MEASUREMENT AND CLASSIFICATION OF URINARY DIELECTRIC PROPERTIES FOR TYPE 2 DIABETES MELLITUS AND CHRONIC KIDNEY DISEASE

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ABSTRACT

Diagnosis and monitoring of diabetes and chronic kidney disease are of crucial importance for preventing end-stage kidney failure. The measurement of dielectric properties has generated interest for clinical utility. In this study, the urinary dielectric properties and behaviour of subjects with Type 2 diabetes mellitus (DM), subjects with chronic kidney disease (CKD), and normal subjects are investigated. The measurements were conducted using open-ended coaxial probe at microwave frequencies between 0.2 GHz and 50 GHz at room temperature (25°C), 30°C and human body temperature (37°C), respectively. The measurement of urinary dielectric properties for DM subjects that obtained dielectric constant increased with glycosuria level of more than 5 g/L at low frequencies and correlated positively with glycosuria level at frequencies above 40 GHz. Loss factor correlated negatively with glycosuria level at frequencies above 15 GHz. The strongest statistically significant difference in urinary dielectric properties was reported at room temperature (25°C) and body temperature (37°C) across different glycosuria and proteinuria levels, respectively. Statistically significant differences were found in the urinary dielectric properties of the CKD subjects compared to those of the normal subjects. Urinary dielectric properties correlated positively and negatively with proteinuria level at frequencies below and above the "cross-over" frequency point, respectively. The experimental data closely matched the single-pole Debye model. The relaxation dispersion and relaxation time increased with the glycosuria and proteinuria level, while decreased with the temperature. Classifications of urinary dielectric properties were conducted using support vector machine (SVM). In two-group classifications, the highest accuracy of 88.72% was obtained by differentiating DM subjects from normal subjects. The highest accuracy was achieved at 67.62% for three-group classifications. The best classification accuracies were obtained at 30°C. This

study demonstrated the potential diagnostic and prognostic value of urinary dielectric properties for Type 2 DM and CKD.

ABSTRAK

Diagnosis dan pemantauan penyakit kencing manis dan penyakit buah pinggang kronik adalah penting untuk mencegah kerosakan buah pinggang peringkat akhir. Pengukuran sifat–sifat dielektrik telah menimbulkan minat untuk utiliti klinikal. Dalam kajian ini, sifat-sifat dielektrik air kencing untuk subjek-subjek yang berpenyakit kencing manis jenis kedua (DM), berpenyakit buah pinggang kronik (CKD), dan normal telah disiasat. Pengukuran telah dijalankan dengan menggunakan prob sepaksi terbuka dalam frekuensi gelombang mikro antara 0.2 GHz dan 50 GHz dalam suhu bilik (25°C), 30°C dan suhu badan manusia (37°C) masing-masing. Pengukuran sifat-sifat dielektrik air kencing untuk subjek-subjek DM memperoleh pemalar dielektrik meningkat dengan tahap glukos kencing yang melebihi 5 g/L dalam frekuensi rendah dan berkorelasi secara positif dengan tahap-tahap glukos kencing dalam frekuensi melebihi 40 GHz. Faktor kehilangan berkorelasi secara negatif dengan tahap-tahap glukos kencing dalam frekuensi melebihi 15 GHz. Perbezaan statistik yang paling signifikan dalam sifat-sifat dielektrik air kencing dilaporkan adalah dalam suhu bilik (25°C) dan suhu badan (37°C) bagi perbezaan tahap-tahap glukos dan protein kencing masing-masing. Perbezaan statistik yang signifikan dalam sifat-sifat dielektrik air kencing adalah antara subjek CKD dan normal. Sifat-sifat dielektrik air kencing berkorelasi positif dan negatif dengan tahap-tahap protein air kencing dalam frekuensi yang mengurangi dan melebihi titk "silang" frekuensi masing-masing. Data eksperimen memadan rapat dengan model Debye kutub tunggal. Penyebaran dan masa santaian meningkat dengan tahap-tahap glukos dan protein air kencing masing-masing, manakala menurun dengan suhu. Sifat-sifat dielektrik air kencing telah dikelaskan dengan menggunakan mesin vektor sokongan (SVM). Dalam pengelasan dua kumpulan, ketepatan tertinggi sebanyak 88.72% telah diperolehi dalam membezakan subjek-subjek berpenyakit DM daripada normal. Ketepatan tertinggi mencapai 67.62% dalam pengelasan tiga kumpulan.

Ketepatan pengelasan yang terbaik telah diperolehi dalam suhu 30°C. Kajian ini menunjukkan nilai berpotensi diagnostik and prognostik sifat-sifat dielektrik air kencing untuk penyakit kencing manis jenis kedua dan penyakit buah pinggang kronik.

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CKD	:	Chronic kidney disease
C	:	Clearance
DM	:	Diabetes mellitus
DKD	:	Diabetic kidney disease
E-Cal	:	Electronic-calibration
GA	:	Genetic algorithm
GFR	:	Glomerular filtration rate
HCG	:	Human Chorionic gonadotropin
HbA1c	:	Haemoglobin A1c
IgA	:	Immunoglobulin A
Q	:	Quality
RBF	:	Radial basis function
RMSPE	:	Root mean square percentage error
SDM	:	Mean of standard deviation
SVM	:	Support vector machine
WHO	:	World Health Organisation

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CHAPTER 1: INTRODUCTION

1.1 Overview

DM is a common public health concern. The prevalence of DM among Malaysian adults aged 18 years and above has increased from 11.6% in 2006 to 16.6% in 2015. Out of 19,887,000 adult population, a total of 3,303,000 patients were found to be diagnosed with Type 2 DM in Malaysia (International Diabetes Federation, 2016). In the United States, 29.1 million (9.3%) people in the population were diagnosed as having diabetes in 2012, compared to 25.8 million (8.3%) in 2010 (American Diabetes Association, 2014). However, Type 2 DM is among the most common causes of CKD. In Malaysia, the prevalence of patients with end stage renal failure on dialysis had increased from 325 per million population in 2001 to 762 per million population in 2009. In 2009, there were about 58% of diabetic kidney disease (DKD) patients who diagnosed with end stage renal failure (Feisul & Azmi, 2012).

Kidney disease is classified into acute and CKD. Patients with clinical conditions such as congestive heart failure, acute kidney disease, DM, hypertension, urinary tract abnormalities, systemic autoimmune disorder or excessive use of known toxins develop higher risk of getting CKD (American Diabetes Association, 1998). Urinary glucose measurement is an essential non-invasive approach to test for DM, while urinary protein is one of the early signs of CKD. Persistent proteinuria followed by progressive decline of renal function (increments in serum creatinine level) are presentations of CKD and this eventually leads to end stage renal failure (Epstein et al., 1998).

Early diagnosis and monitoring of DM and CKD are important for prevention of further complications. As such, targeted screening and early identification are necessary. Urine has diagnostic and prognostic values for DM and CKD, respectively. Current clinical determination uses urinary protein excretion and estimated glomerular filtration

rate (eGFR) to diagnose CKD. Monitoring of urinary protein is required as standard care in the diagnosis and prognostication of patients with CKD. However, test strips that use colour charts to determine glycosuria or proteinuria variability are less accurate compared to tests using numerical readouts, as the former are subjected to numerous interference (Goldstein et al., 2004). Therefore, there is a need for a new application in urinary measurement.

Recently, the measurement of dielectric properties has generated interest for clinical utility. The presence of chemical compounds and biomaterials drastically affects the chemical and physical characteristics of the viable fluid. Studies related to aqueous solutions and biological fluids reported biomaterial dependency of dielectric changes. For instance, the presence of glucose results in different dielectric properties of urine (Lonappan et al., 2004; Lonappan et al., 2007a; Bassey & Cowell, 2013). The respective variations of haematocrit, ionic salt and glucose level also affect the dielectric properties of blood (Alison & Sheppard, 1993; Jaspard et al., 2003; Abdalla et al., 2010). Furthermore, different types of proteins, such as amino acids, horse haemoglobin, bovine serum albumin, and lysozyme from chicken egg solutions have different dielectric properties compared to water. Hence, further investigation of urinary dielectric properties could have an impact on a potentially new diagnostic method for DM and CKD. So far, no studies have been conducted to look into the effects of protein in urine.

1.2 Problem Statement

The drastically increasing CKD prevalence caused by Type 2 DM has become an important health issue. Urinary measurement is an essential non-invasive approach to test for the presence of urinary glucose and protein in DM and CKD, respectively.

However, the presence of limitations in the current methods has motivated the need to propose a new technique in urinary measurement.

Recently, the measurement of dielectric properties has generated interest for clinical utility. From the literature, dielectric properties of biological solutions have been determined to provide informative data. The move to measure dielectric properties of urine arose from the need to address the following problems:

i. Urinary glucose measurement is an essential non-invasive approach for diagnosing and monitoring DM. Current test strips that use colour charts to determine glycosuria variability are less accurate. A comparison between urinary test strips and a laboratory biochemical analysis is always required. Previous studies have found that urinary dielectric properties changed with glycosuria. However, those studies were limited, as the investigations were carried out at low frequencies with no temperature control. The differences in urinary dielectric properties have never been statistically validated. In addition, correlations between urinary dielectric properties and glycosuria level have not been studied. Investigation and validation of the differences of urinary dielectric properties at wider frequencies and different temperatures can provide a reliable and accurate determination of urinary dielectric properties with glycosuria.
ii. Current clinical determination uses urinary protein excretion and eGFR to diagnose CKD. However, this requires a long laboratory determination time. Studies have found that the progression and retardation of CKD closely correlate with the proteinuria level. Test strips that use colour charts to determine proteinuria variability are less accurate. Previous studies have reported changes of dielectric properties in different protein

solutions. However, no study has been conducted to look into the effects of protein in urinary dielectric properties. There is a need to investigate and analyse the dielectric properties of urinary protein at different temperatures and to validate the correlations between dielectric properties and proteinuria levels.

iii. The measurement of dielectric properties offers the potential to determine variability of a solution. However, the accuracy of the determination should be investigated. Data classification has been the most intensively studied method in statistical and decision science. The manual method of approaching dielectric properties classification is not only time-consuming, but may also lead to high possibilities of errors. By using the Artificial Intelligence technique, the approach can be automated to enhance accuracy and to hasten the decision-making process.

1.3 Research Objectives

The purpose of this research is to investigate and classify urinary dielectric properties among subjects with DM, CKD and normal subjects, respectively, at different microwave frequencies.

The detailed objectives are:

i. To determine the significant differences of urinary dielectric properties among subjects with DM, CKD, and normal subjects, respectively at broadband microwave frequencies ranging from 0.2 GHz to 50 GHz.

ii. To investigate the correlation of temperatures and composition of biomaterials with urinary dielectric properties.

iii. To classify the urinary dielectric properties of DM and CKD using SVM.

1.4 Significance of the Study

Urine has diagnostic and prognostic values for DM and CKD, respectively for non-invasive approaches. However, the persistent of limitations in current method to determine glycosuria or proteinuria variability has motivated the need to propose a new technique in urinary measurement.

This study applies a fast, simple and non-destructive method to measure urinary dielectric properties for Type 2 DM and CKD. It determines the effects of different glycosuria and proteinuria levels in urinary dielectric properties at different microwave frequencies. The correlations between respective temperatures and biomaterials with urinary dielectric properties are reported. Besides that, SVM-based classification method is used to determine the accuracy of urinary dielectric properties in distinguishing disease group from normal subjects group.

This study attempts to demonstrate the potential diagnostic and prognostic value of urinary dielectric properties for Type 2 DM and CKD by providing informative data to the literature. It is important for future development of devices for dielectric properties measurement and also application of dielectric properties in clinical determination for disease diagnosis and monitoring.

1.5 Chapter Organisation

This chapter gives a general introduction with an overview of the research by outlining the problem statements, objectives and the correlation among the chapters that lead to the conclusions.

Chapter two provides the literature review of the research. It reviews the current diagnosis and monitoring methods of DM and CKD, respectively, and points out the pro and cons of the methods. Next, the basic concepts of electromagnetics, dielectric

properties and factors affecting dielectric properties are described. Then, it is followed by reviews of the dielectric properties of water, solutions and biological fluids. A description of the techniques of dielectric properties measurement and a comparison of the available measurement techniques are presented. Lastly, the theories for classification using SVM and its applications are described.

Chapter three provides a brief theoretical overview of the current diagnosis and monitoring methods for DM. The selection of an appropriate technique for measuring the dielectric properties of urine is described. The theories of measurement and experimental details are explained. The experimental set up, calibration and measurement of reference solutions are described. The reproducibility and accuracy of the selected technique are validated. The measurement of urinary dielectric properties of glycosuria at microwave frequencies was conducted. The experimental procedures and data processing are described.

Chapter four reports the measurement of urinary dielectric properties of proteinuria at microwave frequencies. A brief theoretical section provides the overview of the current diagnosis and monitoring methods for CKD. The experimental procedures and data processing are described.

Chapter five reports the classifications of urinary dielectric properties at microwave frequencies. Brief explanations about the background theories of non-linear classification and the selection of the classification model are described. Two-group and three-group classifications are presented.

Chapter six summarises the conclusions from the previous chapters and gives suggestions for future possibilities.

CHAPTER 2: LITERATURE REVIEW

2.1 Review of Diagnostic and Monitoring Method for Diabetes Mellitus

The gold standard for diagnosis of DM is the measurement of glucose concentration in blood plasma (hyperglycaemia) due to the dysfunction of carbohydrate metabolism that results in metabolic derangement. The World Health Organization (WHO) has recommended a set of diagnostic criteria comprised of: Haemoglobin (Hb) $A_{1c} \geq 6.5\%$ (48 mmol/mol), fasting plasma glucose $\geq$ 7.0 mmol/L (126 mg/dL), 2 h postload glucose concentration $\geq$ 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test or symptoms of DM and casual plasma glucose concentration $\geq$ 11.1 mmol/L (200 mg/dL). Repeat testing on the following day is necessary to establish the diagnosis if any of the criteria is met by a patient. Testing to detect Type 2 DM is recommended for all asymptomatic people who are above the age of 45 years or for those at high risk of getting DM. Routine measurement of plasma glucose concentrations in an accredited laboratory is not recommended as the primary method of monitoring outpatients with DM. Laboratory glucose testing is used as a supplementary method to confirm the accuracy of self-monitoring (Sacks et al., 2011). Portable glucose meters are recommended to be used by individuals with DM for self-monitoring of blood glucose concentrations due to its ease of use, convenience and accessibility. Self-monitoring is useful for detecting asymptomatic hypoglycaemia or to avoid hyperglycaemia in patients. However, the use of glucose meters generates high possibilities of false positives or false negatives due to certain factors such as haematocrit level (Tang et al., 2000), altitude, environmental temperature or humidity, hypotension, hypoxia and high triglyceride concentrations (American Diabetes Association, 1994).

In laboratory glucose testing, overnight fasting blood (with no caloric intake for a minimum of 8 hours) is drawn in the morning for measurement. Loss of glucose from the sample due to glycolysis is dependent on the glucose concentration, leukocyte and

temperature (Ladenson et al., 1980). Fluoride, which is used as a glycolysis inhibitor, is unable to prevent short-term glycolysis. Glucose concentration was found to decrease for up to 4 hours after sample collection, with or without the presence of fluoride. The glucose concentration in blood with fluoride remains stable for 72 hours at room temperature, but the rate of glycolysis increases if the concentration of leukocyte is high (Chan et al., 1989). Measurement of the concentration of glucose in plasma is highly recommended because plasma can be centrifuged to avoid glycolysis without waiting for the blood to clot (World Health Organization, 2006; American Diabetes Association, 2010). Fasting plasma glucose concentration was found to increase with age, starting with middle age, due to homeostasis and visceral obesity that decreases glucose tolerance (Pekkanen et al., 1999; Imbeault et al., 2003).

Semiquantitative urinary glucose testing is used only for patients who refuse or are unable to go for blood glucose testing due to the fact that it does not reflect plasma glucose accurately (Goldstein et al., 2004). However, it is essential for diagnosing and monitoring DM as a non-invasive approach. It is applicable for situations where blood glucose testing is inaccessible or unaffordable. Diabetes test strips that use oxidase reaction to show glycosuria variability is subject to numerous interference such as drugs and non-glucose sugars (Goldstein et al., 2004).

2.2 Review of Diagnostic and Monitoring Method for Chronic Kidney Disease

CKD is defined as the abnormalities of kidney function that present consistently for more than 3 months. The diagnosis of CKD facilitates the disease classifications. Currently, glomerular filtration rate (GFR) is used as the gold standard to evaluate renal function. Patients with assessed estimated GFR (eGFR) < 90 ml/min/1.73m^2 is diagnosed as having CKD. Clinical determination to classify the disease stages based on eGFR to diagnose overall kidney function is described in Table 2.1:

Table 2.1: Chronic kidney disease stages and GFR

Stage	Details	GFR (ml/min/1.73m2)
1	Kidney damage with normal GFR but protein found in urine	>90
2	Kidney damage with mild decrease in GFR	60 to 90
3	Moderate decrease in GFR	30 to 59
4	Severe reduction in GFR	15 to 29
5	Kidney failure	< 15

GFR is based on Clearance, (*C*) where the rate of an indicator substance being removed from plasma is measured per unit concentration. It specifies the volume of the substance that is removed per unit of time. For example, substance *Z* is cleared by renal elimination:

$$C_Z = \frac{U_Z V}{P_Z} \qquad \textbf{2.1}$$

Where Uz is urinary concentration of *Z*, *Pz* is plasma concentration of *Z*, and *V* is urine flow rate. *Z* refers to the creatinine of measured plasma. Concentration of serum creatinine is affected by factors such as gender, age, ethnicity, medication, muscle mass and protein intake (Lascano & Poggio, 2010). Furthermore, CKD seldom shows clinical symptoms in the early stage as serum creatinine will rise only after the reduction of renal function by at least 50% (Salifu, 2015).

Ultrasound is used to image the renal tract in patients with CKD. It is able to identify renal size and symmetry, renal scarring, uropathy and polycystic disease in a CKD patient. The test is conducted when a renal biopsy is required, when there is persistent haematuria or when a rapid deterioration of renal function is observed (Bush Jr et al., 2000).

Meanwhile, urinary protein has been shown to have diagnostic and prognostic value for CKD. Initial pathophysiological changes in kidneys showed significant changes in urinary proteins, which are potential biomarkers of early stage of CKD (Zürbig et al., 2009). Urinary albumin is the most common type of protein present in urine that is associated with CKD. Ghiggeri et al. (1985) found that protein increases in urine with respect to the stages of CKD. Urinary protein excretion is a modifiable risk factor for CKD progression. The progression and retardation of CKD is closely correlated with proteinuria (Jafar et al., 2001; Atkins et al., 2005; Lea et al., 2005). However, qualitative tests, such as chemical test strips, are subjected to numerous interference factors and are unable to detect small amounts of albuminuria. Hence, results always need to be confirmed with quantitative laboratory tests (Sacks et al., 2011). Monitoring of urinary protein is certainly important as standard care for patients with CKD or those at high risk of developing CKD.

2.3 Overview of Dielectric Properties

2.3.1 Dielectrics

Dielectrics are materials that allow the propagation of electromagnetic waves, which are dependent on their electrical parameters. When potential difference is applied across a material, it results in an electric field. Dielectric materials have virtual energy that is able to create virtual charges in order to support the electric field. Permittivity, ε is the measurement quantity to determine the ability of a material to support ionic and displacement current flow and molecular polarisation. It always refers to the relative permittivity of dielectric material with respect to free space, ε_0.

$$\varepsilon = \varepsilon_r \cdot \varepsilon_0 \qquad \textbf{2.2}$$

The fundamental dimension of permittivity is $T^2Q^2M^{-1}L^{-3}$ where T, Q, M and L are time, charge, mass and length, respectively, expressed as farad per meter (F/m). ε_0 is the permittivity of free space (=8.854191x10^{-12} F/m). The relative permittivity, ε_r is defined as factor of capacitance increases when the volume of a capacitor is filled with dielectric material compared to free space. It varies with different phases of material as it is affected by concentration and bonding. Apart from that, the mixture of dielectric materials, sizes, shapes and structures has created interest in determining the difference of permittivity.

2.3.2 Dielectric Properties Theory

A material is naturally present as an atom, molecule or ion in microscopic form. The atoms, molecules or ions within the material move with the strength of the electrical field applied across the material. Maxwell's equations, as below, are used to determine propagation of microwave energy through a material that relates to current, electrical field, magnetic field and charge.

$$\nabla \times E = -\frac{\partial B}{\partial t} \quad \textbf{2.3}$$

$$\nabla \times H = -\frac{\partial D}{\partial t} \quad \textbf{2.4}$$

$$\nabla \cdot D = \rho \quad \textbf{2.5}$$

$$\nabla \cdot B = 0 \quad \textbf{2.6}$$

where *E, D, H, B,* ρ are electrical field intensity, electrical flux density, magnetic intensity field, magnetic flux density, and charge density, respectively. Faraday's and Ampere's Law are applied to constitutive relations with Maxwell's equations. From the equations shown below, the respective electrical flux density and current density vary linearly with electrical field intensity whereas magnetic flux density is directly proportional to magnetic field intensity.

$$D = \varepsilon E \quad \textbf{2.7}$$

$$B = \mu H \quad \textbf{2.8}$$

$$J = \sigma E \quad \textbf{2.9}$$

Where *J* is current density, ε is permittivity, μ is permeability and σ is conductivity. They are the constant of proportionality. Most materials exist in at least one of the electrical dipoles, where the molecule charges are imbalanced (eg. water), the opposing ions are charged (eg. salt) or the atom nucleus is separated from its electron cloud. When the electrical field is applied, the dipoles rotate to align within the field in the correct polarity. Permittivity describes dielectric properties that influence the reflection of electromagnetic waves at interfaces and the attenuation of wave energy within materials. Dielectric properties of materials can be defined in terms of their relative permittivity, ε_r. Relative permittivity is represented as a complex quantity in terms of

real ($\varepsilon_r^{'}$) and imaginary ($\varepsilon_r^{''}$) parts that are referred to as dielectric constant and loss factor, respectively, as follows:

$$\varepsilon_r = \varepsilon_r' - j\varepsilon_r'' \qquad \textbf{2.10}$$

Where $\varepsilon_r^{'}$ is the energy stored and $\varepsilon_r^{''}$ is the energy lost when exposed to an electrical field. The real and imaginary parts may be described as 90° out of phase (Figure 2.1).

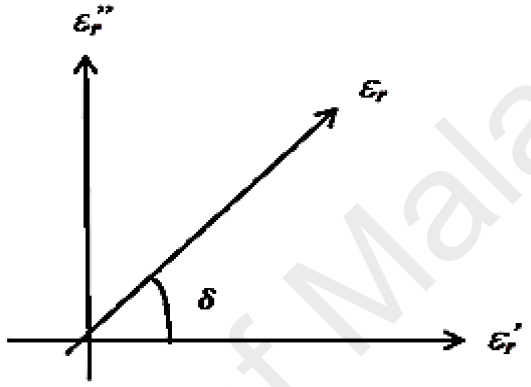


Figure 2.1: Complex permittivity

Loss tangent is defined as the ratio of the imaginary part to the real part:

$$tan\delta = \frac{\varepsilon_r^{''}}{\varepsilon_r} = \frac{\sigma}{w\varepsilon_0\varepsilon_r'} \qquad \textbf{2.11}$$

Loss tangent is the mechanism that causes dielectric loss in heterogenous mixtures that include electronic, polar, atomic and Maxwell-Wagner responses (Metaxas & Meredith, 1983). However, ionic conductance and dipole rotation are the dominant loss mechanisms at microwave frequencies (Ryynänen, 1995). Complex permittivity can also be expressed on the Cole-Cole diagram where $\varepsilon_r^{''}$ is plotted against $\varepsilon_r^{'}$. Figure 2.2 shows the Cole-Cole diagram of pure water at 30°C. Infinite or high frequency relative permittivity, ε_∞ and static or zero-frequency relative permittivity, ε_s can be obtained from the intersecting points on the x-axis, respectively. Dielectric lossy materials convert electrical energy into heat in the presence of microwave frequencies that

increase temperature. The increase of temperature is directly proportional to loss factor (Nelson, 1996).

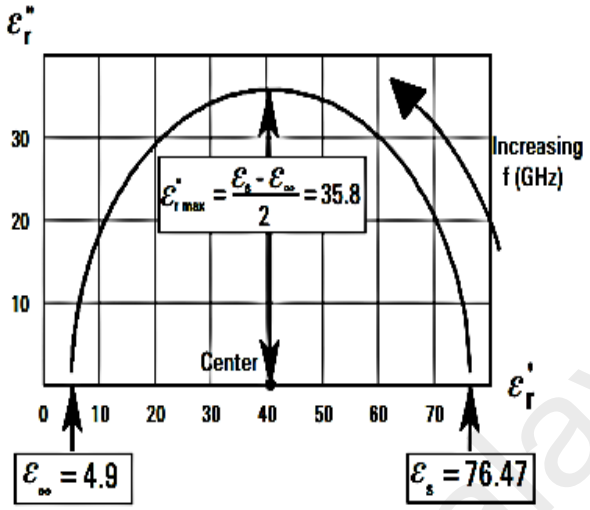


Figure 2.2: Cole-Cole diagram of water at 30°C (Agilent Technologies, 2005)

2.3.3 Frequency Dependence of Dielectric Properties

Dielectric properties are frequency dependent. For moist dielectric materials, ionic conductivity is dominant at frequencies lower than 200 MHz. Ionic conductivity and free water dipole rotation are combined to take part at microwave frequencies. Maximum loss factor at a relaxation frequency (f_c) relates to relaxation time ($\tau = \frac{1}{2\pi f_c}$). Relaxation time is the time required for dipoles to fully orientate in an electrical field.

