5TH CLABIO
LATIN AMERICAN BIOIMPEDANCE
FLORIANOPOLIS – BRAZIL - 2024

Comparative analysis of EBiS instrumentation to determine DNA concentrations

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1 Introduction

Challenges and Limitations in Assessing DNA Concentrations

Accurate determination of human genomic DNA concentrations is essential in molecular biology assays.

Popular techniques, such as:

- Absorbance
- Fluorescent Dye-Binding
- Electrophoresis

are widely utilized for this purpose.

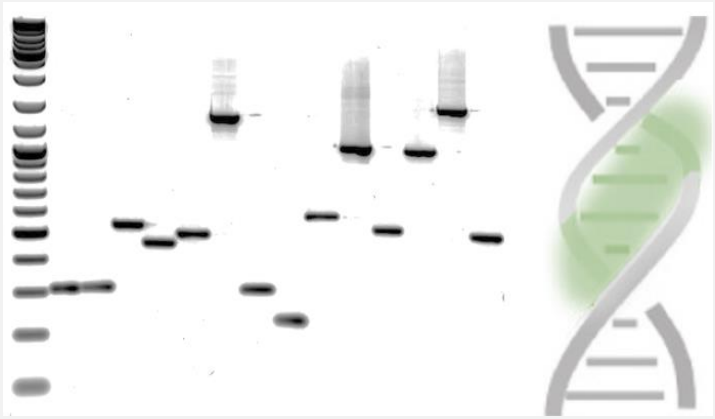


Figure 1. Popular Techniques: Electrophoresis and Fluorescent Dye-Binding.

Nevertheless, recognizing the limitations of current methods is crucial.

1 Introduction

Challenges and Limitations in Assessing DNA Concentrations

Limitations:

- Need for prior purification of raw sample
- Labeling
- Surface functionalization
- Sample immobilization
- Time consumption

Key Limitations:

- Skilled personnel
- Highly expensive equipment

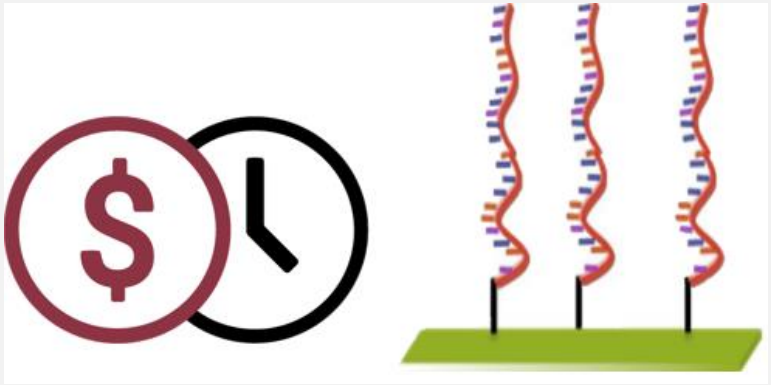
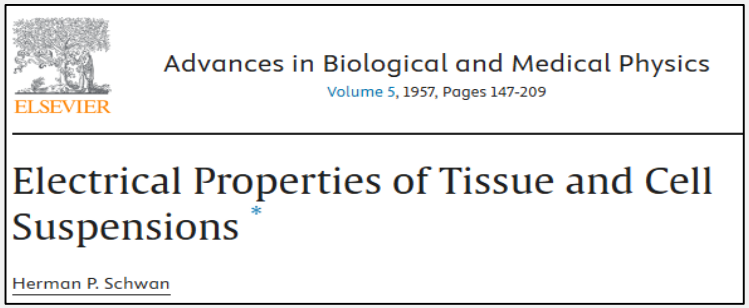


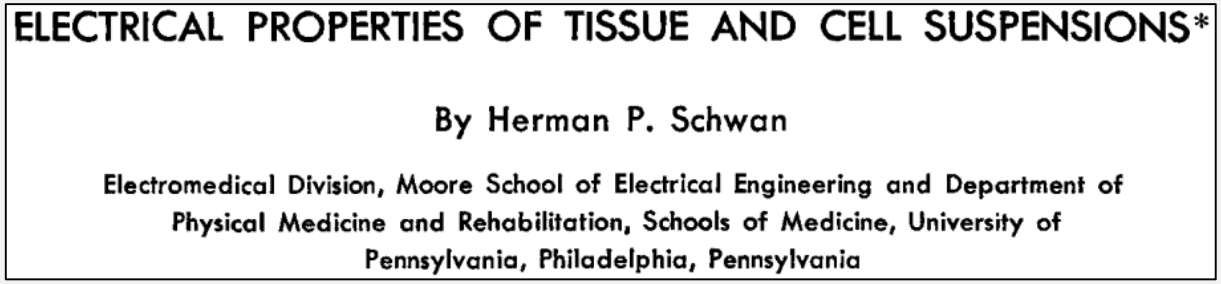
Figure 2. Limitations of Current DNA Concentrations Assessing Methods.

1 Introduction

Electrical Bioimpedance & Electrical Bioimpedance Spectroscopy

Proposed as early as 1957, bioimpedance measurements have emerged as a valuable tool for assessing the electrical properties of biological systems in medical and biomedical applications.





1 Introduction

Electrical Bioimpedance & Electrical Bioimpedance Spectroscopy

Electrical bioimpedance describes the opposition of biological tissue to the flow of an applied alternating current and the resulting voltage signal across the biological system.

Mathematically defined by the complex relationship between a constant, Resistance (R), and a time-dependent variable, Reactance (X).

$$Z = R + jX$$

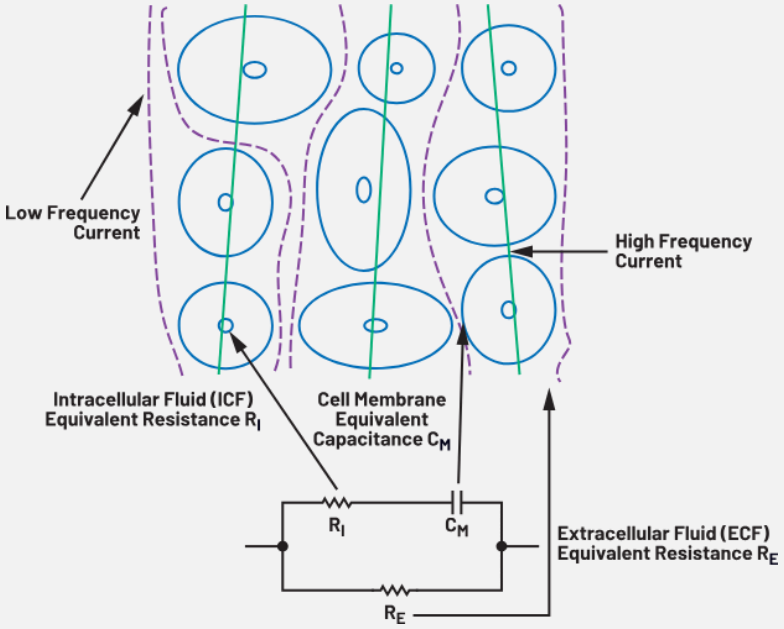


Figure 3. Cole-Cole Modeling of Biological System.

