# Microwave Dielectric and Reflection Characterization on Silver Grunter (Pomadasys hasta) and Tilapia (Oreochromisniloticus) Fish Scale for Potential Use as Scaffold

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***Abstract*— Hydroxyapatite from fish scale was studied and reported of its potential in bone scaffold or regenerative material. Fish scale as a source of collagen and valuable matrix proteins in the pharmaceutical and cosmetic industries is overwhelmingly studied among researchers. In this work, dielectric and reflection measurement was conducted on fish scale from Silver Grunter (Pomadasys hasta) and Tilapia (Oreochromisniloticus) fish ranging from 200 MHz to 20 GHz using Agilent E8362B PNA Network Analyzer in conjunction with an Agilent 85070E High Temperature Probe. The fish scale was prepared as sample under test prior to measurements. Dielectric constant, and loss factor increase with frequency. Meanwhile, the measured magnitude and phase of reflection coefficient that acquired through reflection measurement decrease when frequency increases. On the other hand, both fish scales were characterized as crystalline structure via X-ray diffraction analysis. It is important in analyzing dielectric mechanism occurs in fish scale.**

***Index Terms*— Dielectric Constant; Fish Scale; Loss Factor; Reflection Coefficient; X-Ray Diffraction.**

## I. INTRODUCTION

Hydroxyapatite can be derived from collagen in fish scale [1]. Recently, it becomes spotlight among stem cell biologists due to its possibility as an alternative material in deriving scaffolds. Generally, scaffold contributes to replacement and regeneration of tissues and organs for medical purpose. The frequency-dependent dielectric analysis is important to identify and investigate electrophysiology and biophysics in fish scale. Hence, it can be found that dielectric study was conducted widely on different kind of materials [2]-[4]. The dielectric study of fish scale is necessary to analyze electrophysiological activities in the cell, since one of the raw material of produced scaffold is fish scale.

Fish scale is composed of collagen which covered with calcium salts. Collagen can be extracted from a fish scale. The behavior of ion and molecule in fish scale is important to determine bio-impedance, in order to analyze biocompatibility to ensure no conflict between cell and fish scale. It is crucial in depicting electrophysiological and biophysical activities in the cell. On the other hand, past researchers were conducted on fish scale as potential use as a scaffold [5], e.g. functional hydroxyapatite was synthesized from the fish scale (*Labiorohita* & *Catlacatla*) [6] is explored as scaffold materials. Hence, it is encouraging to explore the potential study of fish scale as a source of scaffold for human cell growth in various methods for characterization as proposed in this work.

In biomedical application, dielectric analysis has been conducted for the human tissue [7, 8]. On the other hand, operating frequency of applied field, temperature, density, chemical composition and structure of materials are a function of dielectric properties. It comes to learn that dielectric properties are very informative in characterizing the state of the biomaterial.

Dielectric properties of a material are depicted by complex permittivity where real part represents dielectric constant, ε'. Meanwhile, imaginary part represents loss factor, ε". ε' and ε" describes the ability of a material to store electrical energy and electrical energy loss in a material subjected to the applied external electric field, respectively. The polarization mechanism elucidates variation of ε' and ε".

Protein that consists of different acids amino can be found in fish scale. Proteins are long chains of amino acids. Amino acids have -COOH group (acidic) and -NH2 group (basic). Amino acid can be classified into three different groups, i.e. polar, charged and hydrophobic group and they are listed in Table 1.

It can be noticed that amino acid can be classified as hydrophobic, polar or charged amino acid. Polar amino acids are responsible for a hydrogen bond with a polar water molecule. When the water molecule is bound with polar amino acid, water molecules become bound water. Bound water is the water that is directly held by chemical bonds to the proteins in fish scale. Hydrophobic amino acid is composed to be the core of protein molecules. This amino acid does not interact with any solvent, including water molecule. Meanwhile, polar and charged amino acid surrounds hydrophobic amino acid to be a protein molecule which exposes to the environment.