Debye model describes the wideband frequency dependence of the dielectric relaxation response of liquids. Equation 2.12 below shows the single-pole Debye equation (Pethig & Kell, 1987).

$$\varepsilon(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + j\omega\tau} = \varepsilon_\infty + \frac{\Delta\varepsilon}{1 + j\omega\tau} \qquad \textbf{2.12}$$

where $\varepsilon(\omega)$ is the dielectric properties and ω is the angular frequency. ε_s is the static relative permittivity, ε_∞ is the infinite relative permittivity and τ is the relaxation time of

the material. Generally, larger molecules have longer relaxation time (Komarov et al., 2005). The model is further expanded to include a static conductivity, σ_s. This indicates that the currents flow at infinite time due to the movement of ions in a constant field (limiting low frequencies).

$$\varepsilon_s = \frac{\sigma_s}{j\omega\varepsilon_0} = -j\frac{\sigma_s}{\omega\varepsilon_0} \quad \textbf{2.13}$$

After including Equation 2.13 into Equation 2.12, the resulting equation is as below:

$$\varepsilon(\omega) = \varepsilon_\infty + \frac{\Delta\varepsilon}{1 + j\omega\tau} - j\frac{\sigma_s}{\omega\varepsilon_0} \quad \textbf{2.14}$$

Since

$$\varepsilon = \varepsilon' - j\varepsilon'' \quad \textbf{2.15}$$

Hence,

$$\varepsilon(\omega) = \varepsilon_\infty + \frac{\Delta\varepsilon}{1 + \omega^2\tau^2} - j[\frac{(\Delta\varepsilon)\omega\tau}{1 + \omega^2\tau^2} + \frac{\sigma_s}{\omega\varepsilon_0}] \quad \textbf{2.16}$$

For two or more relaxations, multi-pole Debye model is applied.

$$\varepsilon(\omega) = \varepsilon_\infty + \frac{\Delta\varepsilon_1}{1 + j\omega\tau_1} + \frac{\Delta\varepsilon_2}{1 + j\omega\tau_2} + \cdots\frac{\Delta\varepsilon_n}{1 + j\omega\tau_n} - j\frac{\sigma_s}{\omega\varepsilon_0} \quad \textbf{2.17}$$

Cole and Cole (1942) modified the Debye model into the Cole-Cole equation as follows, with α for the distribution of relaxation time.

$$\varepsilon(\omega) = \varepsilon_\infty + \frac{\Delta\varepsilon_1}{1 + (j\omega)^{1-\alpha}} - j\frac{\sigma_s}{\omega\varepsilon_0} \quad \textbf{2.18}$$

$\alpha \sim 0$ for water and wet tissue at microwave frequencies. Maxwell-Wagner theory is used to describe dielectric properties of mixtures and tissues, but it is only appropriate for low microwave frequencies (Pethig & Kell, 1987).

2.3.4 Temperature Dependence of Dielectric Properties

Temperature has a significant effect on dielectric properties. For moist materials, ionic conductance increases with temperature, resulting in loss factor increases at low frequencies (<200 MHz) while decreases at high frequencies due to free-water dispersion. Debye explained that viscosity reduces with temperature due to the randomised Brownian movement of molecules. As a result, relaxation time and dielectric constant of pure water decrease with temperature. Combination effects of mechanisms in multi-dispersion materials show the gradual transition of dielectric properties with respect to frequencies (Komarov et al., 2005).

2.4 Review of Dielectric Properties of Solutions

2.4.1 Dielectric Properties of Water

A water molecule consists of one oxygen and two hydrogen atoms. The hydrogen atoms of the molecule is more often present as positive than the oxygen atom due to hydrogen's bonding mechanism that results in net polarity. Interaction and orientation of relevant molecules show how the liquid responds to electromagnetic fields. The dielectric properties of liquids have been well established over the century. A review was conducted by Uematsu and Franck (1980) before the 1980s, on the data regarding static relative permittivity of water and steam by considering the physical variations. They documented and compared the static relative permittivity of water and steam in atmospheric pressure up to 500 MPa and between 0°C and 550°C. From the data obtained, they discovered a new international formulation for static dielectric constant of water and steam. Kaatze (1989) investigated the temperature and frequency

dependence of the dielectric properties of water. He reported that one discrete relaxation time was found in the dielectric properties of water, using the Debye relaxation function at microwave frequency from 1.1 to 57 GHz as well as temperature between -4.1°C and 60°C. Johri and Roberts (1990) employed a resonant microwave cavity to investigate the dielectric response of water. They proposed the Cole-Cole response for the behaviour of water with increased relaxation time at broader frequency ranges. Higher order of water transitions at temperature variations were due to the nonlinear behaviour of hydrogen bonding dipolar molecules and dipoles (Johri & Roberts, 1990). Robert et al. (1993) continued investigating the dielectric responses of water in ice, liquid and vapour forms. They reported various impure states of water with ions affecting the dielectric response and that water molecules displaying temperature transition changed with dielectric properties as the structure changed. Dielectric relaxation of water was found to change with temperature from 0 to 100°C (Robert et al., 1993).

Fernández et al. (1995) further reviewed the static dielectric constant of water and steam up to 600°C and 1189 MPa. The dielectric behaviours of water were evaluated based on difference measurement techniques, frequency dependence and source of error. Overall, at a temperature of about 25°C, the static dielectric constant of water was reported to experience small decreases at frequencies below 1 GHz and strong decreases at frequencies up to 40 GHz respectively. Relaxation time was decreased with increasing temperature. However, the different techniques proposed contributed increased discrepancies of data (Fernández et al., 1995).

In a comparison between the dielectric behaviour of water vapour and organic liquids, Raveendranath and Mathew (1996) reported that the dielectric properties of water vapour was higher than that of methanol and chloroform vapour. The dielectric properties of vapours increased with temperature, unlike that of liquids. This could be

explained by the thermal expansion of liquids, which reduced the amount of molecules per unit volume that resulted in the decrease of molar polarization and thereby the decrease of dielectric properties (Raveendranath & Mathew, 1996). Aqueous solution had the highest dielectric constant, followed by polar organic solvent and non-polar organic solvent at frequencies ranging from 0.2 to 20 GHz at 26°C (Horiuchi et al., 2005).

Ellison (2007) extended the data on the dielectric properties of water up to 25 THz in terms of relaxation and the resonance process. He reported that three dielectric relaxations of water were found in the microwave regions. The three relaxation frequencies of water were at 18.56 GHz, 167.83 GHz and 1.944 THz (Ellison, 2007).

2.4.2 Dielectric Properties of Salt, Glucose and Protein Solution

Electrolytes are substances that dissociate into ionic species in solutions. They can be classified as strong electrolytes (eg. NaCl), which dissociate completely into ionic species, while the weak electrolytes only partially dissociate. Additional ionic substances in solutions result in high decrease of dielectric properties due to dc conductivity. Hence, it is recommended to measure static dielectric properties between the MHz and GHz range, where the proportionate conductivity effect is smaller.

At low frequencies (0.1~100 MHz), the dielectric properties are caused by the water molecules in an applied electrical field. Polarisation in the form of energy is stored. Positive and negative ions move according to the electrical field at the same frequencies. The electrical current corresponds to energy loss.

At higher frequencies (0.1~100 GHz), water molecules no longer change with the electrical field as frequency increases. Higher rotational losses occur and less energy is stored. The mass of the ions prevents water molecules from responding to the change in

the electrical field. At frequencies higher than 100 GHz, water molecules are stretched or even pulled apart.

The decrement of dielectric constant in electrolyte solutions due to the presence of polar ions orientate the water molecules in the solution, reducing the ability of water molecules to re-orientate to the applied electrical field (Craig, 1995). The relationship between the concentration of electrolytes and static dielectric constant is assumed to be linear at the limit between 0.1 mol/l and 1 mol/l, as given by the equation below:

$$\varepsilon_{ss} = \varepsilon_{sw} + \delta_c \qquad \textbf{2.19}$$

Where ε_{ss} and ε_{sw} are the static dielectric constants of sample and water respectively, δ_c is the dielectric decrement that relates to concentration, c of the solute. As the concentration of electrolytes increases, deficit of water molecules redistribute to form ions hydration layers. Hence, the relationship between the static dielectric constant and the concentration of ions is non-linear at higher concentration. The relaxation time also decreases linearly with low levels of electrolyte concentration. The suggested mechanism is due to the breaking effect of hydrogen bonding with the addition of ions (Craig, 1995). Table 2.2 and 2.3 show the changes in dielectric constant and relaxation time for a range of 1M ion aqueous solution.

Table 2.2: Changes in dielectric constant for a range of 1M ion aqueous solution (Pethig & Kell, 1987; Craig, 1995)

Cation	$\Delta\varepsilon_r$' (±1)	Anion	$\Delta\varepsilon_r$' (±1)
H^+	-17	F^-	-5
Li^+	-11	Cl^-	-3
Na^+	-8	I^-	-7
K^+	-8	OH^-	-13
Rb^+	-7	SO_4^{2-}	-7
Mg+	-24		
Ba^{2+}	-22		
La^{3+}	-35		

Table 2.3: **Changes in relaxation time for a range of 1M ion solution (Craig, 1995)**

Cation	$\Delta\tau$ (ps/mol)	Anion	$\Delta\tau$ (ps/mol)
H^+	1.3	F^-	-1.3
Li^+	-1.0	Cl^-	-1.3
Na^+	-1.3	I^-	-5.0
K^+	-1.3	OH^-	-0.7
Rb^+	-1.7		
Mg+	-1.3		
Ba^{2+}	-3.0		
La^{3+}	-5.0		

Besides salt, the presence of glucose also drastically affects the chemical and physical characters of the viable fluid. The dielectric properties of a glucose solution were investigated by Höchtl et al. (2000). They reported that the dielectric constant decreased while loss factor increased with respect to the glucose concentration solution at particular 2.45 GHz. The temperature effect (25°C to 85°C) of glucose solutions in dielectric properties was determined by Liao et al. (2001). The dielectric constant of the glucose solution increased while the loss factor decreased with temperature, respectively. They discovered that the dielectric properties of supersaturated glucose were independent of concentration at a particular concentration range of about 45~56%. Meriakri et al. (2007b) observed that glucose in solution up to 5% showed a decrease in dielectric properties, compared to water, except for frequencies from 92 to 93 GHz which showed slight increase of loss factor with concentration. Kim et al. (2009) investigated the interaction of the electromagnetic field with glucose and NaCl aqueous solutions. They reported that increasing the respective glucose and NaCl concentrations in the solutions yielded decreased intensity of microwave reflection coefficient, S_{11}. Good linear dependency between relative dielectric properties and reflection coefficient, S_{11} was found with increments in glucose concentration and temperature (Kim et al., 2009). Bassey and Cowell (2013) proposed the potential usefulness of dielectric properties to monitor the glucose level of diabetics after measuring diabetic urine with 0.1 to 1 M of urinary glucose concentration between 100 and 300 MHz at room temperature. Smulders et al. (2013) discovered that the changes to the dielectric properties in glucose solution were different compared to the changes in glucose solution with 0.9% NaCl salt. They found that the presence of salt in biological solutions could affect the sensitivity of dielectric properties of the glucose concentration. The presence of 0.9% NaCl salt in glucose solution as a representative of biological solution caused dielectric constant to decrease at frequencies below 20 GHz

and loss factor to increase at frequencies below 40 GHz, while vice versa at frequencies up to 67 GHz (Meriakri et al., 2007b; Smulders et al., 2013).

Meanwhile, the presence of protein causes changes in the dielectric properties of a solution. Initially, Oncley (1938) compared the dielectric properties of different serum protein solutions with pure water. He reported that dielectric properties increased with the concentration of carboxyhaemoglobin solution at radio frequencies. Ferry and Oncley (1938) discovered that serum and urinary albumin protein molecules had relatively faster electrophoresis mobility than other proteins in solutions, which resulted in the increase of dielectric constant. They pointed out that the dielectric constant of the serum protein solution increases with the concentration at radio frequency. Grant et al. (1968) reported that there was a relatively good dielectric dispersions change with temperature for different protein concentrations. The dielectric constant of bovine serum albumin decreased with temperature at frequency less than 1 GHz. Besides that, the dielectric constant decreased with the concentration of bovine serum albumin at 700 MHz. As for broadband frequency measurement, Nandi and Bagchi (1998) found that the dielectric constant increased with whale myoglobin solution at low frequency while decreasing at high frequency. Boresch et al. (2000) and Matyushov (2012) measured the simulation of dielectric properties between the interaction of protein and water. They reported that dielectric properties were affected by the protein-protein, water-water self-term and protein-water cross-term interaction (Boresch et al., 2000). Protein-water interface dramatically reduced their permanent dipoles respectively in order for the proteins to stay in solution (Matyushov, 2012). Rodríguez-Arteche et al. (2012) found two relaxations for amino acids, such as L-glycine, b-alanine, L-alanine, L-proline,L-threonine, L-histidine, L-lysine and L-arginine, in water at room temperature (22°C) from frequencies of 0.2 to 20 GHz. Relaxation time was increased with concentration of protein in solution. They suggested that those relaxations were mainly caused by the

rotation of amino acids in water and reorientation of water molecules, respectively. Wolf et al. (2012) investigated three relaxations in terms of β, γ and δ-relaxation of concentrated aqueous lysozyme solution from 1 MHz to 40 GHz at temperature from 275 K to 330 K. They reported β and γ–-relaxations were strongly correlated with temperature and δ-dispersion was attributed to bound water dynamics with concentration dependent on energy barrier. They suggested single Cole-Cole function for δ-relaxation. Changes in dielectric properties due to protein alter the mobility and linear conduction of a solution (Pethig & Kell, 1987; Abdalla et al., 2010).

2.5 Review of Dielectric Properties of Biological Solutions

The flow of current through the body's pathways provides analysis for a wide range of biomedical applications. Electrical stimulation of various physiological conditions, body composition and radio-frequency hyperthermia leads to important diagnosis or treatment. Knowledge of electrical properties can help improve understanding of biophysics and the underlying basic biological process on either macroscopic or microscopic level. Changes in physiology results in changes to the electrical properties of the biological solutions produced. This principle has been used to diagnose or monitor the presence of various conditions or illnesses with body fluid shift, cardiac output and blood flow by using different impedance measurement techniques. The dielectric properties of microbodies or micro-organs within a biological solution are measured in order to determine the response of biological solutions to electrical stimulation. Observable changes of dielectric properties in biological solutions are dependent on the biomaterials. The dielectric properties of biological solutions such as blood (Alison & Sheppard, 1993; Jaspard et al., 2003; Park et al., 2003; Lonappan et al., 2007d; Abdalla et al., 2010; Abdalla, 2011; Lonappan, 2012; Shim et al., 2013), urine (Lonappan et al., 2004; Lonappan et al., 2007a; Bassey & Cowell, 2013), semen (Lonappan et al., 2007c), cerebrospinal fluid (Rajasekharan et al., 2010) and milk

(Lonappan et al., 2006b) changed with variations in cell type, haematocrit, hormones, ionic salt, protein and glucose level, respectively.

2.5.1 Dielectric Properties of Blood

Blood is a non-Newtonian fluid with dynamic characteristics. The intrinsic and extrinsic molecules, protein, microcells, glucose, bacteria, vitamins, chemicals, hormone and antibodies drastically affect the chemical and physical characteristics of the viable fluid. Jaspard et al. (2003) investigated the haematocrit dependency of dielectric properties in animal blood at 37°C in frequencies ranging from 1 MHz to 1 GHz. The dielectric constant was found to increase and then decrease with haematocrit level. They suggested that it was the influence of cell membranes rather than the electrical component of blood at high frequencies that resulted in the decrease of the dielectric constant. In comparison with Maxwell-Fricke model, more significant divergences were observed at low frequencies between 1 MHz and 10 MHz which may essentially be influenced by erythrocyte capacitance (Jaspard et al., 2003). By considering factors affecting dielectric properties, the dielectric properties of human blood at 25°C and 37°C were measured in Alison and Sheppard's (1993) study. They reported that the relaxation frequency of gamma process in blood increased with temperature (within 5 %) from 25°C to 37°C. The relaxation time of blood was equivalent to pure water in Debye function (Alison & Sheppard, 1993). Lonappan (2012) discovered that different dielectric properties of blood was found between pregnant and non-pregnant women. Pregnant women had higher dielectric constant and conductivity than non-pregnant women at frequencies from 2 GHz to 3 GHz. The results were corroborated with clinical laboratory tests that showed the presence of the hormone Human Chorionic gonadotropin (HCG) in the blood of pregnant women. Shim et al. (2013) showed that the dielectric properties of cell types derived from solid tumours had different dielectric properties compared to peripheral blood cells. These

properties exhibited by tumour cells enable the application of dielectrophoresis for the isolation of circulating tumour cells and the concentration of leukaemia cells from blood (Shim et al., 2013).

Meanwhile, glucose is considerably lighter in mass compared to other components in blood. However, its effects are found in the changes of electrical and dielectric properties. Park et al. (2003) reported that the correlation of dielectric constant changed with hamster blood glucose at a low frequency range. Better accuracy was discovered in dielectric measurement compared to using a blood glucose meter (Park et al., 2003). The influence of human blood glucose in dielectric constant was studied at frequencies up to 3 GHz (Lonappan et al., 2007d). The results showed that dielectric properties varied in different blood glucose concentrations and frequencies, respectively (Lonappan et al., 2007d; Desouky, 2009; Abdalla et al., 2010; Topsakal et al., 2011). Meriakri et al. (2007a) found no visible changes in dielectric properties during the inverse process of glucose loading in blood especially between 41 GHz and 42 GHz. This happened because the temperature increased with the mobility of ions transported in excised blood (Abdalla et al., 2010). The dielectric properties of blood glucose concentration are well-established (Caduff et al., 2003; Hayashi et al., 2003; Karacolak et al., 2013). The application of dielectric spectroscopy has been successfully used in human subjects as a non-invasive approach to monitor changes in blood glucose (Caduff et al., 2003).

2.5.2 Dielectric Properties of Urine

Besides blood, urine is another biological solution that attracts interest in using dielectric properties measurement. Lonappan et al. (2007b) investigated the dielectric properties of urine in pregnant and non-pregnant women. They reported that the urinary dielectric constant of non-pregnant women was higher than that of pregnant women.

They found that the changes in urinary dielectric properties were in conjunction with the presence of HCG in urine which is a marker for pregnancy.

Urinary glucose is essential as a non-invasive approach for DM monitoring and diagnosis. The physiological range of glucose (100-400 mg/dl) was reported to have a direct impact on the impedance modulus of the physiological solution (0.9% NaCl), consequently changing the dielectric properties (Tura et al., 2007; Sbrignadello et al., 2012). Previous studies had looked into the glucose-induced dielectric properties of urine. Lonappan et al. (2007a) and Lonappan et al. (2004) measured the dielectric properties of urine. They reported that different dielectric properties of urine were obtained between diabetic patients and healthy subjects when urine was collected at different intervals of time after meals. Dielectric constant and loss factor were found to increase with glucose level in urine. Bassey and Cowell (2013) reported gradual decrease and increase in dielectric constant and loss factor, respectively, with glycosuria concentration over frequency range of 0.1 to 3 GHz at room temperature (22.5 ± 0.5°C). The effect of glucose was not prominent in the dielectric spectrum within the MHz frequency band (Fuchs & Kaatze, 2001). The changes in dielectric properties with glycosuria showed the potential of using dielectric properties measurement for monitoring and diagnosis of DM.

2.5.3 Dielectric Properties of Urine for Kidney-Related Diseases

Currently, there is limited data on the urinary dielectric properties of kidney-related diseases at the microwave frequency range. CKD is a common complication of DM. The presence of glucose and protein in urine is the essential non-invasive determination for diagnosis and prognosis of DM and CKD, respectively. The influence of glucose on the dielectric dispersion of urine has caught the attention of some researchers.

Raveendranath et al. (1998) investigated the urinary dielectric properties of a normal (117mg/dl) and a diabetic patient (168 mg/dl). They applied cavity perturbation technique to measure the dielectric properties of urine at the frequency range of 2.5 GHz to 11.5 GHz. They found that normal subject showed higher dielectric properties than diabetic patients and reported that changes in urinary dielectric properties were more prominent in normal subjects. Temperature is an important factor that affects dielectric properties measurement, but the study did not include any temperature control.

Lonappan et al. (2004) and (2007a) further investigated the dielectric properties of urine at microwave frequencies. They used rectangular cavity perturbation method to measure the urinary dielectric properties of healthy and diabetic subjects at different intervals of time. The urinary dielectric properties at frequency range of 2.4 GHz to 3 GHz were compared with quantitative urinalysis of clinical biochemistry following consumption of a meal. However, inconsistent results were found compared to Raveendranath et al. (1998). Lonappan et al. (2007a) reported significantly increase of dielectric properties with the presence of glucose in urine. In the study, urinary dielectric properties were measured at low frequency range due to the limitation of the measuring technique that fixes a frequency for each measurement. This resulted in time-consuming investigations and more complications.

Bassey and Cowell (2013) measured the urinary dielectric properties of diabetic patients with different glycosuria molarities. They used a network analyser to conduct measurements between 0.1 MHz and 0.3 MHz at room temperature of 22.5 ± 0.5°C. They reported gradual increase of dielectric properties with glucose molarity. However, the study was limited at the low frequency range and the differences between dielectric properties and glycosuria molarity were not validated statistically.

To date, no studies have reported on the urinary dielectric properties of protein in urine, although it is crucial for the diagnosis and prognosis of CKD. Meanwhile, limited data is available in the literature for dielectric properties with glycosuria. Overall, the previous studies conducted did not include temperature control in their investigations and measurements of the dielectric properties of glycosuria were at the low frequency range. Moreover, the differences and correlations of urinary dielectric properties with glycosuria level had never been validated statistically. Hence, these data remain sparse for determining reliable and accurate urinary dielectric properties of glycosuria and proteinuria.

2.6 Measurement Techniques of Dielectric Properties

Several techniques are available for the measurement of dielectric properties. Dielectric properties measurements are deduced from the reflectivity, impedance or transmission losses of a material under test with respect to the application of electromagnetic waves. Such techniques include open-ended coaxial probe, perturbation resonator, transmission line and free space that have been applied for dielectric properties measurement within the microwave frequency range.

2.6.1 Open-ended Coaxial Probe Technique

Generally, commercial open-ended coaxial probe techniques require a standard vector network analyser (VNA) or impedance analyser to measure dielectric properties at the admittance of the end of the coaxial probe. It generates dielectric properties by calculating the amplitude of the reflected signal at the end of the probe after it was inserted into the sample under test and deemed suitable for measurement of lossy materials. This technique is one of the most convenient ways to take the measurement since almost no sample preparation is required and it is capable of measuring complex biological tissues *in vivo* (Gabriel et al., 1996a; Gabriel et al., 1996b; Gabriel et al.,

1996c; O'Rourke et al., 2007) and biological solutions such as blood (Jaspard et al., 2003) and urine (Bassey & Cowell, 2013). Open-ended coaxial probe technique provides a non-destructive, broadband and high temperature measurement for dielectric properties. The concerns are the calibration of the probe with the dielectric properties of known materials and a reliable short circuit. Gabriel and Peyman (2006) assessed the uncertainties of the open-ended coaxial probe technique. They reported random fluctuations or uncertainties of system measurement that originated from sampling procedures and its natural inhomogeneity. Gabriel and Peyman (2006) proposed that water is probably the best reference material for dielectric properties since the data is well established – a comprehensive list of references can be found in Ellison (2007) and Fernández et al. (1995). The dielectric properties of water were nearly perfect examples of Debye dispersion from 0 to 100 GHz with well-defined parameters. In this technique, the sample must be in good contact with the surface at the end of the probe during measurement. The sample should move to the test probe rather than the other way around to avoid errors. Systemic errors can be obtained from the deviation of reference and calibration drift. For biological materials, the mean of standard deviation (SDM) would be a good measurement of uncertainty. Uncertainty can be improved by increasing the consistency of the sampling technique, increasing the number of samples and ensuring the integrity of samples (Gabriel & Peyman, 2006).