1 Introduction

Electrical Bioimpedance & Electrical Bioimpedance Spectroscopy

Electrical Bioimpedance Spectroscopy (EBiS), a technique for bioimpedance analysis based on the electrophysiological behavior of the dielectric and conductive properties of tissues and cells, analyses electrical properties of biological samples, enabling measurements across a specified alternating current frequency range.

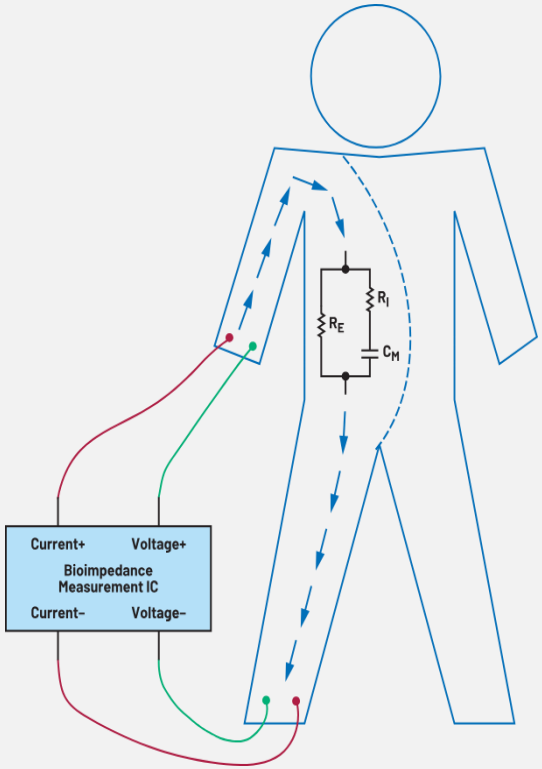


Figure 4. Bioimpedance Measurements and Cole-Cole Model.

1 Introduction

Electrical Bioimpedance & Electrical Bioimpedance Spectroscopy

The application of EBiS has been previously proposed as a sensitive technique for the detection of label-free DNA concentrations, with numerous studies advocating for this approach as an innovative method for gene sensing.

Impedance Measurements Sensitive to Complementary DNA Concentrations

Gerardo Ames[1], R. Gnaim[2], J. Sheviryov[2], A. Goldberg[2], M. Oziel[3], E. Sacristán[1], and César Antonio González[2,4(✉)]

P. Bertemes-Filho (Ed.): ICEBI 2019, IFMBE Proceedings 72, pp. 154–157, 2020.
https://doi.org/10.1007/978-981-13-3498-6_23

4th Latin American Conference on Bioimpedance 2021 (CLABIO 2021) IOP Publishing
Journal of Physics: Conference Series **2008** (2021) 012016 doi:10.1088/1742-6596/2008/1/012016

Bioimpedance Spectroscopy as a potential technique to detect label-free PCR products

G Ames-Lastra[1], V Sánchez[2], E Sacristán-Rock[1], M Gómez-López[2], N Pérez-Vielma[3], I A Castillo-Salazar[4], A Hernández-Nava[5] and C A González-Díaz[2]*

Microsystem Technologies
https://doi.org/10.1007/s00542-020-05074-3

TECHNICAL PAPER

An analysis method to detect the presence of DNA using electrochemical impedance spectroscopy (EIS) for real-time PCR

Juhun Lim[1] · Jinhwan Kim[2] · Eulgi Lee[1] · Hansoo Park[1] · Jong-Hyun Lee[1,2]

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1 Introduction

Bioimpedance-Based Biosensors

Biosensors offer a fast, practical, and cost-effective alternative to large, specialized equipment, making them ideal for hospitals and laboratories in resource-limited settings.

Bioimpedance-based biosensors present a promising substitute for traditional, costly impedance systems.

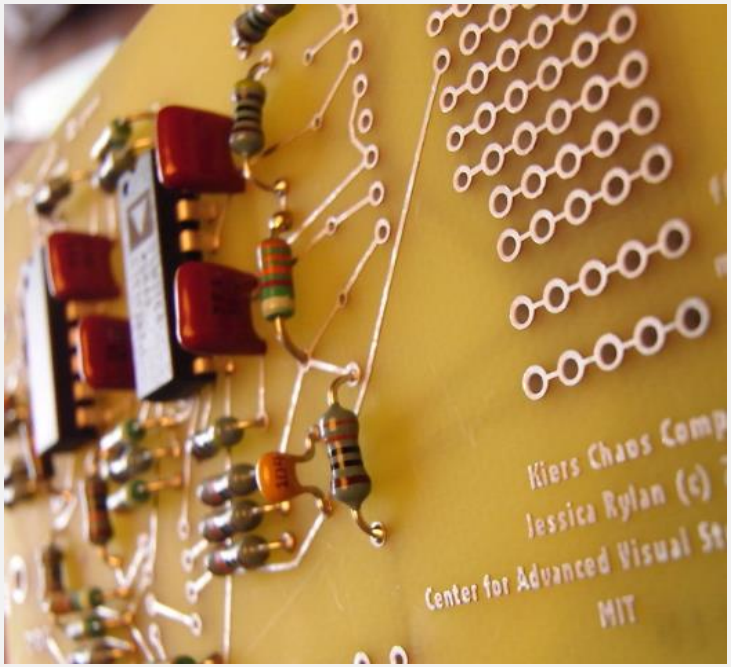

Figure 5. Biosensor Board.

1 Introduction

EVAL-AD5933EBZ

EVAL-AD5933EBZ (Analog Devices, USA) evaluation board serves as an example of commercially available devices for impedance analysis.

AD5933EBZ, as a bioimpedance analyzer, highlights portability and accessibility, aligning with the growing trend in the biomedical field towards portable equipment.

