

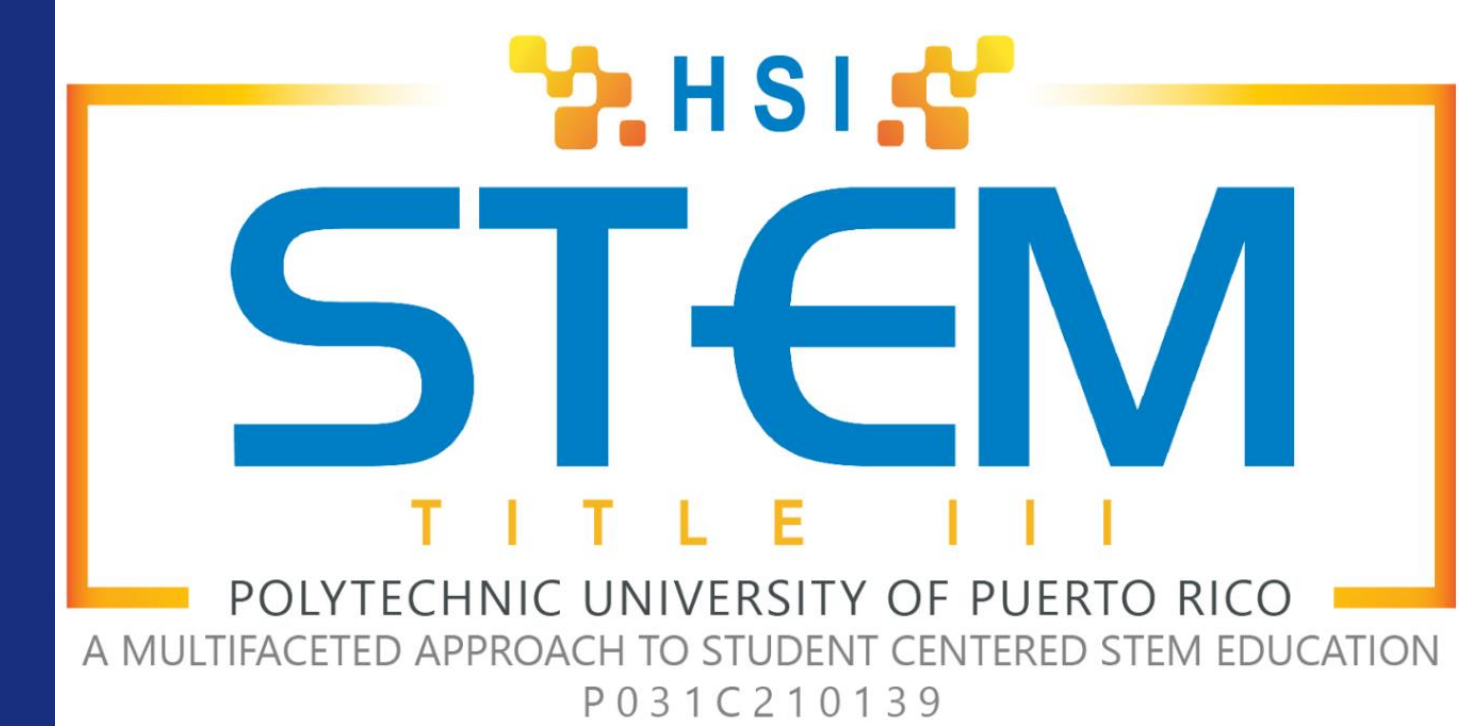
Tissue preservation using simulated seawater and a low percentage of formaldehyde

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Abstract

Formalin at 10% is the most widely used fixative solution for specimen preservation, but it poses risks of respiratory irritation and long-term effects with increased exposure (IARC, 2006). To identify safer, effective alternatives, pig kidneys were preserved for two months in solutions with 2%, 4%, or 10% formalin with 35% salt, and compared with a 10% formalin control. Dielectric spectroscopy measured real permittivity (ϵ') and impedance ($|Z|$) as markers of cell membrane and tissue preservation. According to the data, 4% formalin + 35% salt exceeded all other solutions at preservative efficiency, including the 10% formalin control. This study offers evidence of an alternative that is safer to handle with better preservation indicators.

Introduction

Formaldehyde exposure poses both short-term and long-term health risks. The goal for this investigation is to study whether formaldehyde in lower percentages can preserve the tissue without unpleasant, and possibly hazardous effects. The aim is to find a safer, more cost-effective alternative. We tested whether a simulated seawater solution with lower concentrations of formaldehyde could prove to be a viable solution to this problem. These novel solutions were tested on pig kidneys to assess their effectiveness in preserving tissue color for academic study while reducing formaldehyde exposure. During the study, specimens were monitored for visible signs of decay, such as autolysis and microbial growth. Quantitative variables such as permittivity and impedance were also recorded and analyzed to determine if these solutions could serve as a safer alternative to the current 10% formalin standard.

