size of 0.3 μm , polishing suspension containing Al_2O_3 particles with a size of 0.05 μm and diamond polishing suspension with a particle size of 0.5 μm (Elektron Microscopy Sciences, Hatfield, PA, USA). For modification of the electrode, these chemicals were used: aqueous solution of Nafion™ 10 wt %, zinc oxide Nanopowder (ZnO, 99.9%, 10 – 30 nm) (US Research Nanomaterials, Inc., Houston, TX, USA) and multi-walled carbon nanotubes functionalized with a carboxyl group, average nanotube dimensions 9.5 nm \times 1.5 μm (MWCNTs). All the chemicals used in this study were purchased from Merck KGaA (Darmstadt, Germany) unless otherwise stated. High-purity deionized water (Milli-Q Millipore 18.2 $\text{M}\Omega\cdot\text{cm}^{-1}$, Bedford, MA, USA) was used throughout the study.

2.2 Preparation of modification of electrode

The bare glassy carbon working electrodes were cleaned by the procedure described in previous work [10]. The modification solution was prepared by weighing commercial ZnO nanoparticles in an amount of 10.0 mg along with 6.0 mg of MWCNTs. The sample was dissolved in 1.50 mL of DI water. Finally, 0.500 mL of Nafion™ was added. The resulting electrode modification dispersion was sonicated. Just before use, the dispersion was sonicated for 5 minutes and then briefly vortexed. The dispersion in the amount of 5.00 μl was applied to the surface of the cleaned working electrode and allowed to dry freely at room temperature.

2.3 Condition of characterization of used materials by SEM + EDS

Images of all samples for EDS analysis were made on MIRA 2 SEM from TESCAN (TESCAN Ltd. Brno, Czech Republic, EU). The detector used external SE with an accelerating voltage of 15 kV. Work distance was set to about 15 mm and scanned mode was used UH resolution. 768 \times 858 pixel images were obtained at 50,000 fold magnification. Elemental analysis was made on EDX detector (Oxford Instruments, UK) with Aztec software and with the same condition as photos. Input energy was set at about 20,000 cts, output energy was about 16,000 cts and dead time fluctuated between 19 – 21 %. The time for each analysis was 20 min. The spot size was 100 nm.

2.4 Condition of Electrochemical Measurement

All the electrochemical measurements were performed in a standard three-electrode system on Autolab PGSTAT302N. Cyclic voltammetry (CV) was used to characterize and verify the functionality of the use of individual modifications Fe for the determination of malic acid with the following conditions: Measurement was performed in 5 mM $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$, potential range from -0.5 V to -0.4 V with scan rate $0.05\text{ V}\cdot\text{s}^{-1}$. To verify the functionality of the modification for measuring the concentration of malic acid, 1 M KOH was used, potential range from 0.0 V to 0.0 V with a scan rate of 0.02 V/s . The electrochemical impedance spectroscopy (EIS) method was used to characterize the electrode surface and individual modifications, same solution of 5 mM $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$ was used for measurement with these conditions: applied frequencies from 100 kHz to 0.01 Hz, number of applied frequencies 10 and applied voltage 0.40 V. Sensitivity and reliability were characterised by differential pulse voltammetry (DPV). The parameters of DPV measurement were potential range from -0.3 V to 0.7 V , amplitude 0.025 V and scan-rate $0.015\text{ V}\cdot\text{s}^{-1}$.

3 RESULTS AND DISCUSSION

3.1 Nanozyme characterization

ZnO nanoparticles do not bind to MWCNTs, so a nanocomposite material is not created in the true sense. Also, these two nanomaterials do not form a common stable dispersion, for this reason, Nafion™ was edited to solution to create stable fixed material on the surface of the electrode. **Figure 1** shows ZnONPs MWCNTs in a solution of Nafion™ and DI water. The image clearly shows that the addition of Nafion™ resulted in the formation of a network in which both types of nanoparticles are stabilized. The size of ZnONPs is in the order of tens of nanometres, but after connecting with MWCNTs, they form larger structures that are captured by Nafion™.