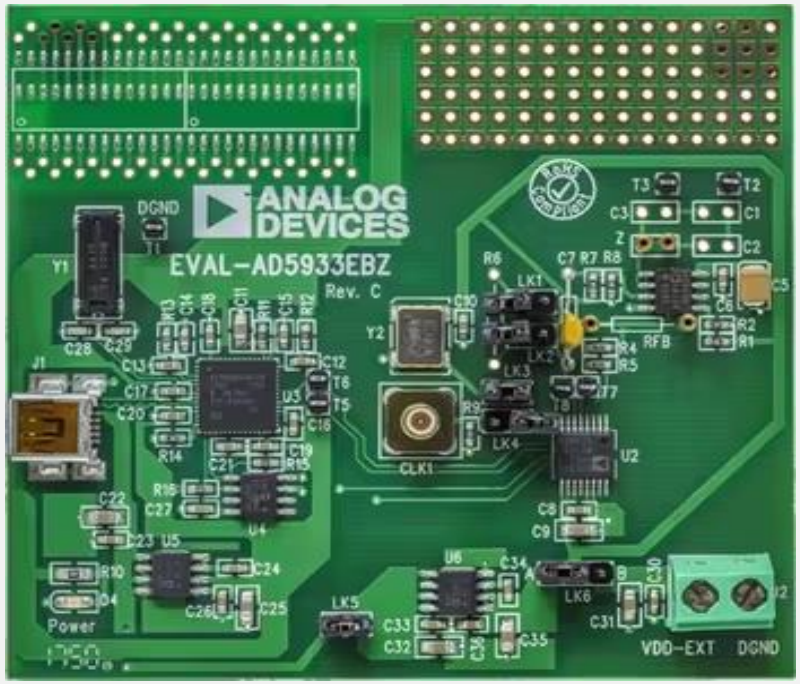

Figure 6. EVAL-AD5933EBZ.

2 Methodology

EBiS Instrumentation & DNA Evaluation

2.1 *DNA Samples*
- DNA samples description

2.2 EVAL-AD5933EBZ
- IC and Evaluation Board, GIU & Calibration

2.3 ScioSpec ISX-3
- ISX-3 description

2.4 DNA Evaluation
- EBiS measurements

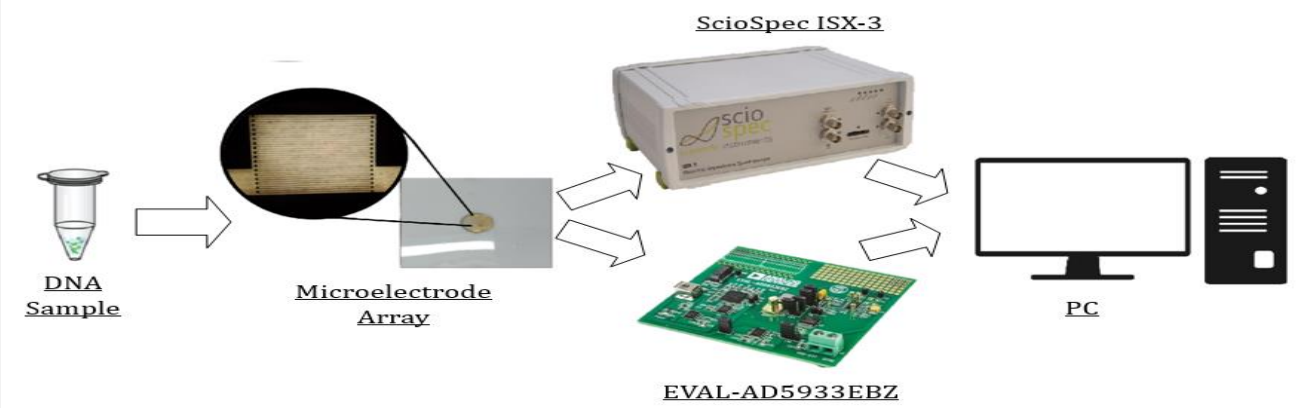

Figure 7. Block diagram of Experimental Setup.

2 Methodology

2.1 DNA Samples

Dental waste DNA samples (*n* = 10) were extracted using a commercial kit QIAGEN QIAamp DNA Micro Kit.

DNA concentrations were measured using UV-Vis Spectrophotometer NanoDrop 2000.

Sample's measured values were grouped within a 10-25 ng/µL concentration group.

Figure 8. Nanodrop 2000.

2 Methodology

2.2 EVAL-AD5933EBZ (IC & Evaluation Board)

AD5933 IC: High precision impedance converter. Combines an on-board 1 kHz to 100 kHz signal generator and ADC.

EVAL-AD5933EBZ: Features a AD5933 IC along with supplementary circuitry to facilitate measurements and manage chip-computer interactions.

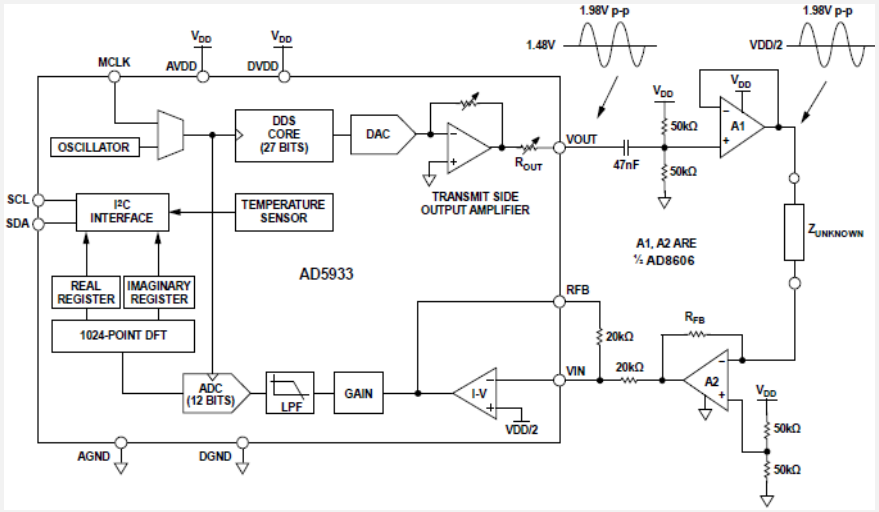

Figure 9. Block diagram AD5933EBZ.

2 Methodology

2.2 EVAL-AD5933EBZ (GUI)

AD5933 GIU: Programming is done through the AD5933 Software Evaluation (Rev1.0) found on the official Analog Devices' website.