Table 1
Classification of Amino Acid Composition in Fish Scale [9]

| Polar | Charged | Hydrophobic |
|---|---|---|
| Glutamine | Arginine | Alanine |
| Asparagine | Lysine | Isoleucine |
| Histidine | Aspartic | Leucine |
| Serine | Glutamic | Phenylalanine |
| Threonine | | Valine |
| Tyrosine | | Proline |
| Cysteine | | Glycine |
| Methionine | | |
| Tryptophan | | |

The polar surface molecule contributes to hydrogen bond which involves donation or acceptance of a proton from an electronegative atom.

When these polar and charged molecules expose to frequency dependent applied a field, orientation polarization occurs. The polar molecules orient itself when interacting with applied field [10]. Relaxation frequency of polar molecule is crucial in determining the fullness of polarization.

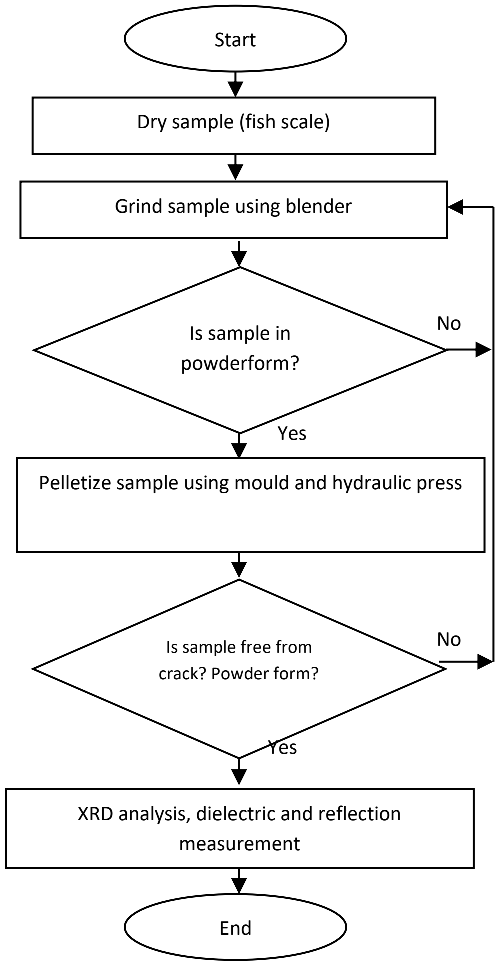



Figure 1: Sample preparation

## II. Methodology

### A. Material and Sample Preparation

In this work, the measured sample needs to be prepared from raw fish scales prior to measurement. These fish scales need to be rinsed with water to remove impurities. The rinsed fish scales were then dried under the sun for few days to ensure that fish scales were fully dried. Next, the dried fish scales were crushed and blended using a mortar and electrical blender, respectively to turn the fish scales into powder form of a sample. The sample needs to be prepared in certain dimension to ensure that it is fully contacted with an aperture of the probe. Therefore, the sample must be pelletized using a mold and hydraulic compressor. The samples need to be prepared in solid cylindrical shape with various thicknesses using a mold, i.e. 1.00 mm, 1.50 mm and 2.00 mm. The procedure of sample preparation can be referred to Figure 1.

### B. Dielectric and Reflection Measurement

A one-port calibration on Agilent 85070E High Temperature Probe must be performed prior to reflection measurement to remove systematic errors. Short-Open-Load calibration (SOL) method was conducted on a coaxial cable that connects the Agilent E8362B PNA Network Analyzer with the probe from 200MHz to 20GHz. Short, Open, and a 50 Ω Broadband Load were used in calibration. Then, the reflection coefficient in terms of magnitude and phase can be measured.