Table 1 Elements representation in the sample by EDS analysis.

Element	Percentage representation
Carbon	84.3 %
Zinck	6,61 %
Oxide	4,50 %
Flour	4,50 %

Figure 1 and **Table 1** show the results of the elemental composition of the ZnONPs and MWCNT sample in the Nafion™ solution created using EDS analysis. The reason for the appearance of flora in the elemental composition is the composition of Nafion™ which is based on fluoropolymer-copolymer.

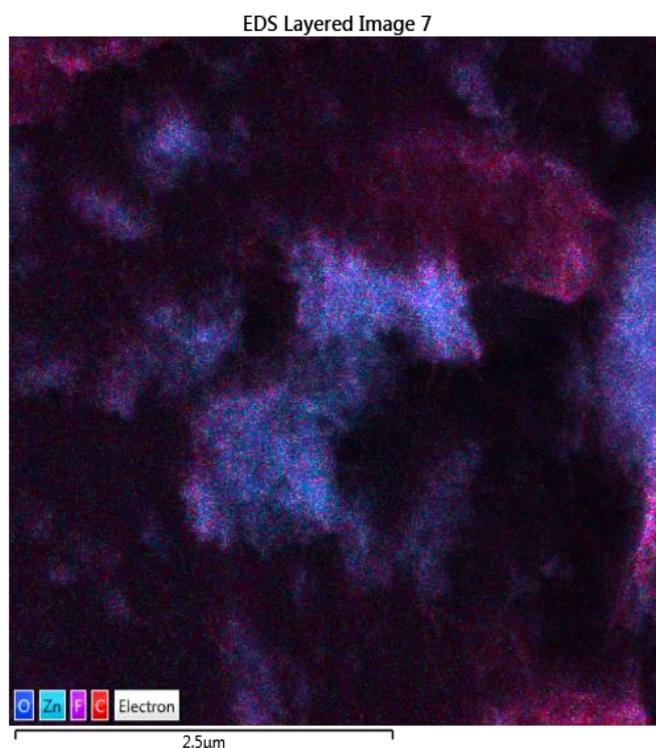


Figure 1 EDS result of modification materials ZnONPs and MWCNTs with Nafion™

3.2 Electrochemical verification of ZnONPs+MWCNTs modification properties

EIS was used to measure the rate of charge transfer. From the EIS result (**Figure 2**) the R_{ct} values for the unmodified and modified electrode can be calculated, based on this value the conductivity properties of the modification can then be determined. The electrode without modification (bare) has $R_{ct} = 350.672 \Omega$. The modified electrode has $R_{ct} = 785.95 \Omega$. Calculated the values of the measured charge transfer resistances are low, which means very fast charge transfer and that the chosen modification is well conductive. At the same time, we can observe a shift of the Nyquist diagram to the left, which usually means an improvement in the electronic contact between the electrode and the electrolyte and a decrease in the total impedance of the electrode, which in this case is very advantageous for us.

