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DNA Characterization Through Nanotechnology-Assisted Bioimpedance and Feature Extraction for Artificial Intelligence

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Abstract. Detecting Deoxyribonucleic Acids (DNA) through their electrical properties is a technique that has not been fully explored. Developing biosensors for detecting label-free DNA without functionalizing transducer surfaces continues to be a relevant technological challenge. Our research group has reported multi-frequency bioimpedance measurements as a potential technique for estimating concentrations of total DNA free of labeling and functionalization. Post-processing techniques, feature extraction, and Artificial Intelligence (AI) algorithms are required for automatic classification and bioimpedance parameters identification that might be used to detect DNA and its concentration. This article explores multi-frequency bioimpedance measurements assisted with magnetic nanoparticles and post-processing AI feature extraction to correlate characteristical bioimpedance parameters with DNA concentrations and purity. Post-processing estimation of the area under the Cole plot seems a relevant feature to correlate with DNA concentration and purity. The results indicate relevant evidence proposing DNA characterization through nanotechnology-assisted bioimpedance and AI.

Keywords. Bioimpedance, DNA, magnetic nanoparticles.

1 Introduction

Electrical impedance spectroscopy has been used in the characterization of materials for a variety of reasons [1] especially because allows for distinguishing interface from bulk phenomena.

It is also helpful for sensing and biosensing since the electrical properties of materials are highly dependent on the interaction with the environment. Indeed, different effects can be interrogated by varying the frequency of the electrical stimulus, which is exploited in determining the interfacial changes induced in sensing experiments [1,2]. This has made impedance spectroscopy a method of choice for much work on sensors and biosensors, particularly with sensing units comprising nanomaterials with large surface area to volume ratios [3] .

An impedance spectroscopy system comprises three main units: signal processing, sensing, and data [1] .

Miniaturized nucleic acid quantification and sizing technologies can serve as point-of-use tools for research and clinical applications ranging from infectious disease and genetic testing to screening environmental samples for virus and pathogen detection [4]. Although much progress has been made in the construction of portable optical systems, ultra-compact [5,6] and portable devices can be more easily achieved using electrical biosensors due to the comparative ease in the miniaturization of electronic systems [5]. The strategy of applying coatings on nanoparticle systems, thus forming a type of nanoparticle called a core-shell, manages to change the surface of this magnetic system so that toxicity or chemical instability is reduced by encapsulating it with another biocompatible material. In this way, this

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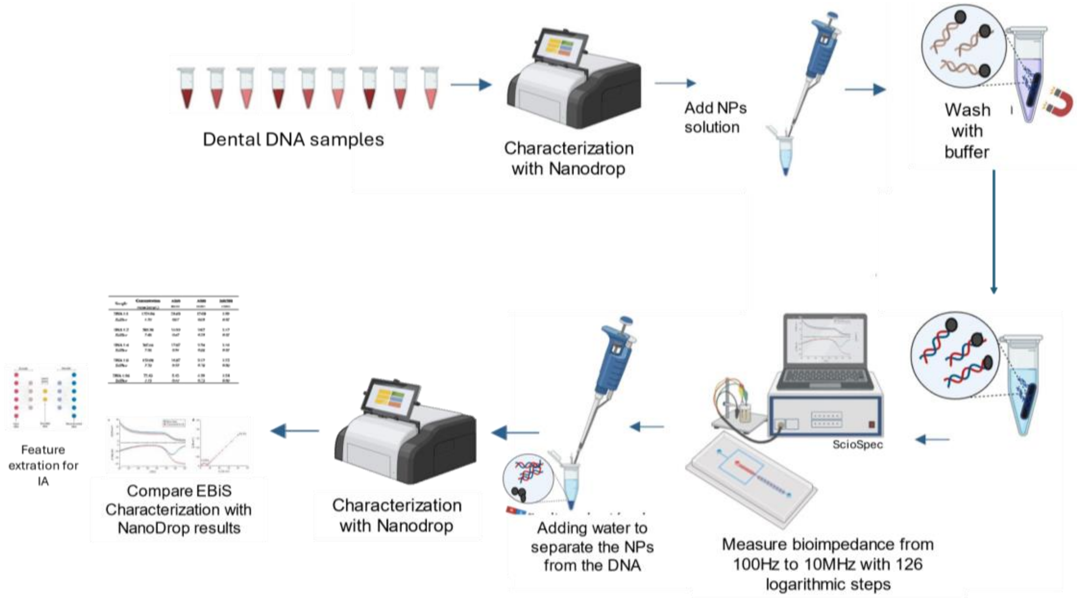

Fig. 1. General methodology

system's properties are taken advantage of [3], since it would retain the magnetic property of the core material. Still, on its surface or shell, it would have specific groups that can physically interact with DNA [4].