Prior to dielectric measurement, calibration Agilent 85070E High Temperature Probe needs to be conducted too. Air, a short circuit, and water were used in calibration because these are known standard. The dielectric measurement was conducted using 85071E Material Measurement Software attached to an Agilent 85070E High Temperature Probe in conjunction with Agilent E8362B PNA Network Analyzer over a frequency range from 200 MHz to 20 GHz.

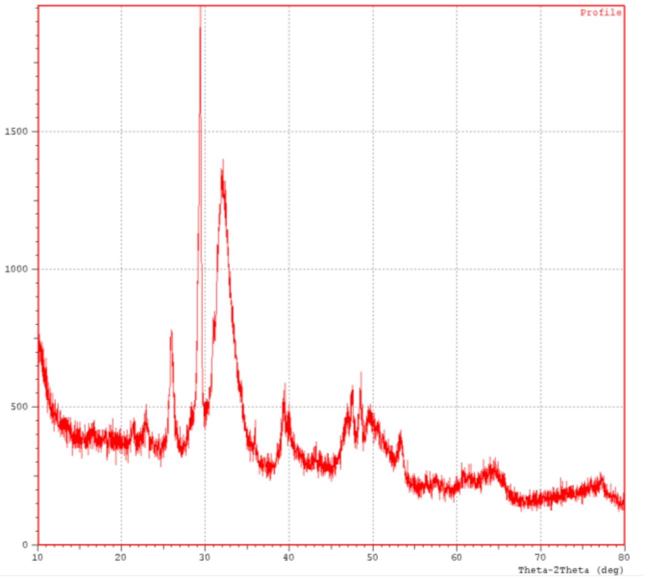
## III. Results and Discussions

### A. XRD Analysis

XRD pattern indicates that crystalline structure elucidates microstructure of these fish scales for coarse and fine fish scale of Silver Grunter and Tilapia fish. It is due to a significant peak that can be observed throughout the patterns shown in Figure 1. The crystalline structure that presents in lattice prepares a massive number of grain boundary in the material. The presence of grain boundary tends to increase dielectric properties of the crystalline material. These grain boundaries elucidate 2D defect in the crystalline structure. Grain boundaries that are found in fish scale are single-phase interface due to its solid state. Crystalline structure in fish scale describes the ordered arrangement of amino acids.

### B. Variation of Dielectric Properties of Fish Scale over Frequency

It can be noticed that Silver Grunter and Tilapia fish scale do not exhibit a significant variation of log ε' as shown in Figure 2. In addition, log ε' exhibit insignificant change too due to the variation of the thickness of sample for both Silver Grunter and Tilapia fish scale.

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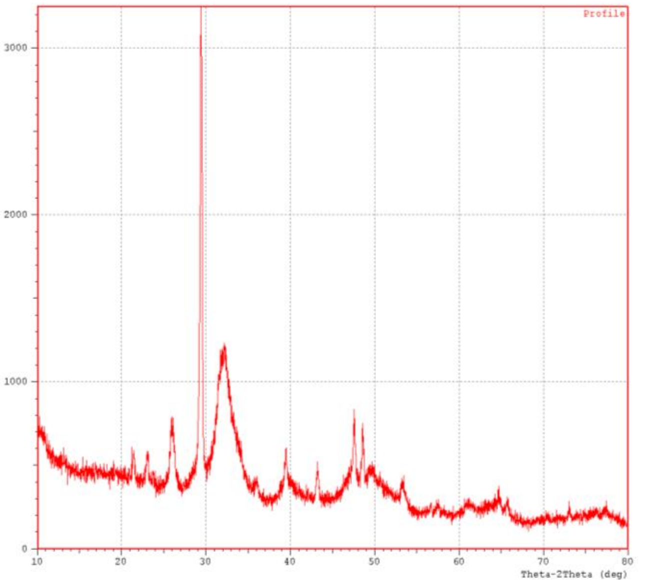
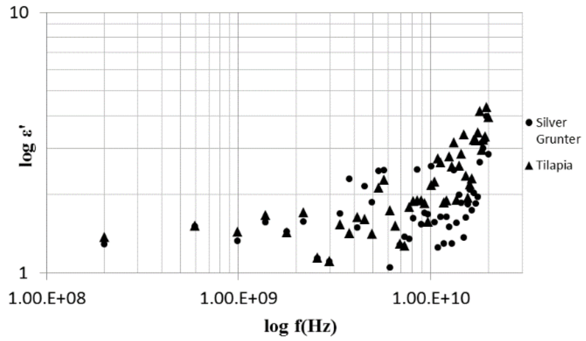