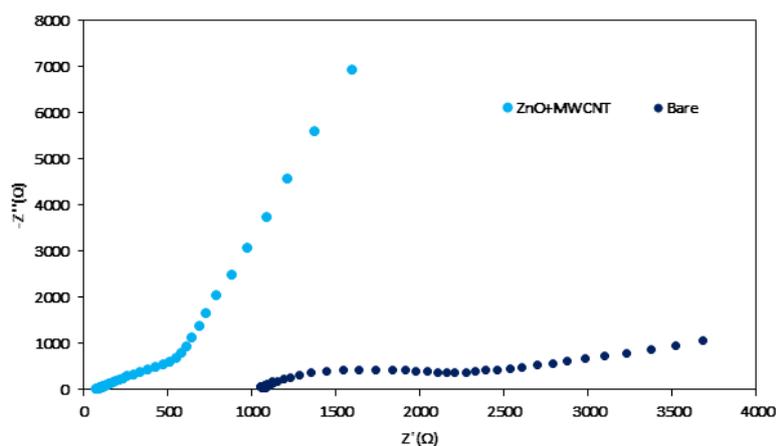


Figure 2 Nyquist diagrams of modification on the electrode

3.3 Measurement of concentration of malic acid

The DPV method was chosen for the detection of malic acid. First, the results of the standard addition method for creating the calibration dependence are presented. The individual volumes and their peak heights are shown in **Figure 3**. We can see from the concentration measurement graph (**Figure 3**) that the blank value is completely different from the rest of the graph, so the selected buffer does not affect the measurement. On the other hand, it can be noticed that a narrow peak is not formed, and the peak peaks are rather spread out, which could cause problems in measurement in a real sample. In a real sample, there are many more substances whose peaks could overlap this one.

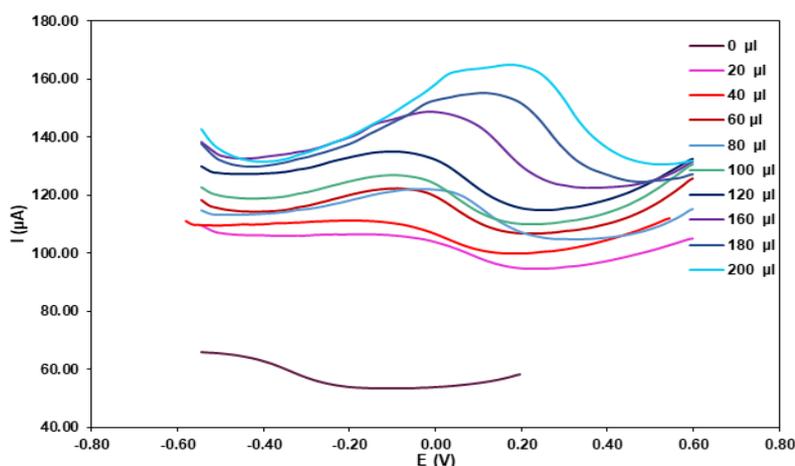


Figure 3 Measurement of DPV during gradual addition of malic acid in PBS solution

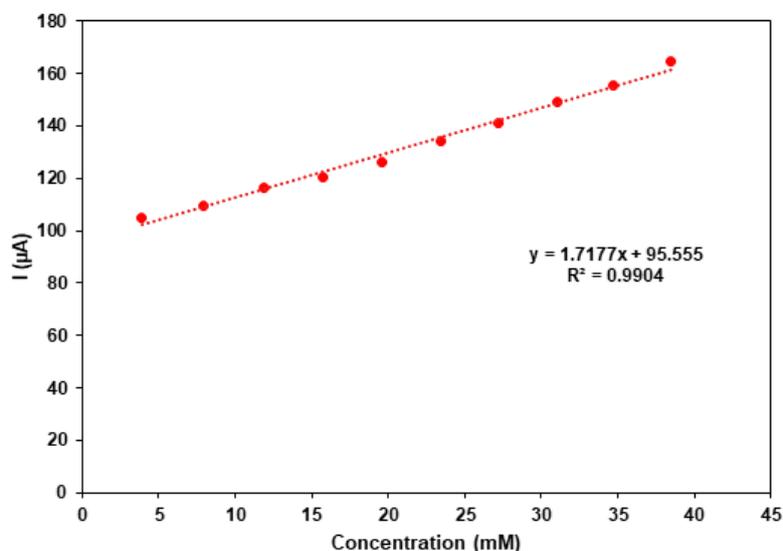


Figure 4 Calibration curve between concentration (mM) and current (µA)

From the calibration curve data (**Figure 4**) and the blank measurement, the detection limit was determined to be 0.79 mM in concentration. In comparison to other sensors designed for the detection of malic acid (**Table 2**), this value may appear relatively high. However, it is important to note that most sensors rely on enzymatic detection methods, whereas the electrode presented here is solely modified with nanomaterials. The average deviation in individual measurements was approximately 0.41, indicating a high degree of precision. Furthermore, the measurement variance was as low as 3.25%. Consequently, it can be inferred that the method exhibits both repeatability and sensitivity.