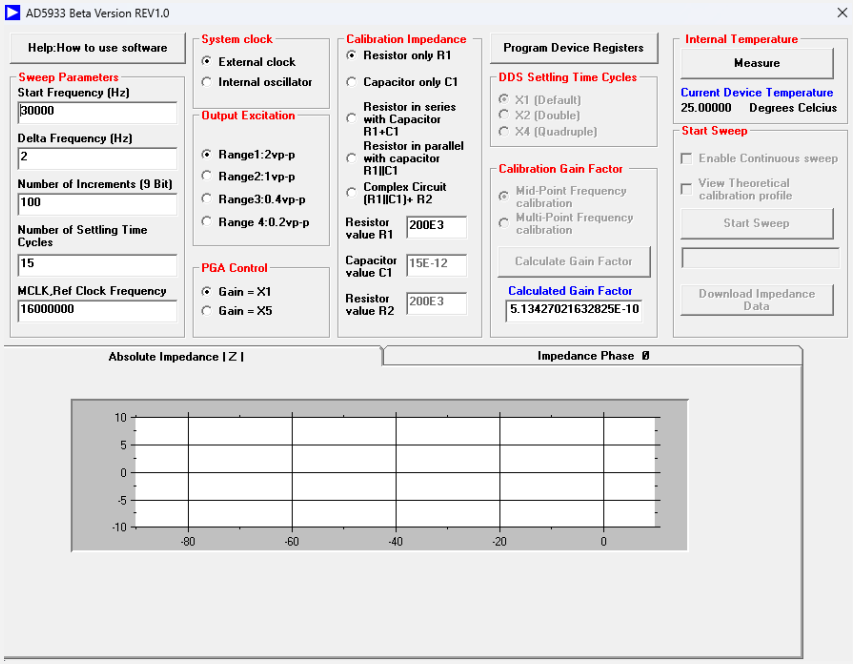

Figure 10. AD5933EBZ Software Evaluation (GIU).

2 Methodology

2.2 EVAL-AD5933EBZ (Calibration)

Prior to acquiring DNA impedance and phase data, the board was calibrated as outlined in the datasheet. The feedback resistor (RFB), required by the manufacturer, ensured accurate measurements and prevented signal clipping. Reliable impedance values were obtained within the 5–50 kHz frequency range.

The RFB (560 Ω) for the development of DNA measurements was determined based on expected minimum impedance values reported in previous studies.

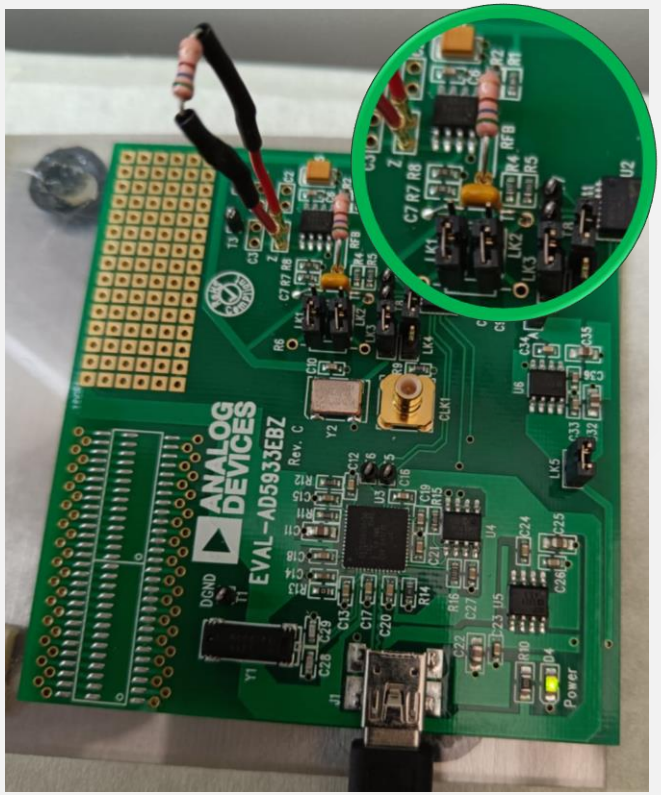

Figure 11. AD5933EBZ Feedback Resistor (*RFB*).

2 Methodology

2.3 ScioSpec ISX-3

The ScioSpec ISX-3 is a versatile impedance analyzer for Electrical Impedance Spectroscopy, EBiS, and Electrical Impedance Tomography. It supports frequencies from 100 mHz to 10 MHz (extendable to 100 MHz) and precision measurements from mΩ to TΩ.

The ISX-3 is well-suited for applications in biological sample analyses, providing a powerful tool for research applications.

Figure 12. ScioSpec ISX-3

2 Methodology

2.4 DNA Evaluation

EBiS measurements were conducted using the EVAL-AD5933EBZ over a 5–50 kHz frequency range with 126 linear steps.

DNA samples (2 µL) were placed on a 10 × 10 mm interdigitated gold-film microelectrode array connected to the load pins, functioning as a transmit-receiver for impedance analysis.

Measurements were performed in triplicate and reported as mean values.

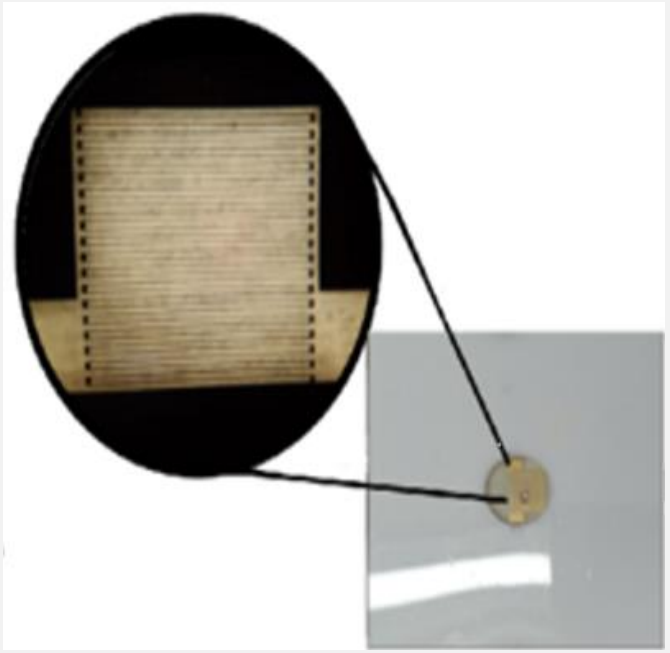

Figure 13. Microelectrode Array

3 Results

DNA Samples

DNA concentrations for the 10-25 ng/µL group, quantified using a UV-Vis Nanodrop spectrophotometer, are shown in Table 1.

Table 1. DNA 10-25 ng/µL concentration group

DNA Sample	Mean (ng/µL)	Std Error
DNA_1	13.2333	0.0105
DNA_2	10.6	0.2810
DNA_3	23.4	0.0795
DNA_4	16.9333	0.0459
DNA_5	16.4	0.0182
DNA_6	17.8	0.0483
DNA_7	14.9666	0.0380
DNA_8	12.3	0.0365
DNA_9	15.8333	0.0459
DNA_10	13.9	0.0316

3 Results

Mean Percentage Error (MPE)

The mean percentage errors (MPE) for Milli-Q water, saline solution, and DNA samples were calculated based on the differences between the magnitude spectra values obtained from the EVAL-AD5933EBZ, used as the measured value, and the Scio-Spec ISX-3 spectrometer, considered the true value, as shown in Table 2.