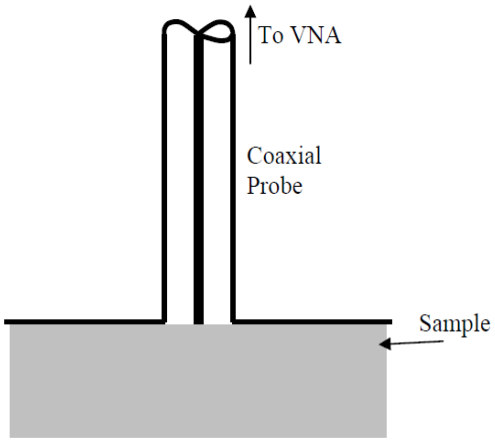


Figure 2.3: Schematic of open-ended coaxial probe technique (Alabaster, 2004)

2.6.2 Resonant Cavity Perturbation Technique

The resonant cavity perturbation technique is the comparative analysis of electromagnetic characteristics between an empty and a partially loaded rectangular or cylindrical resonance cavity. The sample is inserted into the cavity and that is perturbed to resonate at lower frequency with a lower (unloaded) quality (Q) factor suitable for measuring low loss materials. The shift of resonant frequency and absorption characteristics of the cavity provides information for the dielectric constant. Changes in the Q-factor related to ratio of energy stored to energy dissipated indicate dielectric loss estimation. This technique is used to measure biological materials and solutions (Campbell & Land, 1992; Lonappan et al., 2006a). However, it has limitations for measurements at microwave frequencies. The sample size must be smaller than the cavity size especially for materials with high dielectric properties. This causes practical problems at the microwave frequency range because cavity size reduces with increasing frequency. Besides that, this technique only limited to a single frequency at each measurement (Venkatesh & Raghavan, 2005). It is suitable for measuring dielectric properties of medium or low loss materials and provides more accurate results than wave-guiding methods (Komarov et al., 2005).

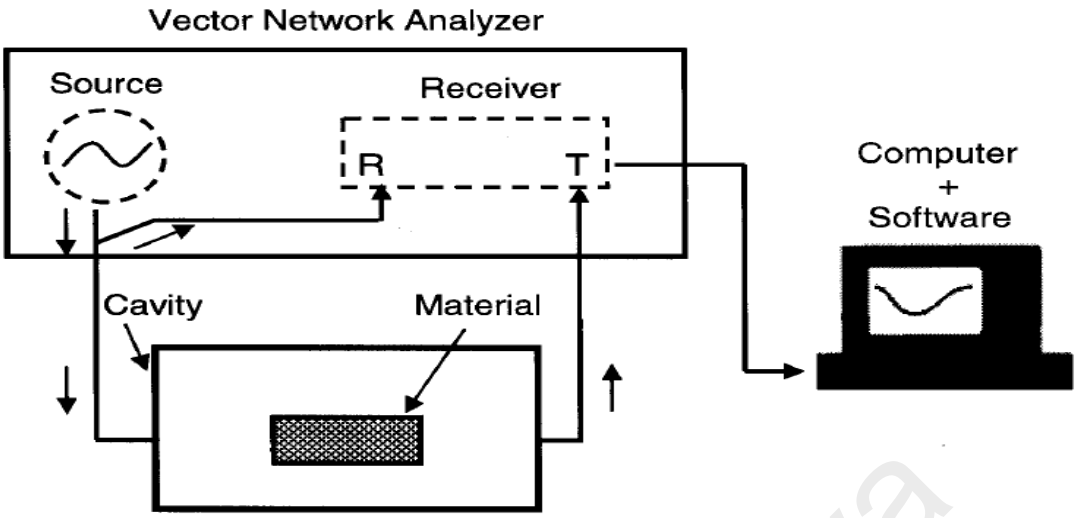


Figure 2.4: Schematic of perturbation resonator technique (Venkatesh & Raghavan, 2005)

2.6.3 Transmission Line Technique

The transmission line technique is related to the non-resonant dielectric properties measuring method. The sample is placed in the middle of the transmission line between two ports of measurement cells so the electromagnetic wave can propagate from the input to output port. This technique measures the phase and amplitude of a reflected microwave from a sample placed against the end of a short-circuit transmission line to determine the dielectric properties. Three main types of transmission lines are used as measurement cells, such as rectangular waveguide, coaxial line and microstrip line (Komarov et al., 2005). For a waveguide structure, a rectangular sample is required to fit into waveguide dimensions for a measured frequency. This method is applied to liquids and solids but not gases due to their low dielectric properties. An annular sample is required to be fabricated for the coaxial line. The sample should have approximately one-quarter thickness of the energy wavelength that has penetrated.

The transmission line technique measurement system is more expensive than the open-ended coaxial probe system at the same range of frequency, and the sample preparation for the former is also more complicated and time-consuming (Komarov et

al., 2005). The transmission line technique is accurate for high-loss materials but rigidity of shape and size is required. In order to determine moisture content using the transmission line method, measurement frequency should be above 5 GHz to prevent influence from bound water relaxation and ionic conductivity (Venkatesh & Raghavan, 2005).

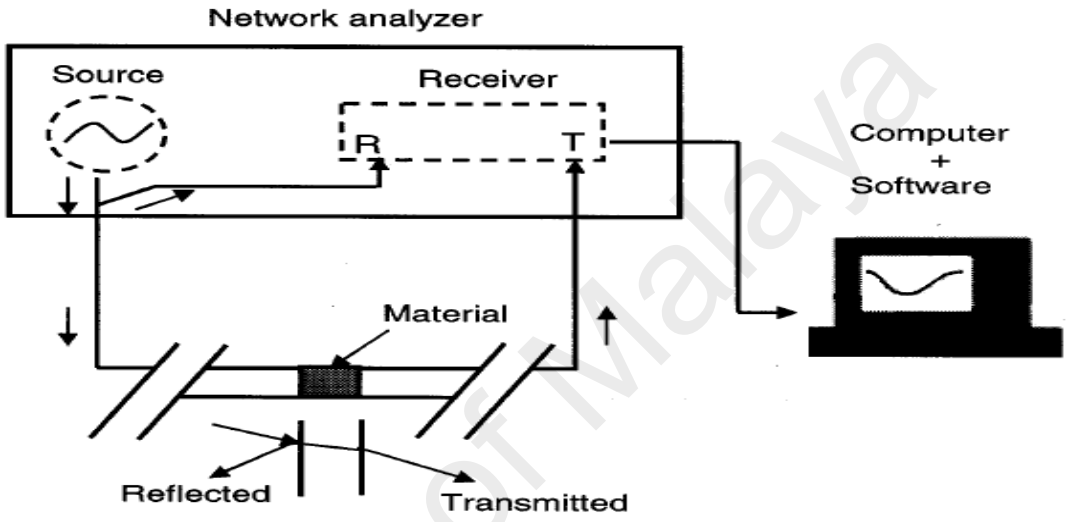


Figure 2.5: Schematic of transmission line waveguide technique (Venkatesh & Raghavan, 2005)

2.6.4 Free Space Transmission Technique

The free space transmission technique is a non-destructive and contact-less method in which no sample preparation is required. The sample is required to be placed between the transmitting and receiving antenna, while the dielectric properties are determined by measuring the attenuation and phase shift of the signal generated. This technique is suitable for measuring inhomogeneous material at high temperature. It requires planar samples with known constant thicknesses that are sufficiently large for interception to the entire beam. It is easily implemented for continuous monitoring and control purposes such as moisture content and density measurement. The assumption is made that the flat surface of a homogenous material is normally incident by a uniform plane

wave, and the planar sample has infinite extent laterally, hence the diffraction effect of the sample's edges can be neglected (Venkatesh & Raghavan, 2005). Alabaster (2004) reported that multiple solutions for the determination of dielectric properties from different methods are fitted to measured data for the free-space transmission technique. It may due to the diffraction of the surrounding edges of the sample or multiple reflections between the sample and horn, or between horn and horn in low loss samples. He suggested that several data points are required to overcome the ambiguity and time-gating techniques to isolate the desired signal path.

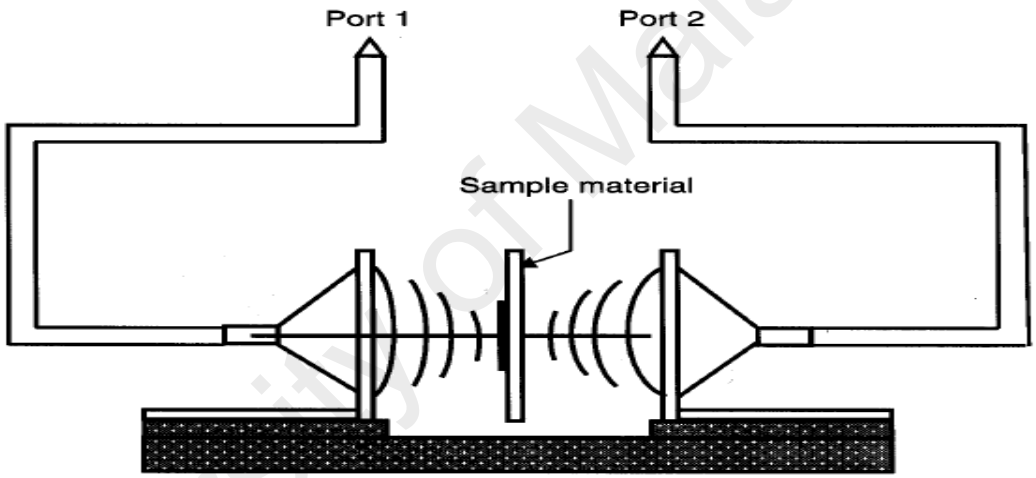


Figure 2.6: Schematic of free space transmission technique (Venkatesh & Raghavan, 2005)

2.6.5 Comparison among Measuring Techniques

Overall comparisons among the measuring techniques for dielectric properties are shown in Table 2.4.

Table 2.4: Comparison between different types of measuring techniques for dielectric properties (Icier & Baysal, 2004; Komarov et al., 2005; Venkatesh & Raghavan, 2005)

	Open-Ended Coaxial Probe	**Transmission Line**	**Resonant Cavity Perturbation**	**Free Space**
Descriptions	This technique is the most popular method. It calculates dielectric properties from the phase and amplitude of the reflected signal at the end of the probe that is placed in contact or immersed into a sample under test.	Sample is connected between cross-sections of an enclosed transmission line to cause impedance changes.	Sample is introduced into a cavity (a high Q resonant structure) and transmission response is calculated.	Sample is placed between the transmitting and receiving antenna. The attenuation and phase shift of the signals are measured.
Frequency Range	200MHz to 100GHz	<100GHz	1MHz to 100GHz	Microwave range
Material	Liquids or semi solids	Solids	Solids or liquids	Solids

Table 2.4, continued

	Open-Ended Coaxial Probe	Transmission Line	Perturbation Cavity Resonator	Free Space
Advantages	It is non-destructive, broadband (RF and microwave ranges), accurate, simple and can withstand high temperatures (<1200°C) with different probes.	It is accurate for high-loss materials with rigid requirements on sample shape and sizes.	It is accurate for low loss materials.	It is non-destructive, contact-less, suitable for high-temperature materials and inhomogeneous dielectrics.
Disadvantages	It is not suitable for extremely low loss materials (plastics, oils, etc) and requires sufficient sample thickness >1cm.	It is more expensive than the open-ended coaxial probe for the same range of frequency. Precise sample shape is required; it is a complicated and time-consuming method.	It provides measurements at a fixed frequency.	Samples require flat parallel faces and large samples at low frequencies.

2.7 Support Vector Machine Classification

SVM is a classification technique that was first pioneered by Vapnik (1995) for pattern classification and nonlinear regression. It implements a method of structural risk minimisation and is able to provide a good generalisation performance on pattern classification (Haykin & Network, 2004). The decision function of a linear hyperplane separation is

$$w^T x + b = 0 \quad \textbf{2.20}$$

where x is an input vector, w is an adjustable weight vector and b is bias. Let the training sample, $X = \{x_1, x_2, \dots x_n\}$and d_i as the desired output.

$$w^T x + b \geq 0 \text{ if } d_i = +1 \quad \textbf{2.21}$$

$$w^T x + b < 0 \text{ if } d_i = -1 \quad \textbf{2.22}$$

The optimal hyperplane function that represents multidimensional linear decision function is

$$g(x) = w_0^T x + b_0 \quad \textbf{2.23}$$

The results of classification must satisfy the following inequality for $d_i = +1$and $d_i = -1$.

$$d_i(w_0^T x_i + b_0) \geq 1, i = 1,2,3, \dots . n \quad \textbf{2.24}$$

Consider support vector $x^{(s)}$ for $d^{(s)}$ = +1. By definition, the following function is obtained:

$$g\left(x^{(s)}\right) = w_0^T x^{(s)} \pm b_0 = \pm 1 \quad \textbf{2.25}$$

when $d^{(s)} = \pm 1$. Given the equation of optimal hyperplane is related to desired algebraic distance, r:

$$r = \frac{g(x^{(s)})}{||w_o||} = \begin{cases} \frac{1}{||w_o||} \text{ if } d_i = +1 \\ -\frac{1}{||w_o||} \text{ if } d_i = -1 \end{cases} \quad \textbf{2.26}$$

The margin of separation is between the hyperplane and the closest data point, p. From the equation above, it follows that

$$p = 2r = \frac{2}{||w_o||} \quad \textbf{2.27}$$

Hence, the Euclidean norm of weight vector, w is maximised as the goal of a SVM.

For nonlinear classification, inner-product Kernel is developed. It converts nonlinear input data into high dimensional linear feature space. The following three types of inner-product Kernel functions, as shown in Table 2.5, are commonly used for SVM.

Table 2.5: Summary of inner Kernel Function

Type of Kernel	Kernel function
Linear	$K(x, x_i) = x^T x_i$
Polynomial	$K(x, x_i) = (x^T x_i + 1)^p$
Radial–basis function (RBF)	$K(x, x_i) = \exp(-\gamma \|x - x_i\|^2)$

2.7.1 Classification Accuracy Analysis

Sensitivity and specificity analysis involved the usage of terms such as true positive (TP), true negative (TN), false positive (FP) and false negative (FN), which are explained in the following:

True positive (TP): A patient is correctly identified as a patient by the expert clinicians.

True negative (TN): A normal subject is correctly identified as a normal (healthy) subject by the expert clinicians.

False positive (FP): A normal subject is incorrectly identified as a patient by the expert clinicians.

False negative (FN): A patient is incorrectly identified as a normal (healthy) subject by the expert clinicians.

$$Sensitivity = \frac{TP}{TP + FN}(\%) \quad \textbf{2.28}$$

$$Specificity = \frac{TN}{FP + TN}(\%) \quad \textbf{2.29}$$

$$Classification\ accuracy = \frac{(TP + TN)}{TP + TN + FN + FP}(\%) \quad \textbf{2.30}$$

2.7.2 Confusion Matrix

Representation of confusion matrix for a two-class classifier is shown in Table 2.6, The confusion matrix provides information about an actual and predicted classification that allows for the performance of a classification system to be visualised. Entries of the confusion data are explained as follows:

a is the number of positive instances that are correctly predicted as positive,

b is the number of positive instances that are incorrectly predicted as negative,

c is the number of negative instances that are incorrectly predicted as positive, and

d is the number of negative instances that are correctly predicted as negative.

Table 2.6: Representation of confusion matrix

Actual	**Predicted**	
	Positive	**Negative**
Positive	a	b
Negative	c	d

2.7.3 Application of Support Vector Machine

Data classification has been the most intensively studied in statistical and decision science. It has been applied in disease diagnosis (Polat et al., 2008; Karabatak & Ince, 2009; Barakat et al., 2010; Ganji & Abadeh, 2011), credit evaluation and image recognition (Michie et al., 1994). The SVM-based classification method has been

widely used to classify urinary proteins of kidney disease (Haubitz et al., 2005; Kistler et al., 2009; Alkhalaf et al., 2010; Gronwald et al., 2011) and DM (Ban et al., 2010; Roshan et al., 2011). Haubitz et al. (2005) used support SVM to classify urinary polypeptide pattern between kidney disease patients and normal people. They mentioned that SVM has fast and stable algorithms that perform well in the evaluation of clinical markers. It is a tool that has the advantage of discriminating data in high dimensional parameter spaces. Classification sensitivity of 100% and 77% were obtained in the discrimination of Immunoglobulin A (IgA) kidney disease from normal people and membranous kidney disease, respectively.

Alkhalaf et al. (2010) reported that 100% sensitivity and 97% specificity were obtained to classify between urinary diabetic kidney disease (DKD) with 65 biomarkers and pure diabetes urine. SVM has also been applied in the classification of autosomal dominant polycystic kidney disease that showed high classification accuracies (>80%) (Kistler et al., 2009; Gronwald et al., 2011).

In the evaluation of gene-gene interaction in diabetes biological pathways, 408 single nucleotide polymorphisms of 87 genes were identified in urinary type 2 DM. SVM achieved 70.9% and 70.6% of prediction rates with different single nucleotide polymorphism combinations by using radial basis function (RBF) kernel (Ban et al., 2010).

The electrical properties of tissues have been well established in the literature. The performance of SVM classifier is proposed to be able to distinguish malignant breast (Kerhet et al., 2006; Laufer & Rubinsky, 2009; Grewal & Golnaraghi, 2014), prostate cancer (Shini et al., 2011) and brain injury tissue (Gonzalez et al., 2013) by measuring its electrical impedance properties. Shini et al. (2011) measured the electrical impedance of prostate tissue using biopsy probes as electrodes. They reported that the SVM

classifier is able to generalise different properties of prostate, while reducing the number of biopsies required and providing information about adjacent tissues of cancer tumours. Gonzalez et al. (2013) applied the first clinical study of volumetric electromagnetics phase shift spectroscopy in patients with brain edema and haematoma. A SVM classifier built within the spectroscopy provided instantaneous diagnosis of the medical condition of brain tissue. Kerhet et al. (2006) concluded that prioritising the dielectric properties of breast tissue in the classification database had increased the classification probability value between tumour and normal breast tissue.

2.8 Summary

This chapter began with a review on the current diagnosis methods for DM and CKD. The limitations and problems present in the current diagnosis methods were pointed out. Next, the overview of dielectric properties which included the relevant concepts of dielectric properties, background theories and factors, such as frequency and temperature that affect changes of dielectric properties, were described. The applications of dielectric properties measurement in different solutions were reviewed, where the changes of dielectric properties with different concentrations of respective salt, glucose and protein in solution were noted. Lately, interest has been generated in measuring the dielectric properties of biological solutions such as blood and urine. There is very sparse dielectric data on human urine at microwave frequency and very little investigation on the temperature effect in the dielectric properties of biological solutions. A few studies related to the measurement of human urine showed that the dielectric properties of urine changed with glucose concentration in diabetic patients. No study had been found related to the measurement of dielectric properties of urinary protein. Various techniques of dielectric properties measurement as well as their respective pros and cons were reviewed and compared for their suitability in measuring

biological solutions at microwave frequency. This indicated the potential application of dielectric properties measurement.

Data classification is proposed to justify the accuracy of dielectric properties measurement. The SVM-based data classification method was reviewed. Background theories related to SVM were described and various inner-kernel functions for non-linear classification were explained. Proper analysis of classification accuracies was demonstrated. Applications of SVM in data classification in terms of urinary proteomics and electrical properties of tissues for disease diagnosis produced high accuracy rates. This has generated interest in using the determination of urinary dielectric properties for glycosuria and proteinuria; the classification of urinary dielectric properties for disease diagnosis will be discussed in later chapters.

CHAPTER 3: THE MEASUREMENT OF URINARY DIELECTRIC PROPERTIES OF GLYCOSURIA FOR TYPE 2 DIABETES MELLITUS

3.1 Introduction

From the review in Chapter two, it can be seen that dielectric properties provide information about the chemical variability in solution. The interest generated in the use of dielectric properties measurement has raised concerns for clinical utility. Prior to this study, limited studies have been published in the literature about the dielectric properties of human urine at microwave frequency.

Urine is an excretory biological solution that reflects the pathophysiology variation of an individual. Diabetic patients who fail to break down glucose in blood for energy will eventually present with glycosuria (Kreisberg, 1978; Sacks et al., 2011). Urinary glucose measurement is an essential non-invasive approach to diagnose and monitor DM. Previous studies had looked into the glucose-induced dielectric properties of urine up to 3 GHz (Lonappan et al., 2004; Lonappan et al., 2007a; Bassey & Cowell, 2013). However, questions still remain regarding temperature effects and broadband frequency measurement in the dielectric properties of glycosuria.

In this chapter, the urinary dielectric properties of subjects with Type 2 DM at room temperature (25°C), 30°C and body temperature (37°C), respectively, at microwave frequency between 0.2 GHz and 50 GHz are investigated and analysed. This chapter determines the significant differences in urinary dielectric properties among subjects with different glycosuria levels. The correlations between urinary dielectric properties and glycosuria level are investigated. The measured data are fitted to Debye model for comparison.

3.2 Background Study

3.2.1 Overview of Diabetes Mellitus

DM, also known as hyperglycaemia, is a common public health concern. It is a disorder of the pancreas that causes abnormal glucose levels in the bloodstream. In Malaysia, the prevalence of DM among Malaysian adults aged 18 years and above has increased from 11.6% in 2006 to 16.6% in 2015 (International Diabetes Federation, 2016). In the United States, 29.1 million (9.3%) people in the population were diagnosed as having DM in 2012 compared to 25.8 million (8.3%) in 2010 (American Diabetes Association, 2014). Generally, there are two types of DM as described in Table 3.1:

Table 3.1: Types of Diabetes Mellitus

Type	Details
1	This type of diabetes is insulin-dependent. It occurs when the immune system destroys the insulin-producing beta cells of the pancreas, causing insufficient insulin to be produced.
2	This type of diabetes is insulin-independent. It occurs when the body becomes less sensitive in detecting the effect of insulin in breaking down glucose, fat and protein into energy that often happen in middle-aged or older people.

DM increases mortality and the risk of macro and microvascular complications, such as blindness, heart disease, stroke, amputation and CKD. Type 2 DM is the most common type of diabetes among the population and it is the primary cause of kidney failure (Feisul & Azmi, 2012; American Diabetes Association, 2014).

3.2.1.1 Diabetes mellitus diagnosis and monitoring

Early diagnosis and monitoring of DM is important for the prevention of further complications. As it is a non-invasive approach, urinary glucose measurement is essential for DM. However, diabetes test strips that use colour charts to determine glycosuria variability is less accurate compared to numerical readouts (Goldstein et al., 2004). Its results may vary with drug intake and non-glucose sugars. Meanwhile, portable glucose meters are recommended to be used by individuals for self-monitoring of blood glucose. However, the application of self-monitoring blood glucose meters is invasive and costly. It also carries a high possibility of false positive or false negative results due to certain factors such as haematocrit level (Tang et al., 2000), altitude, environmental temperature or humidity, hypo-tension, hypoxia and high triglyceride concentrations (American Diabetes Association, 1994). A comparison of blood glucose meter results with laboratory biochemical analysis of glucose is always required (Kabadi et al., 1994).

3.2.2 Selection of Experimental Technique

From the review of Komarov et al. (2005), Venkatesh and Raghavan (2005) and Icier and Baysal (2004), the open-ended coaxial probe has been considered for application in the measurement of urinary dielectric properties. The open-ended coaxial probe technique, based on the measurement of the complex reflection coefficient at the end of the probe, was selected for its simplicity and requires minimum sample preparation. The method used by Blackham and Pollard (1997) and Zhadobov et al. (2012) was, therefore, selected as the basis for the experimental method.

3.2.2.1 Theory of coaxial probe model

The open-ended coaxial probe system has an open-ended coaxial line sensing element that is excited by transverse electromagnetic waves. A network analyser that is

connected to the probe detects the amplitude, phase of incident wave and reflected signal. The inverse model is used to compute the reflection coefficient to dielectric properties. The sample is assumed to have both infinite ground plane and semi-infinite size in the inverse coaxial probe model. Practically, the assumptions are justified if reflections from finite boundaries are not sensed at the probe aperture. Semi-finite sample is stimulated when no-reflected energy is insured at the finite boundaries matched by lossy material. The complex relative permittivity is measured based on the reflected coefficient, $\Gamma = \Gamma' - j\Gamma''$ as shown below (De los Santos et al., 2003):

$$\varepsilon_r' = (A_e f)^{-1}\{\frac{-2\Gamma''}{(1+\Gamma')^2 + \Gamma''^2}\} \quad \textbf{3.1}$$

$$\varepsilon_r'' = (A_e f)^{-1}\{\frac{1-2\Gamma'^2 - \Gamma''^2}{(1+\Gamma')^2 + \Gamma''^2}\} \quad \textbf{3.2}$$

A_e is the empirical coefficient which is dependent on sample size and characteristic impedance of the probe. Calibration measuring three known reflection coefficients solves the systemic errors at the measured frequency. The mathematical equation shows the difference between the actual reflection coefficient, Γ and measured reflection coefficient, Γ_m after removing the effects of systemic errors, as follows (Blackham & Pollard, 1997):

$$\Gamma = \frac{\Gamma_m - a_{11}}{a_{22}(\Gamma_m - a_{11}) + a_{12}} \quad \textbf{3.3}$$

where a_{11} is directivity error, a_{12} is frequency response error and a_{22} is source match error. By considering the propagation constant (γ) and distance between the connector and probe head (z), a_{ij} can be determined in terms of connector S parameters (Komarov et al., 2005):

$$a_{11} = S_{11}; a_{12} = S_{12}S_{21}e^{-2\gamma z}; a_{22} = S_{22}e^{-2\gamma z} \quad \textbf{3.4}$$

The alternative way to explicit systemic error terms which involves cross-ratio invariance of complex numbers is given by the equation below (Blackham & Pollard, 1997):

$$\frac{(\rho_m - \rho_1)(\rho_3 - \rho_2)}{(\rho_m - \rho_2)(\rho_1 - \rho_3)} = \frac{(y_m - y_1)(y_3 - y_2)}{(y_m - y_2)(y_1 - y_3)} \quad \textbf{3.5}$$

ρ_1, ρ_2, and ρ_3 are the respective measured coefficients of the three calibration standards. ρ_m refers to the measured reflection coefficient of the sample. y_1, y_2, and y_3 are the calibration standard admittance at the probe aperture. y_m is the unknown admittance of the dielectric properties calculated.