Table 2 Comparison of the result with other sensors

Type of electrode	Enzyme on surface	LOD (µM)	Reference
platine electrode	yes	0.5	[11]
Clark electrode	yes	2	[11]
carbon electrode with rh modification	yes	18.5	[11]
screen printed electrode	yes	28	[12]
ZnONPs + MWCNTS	no	78.7	this work

4. CONCLUSION

This study introduced an electrochemical sensor based on nanomaterials, offering an alternative to traditional enzyme-based sensors. In this research, a combination of ZnONPs and MWCNTs was employed to detect malic acid. The electrochemical characterization confirmed the modification's conductivity and improved electronic contact between the electrode and the electrolyte. Utilizing differential pulse voltammetry (DPV), the study successfully measured malic acid concentration, establishing a calibration curve and determining a detection limit of 0.79 mM. While this limit may seem relatively high compared to enzymatic sensors, it is essential to recognize that the sensor described here relies solely on nanomaterial modifications, offering advantages in storage and cost-effectiveness. The precision of the method was demonstrated through low average deviation (approximately 0.41) and minimal measurement variance (3.25%), highlighting its repeatability and sensitivity.

ACKNOWLEDGEMENTS

The research was financially supported by the IGA MENDELU AF-IGA2023-IP-027, Mendel University in Brno.

REFERENCES

- [1] ROBLES, A., et al. Determination and identification of organic acids in wine samples. Problems and challenges. *TrAC Trends in Analytical Chemistry*. 2019, vol. 120, p. 115630.
- [2] ONOZATO, M., et al. Column-switching high-performance liquid chromatography-fluorescence detection method for malic acid enantiomers in commercial wines. *Journal of Food Composition and Analysis*. 2022, vol. 106, p. 104282.
- [3] JARAMILLO, E.A., et al. Capillary electrophoresis method for analysis of inorganic and organic anions related to habitability and the search for life. *Electrophoresis*. 2021, vol. 42, no. 19, pp. 1956-1964.
- [4] SOCHOROVA, L., et al. Electrochemical and others techniques for the determination of malic acid and tartaric acid in must and wine. *International Journal of Electrochemical Science*. 2018, vol. 13, no. 9, pp. 9145-9165.
- [5] ADEEL, M., et al. Recent advances of electrochemical and optical enzyme-free glucose sensors operating at physiological conditions. *Biosensors and Bioelectronics*. 2020, vol. 165, p. 112331.
- [6] SMUTOK, O., et al. Biosensor based on peroxidase-mimetic nanozyme and lactate oxidase for accurate L-lactate analysis in beverages. *Biosensors*. 2022, vol. 12, no. 11, p. 1042.
- [7] AHANGARI, H., et al. Latest trends for biogenic amines detection in foods: Enzymatic biosensors and nanozymes applications. *Trends in Food Science & Technology*. 2021, vol. 112, pp. 75-87.
- [8] WANG, X.; S. DONG and H. WEI. Recent advances on nanozyme-based electrochemical biosensors. *Electroanalysis*. 2023, vol. 35, no. 1, p. e202100684.
- [9] ALVARADO-RAMÍREZ, L. et al. Enzyme (single and multiple) and nanozyme biosensors: recent developments and their novel applications in the water-food-health nexus. *Biosensors*. 2021, vol. 11, no. 11, p. 410.
- [10] SEDLACKOVA, E., et al. Label-free DNA biosensor using modified reduced graphene oxide platform as a DNA methylation assay. *Materials*. 2020, vol. 13, no. 21, p. 4936.
- [11] MATTHEWS, C.J.; ANDREWS, E.S. and PATRICK, W.M. Enzyme-based amperometric biosensors for malic acid—a review. *Analytica Chimica Acta*. 2021, vol. 1156, p. 338218.
- [12] ARIF, M., et al. L-Malic acid biosensor for field-based evaluation of apple, potato and tomato horticultural produce. *Analyst*. 2002, vol. 127, no. 1, p. 104-108.