(b)

Figure 2: XRD pattern for a sample from (a) Silver Grunter and (b) Tilapia fish

Collagen is rich in fish scale and it mainly made of protein. Meanwhile, amino acids compose protein. The polar amino acids that cover hydrophobic amino acid (core molecules of protein) play an important role in describing the dielectric behavior. In Figure 3, it can be seen that log ε' for fish scales from both species of fish increase with frequency. When relaxation frequency of polar amino acids is consistent with operating frequency of applied field, full polarization is conducted. The polar molecules are able to orient itself fully at resonance and it causes log ε' rise up. In contrary, it leads to low log ε' due to asynchrony between relaxation frequency and operating frequency of the applied field. The frequency response of log ε' shown in Figure 3 indicates log ε' exhibit insignificant variation with frequency from 200 MHz to 10 GHz. However, when the frequency is exceeding 10 GHz, log ε' rise significantly with frequency. It justified that the relaxation frequency of Silver Grunter and Tilapia fish scale consistent with frequency range exceeding 10 GHz. Polar amino acids are subjected to complete and full orientation polarization. Apart from orientation polarization due to polar molecules, interfacial polarization is probably contributed too in a variation of log ε' at low frequency. This polarization is mainly due to the presence of grain boundaries in fish scales as elucidated through XRD analysis. It plays an insignificant role in dielectric constant in this work. However, it is important in depicting mechanism of energy dissipation or loss in fish scales.

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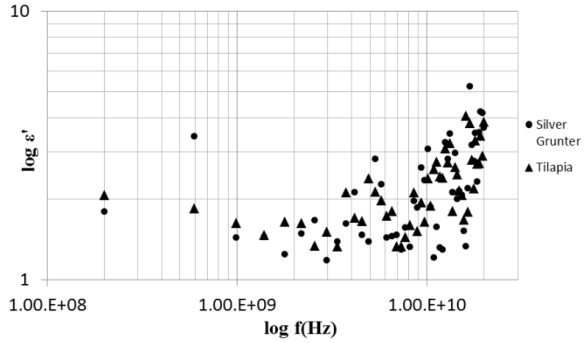


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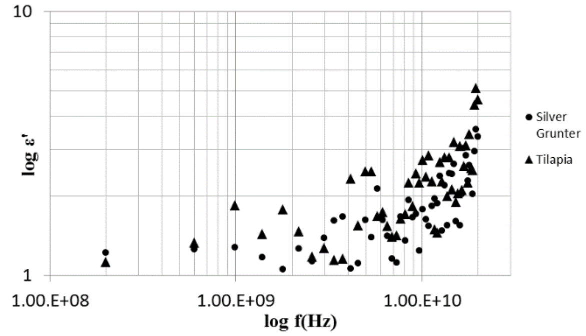
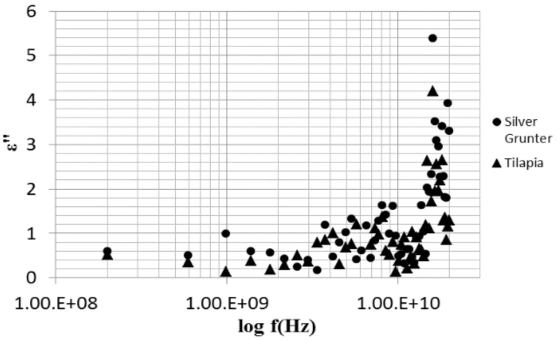