Table 2. MPE for Mili-Q water (MQ), saline solution (SS) and DNA samples (DNA)

Sample	MPE (%)
MQ	19.682
SS	40.243
DNA	12.059

3 Results

Mili-Q water (Magnitude)

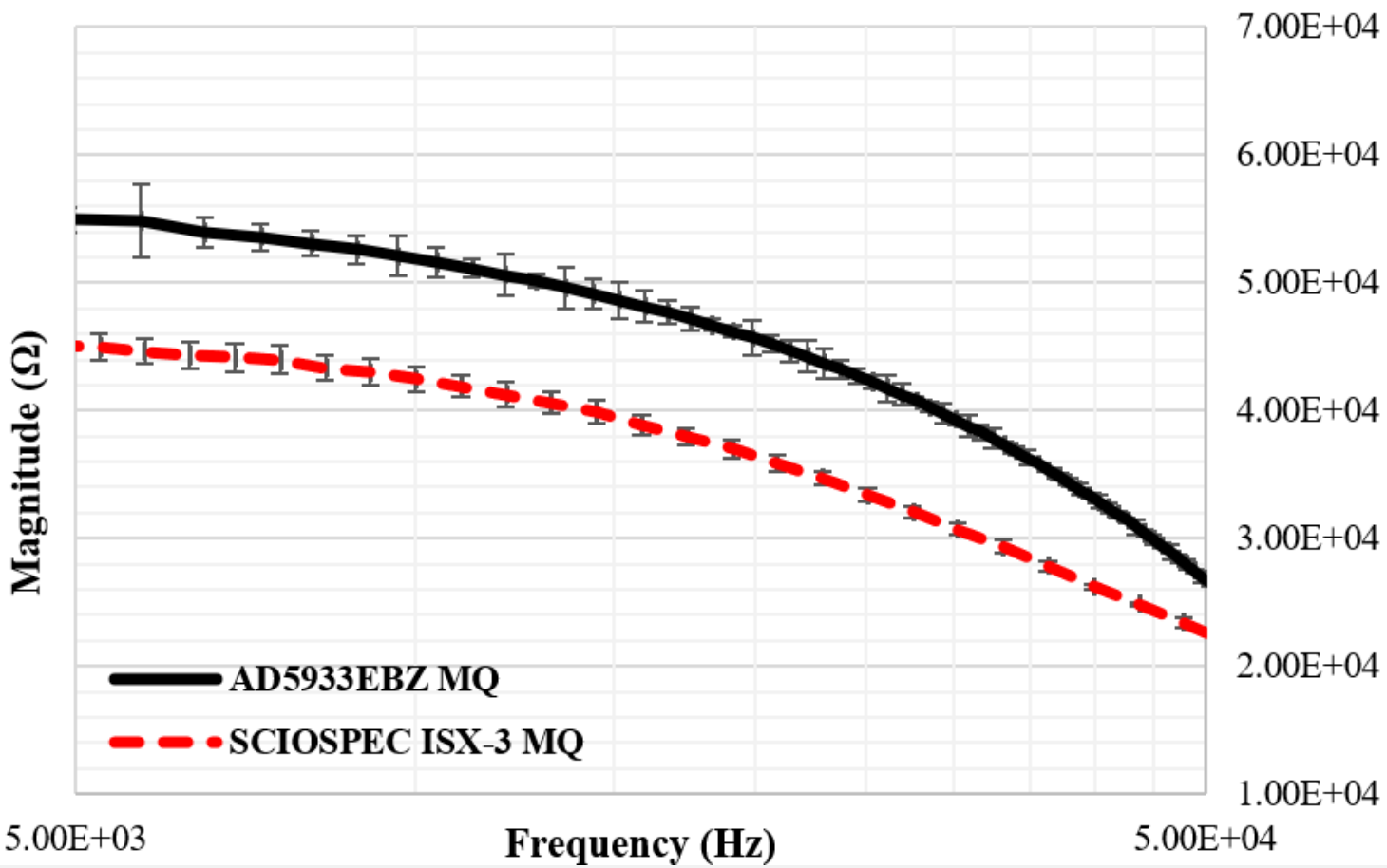

Figure 13 (a). Magnitude spectra for the EVAL-AD5933EBZ and ScioSpec ISX-3 in Milli-Q water (MQ). Data are shown as mean values (measurements taken in triplicate). Error bars represent standard error.

3 Results

Mili-Q water (Phase)