Calibration that is performed using well-known reference liquids as the calibration standard can be easily carried out (Kraszewski et al., 1983). Calibration mapping showed in Equation 3.3 and Equation 3.5 can deduce errors in the probe by measuring materials similar with calibration standards. Short circuit, air and deionised water are well-defined calibration materials. Reflection coefficients of water and air can be obtained from the known value of dielectric properties. Water provides a useful standard for the coaxial probe, which adequately approximates a semi-infinite sample due to dipole of water losses sufficiently at frequencies when radiation from the probe is emitted. Grant et al. (1989) described the difficulties in using deionised water as a calibration standard with a large diameter probe (14 mm) due to the radiation that is prominent at lower frequencies.

3.3 Materials and Methods

3.3.1 Subject Recruitment

A total of 44 subjects with Type 2 DM, with documented disease duration > 3 years, were recruited from the outpatient clinics at the University of Malaya Medical Centre (UMMC). Diabetic subjects were recruited with the following criteria: Haemoglobin A1c (HbA1c) > 6.0% and no diabetic kidney disease (DKD). Exclusion criteria involved diabetic subjects with proteinuria or haematuria. Medical ethics approval was obtained from the Institutional Ethics Review Committee, UMMC**.** All subjects provided written informed consent to participate in this study.

3.3.2 Urine Collection and Storage

Sixty ml of random spot mid-stream urine samples were collected from each subject. For each collected urine sample, 20 ml was used to measure urine clinical chemical variables and microscopy using routine methods at the Division of Laboratory Medicine, UMMC.

Urine samples were collected in sterile urine containers. Fresh urine samples were stored at a temperature of 4°C before measurement for no more than 4 hours. No preservatives were added upon urine collection.

3.3.3 Experimental Setup and Calibration

The dielectric properties measurement system consisted of: (1) Agilent E8364C personal network analyser (PNA; 10 MHz-50 GHz) operated with Agilent 85070 software through Agilent 82357A GPIB interface (Agilent Technologies, Santa Clara, CA); and (2) 50 GHz flexible cables connected to an open-ended coaxial slim probe (nickel) with a diameter of 2.2 mm and length of 200 mm, designed by Agilent Technologies for liquids and semi-solid materials.

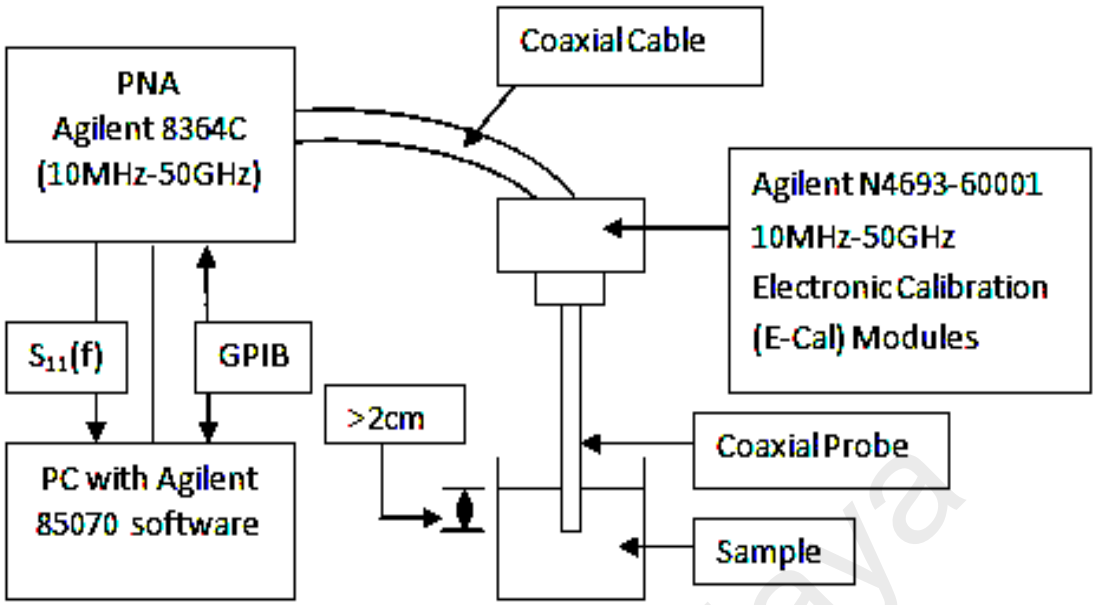


Figure 3.1: Schematic representation of the measurement set-up

A three-term well-defined calibration material was used to correct for the directivity, tracking, and source match errors that can be present in a reflection measurement. The PNA was calibrated with references for air, short circuit and deionised water before measurements took place. These were chosen as the calibration standards for biological solutions to be measured that have > 90% water content; therefore, measurements among available reference liquids are required to determine measurement accuracy (Gabriel & Peyman, 2006; Zhadobov et al., 2012). Electronic-calibration (E-Cal) was used as the standard for refresh calibration. The calibration was recalibrated using E-Cal after no more than 5 measurements. The integrity of the system was checked with repeated measurements on standard liquids (eg. distilled water and methanol) and 0.1M NaCl aqueous solution at different calibration sessions using E-Cal. Uncertainties were reduced by determining the differences between the measured and reference values of the reference liquids (Gabriel & Peyman, 2006; Zhadobov et al., 2012).

3.3.4 Urine Measurements

Before measurements were conducted, urine samples were heated to room temperature (25°C) using WNB 7 water bath (Memmert, Duesseldorf, Germany) with a precision of ± 0.1°C and the samples were gently stirred. Movement of the test table and probe was avoided by adjusting the sample to the probe to remove random errors of the measurements (Gabriel & Peyman, 2006). The measurements were taken when the probe was immersed > 2 cm with perfect contact and no presence of air bubbles under the probe tip as shown in Figure 3.1. The probe was sterilised using alcohol wipes and cleaned with distilled water before each measurement. Experiments were repeated by heating the urine samples to 30°C and 37°C, respectively. For each experiment, three measurement readings were recorded for each urine sample throughout the frequency range of 0.2 to 50 GHz.

3.3.5 Data Analysis

Dielectric properties, in terms of dielectric constant ($\varepsilon_r^{'}$) and dielectric loss factor ($\varepsilon_r^{''}$), were obtained from the measurements. A total of 250 frequency points were measured with an interval of 200 MHz between 0.2 GHz and 50 GHz. Statistical one-way analysis of variance (ANOVA) test of SPSS Statistic 21.0 (IBM Corp, 2012) was conducted to determine the effect of glycosuria across subject groups in the urinary dielectric properties at different microwave frequencies. Tukey post hoc tests were conducted to multi-compare the groups' mean among the subject groups. Apart from that, Pearson correlation test was conducted to determine correlation between glycosuria levels and the urinary dielectric properties. The level selected for statistical significance (p-value) was set at probability value of <0.05.

3.3.6 Curve Fitting

In polar liquids, each type of polar molecule exhibits a particular characteristic response to an imposed electric field. Dielectric relaxation is the delay of molecular polarisation with the change of the electric field at electromagnetic frequencies. Theoretically, Debye model describes the wideband frequency dependence of the dielectric relaxation response (Pethig & Kell, 1987; Komarov et al., 2005). Single-pole Debye model as shown below in Equation 2.14 was applied to fit the experimental data over the frequency range of between 0.2 GHz and 50 GHz using MATLAB (Mathworks, 2011) fitting function:

$$\varepsilon(\omega) = \varepsilon_{\infty} + \frac{\Delta\varepsilon}{1 + j\omega\tau} - j\frac{\sigma_s}{\omega\varepsilon_0}$$

where $\varepsilon(\omega)$ is the complex relative permittivity (dielectric properties) and ω is the angular frequency. Infinite frequency permittivity (ε_{∞}), magnitude of dispersion ($\Delta\varepsilon$), relaxation time (τ), and static conductivity (σ_s) are the parameters of the variables to fit the experimental data. Limits such as $\varepsilon_{\infty} \geq 1$, $\Delta\varepsilon \geq 0$, $\sigma_s \geq 0$ and $\tau \geq 0$ were set on the fitting parameters so that they would remain within physical ranges.

The fitting analysis was conducted using a genetic algorithm (GA) to compute the function score of the complex curve-fitting programme with iterations. GA performs direct search optimised parameters with best fitness from a population. The level of population size was selected at 1500 with cross-over fraction, 0.5 was set. The programme calculates the root mean square percentage error (RMSPE) between the differences of experimental value and the value obtained from the model for fitting. The data were fitted independently for each subject group at respective temperatures.

3.4 Results

3.4.1 Urine Composition

As defined, glycosuria was found in DM subjects. The DM subjects were grouped into three groups based on their urinary glycosuria levels: 0 g/L, >1 g/L and >5 g/L, respectively. The characteristics of the subject groups are presented in Table 3.2.

Table 3.2: Characteristics of the DM subjects.

Group	1	2	3
Glycosuria level (g/L)	0	>1	>5
Total (N)	15	13	16
Age (years)	60 ± 9.7	59 ± 6.4	60 ± 10
HbA_{1c} (%)	6.9 ± 1.0	7.6 ± 0.7	8.2 ± 1.3
Fasting Blood Glucose (mmol/L)	6.5 ± 1.4	7.6 ± 2.6	8.9 ± 2.4

3.4.2 Reproducibility and Accuracy

Reproducibility of the experiment was obtained by measuring the dielectric properties of distilled water repeatedly 5 times at 25°C, 30°C and 37°C, respectively, with independent calibration session as suggested by Gabriel and Peyman (2006) and Zhadobov et al. (2012). According to Agilent Technologies (2012), the accuracy of the measurement with coaxial slim probe is within ± 0.05 or ± 5% at temperature of 23 ± 3°C. To assess the accuracy of the technique, measurements for methanol and 0.1M salt water were conducted. However, it is inappropriate for determining the experimental accuracy at high temperature for methanol, which has different spectra of dielectric properties with water, especially at the millimetre wave range (Zhadobov et al., 2012). Thus, the measurement of methanol was conducted at 25°C. No significant differences ($p>0.05$) were obtained among measured data at different calibration sessions at the

frequency range from 0.2 to 50 GHz. The reproducibility of measurements for urine was the same with those obtained from reference liquids.

Figures 3.2 and 3.3 show the comparison of measured dielectric properties with reference values of distilled water extracted from Ellison's database (Ellison et al., 1996; Ellison, 2007). Overall, the measured data were closely matched to reference data. Maximum deviations compared to reference data were about 4%.

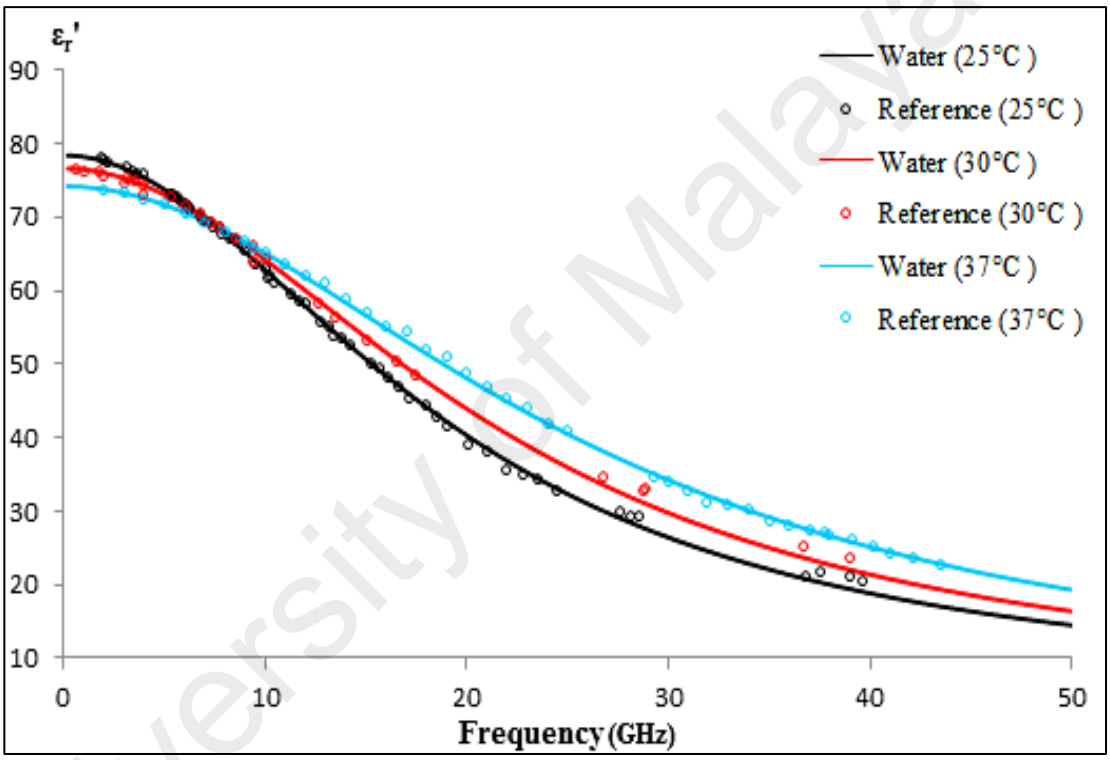


Figure 3.2: Comparison between measured dielectric constant of distilled water and reference data at respective temperatures of 25°C, 30°C and 37°C for frequencies ranging from 0.2 to 50 GHz.

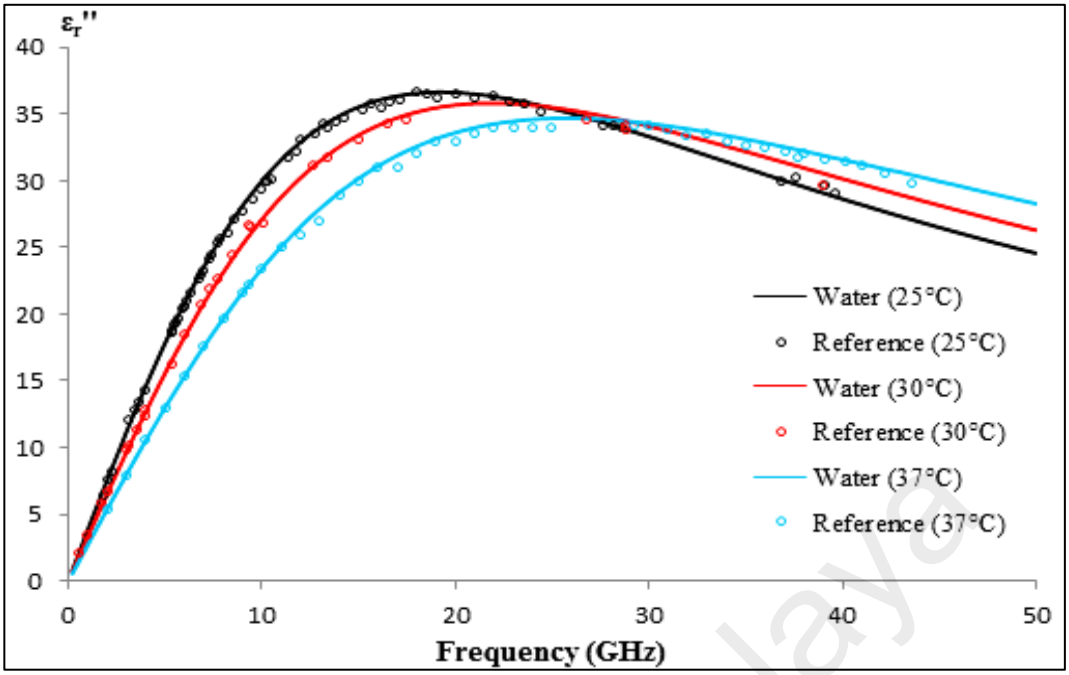


Figure 3.3: Comparison between measured loss factor of distilled water and reference data at respective temperatures of 25°C, 30°C and 37°C for frequencies ranging from 0.2 to 50 GHz.

Tables 3.3 and 3.4 show the comparison of Debye parameters for distilled water and 0.1M salt water with reference data, respectively. Overall, the measured data were close to the reference data with deviation less than 1.5%.

Table 3.3: Comparison of Debye parameters of distilled water with reference data at 25°C, 30°C and 37°C

Temperature (°C)	$\Delta\varepsilon$	τ (ps)	Study
25	73.38	8.20	(This study)
	72.38	8.28	(Ellison, 2007)
	-	7.36	(Johri & Roberts, 1990)
	73.16	8.27	(Kaatze, 1989)
30	71.61	7.30	(This study)
	70.83	7.35	(Ellison, 2007)
	-	6.72	(Johri & Roberts, 1990)
	71.36	7.28	(Kaatze, 1989)
37	69.19	6.22	(This study)
	68.71	6.29	(Ellison, 2007)
	-	5.15	(Johri & Roberts, 1990)
	69.57	6.23	(Kaatze, 1989)

Table 3.4: Comparison of Debye parameters of 0.1M salt water with reference data at 25°C, 30°C and 37°C

Temperature (°C)	Δε	τ (ps)	Study
25	71.67	8.08	(This study)
	71.14	8.07	(Buchner et al., 1999)
30	68.50	7.19	(This study)
	69.58	7.19	(Buchner et al., 1999)
37	67.91	5.98	(This study)
	67.32	5.95	(Buchner et al., 1999)

Figure 3.4 shows the comparison between measured dielectric properties of methanol with reference data presented in Gregory and Clarke (2001), Smith et al. (1998), and Yomogida et al. (2012). Overall, the deviations were within 1~5 %.

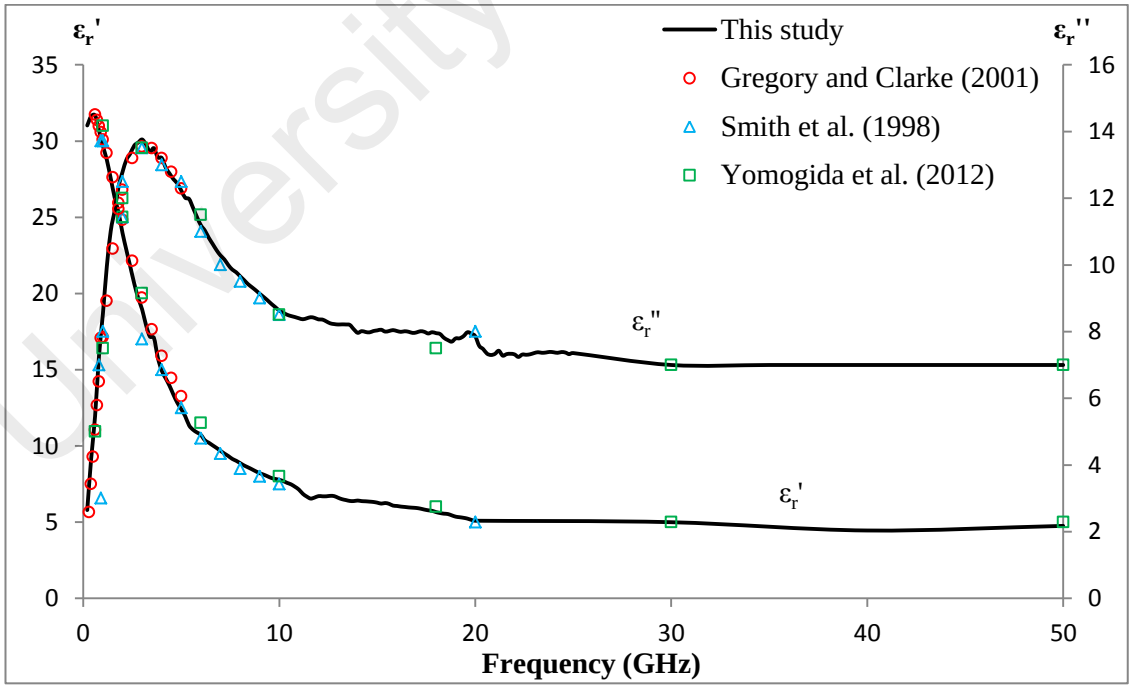


Figure 3.4: The comparison between measured dielectric properties of methanol with reference data at 25°C.

3.4.3 Dielectric Properties of Glycosuria

3.4.3.1 Overview

The urinary dielectric properties were obtained from DM subjects with glycosuria levels: 0 g/L, >1 g/L and >5 g/L at the respective temperatures of 25°C, 30°C and 37°C. A closer look at the measured urinary dielectric properties of the subject groups is presented in Figures 3.5 and 3.6 for wideband analysis of 0.2 GHz to 50 GHz at 25°C. Meanwhile, the same trend was observed for 30°C and 37°C.

At low frequency ranges, less observable changes of urinary dielectric properties are reported with glycosuria level. Subjects with glycosuria level >5 g/L showed the increment of dielectric constant at frequency ranges between 0.2 GHz and 3 GHz, as well as between 25 GHz and 40 GHz (Figure 3.5). The changes in urinary dielectric properties with glycosuria level were prominent at higher frequencies. Dielectric constant was increased, while loss factor was decreased with glycosuria level at frequencies above 40 GHz and 15 GHz, respectively. Relaxation frequency was shifted towards lower values with glycosuria level (Figure 3.6).

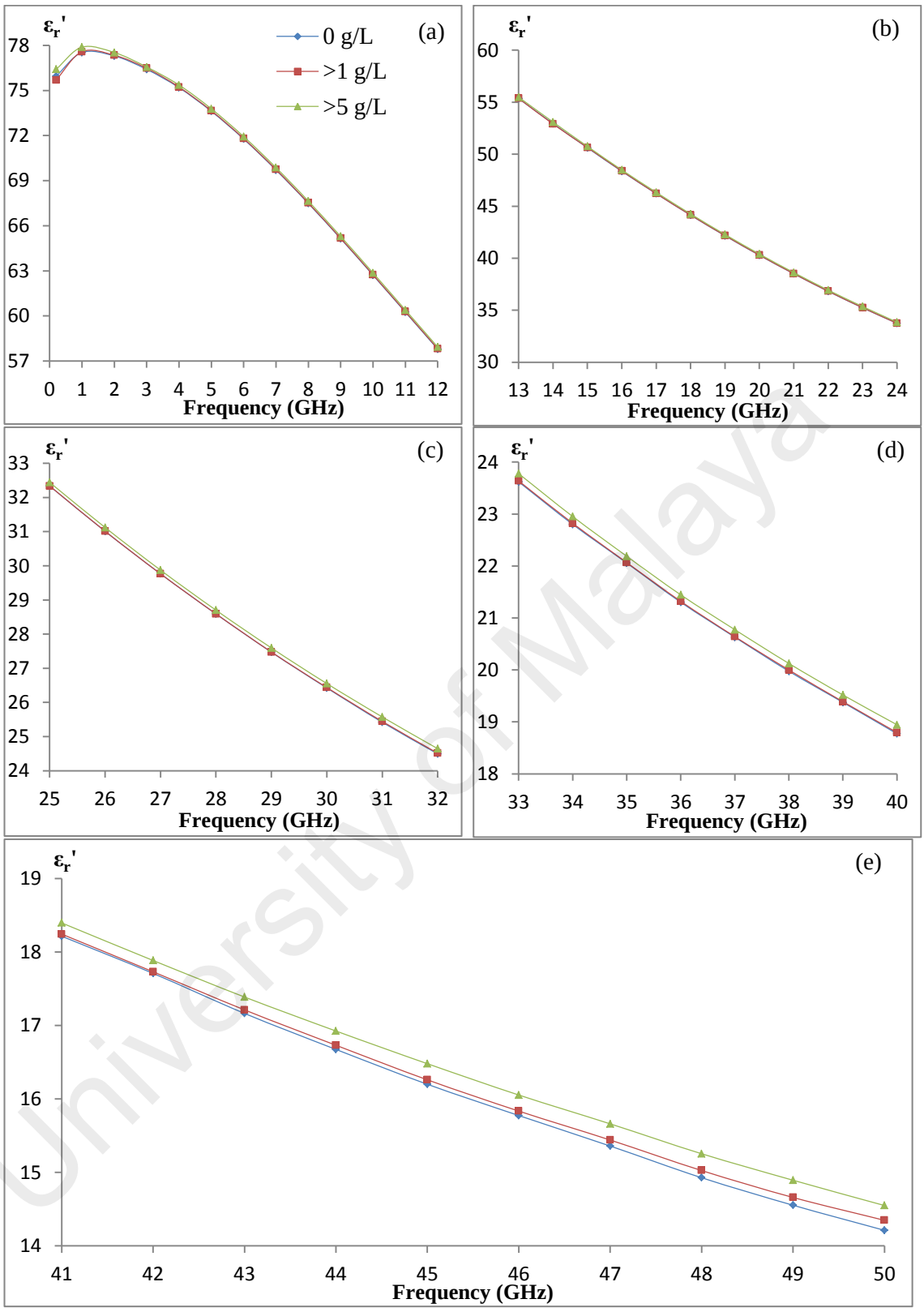


Figure 3.5: Urinary dielectric constant among subject groups at 25°C.