These systems would generate a great advantage over other existing systems since, by applying nanotechnology, a reduction in particle size causes an increase in the contact area of the system; in other words, the smaller the system, the more absorption capacity it can have. Moreover, there is a novel approach to identifying DNA fragments based on their frequency-dependent dielectric properties. Multi-frequency excitation of electric fields within a small microfluidic channel is used to detect changes in electrical impedance as DNA fragments, which couple into nano-sized particles, pass through the channel [4].

Accurate detection and quantification of DNA is a crucial part of several applications in biomedicine and molecular diagnostics. Although much progress has been made in sequencing and genetic analysis technologies, there are substantive challenges with direct DNA detection. Multi-frequency bioimpedance, magnetic nanoparticles, and modern artificial intelligence techniques form a comprehensive approach to overcoming these challenges. Multifrequency bioimpedance is a non-invasive technique for DNA detection that can monitor changes in the electrical properties of the biological medium. It is, therefore, suitable for direct and non-invasive detection of DNA concentration and is an alternative to overcome problems related to the labeling technique [4].

Using nanoscale magnetic particles for DNA immobilization allows for a viable approach to many modern approaches. The combination of multifrequency bioimpedance measurements assisted with magnetic nanoparticles anchored to genetic material by the effect of electrostatic-type interactions and advanced post-treatment artificial intelligence techniques allows the detection and quantification of DNA.

This methodology can potentially improve the sensitivity and specificity of DNA detection and facilitate the development of biosensors applicable to medical diagnosis. This work explores multi-frequency bioimpedance measurements assisted with magnetic nanoparticles and post-processing feature extraction based on AI to correlate characteristical bioimpedance parameters with DNA concentrations and purity.

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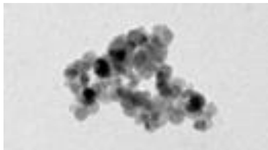

Fig. 2. TEM-multi-domain magnetite core [7]

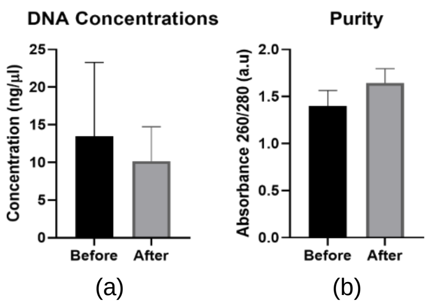

Fig. 3. Bar graphs result of UV-Vis spectrophotometry measurements (a) Concentrations before and after purification (b) Purity before and after purification

2 Materials and Methods

2.1 Experimental Design

To evaluate the bioimpedance's capacity to identify samples with pure DNA from one without a certain purity level or concentration, we developed a simple test using thirty waste samples consisting of dental DNA: instead, magnetic nanoparticles were added for DNA purification. **Fig. 1**. Shows a scheme of experimental design from DNA extraction to DNA purification with nanotechnology, as well as its Electrical Bioimpedance Spectroscopy (EBiS) and UV-Vis Spectroscopy characterization.

2.2 DNA Concentration and Purification

DNA concentration and purity were assessed by UV-Vis spectroscopy and segmented into three specific ranges for molecular biology assays: concentration (<10 ng/µL, 10-25 ng/µL, >25 ng/µL) and purity (<1.4, 1.4-1.8, >1.8). These ranges allowed to evaluate the impact of DNA purity and concentration on the experimental results.

For DNA purification, the geneMAG 1000 kit (Cat. No.: 3601-1000) was used. Which is a magnetic purification system specifically designed to efficiently remove unincorporated staining terminators and residual salts from sequencing reactions. This system is based on using magnetic nanoparticles functionalized on its surface with a hydrodynamic diameter of 100-200 nm, which have a multidomain core and can be easily separated in batches by applying an external magnetic field [7]. This feature allows rapid and simple isolation of nanoparticles loaded with purified DNA.

This study included the vehicle with magnetic nanoparticles and buffer. All samples were purified, and all DNA samples were subjected to this purification process. This vehicle system was developed to work with DNA samples, helping to eliminate impurities and optimizing the quality of the genetic material collected.

2.3 EBiS Measurements

EBiS measurements were developed using the ScioSpec ISX-3 system (Sciospec Scientific Instruments Inc, Germany) in bipolar configuration at a frequency range from 100 Hz to 10MHz in 126 logarithmically spaced steps. A 2µl sample was gently dropped on an interdigitated microelectrode array. EBiS measurements were made before and after DNA purification to study the changes in EBiS caused by impure vs. pure DNA.

3 Results

3.1 UV-Vis Measurements

Fig. 3. (a) and (b) show the UV-Vis measurements. The bar graphs in the case of Fig. 3. (a) show the remarkable decrease of the concentration after the purification procedure with magnetic nanoparticles, on the other hand in Fig.2. (b) the increase of DNA purity is observed.