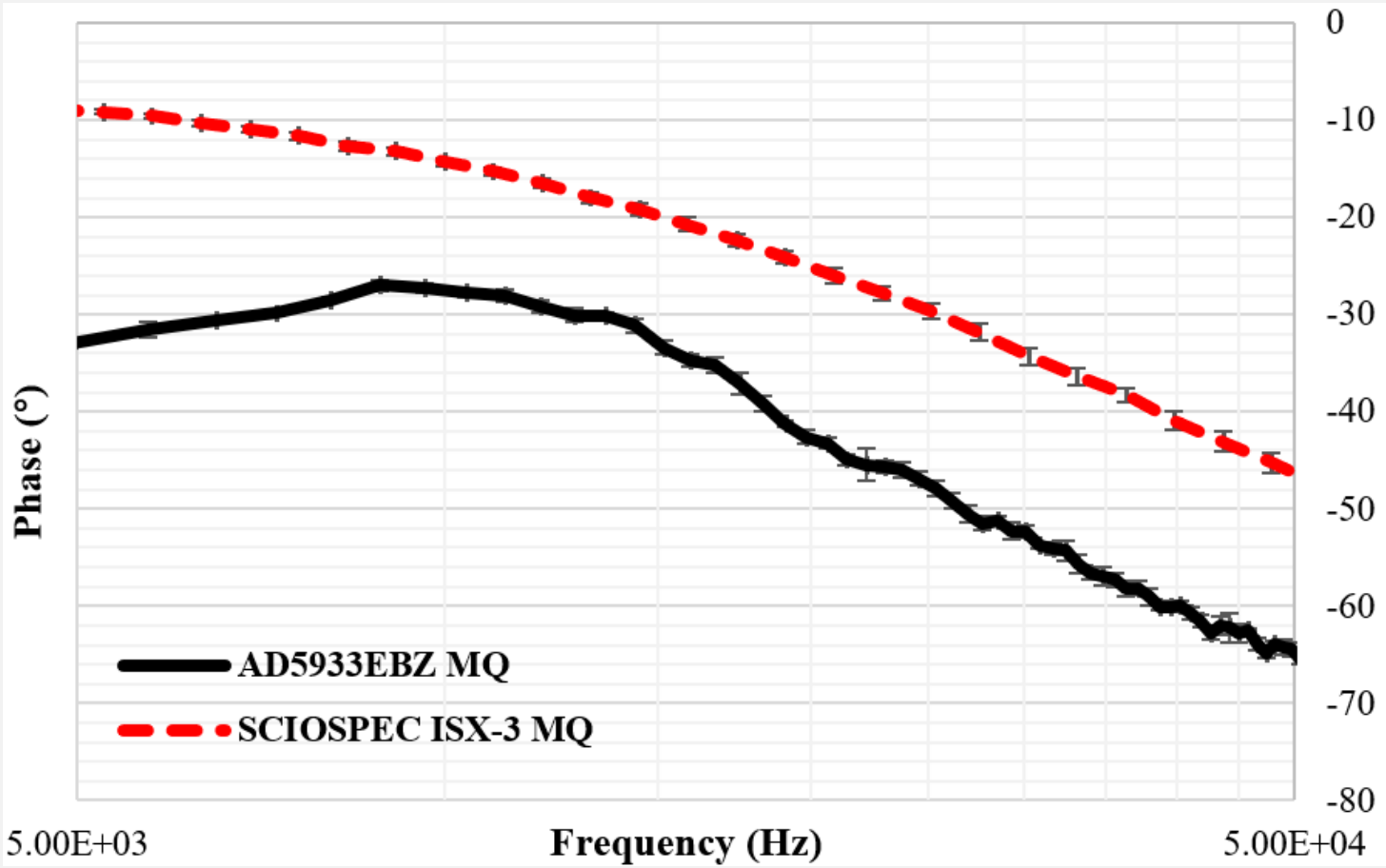

Figure 13 (b). Phase spectra for the EVAL-AD5933EBZ and ScioSpec ISX-3 in Milli-Q water (MQ). Data are shown as mean values (measurements taken in triplicate). Error bars represent standard error.

3 Results

Saline Solution (Magnitude)

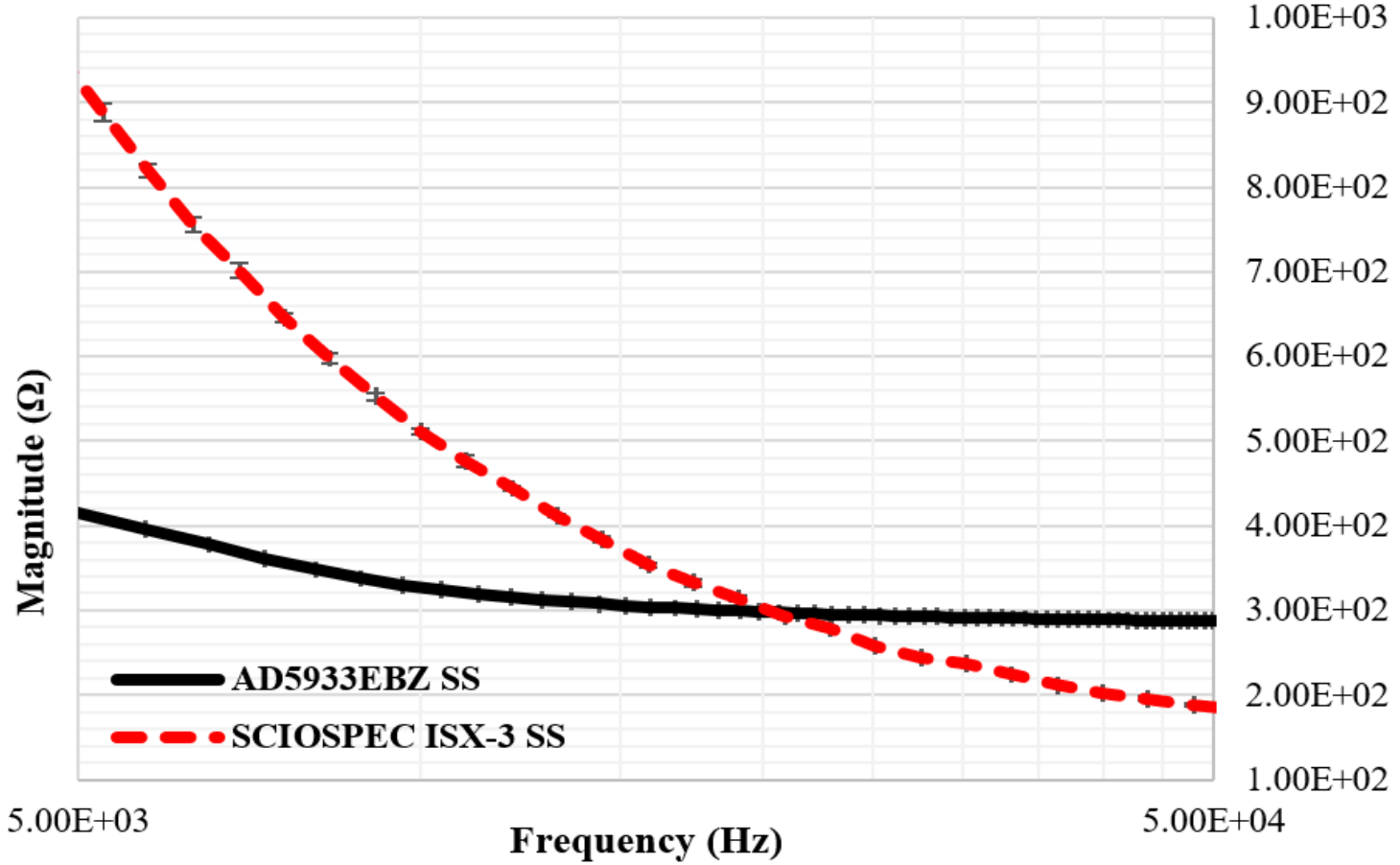

Figure 14 (a). Magnitude spectra for the EVAL-AD5933EBZ and ScioSpec ISX-3 in saline solution (SS). Data are shown as mean values (measurements taken in triplicate). Error bars represent standard error.