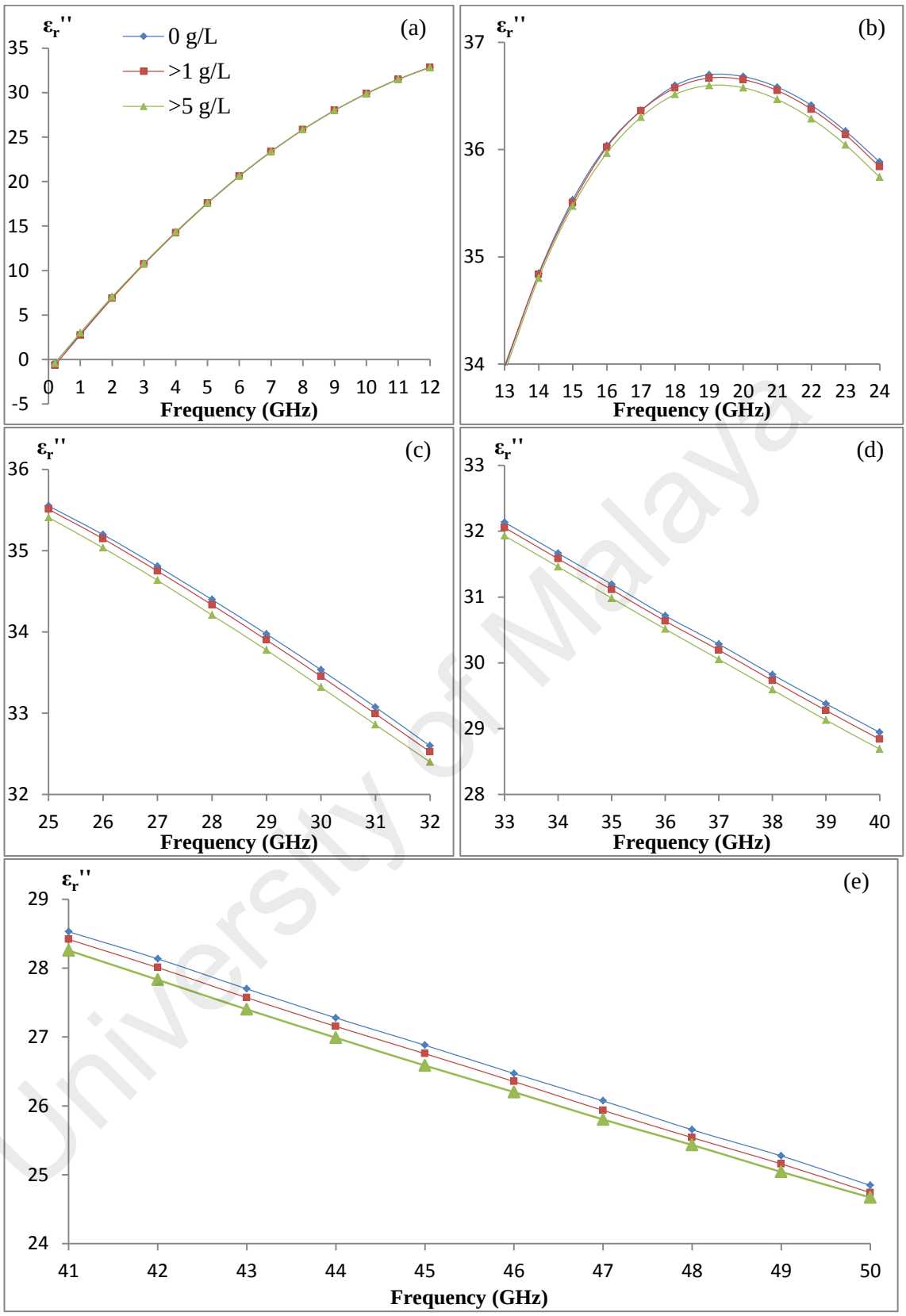


Figure 3.6: Urinary loss factor among subject groups at 25°C.

3.4.3.2 Statistical analysis

Overall, the temperature of 25°C produced the strongest statistically significant differences ($p < 0.05$) across subject groups compared to 30°C and 37°C. The highest observed F number, $F(2, 41) = 8.681$; $p < 0.01$ was obtained at the frequency of 35.2 GHz between measured frequency range of 0.2 to 50 GHz. The statistically significant difference was defined at critical F value, $F_{crit} \geq 3.226$. Table 3.5 shows the observed F number and p-value of urinary dielectric properties across subject groups at different microwave frequencies. There were stronger significant differences in the dielectric constant across subject groups, compared to loss factor.

Table 3.5: F number and p-value of urinary dielectric properties across DM subject groups at different microwave frequencies

Frequency (GHz)	25°C				30°C				37°C			
	ε_r'		ε_r''		ε_r'		ε_r''		ε_r'		ε_r''	
	F number	p-value	F number	p-value	F number	p-value	F number	p-value	F number	p-value	F number	p-value
0.2	1.045	0.359	0.597	0.554	1.267	0.290	0.536	0.588	1.635	0.205	0.633	0.535
0.4	1.308	0.279	0.788	0.460	1.153	0.324	1.754	0.183	1.664	0.199	0.824	0.444
0.6	1.577	0.216	0.954	0.392	1.104	0.339	2.274	0.113	1.796	0.176	1.170	0.318
0.8	1.708	0.191	0.789	0.460	0.836	0.439	1.593	0.213	1.969	0.150	1.717	0.189
1	2.051	0.139	0.877	0.422	0.793	0.458	1.977	0.149	2.038	0.140	1.576	0.216
3	0.201	0.818	0.654	0.524	1.429	0.249	3.012	0.058	0.749	0.478	2.056	0.138
5	3.898	0.026	0.422	0.658	0.539	0.586	1.145	0.326	1.555	0.221	1.397	0.256
10	4.886	0.011	0.056	0.945	2.388	0.102	0.468	0.629	1.045	0.359	1.303	0.280
30	7.648	<0.01	2.453	0.096	4.024	0.024	1.530	0.226	2.786	0.071	0.844	0.436
50	2.964	0.060	0.669	0.517	0.038	0.968	4.483	0.016	1.082	0.346	1.696	0.193

Table 3.6 shows the group-pairs that have significant differences in urinary dielectric properties. Subjects with glycosuria level 0 g/L and >5 g/L showed the best pair group significant differences of dielectric constant at 25°C, followed by subjects with glycosuria level >1 g/L and >5 g/L. Meanwhile, subjects with glycosuria level 0 g/L and >1 g/L showed no significant pair differences in the measured microwave frequency range. The Pearson correlation test indicated that dielectric constant showed significant positive correlation ($r = 0.559$; $p < 0.01$) with glycosuria level. Loss factor had significant negative correlation ($r = -0.491$; $p = 0.015$) at >15 GHz.

Table 3.6: Significant differences in urinary dielectric properties of group-pairs

Frequency (GHz)	**25°C**		**30°C**		**37°C**	
	ε_r'	**ε_r''**	**ε_r'**	**ε_r''**	**ε_r'**	**ε_r''**
0.2	-	-	-	-	-	-
0.4	-	-	-	-	-	-
0.6	-	-	-	-	-	-
0.8	-	-	-	-	-	-
1	-	-	-	-	-	-
3	-	-	-	-	-	-
5	1-3	-	-	-	-	-
10	1-3, 2-3	-	-	-	-	-
30	1-3, 2-3	-	1-3	-	-	-
50	-	-	-	1-3	-	-

3.4.3.3 Comparison with Debye Model

Figure 3.7 shows the Cole-Cole diagram of experimental urinary dielectric properties data fit to the Debye model for glycosuria subjects at 25°C. Overall, the experimental data was fitted to the single-pole Debye model with deviations of about 0.5% ~ 2%.

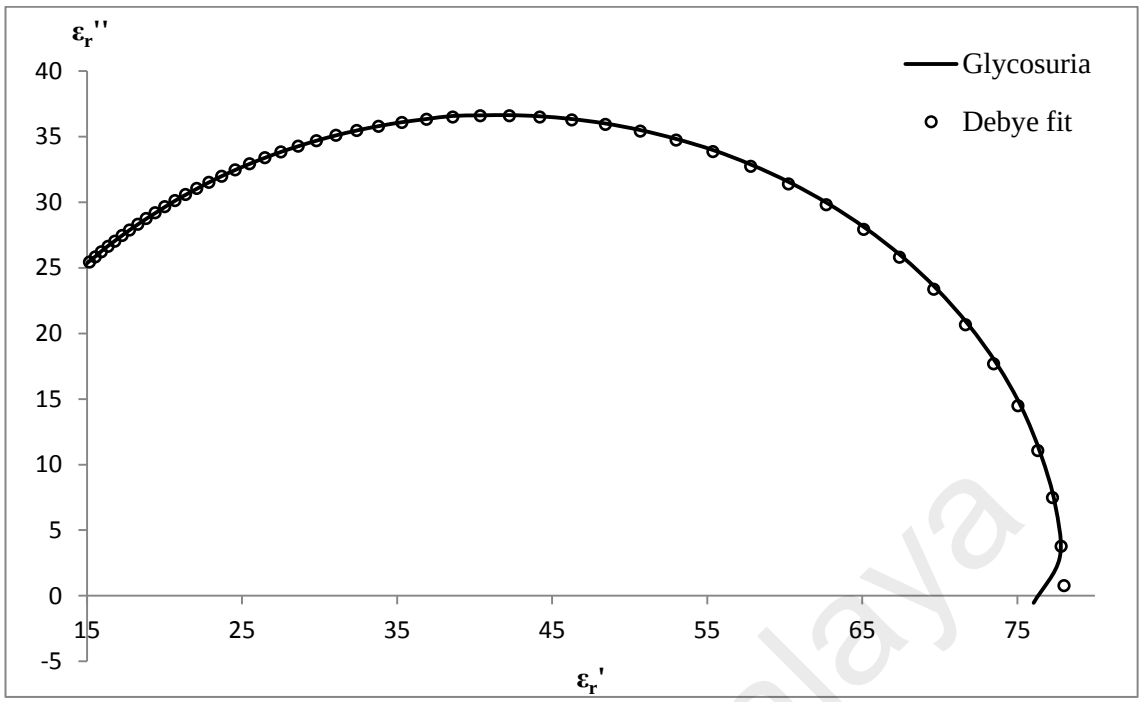


Figure 3.7: Cole-Cole diagram of experimental urinary dielectric properties data fit to the Debye model for glycosuria subjects at 25°C

Table 3.7 shows the Debye dielectric parameters of different glycosuria levels at 25°C, 30°C and 37°C, which were calculated using Equation 2.14. $\Delta\varepsilon$ and τ increased, and decreased with glycosuria level and temperature, respectively.

Table 3.7: Debye dielectric parameters of different glycosuria levels at 25°C, 30°C and 37°C

Glycosuria Level (g/L)	25°C					30°C					37°C				
	ε_∞	$\Delta\varepsilon$	τ (ps)	σ_s (S/m)	RMSPE (%)	ε_∞	$\Delta\varepsilon$	τ (ps)	σ_s (S/m)	RMSPE (%)	ε_∞	$\Delta\varepsilon$	τ (ps)	σ_s (S/m)	RMSPE (%)
0	4.60	73.13	8.16	0.03	0.25	4.92	71.56	7.23	0.01	0.11	4.68	69.35	6.14	0.01	0.12
>1	4.77	74.30	8.69	0.02	0.13	4.85	71.73	7.55	0.01	0.11	4.57	69.46	6.22	0.02	0.18
>5	4.87	76.50	9.25	0.09	0.11	4.84	72.21	8.09	0.06	0.17	4.74	69.77	6.65	0	0.22

3.4.3.4 Classifications accuracy between glycosuria and non-glycosuria groups

In order to determine the accuracies of urinary dielectric properties to distinguish between glycosuria and non-glycosuria groups, classifications were conducted based on the threshold from the mean values of subject groups. Table 3.8 shows the classification accuracies of urinary dielectric properties between glycosuria and non-glycosuria groups at different temperatures. Overall, the deviations were about 7~17% and 3~15% across different urinary dielectric properties and temperatures, respectively.

Table 3.8: Classification accuracies between non-glycosuria and glycosuria groups at 25°C, 30 °C and 37°C

Dielectric Properties	Classification Accuracy (%)		
	25°C	30°C	37°C
ε_r'	72.0	77.08	66.67
ε_r''	54.9	69.39	66.67

3.5 Discussion

3.5.1 Reproducibility and Accuracy

To report the urinary dielectric properties of glycosuria, additional reproducible and accurate procedures were conducted at 30°C and 37°C in order to determine strong temperature dependency of dielectric properties in high water content urine. Arai et al. (1995) and Chen et al. (2004) reported that dielectric properties measured at temperatures higher than room temperature produced lower accuracies due to technical problems in thermal expansion and temperature gradient of the coaxial probe. To overcome this issue, the coaxial slim probe was heated to 30°C and 37°C in a water bath

before measurements were taken at the respective temperatures. This increased the reproducibility of measurements >90%.

In comparison between the measured data with reference data, the data of Johri and Roberts (1990) shows the highest deviation with the measured data for water. They used microwave resonant cavity perturbation technique to determine the dielectric response of water. However, the technique is more suitable for measuring solids and low loss materials as suggested by Venkatesh and Raghavan (2005), Komarov et al. (2005) and Icier and Baysal (2004). Hence, measurement accuracy may be considered to differ between two different techniques, cavity perturbation and open-ended coaxial probe technique. Meanwhile, less comparison data was observed at high frequencies for dielectric properties of methanol. The measurements of methanol are mostly available at frequencies below 10 GHz, as the dielectric properties of methanol at milimeter waves are considered too low to obtain reliable results from the technique in this study (Kaatze, 2007). The appropriate method for measuring dielectric properties of biological solutions with approximately 0.1M salt content has been validated by using open-ended coaxial probe technique with accuracy > 95% (Table 3.4).

3.5.2 Effect of Temperature

In fact, the urine temperature may vary between the body and room temperature in the process of measurement. Temperature variation is an important factor for dielectric properties measurement. The spectra of dielectric properties of urine were observed to be close to that of water since > 90% of urine is water content. Urinary dielectric properties of subject groups were observed to change across temperatures from 25°C to 37°C. This could be due to the increase in temperature, which affects the stretching of intramolecular hydrogen bonds and Brownian movement in solution that accounts for the changes of dielectric properties (Robert et al., 1993; Komarov et al., 2005; Ellison,

2007). However, randomising the agitation of molecules at high temperature reduced the significant effect of glucose. This result in the strongest statistically significant differences ($p < 0.05$) across subject groups that were found at 25°C compared to 30°C and 37°C. This study validated the reliability of dielectric measurement of glycosuria at room temperature.

3.5.3 Effect of Glucose

Zhadobov et al. (2012) reported that glucose solutions below concentration 1% had similar dielectric properties with distilled water. In this study, statistical analysis showed that urinary dielectric properties had significant differences ($p < 0.05$) across subject groups with different glycosuria levels at high frequencies. Correlation tests validated the relationship of urinary dielectric properties with different glycosuria levels.

According to Figure 3.5, dielectric constant showed an increasing trend at frequency of 0.2 to 1 GHz, a finding which is in agreement with the study by Smulders et al. (2013). The presence of salt content in urine (physiological solution) causes a decrease of the dielectric constant compared to pure water at low frequencies (Smulders et al., 2013). Different trends in glycosuria dielectric properties were observed compared to glucose solution (Liao et al., 2001; Smulders et al., 2013). Lonappan et al. (2004) and Lonappan et al. (2007a) reported that dielectric constant increased with glycosuria level more than 1.5% at frequency of 2.4 to 3 GHz. The results are in agreement with Lonappan et al. (2004) and Lonappan et al. (2007a). The dielectric constant was found increased at physiological glycosuria level more than 5 g/L (> 0.5%) at frequency range of 0.2 to 3 GHz, and 20 to 40 GHz, respectively. Significant correlation between urinary dielectric properties and glycosuria levels were prominent at high frequencies (as shown in Figure 3.5 and Figure 3.6). Maritim et al. (2003) found the presence of oxidative

stress in biological solution due to free radicals formed by glucose oxidation under normal physiological condition that increases free charges. Studies reported higher free radicals (8-iso prostaglandin $F_{2\alpha}$) in the urine of diabetic patients with glucose variability compared to normal subjects (Monnier et al., 2006; Wentholt et al., 2008). Increments in dielectric constant and relaxation time are due to the free radicals of glucose bio-oxidation process that increases the density of dipoles (Abdalla et al., 2010).

3.5.4 Comparison with Debye model

There is limited data available to compare the dielectric properties of urine. Peyman and Gabriel (2012) compared the dielectric properties of porcine urine with Debye model for frequency between 50 MHz and 20 GHz. Thus, comparison between experimental data and Debye model is proposed. It is sufficient to model the experimental dielectric data with single-pole Debye model using Equation 2.14, as a main dispersion was observed at the measured frequency between 0.2 GHz and 50 GHz. Overall, the experimental data were well fitted to the Debye model. The model appears to be sufficient to estimate the dielectric evolution of urine. The deviations between measured data and Debye fit data were mostly observed at low frequencies with about 2% (Figure 3.7). This may be due to the instability of the high frequency dielectric system to measure low frequencies below 1 GHz. However, the variations were within the acceptable range of standard error of ± 5%.

According to Table 3.7, ε_∞ was close to 5 across all the subject groups and temperatures as the major component of urine is water molecules. This indicates that the water content of urine was not affected in glycosuria. Peyman and Gabriel (2010) and (2012) reported that the variation of up to 25% for the value of ε_∞ has very little impact on the other fitted parameters. $\Delta\varepsilon$ and τ were found to increase with glycosuria level, but

decrease with temperature. The strength of changes across subject groups was observed to decrease with temperature from 25°C to 37°C. Dielectric properties of glycosuria were more prominent at room temperature (25°C) rather than at body temperature (37°C). Static conductivity, σ_s was negligible across subject groups and temperatures. Conductivity of urine was relatively small in a constant field as human physiological solution is quoted to have only about 0.9% of salt content (Nörtemann et al., 1997; Smulders et al., 2013). The temperature and glycosuria level affect the overall urinary dielectric dispersion and relaxation.

3.5.5 Classification across Glycosuria and Non-glycosuria Groups

According to Table 3.8, temperature effects were observed in the classification accuracies of overall urinary dielectric properties. Among the three measured temperatures, 30°C showed the highest classification accuracies. Meanwhile, dielectric constant was found to produce higher classification accuracies than loss factor at 25°C and 30°C. However, bias may occur in this classification method due to its dependence on the threshold (mean) differences across subject groups. This remains a challenge in carrying out cross-comparison with other methods.

3.6 Summary

In this chapter, the urinary dielectric properties of subjects with Type 2 DM at microwave frequency between 0.2 GHz and 50 GHz were investigated. Glycosuria variability among subjects groups was described. The effect of temperature was determined in the changes of urinary dielectric properties with respective glycosuria level. Statistical correlation between urinary dielectric properties and glycosuria level was identified. Additional classifications were conducted to distinguish between glycosuria and non-glycosuria groups.

Room temperature (25°C) produced the strongest statistically significant difference in urinary dielectric properties for the change of glycosuria level. The results obtained showed that dielectric constant increased with glycosuria level more than 5 g/L (0.5%) at low frequencies. Dielectric constant correlated positively with glycosuria level at frequencies above 40 GHz. Loss factor correlated negatively with glycosuria level at frequencies above 15 GHz. The experimental data were fitted to the single-pole Debye model. This found that the experimental data were closely matched with the Debye model. The urinary dielectric dispersion and relaxation time increased with glycosuria level, while decreased with temperature. In classifications, dielectric constant produced higher accuracies than loss factor. Different temperatures were observed affecting the overall classification accuracies. However, this remains a challenge in cross-comparison with other classification methods.

CHAPTER 4: THE MEASUREMENT OF URINARY DIELECTRIC PROPERTIES OF PROTEINURIA FOR CHRONIC KIDNEY DISEASE

4.1 Introduction

Urinary protein is defined as an early sign of CKD. Persistent proteinuria followed by progressive decline of renal function (increments in serum creatinine level) are presentations of CKD and this eventually leads to end stage renal disease (Epstein et al., 1998). The presence of proteinuria has been identified in patients with increased risk of CKD progression (Iseki et al., 2004; Ishani et al., 2006). The changes in dielectric properties of solutions with respect to animal protein concentration indicate the potential application of dielectric properties measurement in proteinuria. So far, no studies have been conducted to look into the urinary dielectric properties of proteinuria. No data has been reported for changes in the urinary dielectric properties of proteinuria with temperature.

In this chapter, the urinary dielectric properties of normal subjects and subjects with CKD at room temperature (25°C), 30°C and body temperature (37°C), respectively, at microwave frequency between 0.2 GHz and 50 GHz are investigated. This chapter determines the significant differences in urinary dielectric properties among subjects with different proteinuria levels. The correlations between urinary dielectric properties and proteinuria level are investigated. The measured data are fitted to Debye model for comparison.

4.2 Background Study

4.2.1 Overview of Kidney Disease

The kidneys are organs of blood purification. They remove metabolic waste such as excessive water, salt and toxins in the form of urine from the human body and perform homeostatic functions. Patients with either DM, hypertension, urinary tract

abnormalities, systemic autoimmune disorder, excessive use of known toxins and systems suggestive of systemic illness develop higher risk of getting CKD (American Diabetes Association, 1998).

Kidney disease is classified into acute and CKD. Acute kidney disease such as polycystic kidney disease occurs suddenly due to many cysts or cavities being formed in the kidneys while pyelonephritis is caused by kidney infection. Meanwhile, CKD occurs when there is a long period of kidney deficiency. Patients seldom have symptoms at the early stage of CKD.

Overall, CKD is classified in terms of diabetic kidney disease (DKD) and non-diabetic kidney disease (non-DKD). Type 2 DM is the primary cause of kidney failure among the population (Feisul & Azmi, 2012; American Diabetes Association, 2014). High level of blood glucose causes kidneys to produce extra force to maintain the necessary filtering processes. The extra forces required may cause the capillaries in the kidneys to leak, allowing loss of glucose and protein in the urine. Eventually, the kidneys lose their ability to function and waste products will accumulate in the blood (American Diabetes Association, 1998; Kanwar et al., 2008).

4.2.1.1 Chronic kidney disease diagnosis and monitoring

Current clinical determination uses estimated glomerular filtration rate (eGFR) to diagnose the overall kidney function and stages of CKD. However, eGFR that uses concentration of serum creatinine may be affected by factors such as gender, age, ethnicity, medication, muscle mass and protein intake (Lascano & Poggio, 2010). Furthermore, CKD seldom has clinical symptoms in the early stage.

Urinary protein is defined as an early sign of CKD. Urinary protein excretion is important to identify, characterise and monitor the progression of CKD. Zürbig et al.

(2009) reported initial pathophysiological changes in kidneys, resulting in significant changes of urinary proteins, which are potential biomarkers at the early stage of CKD. Protein increases in urine with respect to the stages of CKD (Ghiggeri et al., 1985). Monitoring of urinary protein is required as standard care in diagnosis and prognostication of patients with risk factors of CKD.

4.3 Materials and Methods

4.3.1 Subject Recruitment

A total of 43 subjects with CKD (disease duration of >3 years) were recruited from the outpatient clinics at the University of Malaya Medical Centre (UMMC). Exclusion criteria involved DKD subjects and bladder-related diseases such as presence of glycosuria or haematuria. A total of 40 subjects were recruited as normal, which involved healthy subjects who were recruited at the University of Malaya, Malaysia. Normal subjects without history of kidney or bladder-related diseases were recruited. Medical ethics approval was obtained from the Institutional Ethics Review Committee, UMMC**.** All subjects provided written informed consent to participate in this study.

4.3.2 Urine Collection and Storage

Sixty ml of random spot mid-stream urine samples were collected from each subject. For each collected urine sample, 20 ml was used to measure urinary clinical chemical variables and microscopy using routine methods at the Division of Laboratory Medicine, UMMC.