Objectives

Determine which solution is most effective for tissue preservation.

Characterize the fixative solutions with and without formalin by measuring pH and temperature.

Visually monitor the specimen jars for contamination.

Analyze the specimen's post-fixation dielectric values as indicators of preservation using bioimpedance and permittivity.

Compare tissue's physical qualities after fixation.

Methodology

Material Acquisition

Reactives and specimens

- Pig kidneys
- Formaldehyde

Simulated seawater components:

- Sodium chloride (30g)
- Magnesium sulfate (12g)
- Deionized Water (1L)

Equipment

- Fume Hood
- Keysight 16451B Dielectric Test Fixture (Electrode A)
- pH Meter

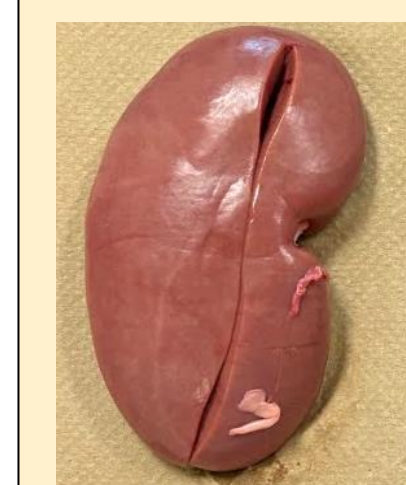


Figure 1: Fresh pig kidney.



Figure 2: Keysight dielectric test fixture seen with electrode and kidney sample

Specimen and solution preparation

Preservative Solutions:

Five solutions were prepared to assess fixative efficiency

- Negative control: 35% Simulated seawater (SSW)
- Positive control: 10% formaldehyde
- 2% formaldehyde + SSW
- 4% formaldehyde + SSW
- 10% formaldehyde + SSW

Specimen Preparation

Immersion was the chosen method to preserve the kidneys due to its convenience and efficiency (McFadden et al. (2019)) Ten kidneys were placed in separate, sealed glass jars with different preservative solutions for 2 months.

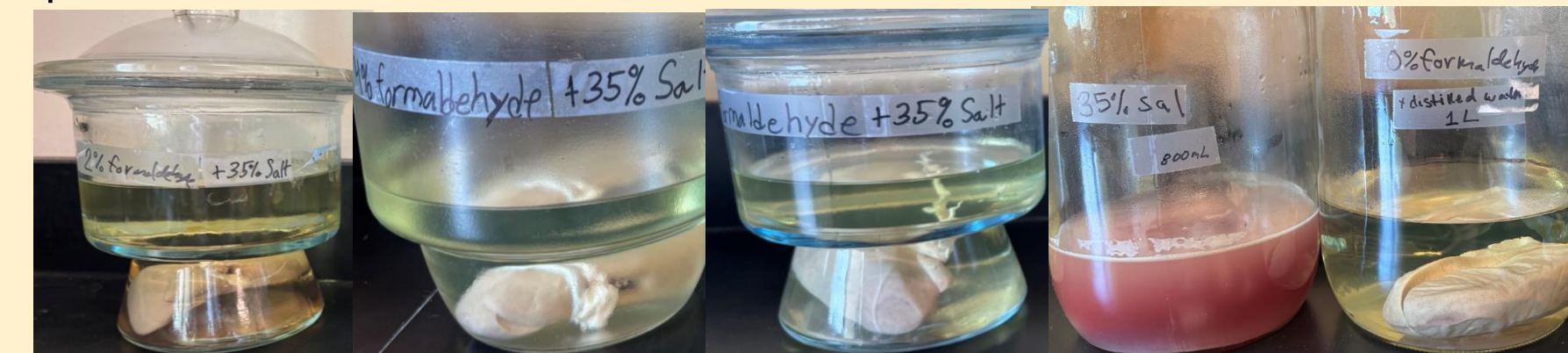


Figure 3: Immersed kidneys with 2% formalin+SSW, 4%+SSW, 10%+SSW, SSW (negative control), and 10% formaldehyde (positive control), respectively

Data Collection

Solution characterization

Solutions	pH	Temperature
2% formalin + 35% salt	6.89	19.6
4% formalin + 35% salt	6.91	19.6
10% formalin + 35% salt	6.38	19.6
35% salt	7.75	19.7
10% formalin (control)	4.23	19.6

Table 1: Measured pH for solutions and the temperature at which they were taken.