(c)

Figure 3:The variation of log ε' for Silver Grunter and Tilapia fish scale with thickness (a) 1.00 mm, (b) 1.50 mm and (c) 2.00 mm over frequency

The presence of hydrogen bond disperses relaxation frequency among bound and free polar molecules, since the inertia between bound and free polar molecules is different. Hence, it increases the asynchronous of rotation when frequency increases and lead to the rotational losses or friction among the polar molecules increases. It can be justified by Figure 4 that ε" increases with frequency for all thicknesses. The frequency responses of ε" are similar among1.00 mm, 1.50 mm and 2.00 mm thick of samples. It elucidates that 1.00 mm is longer than the penetration depth of samples.

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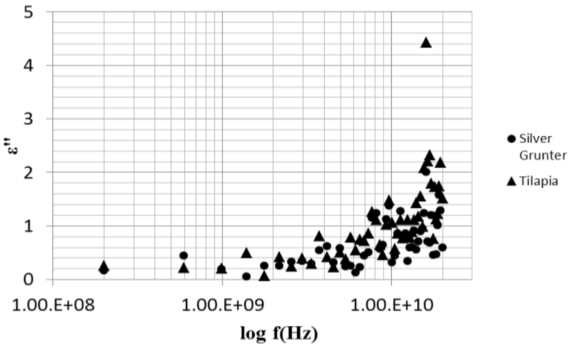


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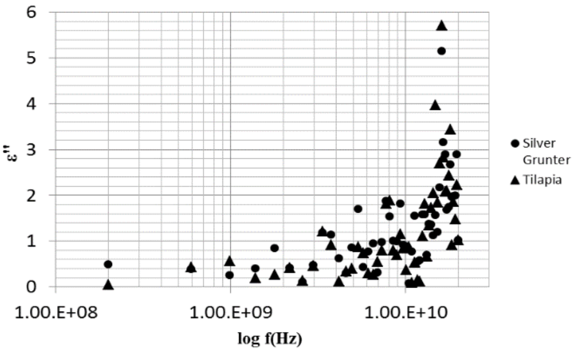


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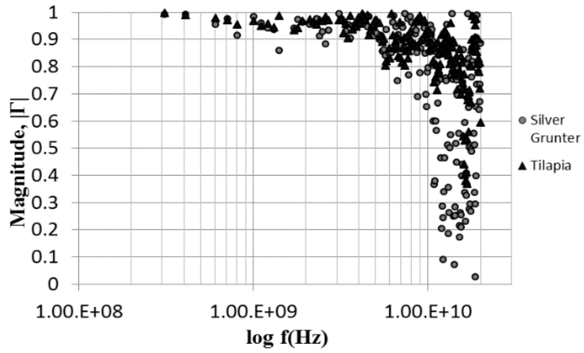
Figure 4:The variation of ε” for Silver Grunter and Tilapia fish scale with thickness (a) 1.00 mm, (b) 1.50 mm and (c) 2.00 mm over frequency