Urine samples were collected in sterile urine containers. Fresh urine samples were stored at a temperature of 4°C within 4 hours before the measurement of dielectric properties was conducted. No preservatives were added upon urine collection.

4.3.3 Urine Measurements

The measurement setup is as described in the previous chapter (Chapter 3). Before measurements were conducted, urine samples were heated to room temperature (25°C) using a WNB 7 water bath (Memmert, Duesseldorf, Germany) with a precision of ± 0.1°C and the samples were gently stirred. Random and systemic errors were taken into account to reduce uncertainties as reported by Gabriel and Peyman (2006). Experiments were repeated by heating the urine samples to 30°C and 37°C, respectively. For each experiment, three measurement readings were recorded for each urine sample throughout the frequency range of 0.2 to 50 GHz.

4.3.4 Data Analysis

Dielectric properties, in terms of dielectric constant (ε_r') and loss factor (ε_r''), were obtained from the measurements. A total of 250 frequency points were measured with an interval of 200 MHz at the microwave frequency range of 0.2 to 50 GHz. The Independent Samples T test of SPSS Statistic 21.0 (IBM Corp, 2012) was used to determine the statistically significant differences between urinary dielectric properties and urine composition of normal and CKD subjects. The statistical one-way analysis of variance (ANOVA) test was conducted to determine the respective effects of proteinuria and temperature in the urinary dielectric properties at different microwave frequencies. Pearson correlation test was conducted to determine correlation between proteinuria levels and urinary dielectric properties. The level selected for statistical significance (p-value) was set at probability value <0.05.

4.3.5 Curve Fitting

Single-pole Debye model as shown below in Equation 2.14, was applied to fit the experimental data over the frequency range of between 0.2 GHz and 50 GHz using MATLAB (Mathworks, 2011) fitting function:

$$\varepsilon(\omega) = \varepsilon_{\infty} + \frac{\Delta\varepsilon}{1 + j\omega\tau} - j\frac{\sigma_s}{\omega\varepsilon_0}$$

where ε(ω) is the complex relative permittivity (dielectric properties) and ω is the angular frequency. Infinite frequency permittivity (ε_∞), magnitude of dispersion (Δε), relaxation time (τ) and static conductivity (σ_s) are the parameters of the variables to fit the experimental data. Limits such as $\varepsilon_\infty \geq 1$, $\Delta\varepsilon \geq 0$, $\sigma_s \geq 0$ and $\tau \geq 0$ were set on the fitting parameters so that they would remain within physical ranges.

The fitting analysis was conducted using a genetic algorithm (GA) to compute the function score of the complex curve-fitting programme with iterations. GA performs direct search optimised parameters with best fitness from a population. The level of population size was selected at 1500 with cross-over fraction, 0.5 was set. The programme calculates the root mean square percentage error (RMSPE) between the differences of experimental value and the value obtained from the model for fitting. The data were fitted independently for each subject group at respective temperatures.

4.4 Results

4.4.1 Urine Composition

As defined, proteinuria was found in CKD subjects. Overall, lower levels of mean creatinine, urea and salt ions were found in the urine of CKD subjects compared to normal subjects (Table 4.1). This could be explained by the fact that CKD patients suffer from kidney malfunction, which affects the body's efficiency in removing waste and also causes leakage of protein into the urine. CKD subjects were grouped based on their urinalysis proteinuria levels: >0.25 g/L, >0.75 g/L, >1.5 g/L and >5 g/L, respectively. The characteristics of the subject groups are presented in Table 4.2.

Table 4.1: Chemical variables of normal and CKD subjects

Chemical Variables	Normal	CKD
Creatinine (μmol/L)	8846 ± 2677	6032 ± 1951
Urea (mmol/L)	183 ± 25	118 ± 46
Cl^- (mmol/L)	103 ± 66	85 ± 33
Na^+(mmol/L)	90 ± 59	83 ± 31
K^+ (mmol/L)	32 ± 26	25 ± 13

Table 4.2: Characteristics of the CKD subjects

Characteristics	Proteinuria level (g/L)			
	>0.25	>0.75	>1.5	>5
Total (N)	16	10	10	7
Age (years)	70 ± 11	67 ± 6	67 ± 10	54 ± 19
Serum creatinine (μmol/L)	184 ± 72	209 ± 74	219 ± 83	248 ± 68

4.4.2 Dielectric Properties of Proteinuria

4.4.2.1 Overview

The measurements of urinary dielectric properties were obtained from normal and CKD subjects, and are presented in Figures 4.1 and 4.2. Urinary dielectric properties showed different trends with temperature over the frequencies for normal and CKD subjects. A 'cross-over' frequency point was observed at about 7 GHz for the dielectric constant and 27 GHz for the loss factor. The urinary dielectric properties were constant over the changes of temperature and subject groups at the respective 'cross-over' frequency point. Below this frequency point, the urinary dielectric properties decreased with temperature. Urinary dielectric properties of CKD subjects were found to be higher

than those of normal subjects. Meanwhile, urinary dielectric properties increased with temperature above the 'cross-over' point. The CKD subjects had lower urinary dielectric properties than those of the normal subjects above the frequency point. Generally, the urinary dielectric properties showed more differences between the normal and CKD subjects as the temperature increased from 25°C to 37°C.

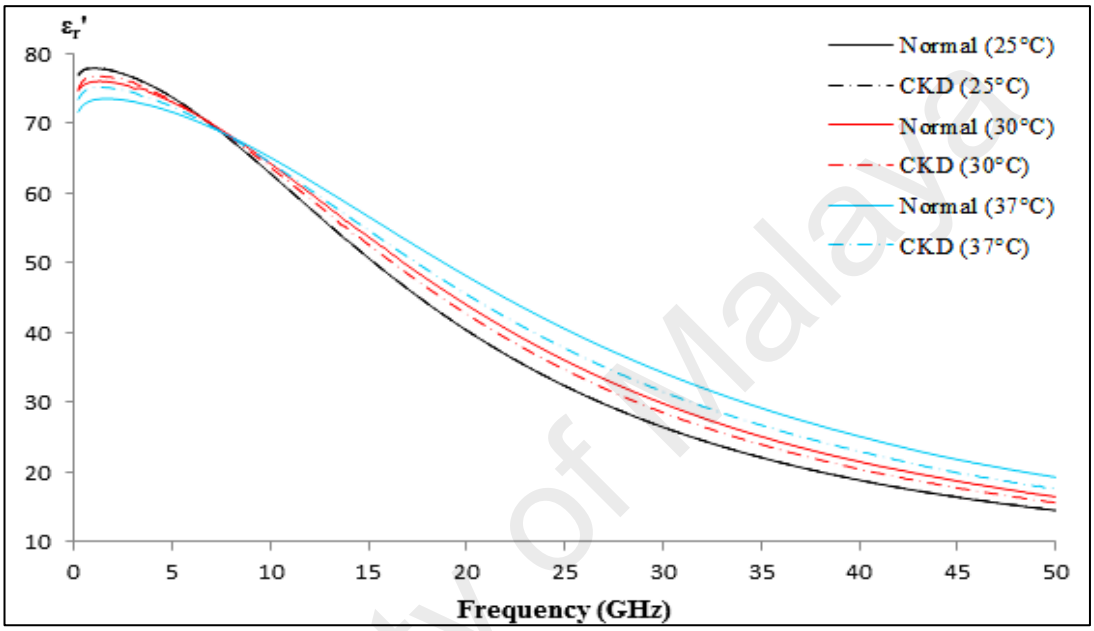


Figure 4.1: Urinary dielectric constant of normal and CKD subjects at respective temperatures of 25°C, 30°C and 37°C for frequencies ranging from 0.2 to 50 GHz.

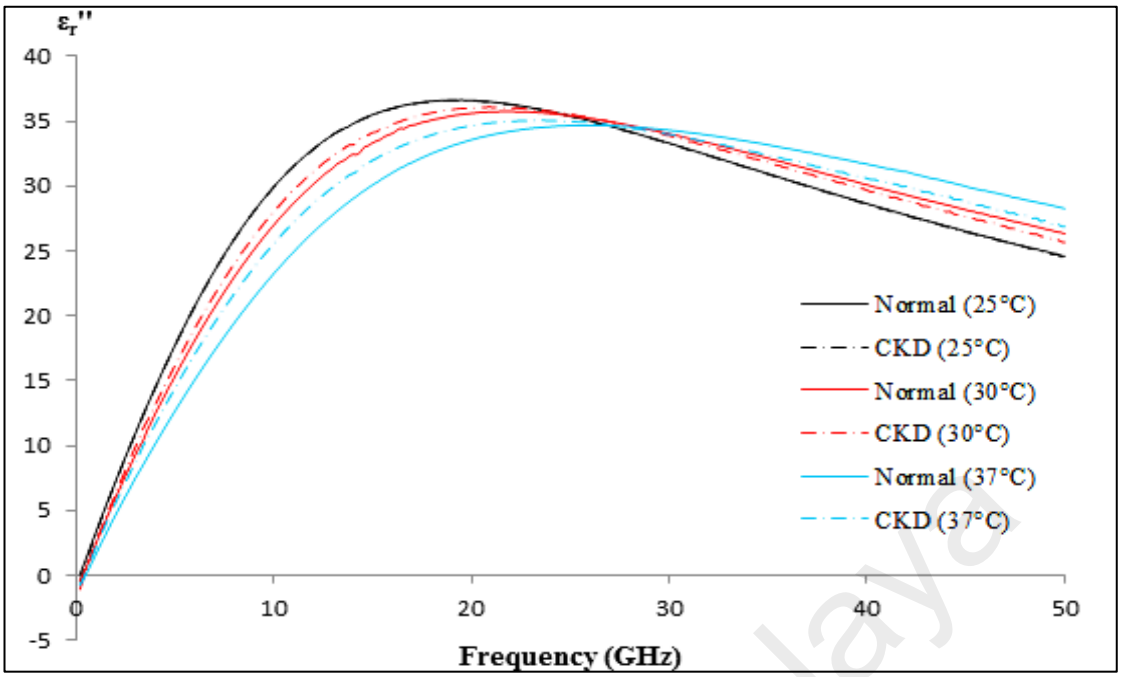


Figure 4.2: Urinary loss factor of normal and CKD subjects at respective temperatures of 25°C, 30°C and 37°C for frequencies ranging from 0.2 to 50 GHz.

4.4.2.2 Statistical analysis

Table 4.3 shows the T number and p-value of urinary dielectric properties for CKD subjects in comparison with normal at three different temperatures and different microwave frequencies. Stronger statistically significant differences between normal and CKD subjects were reported in dielectric constant compared to loss factor. Statistically significant differences in urinary dielectric properties ($p<0.05$) were found at most of the frequencies for 30°C and 37°C. The T-test results showed no statistically significant differences ($p>0.05$) in mean urinary creatinine, urea and salt ions (Cl^-, Na^+ and K^+) between normal and CKD subjects. The presence of urinary protein in CKD subjects caused the urinary dielectric properties to change significantly.

Table 4.3: T number and p-value of urinary dielectric properties for CKD subjects in comparison with normal at three different temperatures and different microwave frequencies

Frequency (GHz)	25°C				30°C				37°C			
	ε_r'		ε_r''		ε_r'		ε_r''		ε_r'		ε_r''	
	T number	p-value	T number	p-value	T number	p-value	T number	p-value	T number	p-value	T number	p-value
0.2	-1.465	0.147	-1.592	0.115	1.201	0.233	-1.428	0.157	3.880	<0.01	-0.663	0.509
0.4	-0.330	0.742	-2.564	0.012	2.688	<0.01	-1.468	0.146	4.497	<0.01	-0.013	0.989
0.6	0.562	0.576	-2.182	0.032	3.861	<0.01	-0.596	0.553	4.939	<0.01	0.972	0.334
0.8	1.237	0.220	-1.192	0.059	4.125	<0.01	-0.068	0.946	4.911	<0.01	2.078	0.041
1	1.592	0.115	-1.428	0.157	4.381	<0.01	0.847	0.400	4.903	<0.01	2.954	<0.01
3	-0.848	0.399	0.127	0.899	4.108	<0.01	3.760	<0.01	4.187	<0.01	4.552	<0.01
5	0.749	0.456	-0.613	0.542	2.626	0.01	4.476	<0.01	4.443	<0.01	4.568	<0.01
10	1.276	0.206	-0.174	0.862	-4.868	<0.01	4.474	<0.01	-4.185	<0.01	4.416	<0.01
30	-0.558	0.578	-0.880	0.381	-4.728	<0.01	-2.065	0.042	-4.282	<0.01	-4.653	<0.01
50	-0.582	0.562	-1.546	0.126	-4.575	<0.01	-3.724	<0.01	-4.213	<0.01	-4.191	<0.01

Table 4.4 indicates the F number and p-value of urinary dielectric properties across proteinuria levels at different microwave frequencies. Overall, the temperature of 37°C produced the strongest statistically significant differences ($p<0.05$) across proteinuria subject groups compared to 25°C and 30°C. The highest observed F number, $F(3, 39) = 9.598$; $p < 0.01$ was obtained at 30 GHz of measured frequency range from 0.2 to 50 GHz. The statistically significant differences were defined at critical F value, $F_{crit} \geq 2.845$. Dielectric constant had stronger significant differences than loss factor across proteinuria subject groups.

Table 4.4: F number and p-value of urinary dielectric properties across proteinuria levels at different microwave frequencies

Frequency (GHz)	25°C				30°C				37°C			
	ε_r'		ε_r''		ε_r'		ε_r''		ε_r'		ε_r''	
	F number	p-value	F number	p-value	F number	p-value	F number	p-value	F number	p-value	F number	p-value
0.2	0.288	0.834	2.533	0.115	1.107	0.107	1.135	0.346	5.997	<0.01	1.108	0.359
0.4	1.041	0.385	6.574	0.012	4.267	<0.01	1.575	0.189	8.242	<0.01	1.672	0.165
0.6	1.077	0.370	4.760	0.032	6.600	<0.01	1.527	0.202	9.293	<0.01	2.450	0.053
0.8	1.084	0.367	3.657	0.059	7.065	<0.01	1.815	0.134	8.994	<0.01	3.490	0.11
1	0.314	0.815	2.039	0.157	7.730	<0.01	1.997	0.103	8.819	<0.01	4.893	<0.01
3	1.046	0.383	0.016	0.899	6.425	<0.01	7.993	<0.01	6.421	<0.01	8.844	<0.01
5	0.773	0.516	0.376	0.542	3.804	<0.01	8.521	<0.01	8.014	<0.01	8.349	<0.01
10	2.587	0.067	0.030	0.862	7.651	<0.01	7.806	<0.01	7.272	<0.01	7.728	<0.01
30	0.300	0.585	1.956	0.109	7.740	<0.01	3.779	<0.01	7.242	<0.01	9.598	<0.01
50	1.391	0.245	1.436	0.230	6.466	<0.01	6.013	<0.01	6.681	<0.01	7.566	<0.01

Figure 4.3 shows observable changes in urinary dielectric properties with proteinuria level at 37°C. Pearson correlation test showed positive correlation between proteinuria level and urinary dielectric properties [$r_{\text{dielectric constant}}(43) = 0.568$, $p < 0.01$; $r_{\text{loss factor}}(43) = 0.564$; $p < 0.01$] below the "cross-over" frequency point, while negative correlation [$r_{\text{dielectric constant}}(43) = -0.535$, $p < 0.01$; $r_{\text{loss factor}}(43) = -0.505$; $p < 0.01$] was found above the "cross-over" frequency point at 37°C. Less significant correlation was found at 25°C.

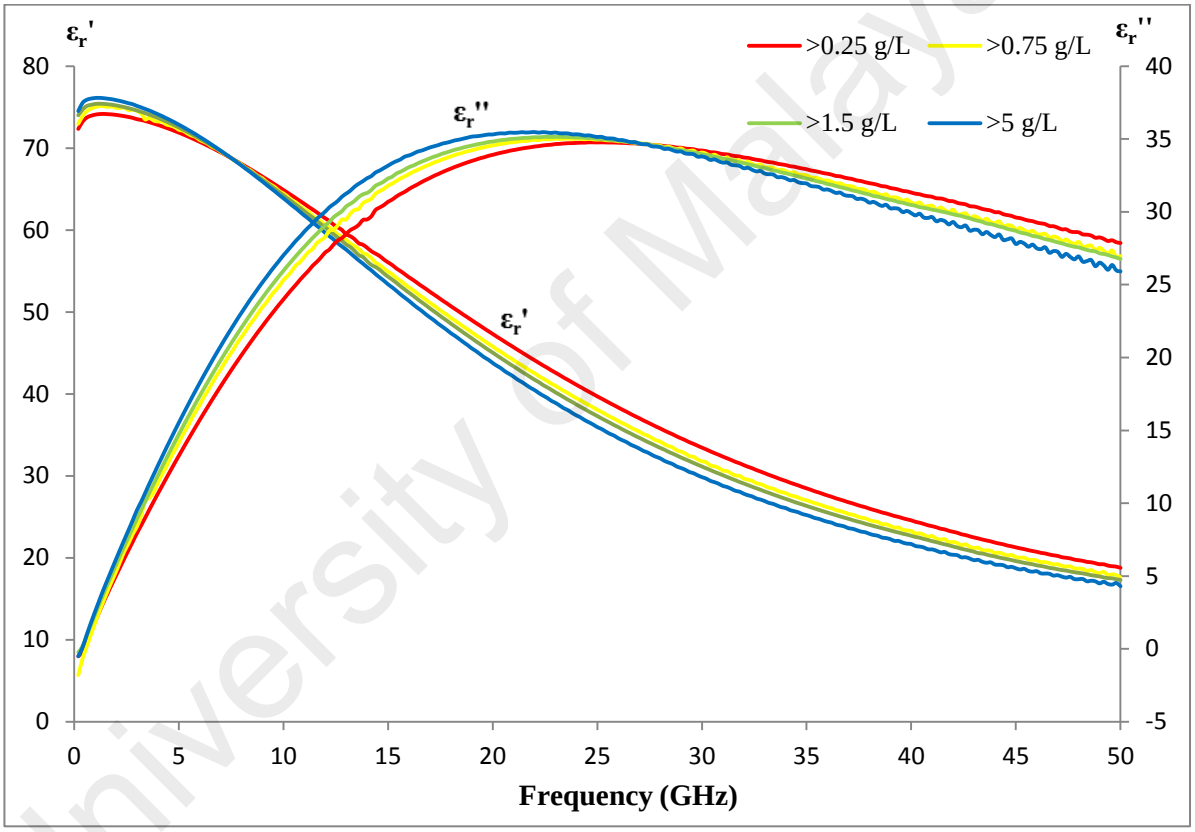


Figure 4.3: Urinary dielectric properties of different proteinuria levels at 37°C

4.4.2.3 Comparison with Debye model

Figure 4.4 shows the Cole-Cole diagram of experimental urinary dielectric properties data fit to the Debye model for normal and CKD subjects at 37°C. The experimental data was fitted to the single-pole Debye model with deviations of about 0.5%~3%.

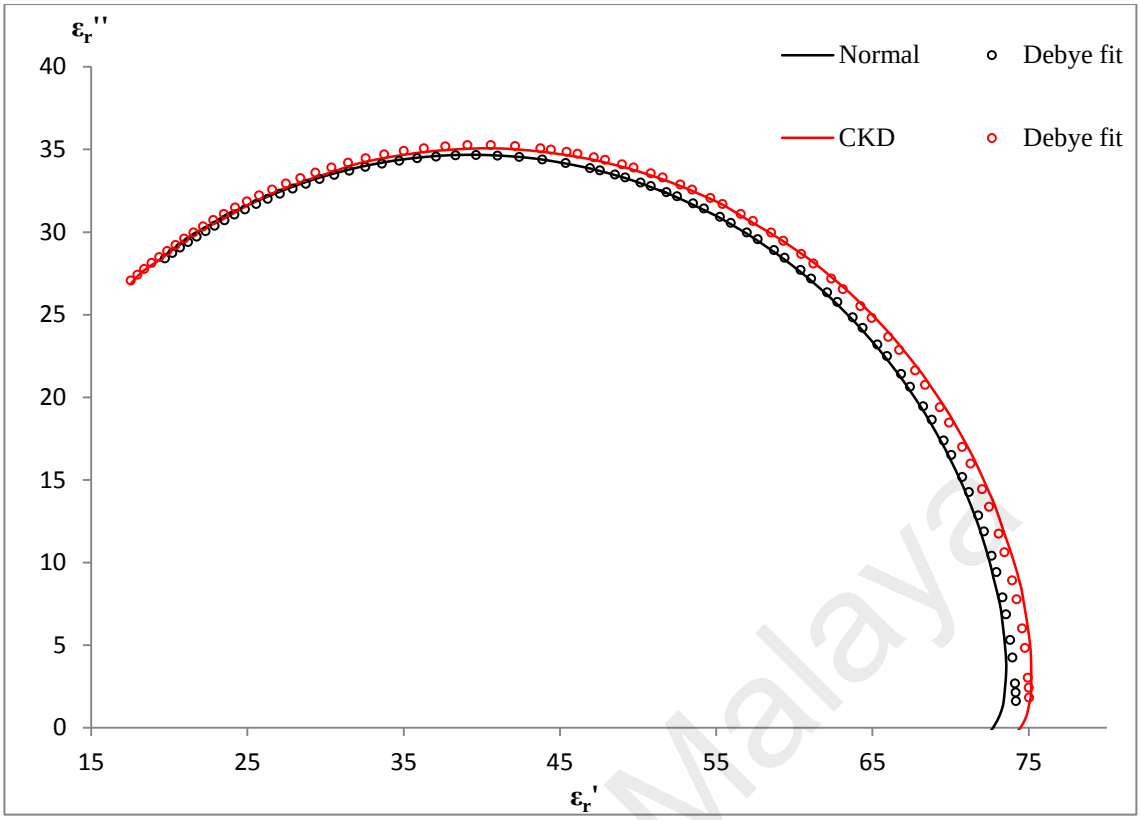


Figure 4.4: Cole-Cole diagram of experimental urinary dielectric properties data fit to the Debye model for normal and CKD subjects at 37°C

Table 4.5 shows Debye parameters of different proteinuria levels at 25°C, 30°C and 37°C, respectively. $\Delta\varepsilon$ and τ were found to increase with proteinuria level, while decreasing with temperature.

Table 4.5: Debye dielectric parameters of different proteinuria levels at 25°C, 30°C and 37°C

Proteinuria Level (g/L)	25°C					30°C					37°C				
	ε_∞	$\Delta\varepsilon$	τ (ps)	σ_s (S/m)	RMSPE (%)	ε_∞	$\Delta\varepsilon$	τ (ps)	σ_s (S/m)	RMSPE (%)	ε_∞	$\Delta\varepsilon$	τ (ps)	σ_s (S/m)	RMSPE (%)
0 (Normal)	4.99	72.75	8.20	0.02	0.22	4.98	71.31	7.15	0.02	0.19	4.94	69.30	6.11	0.06	0.28
>0.25	5.06	72.83	8.23	0.01	0.17	4.86	71.87	7.26	0.01	0.22	4.89	69.83	6.35	0.02	0.18
>0.75	5.03	73.17	8.26	0.01	0.48	4.96	72.26	7.43	0.02	0.47	4.92	70.56	6.73	0.06	0.45
>1.5	4.97	73.19	8.27	0.05	0.18	5.02	72.59	7.64	0.07	0.21	5.08	71.55	7.11	0.08	0.26
>5	5.02	73.22	8.30	0.11	0.20	4.93	73.01	7.96	0.12	0.42	5.02	72.91	7.63	0.14	0.39

1.1.1.4 Accuracy of classifications between normal and CKD subjects

In order to determine the accuracy of urinary dielectric properties to distinguish between normal and CKD subjects, additional classifications were conducted based on the threshold from the mean values of subject groups. Table 4.6 shows the classification accuracies of urinary dielectric properties between normal and CKD subjects at different temperatures.

Table 4.6: Classification accuracies between normal and CKD subjects at 25°C, 30 °C and 37°C

Dielectric Properties	Classification Accuracy (%)		
	25°C	30°C	37°C
ε_r'	59.72	68.67	79.52
ε_r''	72.29	69.88	51.81

4.5 Discussion

4.5.1 Effect of Temperature

Significant differences in urinary dielectric properties between normal and CKD subjects were observed to be affected by the measured temperatures. The dielectric properties of solutions change with temperature, due to the stretching of intramolecular hydrogen bonds between water molecules (Ellison, 2007). As the temperature increased, the relaxation time ($\tau = 1/2\pi fc$) of urine decreased. The increment in molecular movement and ion mobility decreased the viscosity of the solution when the temperature increased. Relaxation frequency is inversely proportional to the viscosity of the solution (Komarov et al., 2005).

The temperature of 37°C produced the strongest statistically significant differences ($p<0.05$) in urinary dielectric properties between normal and CKD subjects (Table 4.3) and across proteinuria subject groups (Table 4.4). The effect of urinary protein is more prominent at higher temperatures compared to 25°C and 30°C. This could be explained by the urinary protein results in linear conduction rather than the oscillation motion of the electrical field when the temperature changes (Pethig & Kell, 1987; Abdalla et al., 2010).