Observation period (2 months)

During the observation period, the specimens were examined for:

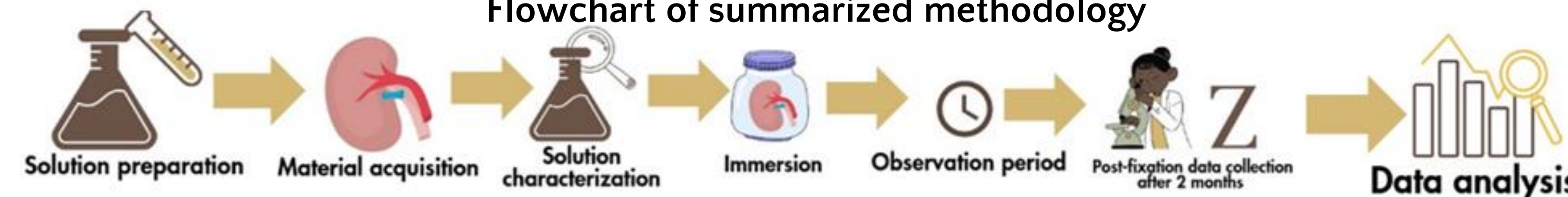
- Specimen discoloration
- Murky solution
- Microorganism activity

Which would indicate failure to preserve.

Tissue analysis

The dielectric properties of each kidney were measured after preservation using Keysight 16451B Dielectric Test Fixture and then analyzed.

Flowchart of summarized methodology



Results

Dielectric properties recorded from specimens vs frequency

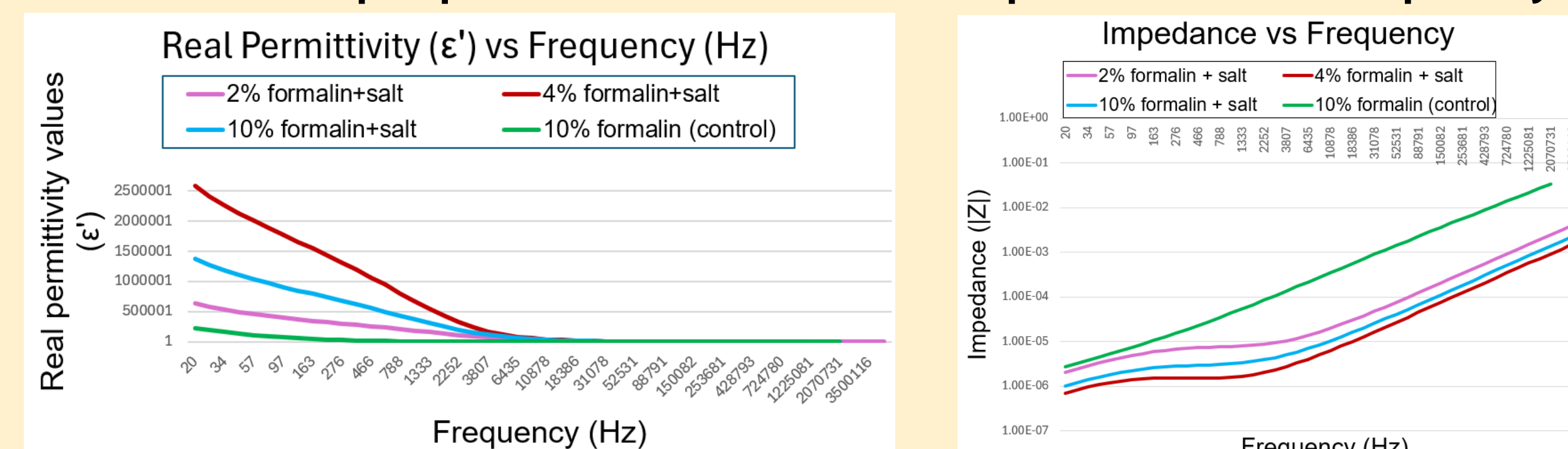


Table 2: Real Permittivity vs Frequency

Table 3: Impedance vs Frequency

Kidneys' physical changes before and after preservation

Observed physical properties pre- and post-fixation				
Pre-fixation	2% formalin + 35% salt	4% formalin + 35% salt	10% formalin + 35% salt	10% formalin (control)
Fleshy, firm consistency	Rubber-like consistency, increasing radially	Rubber-like consistency, increasing radially	Rubber-like consistency	Rubber-like consistency
Bright colors, clearly visualized internal structures	Bright colors, clearly visualized internal structures	Dull colors, clearly visualized internal structures	Dull colors, clearly visualized internal structures	Dull colors, clearly visualized internal structures
Homogenous hydration throughout the whole organ.	Moderate hydration present, decreasing radially.	Slight hydration present, decreasing radially.	Dehydrated throughout. Stiff, hard to cut. Very irritating, suffocating smell	Dehydrated throughout. Stiff, hard to cut. Very irritating, suffocating smell

Table 4: Observations of the kidney's physical properties across preservative solutions.