3 Results

Saline Solution (Phase)

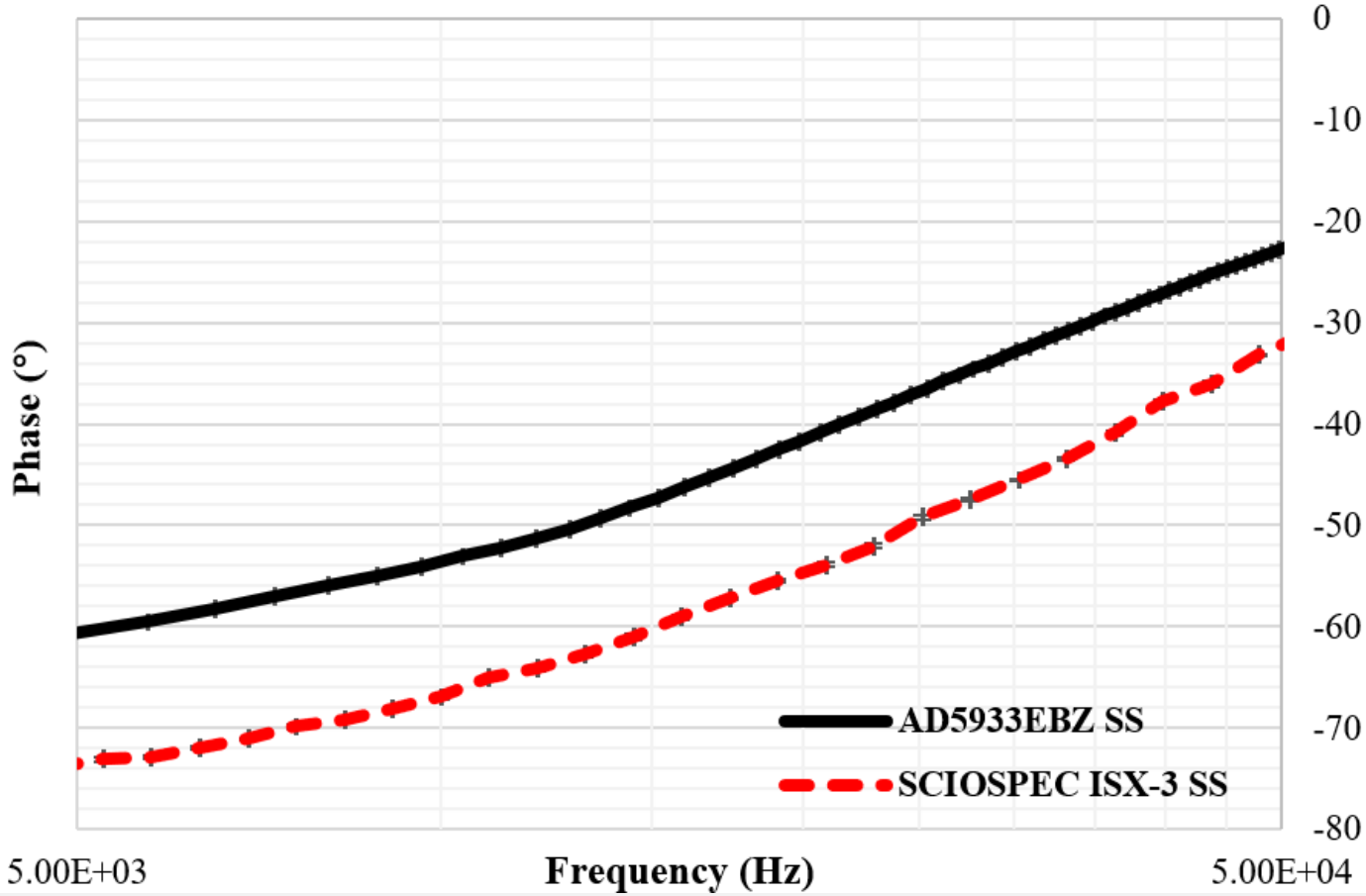

Figure 13 (b). Phase spectra for EVAL-AD5933EBZ and ScioSpec ISX-3 for saline solution (SS). Data are shown as mean values (measurements taken in triplicate). Error bars represent standard error.

3 Results

DNA (Magnitude)

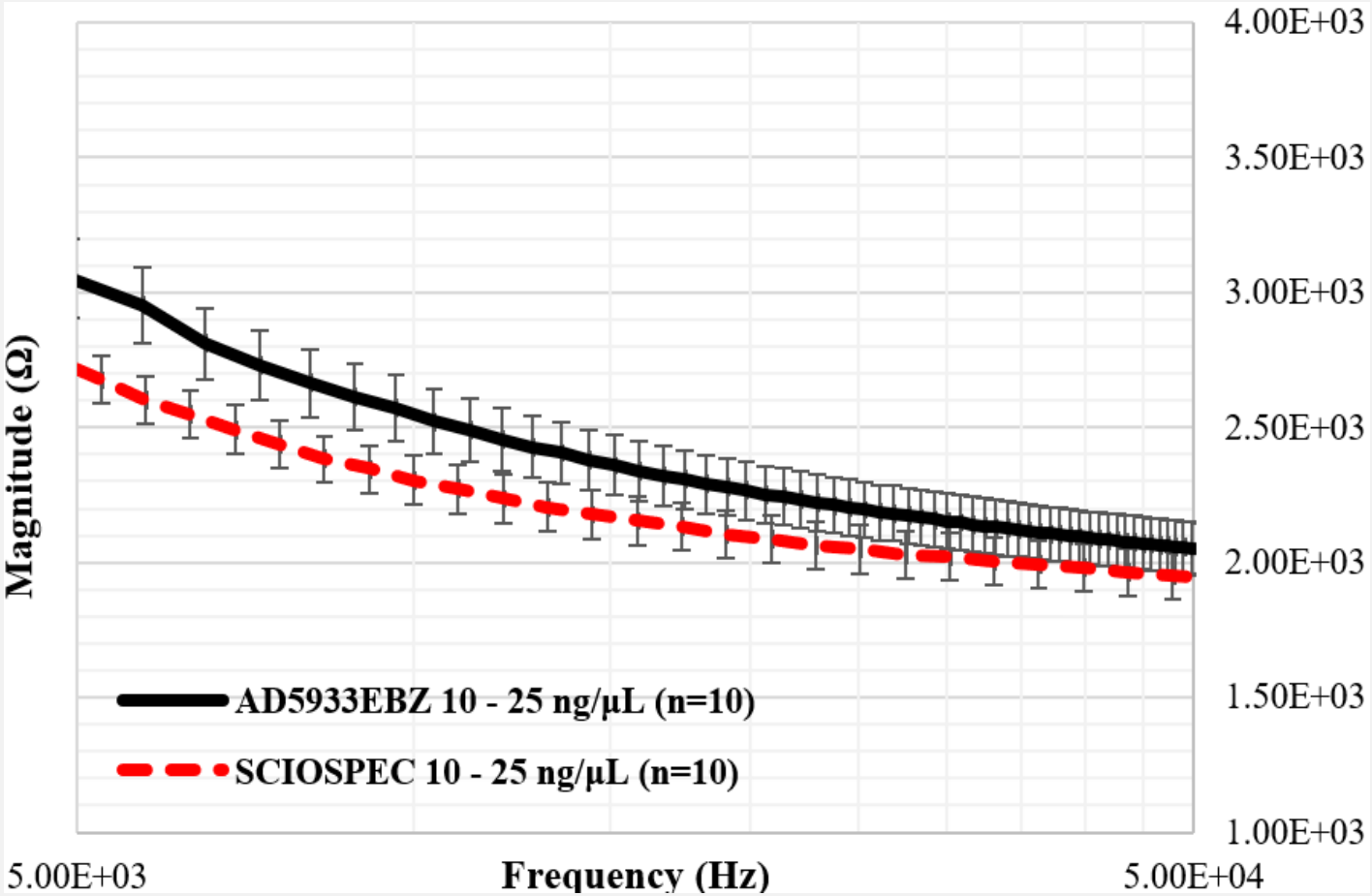

Figure 14 (a). Magnitude spectra for the EVAL-AD5933EBZ and ScioSpec ISX-3 for 10-25 ng/µL concentration. Data are shown as mean values (measurements taken in triplicate). Error bars represent standard error.