4.5.2 Effect of Protein

Statistical analyses showed that the presence of proteinuria in CKD subjects caused the urinary dielectric properties to change significantly ($p<0.05$), especially at 30°C and 37°C. Relaxation frequency was found to shift to lower frequencies when proteinuria levels increased (Figure 4.3). As the proteinuria level increases, it decreases the bulk water concentration that causes slower relaxation time scale to replace the faster relaxation time scale of bulk water (Nandi & Bagchi, 1998). Previous studies have reported the changes in dielectric properties of extracted protein solutions in radio (Ferry & Oncley, 1938; Oncley, 1938) and microwave frequencies (Zhadobov et al., 2012). Proteinuria was observed to have similar effects as protein solution in the changes of dielectric properties. Although protein may not be as massive as other components in complex biological solutions, however, additional substances significantly alter the electrical properties of the solution (Abdalla et al., 2010). Desouky (2009) discovered that glucose in blood (DM) drastically changed the dielectric properties of blood. Urine is a complex biological solution similar to blood. When water is protein-bound, it affects the linear conduction in urine, and this causes drift scatter motion along the electrical field side that changes the overall dielectric properties (Pethig & Kell, 1987; Abdalla et al., 2010).

4.5.3 Comparison with Debye Model

A main dielectric dispersion was observed at the measured frequency between 0.2 GHz and 50 GHz. Hence, it is sufficient to model experimental data with single-pole Debye model using Equation 2.14. Overall, the experimental data were well fitted to the Debye model for normal and CKD subjects. The deviations between experimental and theoretical data were mostly observed at low frequencies, at about 2%~3%. This may be due to the instability of the dielectric system to measure low frequencies at below 1 GHz. However, the variations were within the acceptable range of standard error of ± 5%.

According to Figure 4.4, observable different static permittivity, ε_s between normal and CKD subjects were obtained. ε_∞ was assumed to reach a constant for normal and CKD subjects, although it was not within our measured frequency range. The temperature and proteinuria level affect the overall urinary dielectric dispersion and relaxation (Table 4.5). ε_∞ was obtained as approximately constant ($\varepsilon_\infty \approx 5$) across all the proteinuria levels and temperatures. This indicates that the water content of urine remained unchanged for different proteinuria levels. Peyman and Gabriel (2010, 2012) mentioned that the variation of about 25% on the value of ε_∞ has very little effect on the other fitted parameters. The relaxation amplitude, $\Delta\varepsilon$ and relaxation time, τ increased with proteinuria level, but decreased with temperature. The changes were observed to be stronger at a higher temperature (37°C). The urinary dielectric properties of proteinuria were certainly more prominent at body temperature (37°C) rather than at room temperature (25°C). Static conductivity, σ_s was negligible ($\sigma_s \approx 0$) at low proteinuria levels. Conductivity of urine was relatively small in a constant field as biological solution is quoted to have only about 0.9% (0.1 M) of salt content (Nörtemann et al., 1997; Smulders et al., 2013). However, slight increase of σ_s was observed with temperature and proteinuria level reaching 1.5 g/L and 5 g/L, respectively. This remains

a challenge for conductivity determination from measured spectra at limiting low frequencies.

4.5.4 Classifications between Normal and CKD Subjects

According to Table 4.6, different temperatures were observed affecting the overall urinary dielectric properties classification accuracies. The classification accuracy of dielectric constant was observed to increase, while the loss factor decreased with temperature. The deviations were about 1~27% across different urinary dielectric properties. However, bias may occur in this classification method due to its dependence on the threshold (mean) differences across subject groups. Cross-comparison with other classification methods is required.

4.6 Summary

In this chapter, the urinary dielectric properties of normal subjects and those with CKD at microwave frequency between 0.2 GHz and 50 GHz were investigated. The urine composition variations of normal and CKD subjects groups were described. Proteinuria was found in CKD subjects. Overall, lower levels of urinary creatinine, urea and salt ions were reported in CKD subjects compared to normal subjects. The effect of temperature was determined in the changes of urinary dielectric properties with proteinuria level. Statistical correlation between urinary dielectric properties with proteinuria level was identified. Additional classifications were conducted to distinguish between normal and CKD subjects.

The strongest statistically significant differences across proteinuria levels were observed at body temperature (37°C). Urinary dielectric properties correlated positively and negatively with proteinuria level below and above its “cross-over” frequency points, respectively. The experimental data were fitted to the single-pole Debye model. This found that the experimental data closely matched the Debye model. The urinary

dielectric dispersion and relaxation time increased with proteinuria level, but decreased with temperature. The classification accuracy of dielectric constant increased, while loss factor decreased with temperature. However, cross-comparison with other classification methods remains a challenge.

CHAPTER 5: CLASSIFICATION OF URINARY DIELECTRIC PROPERTIES FOR TYPE 2 DIABETES MELLITUS AND CHRONIC KIDNEY DISEASE

5.1 Introduction

From the previous chapter, it can be seen that the measurement of dielectric properties offers the potential to determine the variability of glycosuria and proteinuria in a simple and non-destructive manner. The accuracy of the determination should be investigated in order to show the potential of urinary dielectric properties to differentiate among DM, CKD and normal subjects. Data classification has been used as the most intensively studied method in statistical and decision science. Applications of SVM classification for kidney disease (Haubitz et al., 2005; Kistler et al., 2009; Alkhalaf et al., 2010; Gronwald et al., 2011), DM (Ban et al., 2010; Roshan et al., 2011) and cancers (Kerhet et al., 2006; Laufer & Rubinsky, 2009; Shini et al., 2011; Gonzalez et al., 2013; Grewal & Golnaraghi, 2014) have validated the capability of SVM as a classifier. Ideally, a desirable diagnostic tool for DM and CKD would be one that is non-invasive and highly accurate by measuring urinary dielectric properties.

In this chapter, the urinary dielectric properties of normal subjects, subjects with Type 2 DM and subjects with CKD at room temperature (25°C), 30°C and body temperature (37°C) between microwave frequencies ranging from 1 GHz to 50 GHz are classified. SVM-based classification was applied and the effect of temperature in classification accuracy is determined.

5.2 Background Study

5.2.1 Inner-Product Kernel

For nonlinear classification, inner-product Kernel function is used in SVM. It converts nonlinear input data into high dimensional linear feature space. Theoretically, x is a vector from the input space with assumed dimensional m_0 and $\{\varphi(x)\}_{j=1}^{m_i}$ is a set of

non-linear transformation from input space to feature space, m_1. Hyperplane for the decision surface of nonlinear transformation is as follows:

$$\sum_{j_1}^{m_1} w_j \varphi_j(x) + b = 0 \qquad \textbf{5.1}$$

where $\{w_j\}_{j=1}^{m_1}$ is a set of linear weights that connects feature space to output space and b is the bias. $\varphi_j(x)$ of the inputs to the weight, w_j through the feature space. The vector is defined as follows:

$$\varphi(x) = [\varphi_0(x), \varphi_1(x), \ldots . \varphi_{m_i}(x)]^T \qquad \textbf{5.2}$$

where $\varphi_0(x) = 1$ for all x. The decision surface may be defined in compact form since $\varphi(x)$ represents the "image" in feature space due to x.

$$w^T \varphi(x) = 0 \qquad \textbf{5.3}$$

where $w = \sum_{i=1}^{N} \alpha_i d_i \varphi(x_i)$ and $\varphi(x_i)$ is the feature vector representing input pattern x_i in ith examples. Hence,

$$\sum_{i=1}^{N} \alpha_i d_i \varphi^T(x_i)\varphi(x) = 0 \qquad \textbf{5.4}$$

The inner-product Kernel denoted by K(x,x_i) and defined by

$$K(x, x_i) = \varphi^T(x)\varphi(x_i) = \sum_{j=0}^{m_i} \varphi_j(x)\varphi_j(x_i) \text{ for } i = 1,2, \dots n \qquad \textbf{5.5}$$

Inner-product Kernel, K(x,x_i) is used to construct optimal hyperplane in the feature space, considering feature space in the explicit form. Figure 5.1 shows the architecture of SVM that constructs a decision classification for non-linear input space.

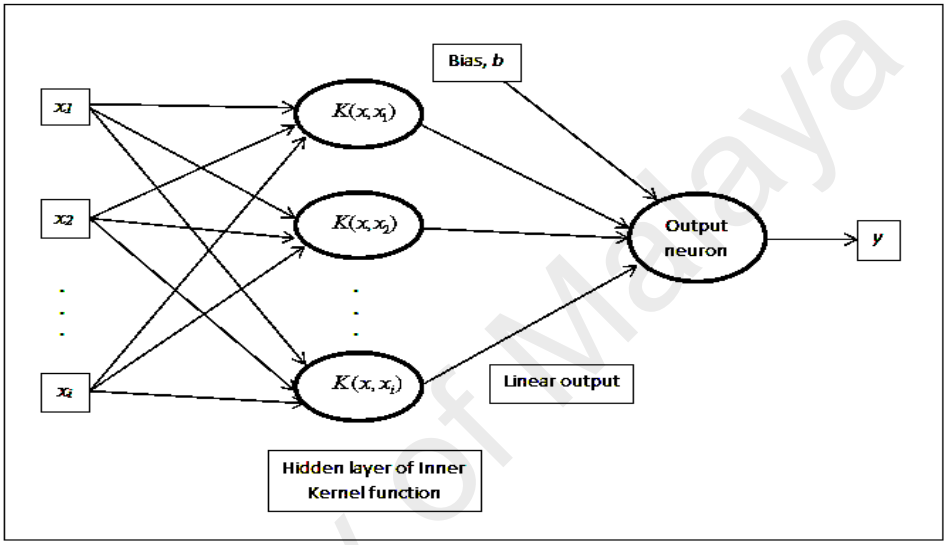


Figure 5.1: Architecture of SVM for non-linear classification.

5.2.2 Model Selection

Inner-product Kernel must satisfy the Mercer's theorem (Haykin & Network, 2004). Classifier accuracy on the hyperparameters is dependent on the decision boundary of the SVM. A set of parameters called hyperparameters that includes the soft-margin, C and other parameters of the kernel functions dependence (Table 2.5) for SVM are needed to be tuned. Soft-margin constant, C is assigned to margin errors. It represents the two points closest to the hyperplane that affect the orientation. When C value is decreased, it provides a larger margin for the data and allows ignoring points that are close to the boundary. For Kernel parameters, the degree of polynomial kernel, p and the width parameter, γ of the radial basis function (RBF) kernel control the flexibility of the classifier. The increase of p value yields greater curvature of the decision boundary.

When γ value is less, the boundary is nearly linear. The flexibility of the boundary increases with γ value. However, the large value of γ causes overfitting of data where the discriminant function is constant over the outer side in close proximity to the region. Ben-Hur and Weston (2010) suggested that RBF and polynomial kernel may cause overfitting in high-dimensional database with a small number of samples. RBF kernel usually performs better in terms of accuracy and time convergence compared to polynomial kernel.

5.3 Materials and Methods

5.3.1 Data Source

The database was obtained from the urinary dielectric properties of Type 2 DM, CKD and normal subjects at microwave frequency ranging from 1 GHz to 50 GHz. Subjects involved a total of 102 subjects with DM, 232 subjects with CKD and 97 healthy subjects. Subjects with CKD were further classified into diabetic kidney disease (DKD) with n=102 and non-DKD (n=130). The description of the database and clinical characteristics of the subjects are shown in Table 5.1 and Table 5.2, respectively.

Table 5.1: Database of DM, CKD and normal subjects

Subject	DM	DKD	Non-DKD	Normal
Male	54	68	79	51
Female	48	34	51	46
Total	102	102	130	97

Table 5.2: Clinical characteristics of DM, DKD, non-DKD and normal subjects.

Subject	DM	DKD	Non-DKD	Normal
Age (years)	61 ± 9	65 ± 10	64 ± 12	27 ± 8
Systolic BP (mmHg)	146 ± 26	147 ± 20	137 ± 19	115 ± 10
Diastolic BP (mmHg)	77 ± 17	74 ± 13	72 ± 14	72 ± 7
Serum creatinine (μmol/L)	<115	248 ± 149	218 ± 107	<115
eGFR (ml/min per 1.73m^2)	>90	21 ± 12	27 ± 16	>90
Proteinuria level (g/L)	-	2.71 ± 1.11	2.15 ± 1.30	-
Glycosuria level (g/L)	4.62 ± 2.18	3.08 ± 2.70	-	-

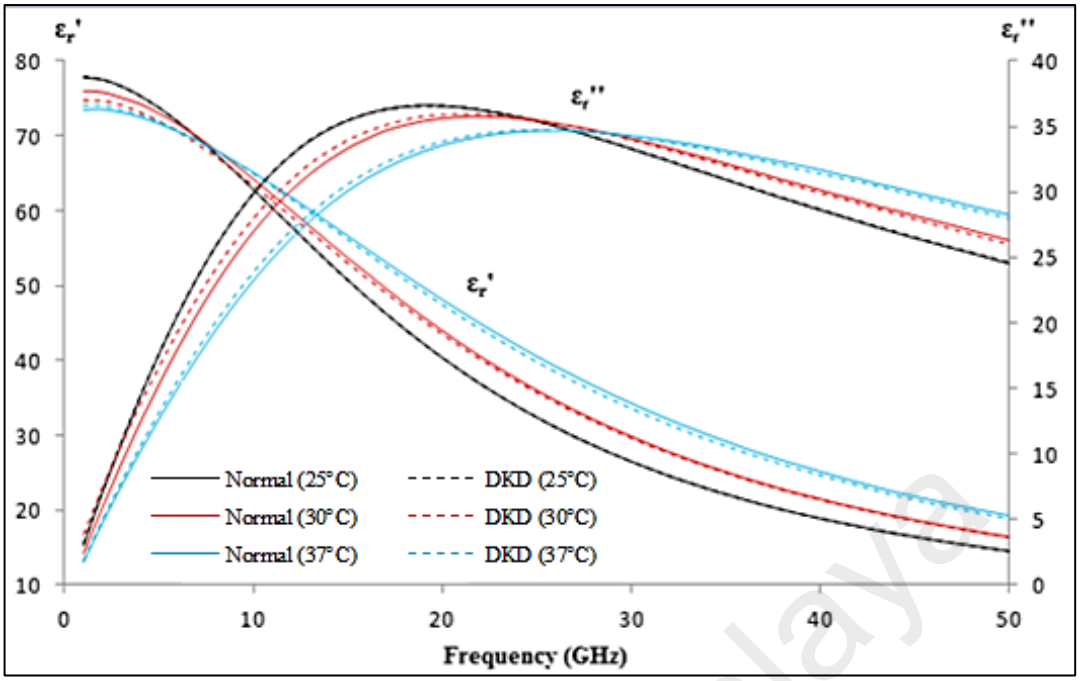
5.3.2 Classification Model

SVM-based classification of urinary dielectric properties was performed using LIBSVM tool (Chang & Lin, 2011) in MATLAB software (Mathworks, 2011). A total of 50 variables (microwave frequency from 1 GHz to 50 GHz) of urinary dielectric properties were used as the inputs to the classification model. Classification was accomplished based on 3-fold cross validation method. Two-thirds of the database was used as training set and the remaining one-third was used for validation. The classification was repeated three times based on different training and validation sets from the same database. The prediction accuracies were obtained from the average of three test sets. RBF kernel, $K(x, x_i) = \exp(-\gamma \|x - x_i\|^2)$ was applied with the parameter, $\gamma \in [0.1, 10]$ and was tested in the system to determine the optimal classification accuracy. Classification models were generated based on the respective urinary dielectric constant and loss factor at 25°C, 30°C and 37°C, respectively. The classifiers were applied to the database to predict the disease of the subjects based on different urinary dielectric properties.

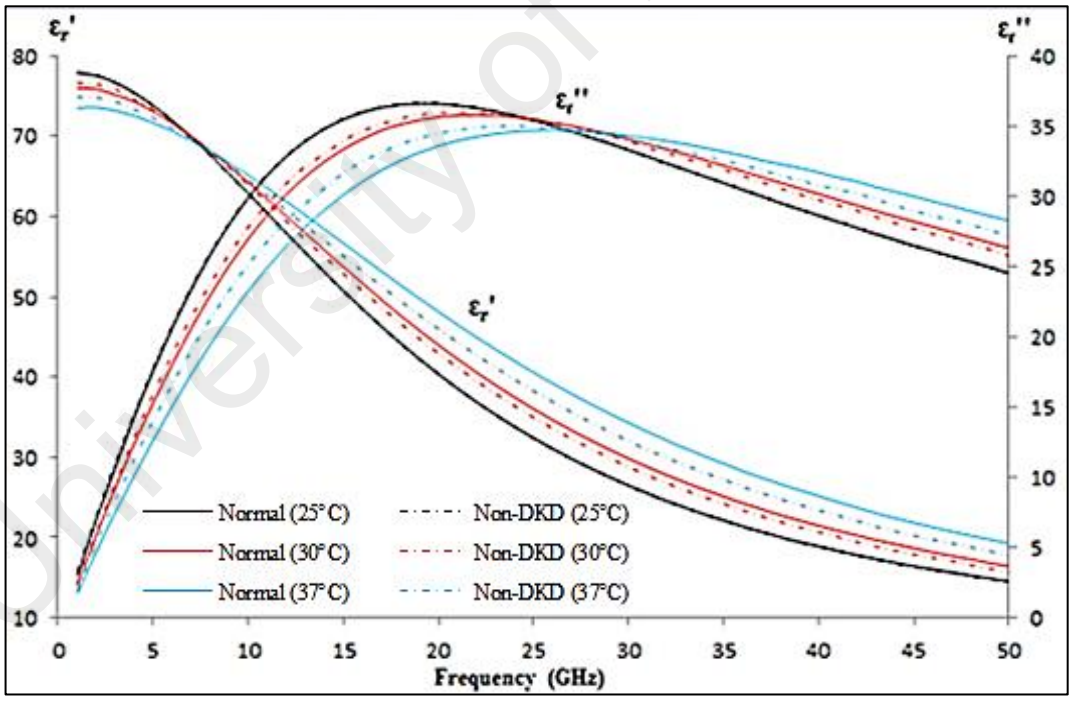
5.4 Results

5.4.1 Overview

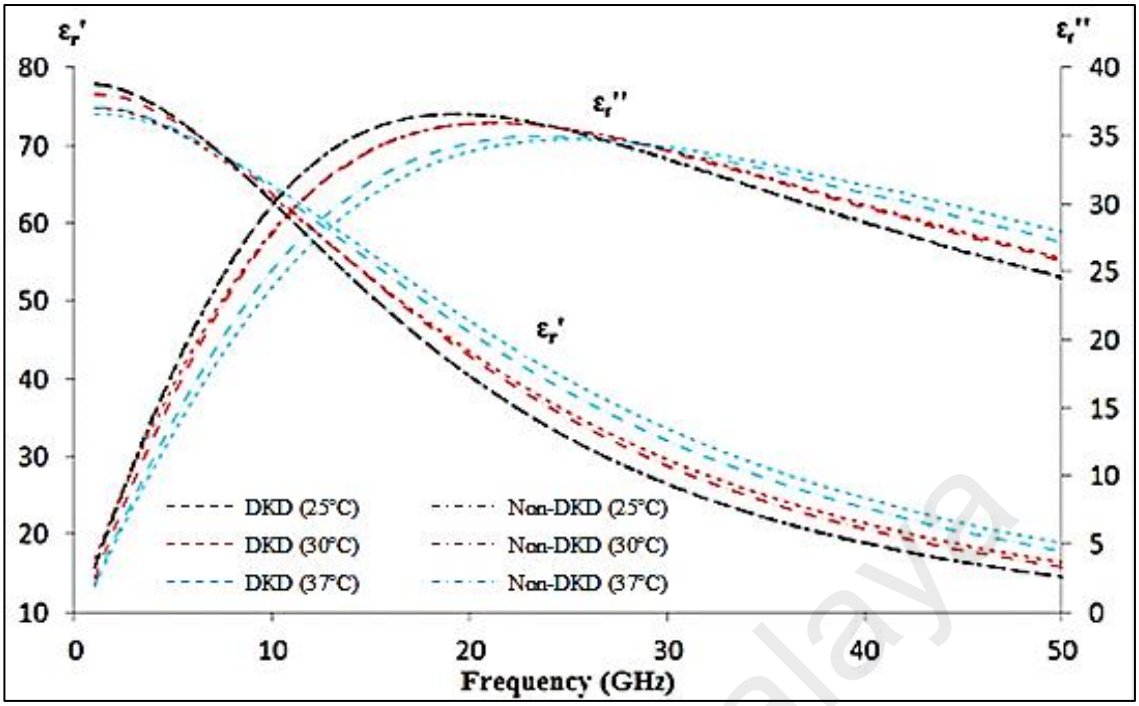
The cross-comparisons of mean urinary dielectric properties for different subject groups are presented in Figure 5.2 at respective temperatures of 25°C, 30°C and 37°C. Differences in urinary dielectric properties were observed in comparison between DKD and normal, non-DKD and normal, and DKD and non-DKD subject groups, respectively. DKD subjects had lower and higher urinary dielectric properties than non-DKD subjects below and above its 'cross-over' frequency point, respectively.



(a)



(b)



(c)

Figure 5.2: Comparison of mean urinary dielectric properties among DKD, Non-DKD and normal subject groups.

5.4.2 Two-group Classification: DM vs Normal, DKD vs Normal and Non-DKD vs Normal

The classifications were performed using urinary dielectric properties database with 50 variables and 431 records for dielectric constant and loss factor, respectively. RBF kernel with $\gamma = 0.5$ was found to show optimal performance in classifications. Classification results of urinary dielectric properties between DM vs normal, DKD vs normal and non-DKD vs normal group at 25°C, 30°C and 37°C, respectively are summarised in Table 5.3 to Table 5.5.

Table 5.3: Classification accuracy, sensitivity and specificity between DM, DKD and non-DKD with normal group, respectively, at 25°C

Comparison Group	ε_r'			ε_r''		
	Classification Accuracy (%)	Sensitivity (%)	Specificity (%)	Classification Accuracy (%)	Sensitivity (%)	Specificity (%)
DM vs Normal	84.02	76.47	92.39	77.20	67.65	87.91
DKD vs Normal	81.96	82.35	81.52	75.92	86.0	64.84
Non-DKD vs Normal	79.30	83.21	73.95	71.80	78.63	62.50

Table 5.4: Classification accuracy, sensitivity and specificity between DM, DKD and non-DKD with normal group, respectively, at 30°C

Comparison Groups	ε_r'			ε_r''		
	Classification Accuracy (%)	Sensitivity (%)	Specificity (%)	Classification Accuracy (%)	Sensitivity (%)	Specificity (%)
DM vs Normal	88.72	88.23	89.24	80.51	78.43	82.80
DKD vs Normal	84.10	85.29	82.79	78.46	75.49	81.72
Non-DKD vs Normal	79.30	85.38	71.13	79.30	80.15	78.35

Table 5.5: Classification accuracy, sensitivity and specificity between DM, DKD and non-DKD with normal group, respectively, at 37°C

Comparison Groups	ε_r'			ε_r''		
	Classification Accuracy (%)	Sensitivity (%)	Specificity (%)	Classification Accuracy (%)	Sensitivity (%)	Specificity (%)
DM vs Normal	82.99	74.25	92.47	80.0	83.33	76.34
DKD vs Normal	77.93	78.43	77.41	77.43	90.19	63.44
Non-DKD vs Normal	77.29	83.33	69.07	78.12	88.64	63.92

At 25°C, classification model obtained 84.02%, 81.96% and 79.30% accuracy to distinguish DM, DKD and non-DKD, respectively, from the normal group using urinary dielectric constant. Meanwhile, loss factor produced classification accuracies at 77.20%, 75.92% and 71.80% to classify DM, DKD and non-DKD, respectively from the normal group. Overall, dielectric constant produced higher classification accuracies than loss factor except for classification between non-DKD and normal group at 37°C. The highest classification accuracies were observed at 30°C.

5.4.3 Three-group Classifications

5.4.3.1 Classification of DM vs DKD vs normal

Table 5.6 and 5.7 show the confusion matrix of the classifications for urinary dielectric properties among DM, DKD and normal group at 25°C, 30°C and 37°C. The classification model showed overall prediction accuracy of 67.0%, 67.64% and 65.55% using urinary dielectric constant at 25°C, 30°C and 37°C, respectively. Urinary loss factor produced 61.49%, 66.02% and 61.79% accuracy at 25°C, 30°C and 37°C, respectively. Urinary dielectric constant produced higher classification accuracies than loss factor. The highest classification accuracy was shown at 30°C. The overall deviation of accuracies was determined at about 2~5% across temperatures.