3.2 Bioimpedance Measurements

Fig. 4. illustrates the Nyquist plots obtained under different experimental conditions, showcasing the classification based on DNA purity and

Table 1. The area under the Bode magnitude curve dependent on the purity

DNA purity	<1.4	1.4-1.8	>1.8
$\int_{1}^{126} Z_{\|Z\|}$	2.4095E10	2.347E10	3.2995E10

Table 2. The area under the Bode magnitude curve dependent on the concentration

DNA concentration	0-10 ng/μl	10-15 ng/μl
$\int_{1}^{126} Z_{\|Z\|}$	2.383422E10	2.315623E10

concentration before and after purification. Fig. 4 (a) and (b) show Nyquist plots classified based on (a) DNA purity, and (b) DNA concentrations, in both cases assisted with magnetic nanoparticles.

Note that the post-purification concentration ranges were divided into only two groups of concentrations between 0-10 ng/μl and 10-25ng/μl as it was decreased.

4 Discussion

4.1 UV-Vis Characterization

The remarkable decrease of the concentration was observed after the purification procedure with magnetic nanoparticles possibly due to a bad untethering of the DNA-NPs complex, however if the increase of the DNA purity index is observed comparing the before and after of the absorbance values measured at 260 nm and 280 nm obtained. Which indicates that there was a satisfactory purification process.

4.2 EBiS Characterization

In this study, the EBiS characterization of DNA assisted with magnetic nanoparticles, in addition to allowing DNA purification, allows the genetic material to be anchored to a microelectrode system for an EBiS characterization in which we can observe a region of sensitivity dependent on concentration or purity. Therefore, all DNA samples were analyzed, and their impedance spectra were observed. The process was identified according to the graphical features of impedance data in the Nyquist plot.

By comparing the impedance data, one can find semicircles in the middle-frequency range centered around 20 kHz.

In the characterization of DNA anchored to magnetic nanoparticles at frequencies between 100 Hz and 10 MHz, it is possible to observe different frequency-dependent processes. At low frequencies, the contributions are usually related to the interfacial polarization and the charge effects on the surface of the nanoparticles. However, at higher frequencies, in the MHz range, the dominant effects may be related to the dipole relaxation of DNA molecules.

4.2.1 Nyquist Plot Analysis

Higher-purity samples anchored to nanoparticles before purification exhibit larger semicircles, indicating higher resistance, which may be attributed to contaminants interfering with the electrochemical process at the electrode interface or the effect of the nanoparticles.

After purification, the Nyquist plot sorted by DNA purity shows a marked improvement in homogeneity, with well-defined semicircles observed. Lower impedance values correlate to higher DNA concentrations.

The NPs-assisted purification process evidence reduced semicircle diameters, indicating a decrease in charge transfer resistance, probably due to removing contaminating or non-specific binding molecules.

This improved sensitivity after purification suggests that the NPs facilitate the removal of impurities.

4.3 Sensibility Features Extraction

As far as parameter identification is concerned, the semicircles observed in the mid-frequency range serve as fingerprints for different concentration levels, allowing a qualitative assessment of DNA concentration from impedance data.

The difficulties encountered stemmed from the overlapping of the semicircles in the Nyquist plot