### C. *Complex Reflection Coefficient, Γ of Fish Scales with Different Thicknesses over Frequency*

Increasing ε’ results in the decrease of load impedance, $Z_L$ which, in turn, causes the |Γ| to decrease. It was depicted by admittance model and capacitance model [11] where decrement of $Z_L$ due to greater ε’ results in a decrement in the |Γ|. In other words, capacitive impedance of fish scales is a function of ε’ and they are in an inverse relationship. The decrement of $Z_L$ is attributed to the discrepancy of ε’ between the open-ended coaxial sensor (coaxial line) and fish scales. The variation of thickness seems insignificant as they have similar frequency response for all thicknesses. The drastic increment in log ε’ at high-frequency causes |Γ| to plummet drastically as shown in Figure 5. This drastic increment of log ε’ leads to a drastic decrement in term of |Γ| because of capacitive impedance increases. When frequency increases, charging phase in fish scale can be conducted continuously because a full wave takes a shorter period than the steady-state condition in charging phase. Fish scale is equivalent to a capacitive lump-element circuit in this work. As a result, the capacitive impedance decreases because capacitive load fail or very short time to maintain steady-state condition. The full polarization is not being pertained at a long period of time where next polarization at opposite direction will be taken place shortly at the interface between a coaxial line and media (fish scale) when interacting with a full wave. Subsequently, it would highly mitigate the discontinuity of electrical impedance or mismatch impedance along the interface between two media. On the other hand, the consistency between relaxation frequency and high operating frequency provide less mismatch impedance on the interface between a coaxial line and material under test as load impedance might approach characteristic impedance of coaxial line (typical value is 50 Ω) progressively as frequency increases. Withal, it can be noticed that both log ε’ and |Γ| exhibit high similarity as both parameters are indistinguishable over frequency. It can be justified by the high consistency of frequency response between log ε’ and |Γ|.

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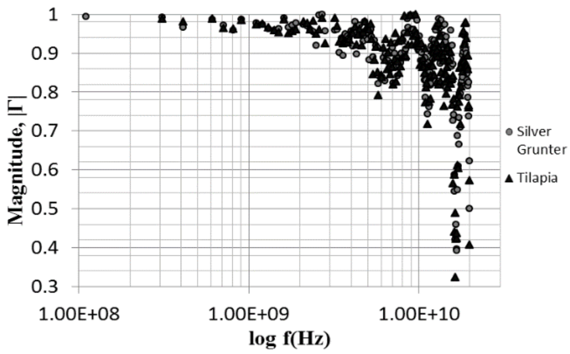


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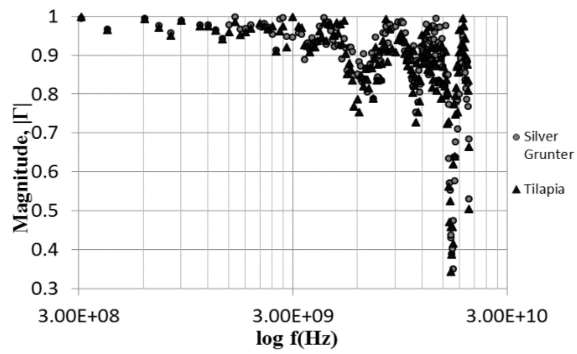


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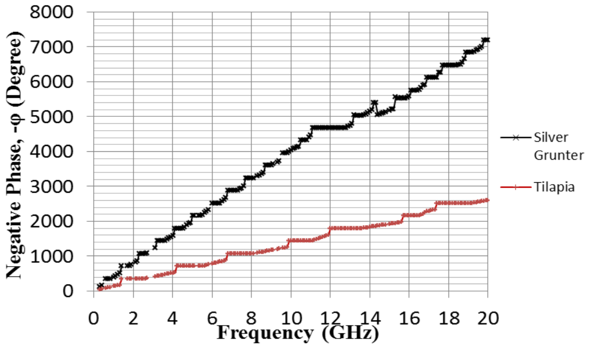
Figure 5:The variation of |Γ| for Silver Grunter and Tilapia fish scale with thickness (a) 1.00 mm, (b) 1.50 mm and (c) 2.00 mm over frequency