3 Results

DNA (Phase)

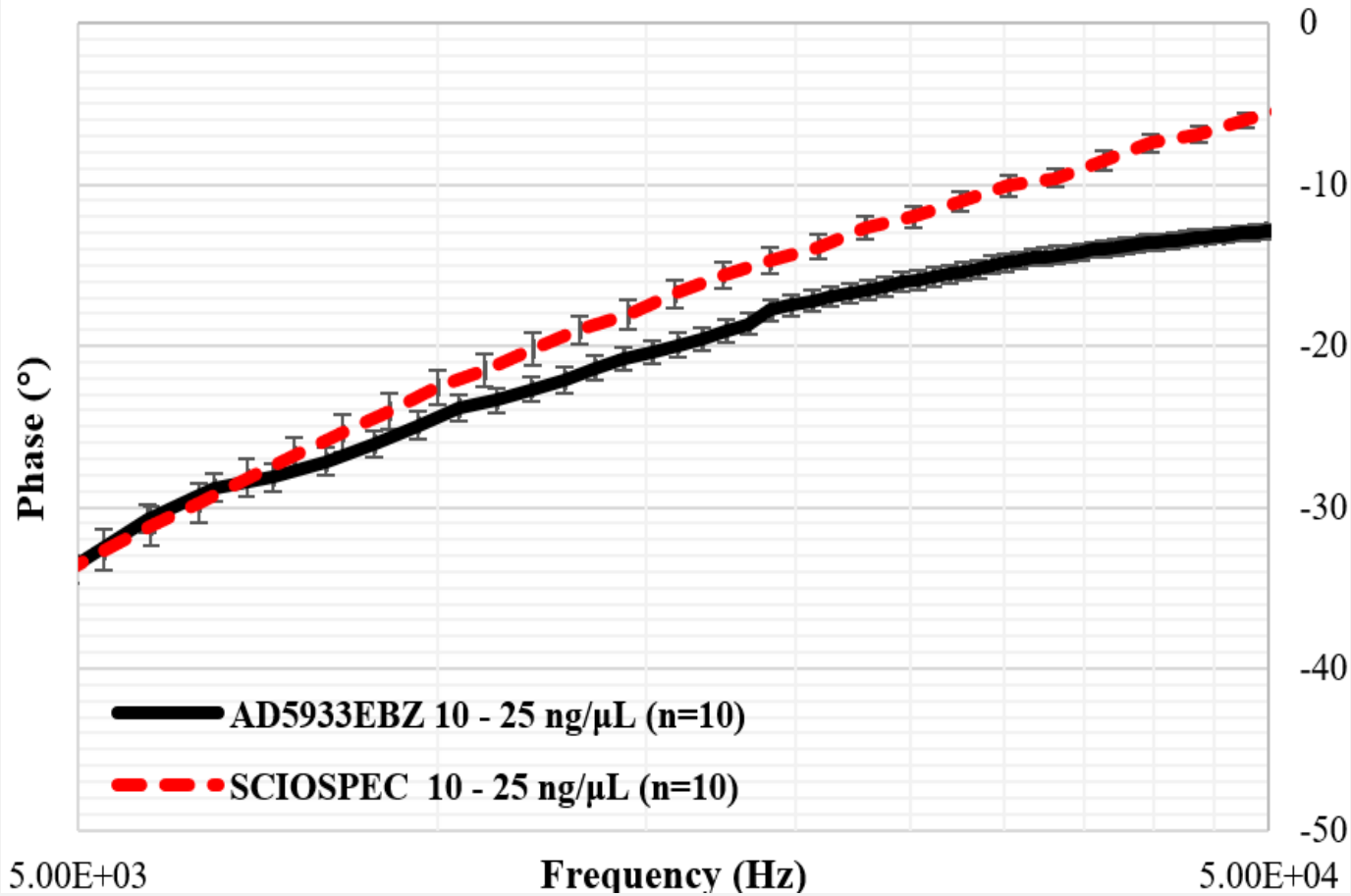

Figure 14 (b). Phase spectra for the EVAL-AD5933EBZ and ScioSpec ISX-3 for 10-25 ng/µL concentration . Data are shown as mean values (measurements taken in triplicate). Error bars represent standard error.

4 Discussions

Impedance magnitudes across the bandwidth showed strong similarity between the ScioSpec ISX-3 and EVAL-AD5933EBZ, particularly in DNA and Milli-Q water studies.

EBiS error decreased with frequency for magnitude but increased for phase. EBiS magnitude appeares to be influenced by electrode-analyte capacitive effects at low frequencies. As the frequency increases, a capacitive effect intrinsic to the AD5933EBZ system emerges.

Saline solution measurements differed significantly due to the AD5933EBZ's minimum measurable impedance of 1 kΩ.

4 Discussions

DNA bioimpedance values for both devices demonstrated comparable sensitivity over 5–50 kHz, with a mean percentage error (MPE) of 12.059%. Milli-Q water showed favorable MPE, but technical constraints affected saline solution accuracy.

The EVAL-AD5933EBZ exhibits sensitivity comparable to ScioSpec ISX-3 for 10–25 ng/µL DNA concentrations, suggesting its potential as a cost-effective DNA analysis tool. However, its bandwidth limitations restrict higher-frequency measurements, precluding exploration of DNA-polar water interactions and related bioimpedance effects.

5 Conclusion

Despite technical barriers, EBiS measurements using the EVAL-AD5933EBZ provide bioimpedance measurements with sufficient sensitivity, comparable to that of specialized equipment such as the ScioSpec ISX-3 impedance analyzer.

Thus, the EVAL-AD5933EBZ demonstrates significant potential for developing cost-effective tools to determine DNA concentrations through Electrical Bioimpedance Spectroscopy.

Acknowledgment

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