Ranking the solution's preservative efficiency

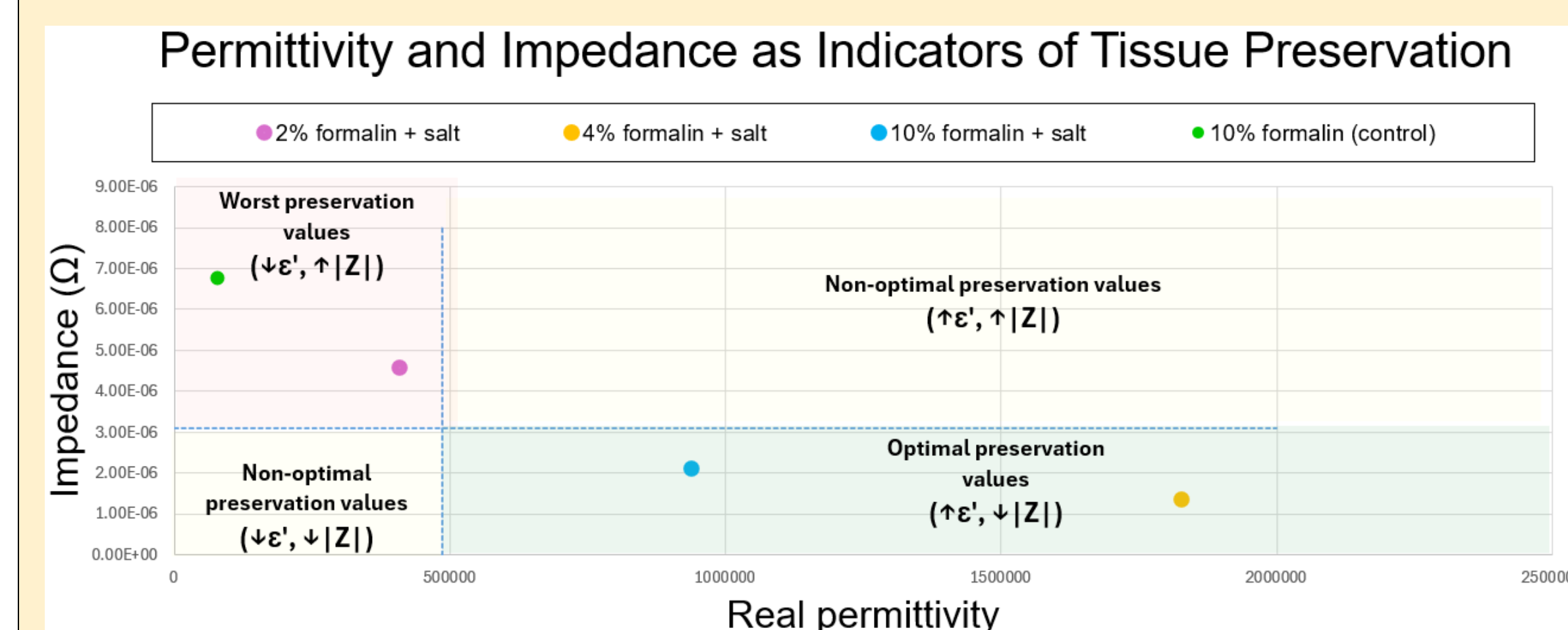


Table 5: Preservation quality map created using low frequency band analysis at 20–359Hz. High permittivity and low impedance value represent ideal preservation, 4% formalin was found to be most effective at preservation and 10% formalin (control) was least effective.

Conclusion

The percentage of formaldehyde showed a direct impact on the physical properties of the tissue specimens, with higher tissue dehydration as formaldehyde concentration increased. While all the formaldehyde-containing specimens were preserved, the solution with 4% formaldehyde and simulated seawater provided the best conservation of tissue structure and cellular membranes. It also provided hydrated tissues with good color visualization. In contrast, both the solutions containing 10% formaldehyde resulted in dehydrated tissues. Additionally, both 10% formaldehyde solutions created specimens that when handled produced a suffocating smell and caused both airway and ocular irritation. The findings indicate that implementation of 4% formaldehyde with SSW as a preservative solution could better the tissue handling experience, improve tissue visualization, and lower institutional costs.

Future work

Microscopic assessment of the glomeruli and other cellular structures

Testing a wider range of concentrations for efficiency to further study the trend of formalin efficiency at concentrations between 1%–10%.

Evaluate the preservative solutions' efficiency with varying degrees of salt.

Repeat the same methodology, but with striated cardiac muscle.

References

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