In addition, increment of log ε' and ε" over frequency lead to increment of negative phase, -φ as shown in Figure 6 for Silver Grunter and Tilapia. The -φ implies that reflected wave lag behind incident wave. The lagging phase is increase over frequency. Increment of log ε' and ε" suggest that polarization and energy dissipation become vigorous when frequency increases. As a result, time delay become severe and it causes high –φ. It can be justified through Figure 5. Fish scale of Silver Grunter exhibit higher –φ than Tilapia with thickness of 1.00 mm. When thickness > 1.00 mm, it can be observed that –φ of Silver Grunter and Tilapia is indistinguishable. It can be inferred that the incident wave is unreachable when thickness > 1.00 mm. In other words, the penetration depth is speculated to be less than 1.50 mm. When the incident field impinges on interface, it might cause time delay during reflection. In Figure 6, time delay between incident and reflected field increases over frequency. Therefore, -φ suggests that time delay is lengthened during polarization when frequency increases. This delay is due to process of oscillation and friction when polarization occurs. When the applied field interacts with polar molecules, the energy is stored during oscillation. Subsequently, oscillation causes friction among molecules.



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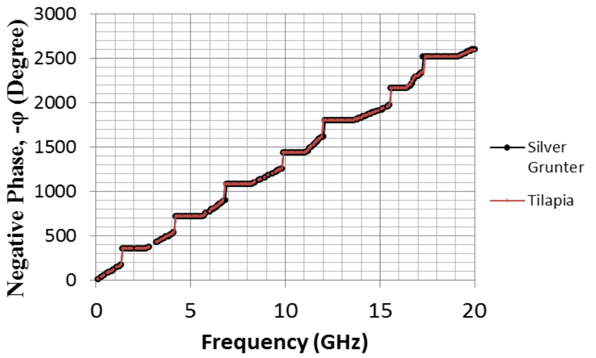

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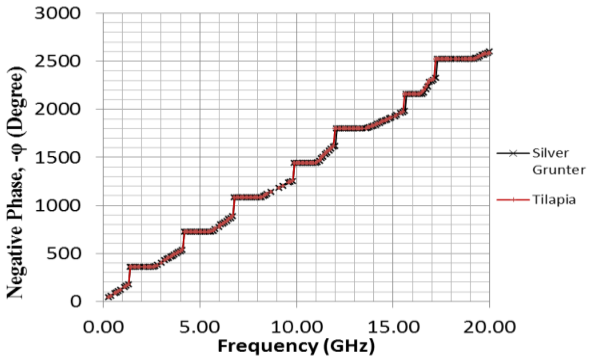

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Figure 6:The variation of -φ for Silver Grunter and Tilapia fish scale with thickness (a) 1.00 mm, (b) 1.50 mm and (c) 2.00 mm over frequency

The friction among molecules can cause immense loss of energy if frequency of applied field is high. The storage of energy and friction causes the time delay to restore idle state. The excited state would release energy to reach idle state. Hence, energy will be carried by reflected wave. In Figure 5(a), fish scale of Silver Grunter exhibit higher level and sensitivity of –φ than Tilapia. It is most probably due to higher density of polar molecules which have greater dipole moment in fish scale of Silver Grunter than Tilapia. The prepared samples from Silver Grunter and Tilapia might be inhomogeneous that lead to dispersive density.

## IV. CONCLUSION

In this work, a dielectric and reflection measurement system which consists of Agilent 85070E High Temperature Probe and Agilent E8362B PNA Network Analyzer was developed for the fish scale of Silver Grunter and Tilapia from 200MHz to 20GHz. Dielectric constant, ε' and loss factor, ε" of both fish scales were measured using Agilent 85070E High Temperature Probe during dielectric measurement. In the meantime, reflection measurements using High Temperature Probe were conducted too. ε' and ε" decrease when frequency increases. Likewise, |Γ| decreases when frequency increases. In contrary, –φ increases over frequency. The polar molecules, e.g. amino acid play an important role in determining dielectric behavior. This dielectric behavior is dominated by orientation polarization due to the presence of polar molecules in amino acid (protein). The corresponding dielectric responses over frequency lead to a variation of |Γ| and –φ. |Γ| and –φ of fish scale for Silver Grunter and Tilapia is indistinguishable in terms of ε', ε", |Γ| and –φ with a thickness of 1.00 mm, 1.50 mm and 2.00 mm due to inhomogeneity in the prepared sample.

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