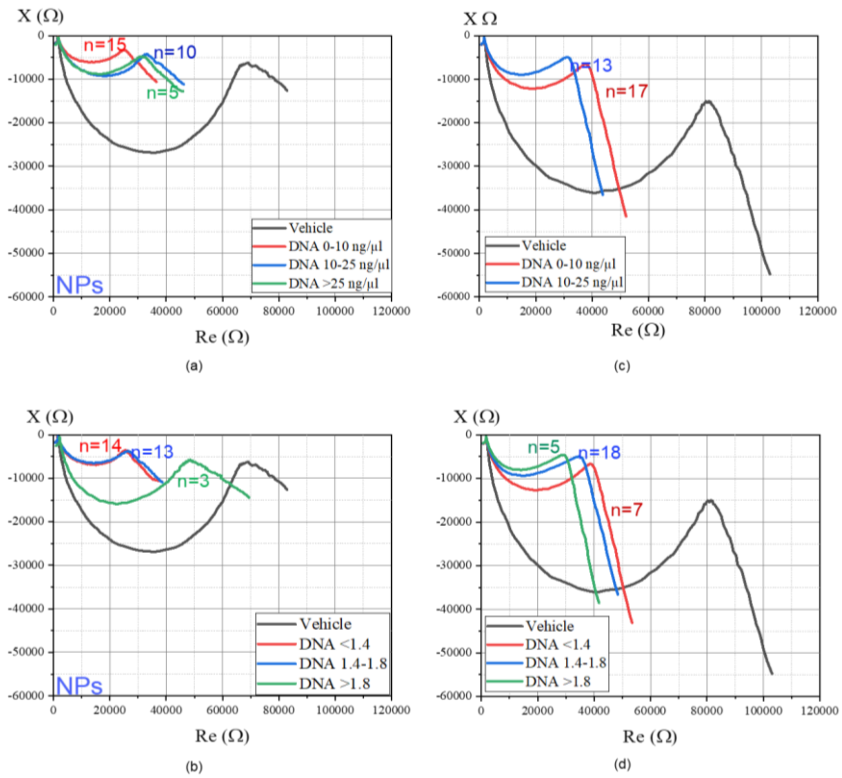

Fig. 4. (a) Nyquist diagram classified based on DNA concentration assisted with nanotechnology (b) Nyquist diagram classified based on DNA purity assisted with nanotechnology, (c) Nyquist diagram classified based on DNA concentration after the purification with nanotechnology (d) Nyquist diagram classified based on DNA purity after the purification with nanotechnology

due to the proximity of characteristic frequencies. Therefore, it was decided to extract specific data from the post-purification Bode plots.

This choice is due to the fact that Bode diagrams provide a clearer representation of the relationship between impedance and frequency, which facilitates the evaluation of changes in the system after the purification process. In this way, the data presented are consistent with the methodology applied to evaluate the behavior of DNA anchored to magnetic nanoparticles.

Table 1 and Table 2 show the areas under the curve of the Bode plots of the post-purification results for both purity and characterization, these areas provide sensitive data that can be integrated into AI algorithms for more accurate analysis since AI requires accurate and relevant data to function optimally, proceeding with automation in turn reducing the time and effort needed for DNA characterization, whether based on purity or concentration.

5 Conclusions

In this study, we propose to design a gene-sensor based on nanotechnology-assisted relative electrical bioimpedance measurements, specifically in this study with magnetic nanoparticles. The characterization of DNA anchored to magnetic nanoparticles by EBiS provides a solid basis for using AI algorithms to determine DNA concentration or purity.

AI algorithms can analyze the ranges of curves in Nyquist plots obtained during this characterization to identify specific patterns associated with different DNA concentrations and purity levels.

This combination of advanced techniques enables accurate and efficient evaluation, improving the precision and speed of biomolecular analysis, which has important implications in areas such as biomedicine and genetic research. Not to mention that the complete extraction of all associated parameters, such as the identification of characteristic frequency and slope, among others, not obtained in this work, are crucial to improve the accuracy and efficiency of AI models applied in DNA characterization.

A key aspect of this research is the reproducibility of the technique used. Multiple measurements were performed under controlled conditions and consistent results were obtained in each experiment, which supports the robustness of the method for characterizing DNA anchored to magnetic nanoparticles. In addition, the accuracy of the analyses, such as obtaining the area under the curve in the Bode diagrams, reinforces the reliability of the proposed approach. The ability to replicate these results in different samples and under various experimental conditions suggests that this technique has significant potential to be used in future studies related to the interaction between biomolecules and nanomaterials

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References

1. **Buscaglia, L.A. (2022).** Development of a Portable Impedance Spectrometer. Instituto de Física de São Carlos, Universidade de São Paulo. DOI: 10.11606/D.76.2022.tde-19072022-120227.
2. **Navarro, K. (2018).** Nanomateriales: Optimización en la extracción de ADN. México Ciencia y Tecnología. https://www.cienciamx.com/index.php/tecnologia/nanotecnologia/20348-cnyn-unam-uabc -nanoparticulas-ald.
3. **Chengzhou, Z., Guohai, Y., Él, L., Dan, D. (2014).** Electrochemical Sensors and Biosensors Based on Nanomaterials and Nanostructures. ACS Publications, Vol. 71, No. 1. DOI: 10.1021/ac5039 863.
4. **Sui, J., Gandotra, N., Xie, P., et al. (2021).** Multi-frequency impedance sensing for detection and sizing of DNA fragments. Sci Rep, Vol. 11, No. 6490. DOI: 10.1038/s41598-021-85755-9.
5. **Buscaglia, L.A., Oliveira, O.N., Carmo, J.P. (2021).** Roadmap for Electrical Impedance Spectroscopy for Sensing: A Tutorial. IEEE Sensors Journal, pp. 22246–22257. DOI: 10.1109/JSEN. 2021.3085237.
6. **Bhatt, G., Bhatt, M., Bhattacharya, S. (2023).** Impedance Spectroscopy and its Application in Biological Detection, CRC Press. DOI: 10.1201/978 1003358091.
7. **Chemicell Media, K. (2007).** Ferrofluids: Ferromagnetic Particles in Carrier Fluid: Fluidmag. www.chemicell.com/products /Magnetic_Nanoparticle/magnetic_Nanoparticles.html [retrieved on 2024-07-22].

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