Table 5.6: Confusion matrix for the classification of urinary dielectric constant among DM, DKD and normal group at 25°C, 30°C and 37°C

	25°C				30°C				37°C			
	DM	**DKD**	**Normal**	**Accuracy (%)**	**DM**	**DKD**	**Normal**	**Accuracy (%)**	**DM**	**DKD**	**Normal**	**Accuracy (%)**
DM	54	36	12	52.94	55	41	6	53.92	54	34	14	52.94
DKD	12	69	21	67.65	20	71	11	69.61	18	73	11	71.56
Normal	5	14	78	80.41	8	12	77	79.38	6	21	70	72.16
Overall Prediction Accuracy				67.0				67.64				65.55

Table 5.7: Confusion matrix for the classification of urinary loss factor among DM, DKD and normal group at 25°C, 30°C and 37°C

	25°C				30°C				37°C			
	DM	**DKD**	**Normal**	**Accuracy (%)**	**DM**	**DKD**	**Normal**	**Accuracy (%)**	**DM**	**DKD**	**Normal**	**Accuracy (%)**
DM	57	32	13	55.88	62	26	14	60.78	51	38	13	50.0
DKD	24	66	12	65.71	27	58	17	56.86	21	75	6	73.52
Normal	7	29	61	62.89	10	9	78	80.41	11	26	60	61.85
Overall Prediction Accuracy				61.49				66.02				61.79

5.4.3.3 Classification of DM vs non-DKD vs normal

Tables 5.8 and 5.9 show the confusion matrix for the classification of urinary dielectric properties among DM, non-DKD and normal groups at 25°C, 30°C and 37°C. The classification model showed overall prediction accuracy of 62.14%, 63.68% and 64.50% using urinary dielectric constant at 25°C, 30°C and 37°C, respectively. Urinary loss factor produced 59.20%, 63.87% and 63.22% accuracy at 25°C, 30°C and 37°C, respectively. Overall, urinary dielectric constant showed higher classification accuracies than loss factor except for classification at 30°C that had deviation <0.5 %.

Table 5.8: Confusion matrix for the classification of urinary dielectric constant among DM, non-DKD and normal group at 25°C, 30°C and 37°C

	25°C				30°C				37°C			
	DM	**Non-DKD**	**Normal**	**Accuracy (%)**	**DM**	**Non-DKD**	**Normal**	**Accuracy (%)**	**DM**	**Non-DKD**	**Normal**	**Accuracy (%)**
DM	42	48	12	41.18	58	37	7	56.86	46	41	15	45.10
Non-DKD	14	94	22	72.31	30	86	14	66.15	13	103	14	79.23
Normal	3	23	71	72.92	5	26	66	68.04	6	24	67	69.07
Overall Prediction Accuracy				62.14				63.68				64.50

Table 5.9: Confusion matrix for the classification of urinary loss factor among DM, non-DKD and normal group at 25°C, 30°C and 37°C

	25°C				30°C				37°C			
	DM	**Non-DKD**	**Normal**	**Accuracy (%)**	**DM**	**Non-DKD**	**Normal**	**Accuracy (%)**	**DM**	**Non-DKD**	**Normal**	**Accuracy (%)**
DM	47	43	12	46.07	51	34	17	50.0	53	31	18	51.96
Non-DKD	12	96	22	73.84	18	101	11	77.69	16	100	14	76.90
Normal	8	34	55	57.70	7	28	62	63.92	7	31	59	60.82
Overall Prediction Accuracy				59.20				63.87				63.22

5.4.3.5 Classification of DKD vs non-DKD vs normal

Tables 5.10 and 5.11 show the confusion matrix for the classification of urinary dielectric properties among DKD, non-DKD and normal groups at 25°C, 30°C and 37°C. The classification model showed prediction accuracy of 60.92%, 61.12% and 59.63% using urinary dielectric constant at 25°C, 30°C and 37°C, respectively. As for urinary loss factor, the classification accuracy was reported at 60.27%, 61.23% and 60.41% at 25°C, 30°C and 37°C, respectively. Again, the highest classification accuracy was achieved at 30°C (61.23%).

Table 5.10: Confusion matrix for the classification of urinary dielectric constant among DKD, non-DKD and normal group at 25°C, 30°C and 37°C.

	25°C				30°C				37°C			
	DKD	**Non-DKD**	**Normal**	**Accuracy (%)**	**DKD**	**Non-DKD**	**Normal**	**Accuracy (%)**	**DKD**	**Non-DKD**	**Normal**	**Accuracy (%)**
DKD	54	40	8	52.94	58	38	6	56.86	49	44	9	48.04
Non-DKD	32	83	15	63.85	39	72	19	55.38	34	83	13	63.85
Normal	16	17	64	65.98	12	16	69	71.13	13	19	65	67.01
Overall Prediction Accuracy				60.92				61.12				59.63

Table 5.11: Confusion matrix for the classification of urinary loss factor among DKD, non-DKD and normal group at 25°C, 30°C and 37°C.

	25°C				30°C				37°C			
	DKD	**Non-DKD**	**Normal**	**Accuracy (%)**	**DKD**	**Non-DKD**	**Normal**	**Accuracy (%)**	**DKD**	**Non-DKD**	**Normal**	**Accuracy (%)**
DKD	52	42	8	50.98	59	35	8	56.19	53	45	4	51.96
Non-DKD	40	79	11	60.76	32	84	14	64.62	38	85	7	65.38
Normal	12	18	67	69.07	11	25	61	62.88	12	23	62	63.91
Overall Prediction Accuracy				60.27				61.23				60.41

5.5 Discussion

5.5.1 Effect of Temperature in Classifications

In fact, urine temperature may vary between the body (37°C) and room temperature (25°C) in the process of collection to measurement. Temperature variation is an important factor that affects the dielectric properties. Overall, the highest classification accuracy was shown at 30°C for two-group and three-group classifications. The maximum accuracy deviation was about 8% across temperatures of 25°C, 30°C and 37°C. Urinary dielectric properties changed with temperature, due to the stretching of intramolecular hydrogen bonds between water molecules of solutions (Ellison, 2007). Urine is a complex biological solution that consists of different chemical constituents that are broadly categorised as electrolytes, organic and in-organic compounds. The combination effects of ionic conductance, molecular Brownian movement and hydrogen bonds increased with temperature in urine. However, randomising the agitation of molecules at high temperature reduced the significant effect of the biomaterials present in urine that could affect the classification accuracy. The classifications of urinary dielectric behaviour were optimal at 30°C for urine, while the accuracies decreased when the temperature reached 37°C.

5.5.2 Two-group Classifications

In two-group classifications, the highest classification accuracies for urinary dielectric properties were obtained to differentiate DM from normal group. The deviations were about 2~5% in comparison between DM and CKD two-group classifications of urinary dielectric properties. Overall, the classification model produced considerably high accuracies, which appear to be sufficient to classify the urinary dielectric properties as a function between disease and normal group. These findings suggest the existence of unique behaviours related to urinary dielectric properties between normal and disease conditions at microwave frequency between

1GHz and 50GHz. The presence of glycosuria and proteinuria in DM and CKD subject groups, respectively, significantly affects the behaviour of urinary dielectric properties.

5.5.3 Three-group Classifications

In three-group classifications, the lowest accuracy was obtained when classifying the urinary dielectric properties of DM subjects among CKD and normal subject groups. High misclassifications in prediction output of DM subjects were classified as CKD subjects. The highest misclassification was found in about 41 and 48 subjects from a total of 102 DM subjects who were classified in the DKD and non-DKD groups, respectively. The confusion classification between DM and CKD group was about 35 ~ 45%. It may be due to the possible existence of DM subjects with high risk of getting CKD, who have absence of clinical symptoms with estimated glomerular filtration rate (eGFR) < 90 ml/min per $1.73m^2$. As reported by Zürbig et al. (2009), initial pathophysiological changes in kidneys resulted in different urinary constituents such as micro-albuminuria as a potential biomarker for early stage of CKD. Based on the pathological changes, the measurement of urinary dielectric properties would potentially be able to distinguish diabetic subjects with high risk of CKD. However, this study remains limited for changes in urinary dielectric properties for albuminuria. Meanwhile, Alkhalaf et al. (2010) discovered that different biomarkers were found between Type 1 and 2 DM patients due to the different pathophysiology of the particular DKD. Type 1 DM still remains a challenge in this study and requires future investigation.

5.5.4 Comparison Accuracy between Classification Methods

Table 5.12 and Table 5.13 show the comparison of accuracies between threshold and SVM classification method for urinary dielectric properties. Overall, classification accuracies produced by SVM were significantly higher than the threshold method for 25°C, 30°C and 37°C. The accuracy deviations were >10% in comparison between the

two classification methods except for CKD vs normal group at 37°C. This shows that SVM enhanced the accuracy of urinary dielectric properties measurement in distinguishing DM and CKD, respectively, from normal subjects.

Table 5.12: Comparison of classification accuracies between threshold and SVM classification method for urinary dielectric constant.

Comparison Groups	Threshold			SVM		
	25°C	30°C	37°C	25°C	30°C	37°C
DM vs Normal	72.0	77.08	66.67	84.02	88.72	82.99
CKD vs Normal	59.72	68.67	79.52	79.30	79.30	77.29

Table 5.13: Comparison of classification accuracies between threshold and SVM classification methods for urinary loss factor.

Comparison Groups	Threshold			SVM		
	25°C	30°C	37°C	25°C	30°C	37°C
DM vs Normal	54.90	69.39	66.67	77.20	80.51	80.0
CKD vs Normal	72.29	69.88	51.81	71.80	79.30	78.12

5.6 Summary

In this chapter, the urinary dielectric properties of Type 2 DM, CKD and normal group were classified by using the SVM classification model at microwave frequency from 1 GHz to 50 GHz. The CKD group was further categorised into DKD and non-DKD for classification. Differences in urinary dielectric properties were observed between DKD and non-DKD compared to the normal group, and between DKD and non-DKD groups. Two-group and three-group classifications were conducted. In two-group classifications, the highest classification accuracy was achieved at 88.72% for differentiating DM from the normal group. As for the three-group classifications, the highest accuracy was achieved at 67.64%. The effect of temperature in the classification of urinary dielectric properties was determined. The highest classification accuracy was obtained at 30°C for two-group and three-group classifications. SVM classification enhanced the accuracy of urinary dielectric properties measurement in distinguishing Type 2 DM and CKD from normal subjects.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study investigated the urinary dielectric properties of Type 2 DM, CKD and normal subjects using the open-ended coaxial probe technique at microwave frequency between 0.2 GHz and 50 GHz. Dielectric constant increased with glycosuria level of more than 5 g/L (0.5%) at low frequencies, while correlated positively with glycosuria level at frequencies above 40 GHz. Loss factor correlated negatively with glycosuria level at frequencies above 15 GHz. Relaxation frequency shifted towards lower values with higher glycosuria levels. The strongest statistically significant differences in urinary dielectric properties across different glycosuria levels were reported at 25°C.

The urinary dielectric properties of CKD subjects were higher and lower than those of normal subjects at frequencies below and above the "cross-over" frequency points, respectively. The strongest statistically significant differences in the urinary dielectric properties of CKD subjects compared with normal subjects were reported at 37°C. Urinary dielectric properties correlated positively and negatively with proteinuria level at frequencies below and above the "cross-over" frequency point, respectively.

The experimental data closely matched the single-pole Debye model. The urinary relaxation dispersion and relaxation time increased with the glycosuria and proteinuria levels, respectively, while decreased with temperature. As for static conductivity, it increased with proteinuria levels higher than 1.5 g/L.

Classifications of urinary dielectric behaviour of DM, CKD and normal groups were conducted using the SVM-based classification model at microwave frequencies from 1 GHz to 50 GHz. In two-group classifications, the highest classification accuracy was achieved at 88.72%, 84.10% and 79.38% for differentiating DM, DKD and non-DKD groups, respectively, from the normal group. The highest accuracy was achieved at

67.64% in three-group classifications. The temperature of 30°C was found to be optimal for the classification of urinary dielectric behaviour, compared to 25°C and 37°C. SVM enhanced the classification accuracy of urinary dielectric properties measurement in distinguishing DM and CKD from normal subjects. This study demonstrated the potential diagnostic and prognostic value of urinary dielectric properties for Type 2 DM and CKD.

6.2 Recommendations

At the current stage, the differences in urinary dielectric properties of glycosuria and proteinuria, involving DM and CKD, respectively, are insufficient for applying dielectric spectroscopy as a diagnostic or monitoring tool. Cross-comparison of dielectric techniques may be required for determining the validation of differences between subject groups. Collection and measurement of urine must be particularly careful to avoid contamination that may affect the results. Future studies will require larger patient groups with broader glycosuria or proteinuria variability, and measurement taken over a wider microwave frequency range. In this study, the urinary dielectric properties were found would potentially be able to distinguish diabetic subjects with high risk of CKD. However, this study highlights the need for future investigation of the changes in urinary dielectric properties changes with albuminuria.

On the other hand, different biomarkers reported between Type 1 and 2 DM patients showed different pathophysiology of the resulting diabetic kidney disease (Alkhalaf et al., 2010). This poses a challenge for future investigation into dielectric properties for Type 1 DM.

Moreover, future studies on correlations between serum glucose level and creatinine with urinary dielectric properties are recommended. This is certainly important to

determine the indirect impact of serum glucose or creatinine that causes differences in urine composition, which subsequently changes urinary dielectric properties.

The measurement of dielectric properties could be extended to other diseases, such as leukaemia, bladder stone, bladder cancer and oral cancer that strongly require clinical determination in biological solutions. Dielectric properties would work as powerful parameters to determine variations of biomaterials in a solution for clinical utility.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

i. Mun, P. S., Ting, H. N., Ong, T. A., Wong, C. M., Ng, K. H. & Chong, Y. B. (2015) A study of dielectric properties of proteinuria between 0.2 and 50 GHz. *Plos One*, 10(6), e0130011.

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RESEARCH ARTICLE

A Study of Dielectric Properties of Proteinuria between 0.2 GHz and 50 GHz

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Abstract

This paper investigates the dielectric properties of urine in normal subjects and subjects with chronic kidney disease (CKD) at microwave frequency of between 0.2 GHz and 50 GHz. The measurements were conducted using an open-ended coaxial probe at room temperature (25°C), at 30°C and at human body temperature (37°C). There were statistically significant differences in the dielectric properties of the CKD subjects compared to those of the normal subjects. Statistically significant differences in dielectric properties were observed across the temperatures for normal subjects and CKD subjects. Pearson correlation test showed the significant correlation between proteinuria and dielectric properties. The experimental data closely matched the single-pole Debye model. The relaxation dispersion and relaxation time increased with the proteinuria level, while decreasing with the temperature. As for static conductivity, it increased with proteinuria level and temperature.

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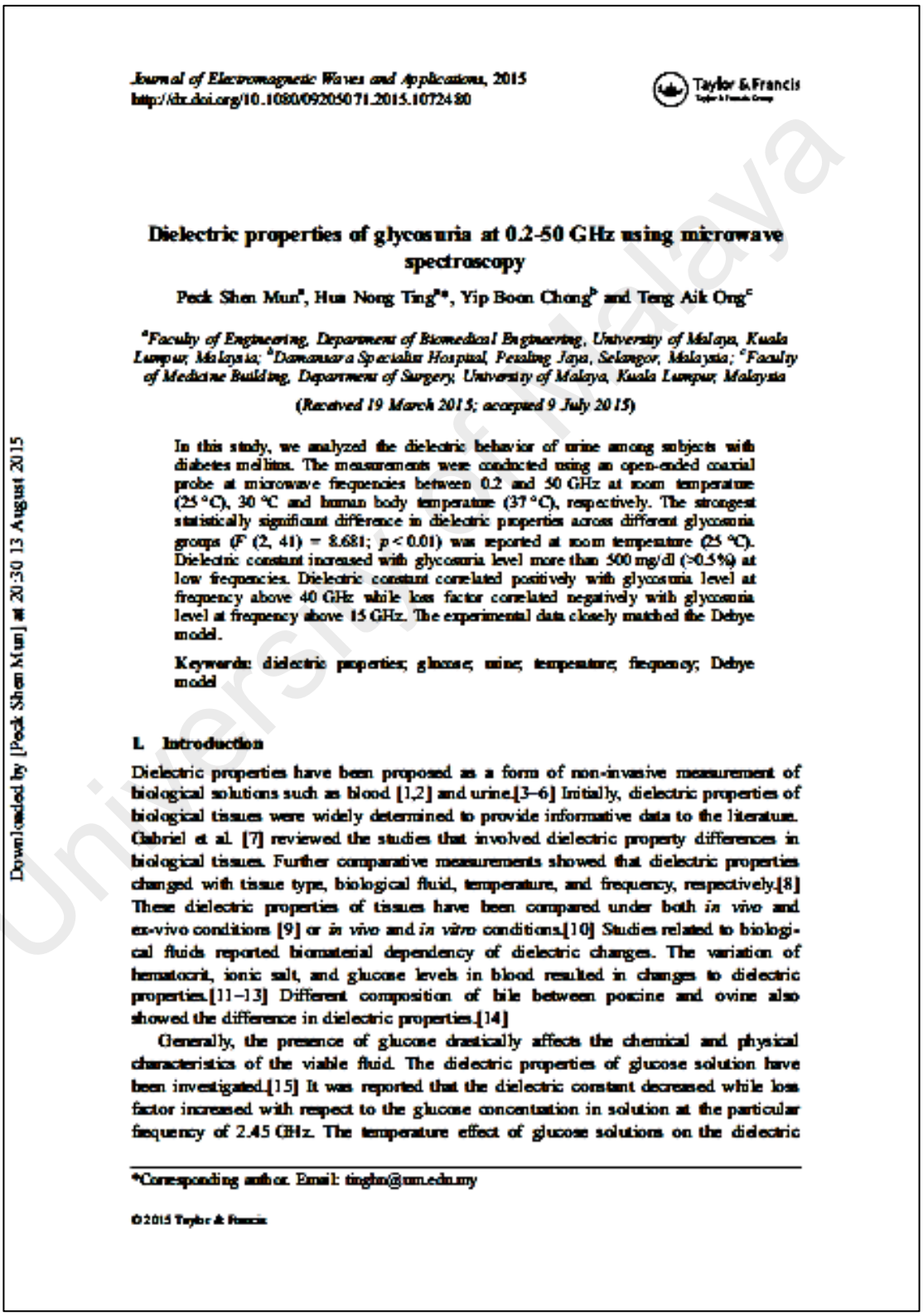
Introduction

The measurement of dielectric properties is generating interest for clinical utility. Dielectric properties of tissues have been widely determined to provide informative data to the literature related to the application of measurements for biological dielectric properties. Stuchly et al. [1] measured the dielectric properties of animal tissues following the time of tissue death in radio frequency. They concluded that the concentration of cell-derived ions in tissues exhibits changes in dielectric properties. Recently, studies have been conducted in microwave frequencies due to stronger observable interaction with polarization of ions. Gabriel et al. [2] and Gabriel et al. [3] reviewed the studies of differences in dielectric properties among biological tissues and further comparative measurement changes with tissue type, biological fluid, temperature and frequency, respectively. They reported that frequency dependence of human and animal tissues corresponded to the temperature of tissue extraction at frequencies up to 20 GHz. Dielectric behavior was affected by water content and blood infiltration within the tissues [2, 3]. Previous studies related to biological fluids reported biomaterial dependency of dielectric

ii. Mun, P. S., Ting, H. N., Chong, Y. B. & Ong, T. A. (2015) Dielectric properties of glycosuria at 0.2-50 GHz using microwave spectroscopy. *Journal of Electromagnetic Waves and Applications*, 1-15. doi: 10.1080/09205071.2015.1072480



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Dielectric properties of glycosuria at 0.2-50 GHz using microwave spectroscopy

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In this study, we analyzed the dielectric behavior of urine among subjects with diabetes mellitus. The measurements were conducted using an open-ended coaxial probe at microwave frequencies between 0.2 and 50 GHz at room temperature (25 °C), 30 °C and human body temperature (37 °C), respectively. The strongest statistically significant difference in dielectric properties across different glycosuria groups (F (2, 41) = 8.681; $p < 0.01$) was reported at room temperature (25 °C). Dielectric constant increased with glycosuria level more than 500 mg/dl (>0.5%) at low frequencies. Dielectric constant correlated positively with glycosuria level at frequency above 40 GHz while loss factor correlated negatively with glycosuria level at frequency above 15 GHz. The experimental data closely matched the Debye model.

Keywords: dielectric properties; glucose; urine; temperature; frequency; Debye model

1. Introduction

Dielectric properties have been proposed as a form of non-invasive measurement of biological solutions such as blood [1,2] and urine.[3–6] Initially, dielectric properties of biological tissues were widely determined to provide informative data to the literature. Gabriel et al. [7] reviewed the studies that involved dielectric property differences in biological tissues. Further comparative measurements showed that dielectric properties changed with tissue type, biological fluid, temperature, and frequency, respectively.[8] These dielectric properties of tissues have been compared under both *in vivo* and ex-vivo conditions [9] or *in vivo* and *in vitro* conditions.[10] Studies related to biological fluids reported biomaterial dependency of dielectric changes. The variation of hematocrit, ionic salt, and glucose levels in blood resulted in changes to dielectric properties.[11–13] Different composition of bile between porcine and ovine also showed the difference in dielectric properties.[14]

Generally, the presence of glucose drastically affects the chemical and physical characteristics of the viable fluid. The dielectric properties of glucose solution have been investigated.[15] It was reported that the dielectric constant decreased while loss factor increased with respect to the glucose concentration in solution at the particular frequency of 2.45 GHz. The temperature effect of glucose solutions on the dielectric

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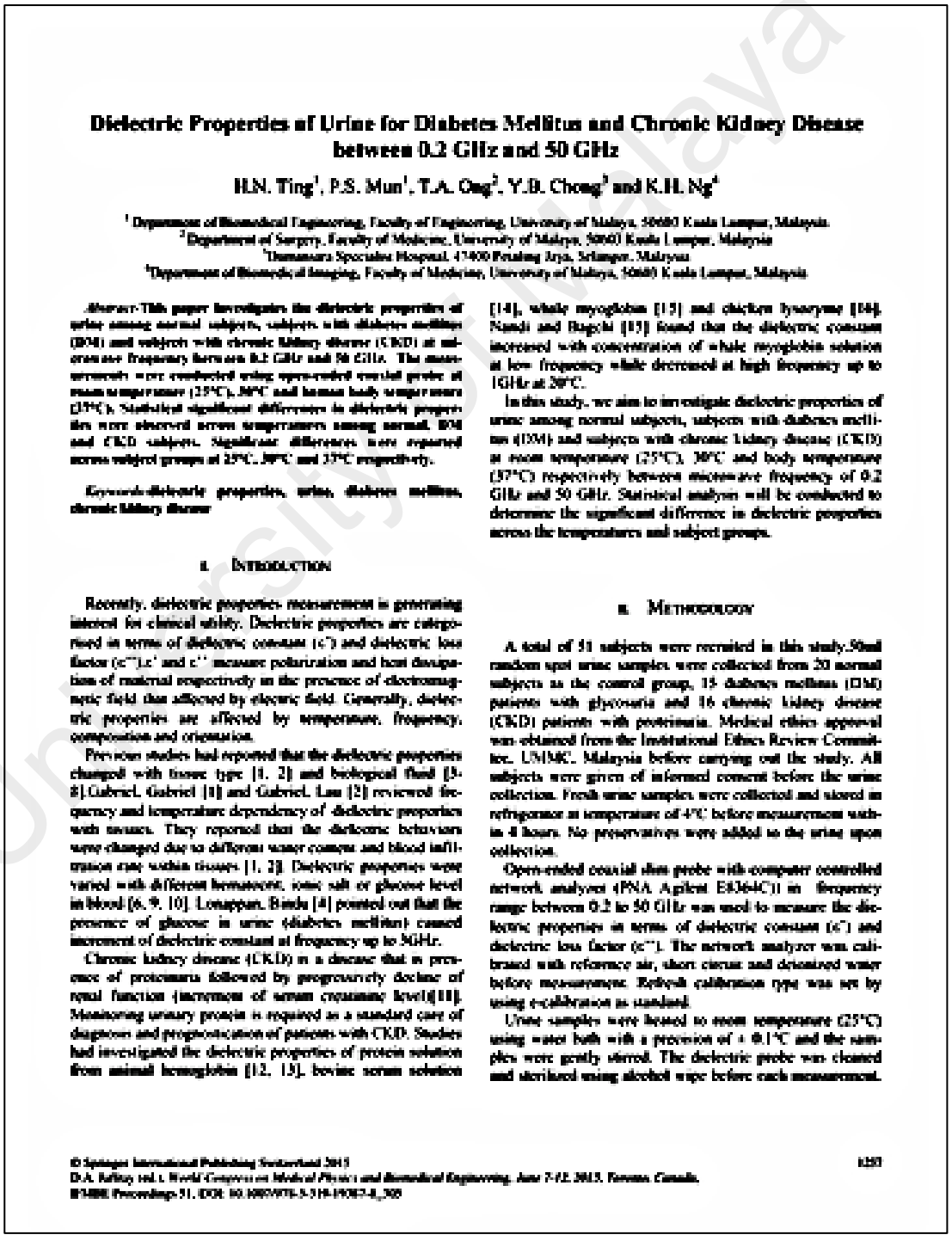

Dielectric Properties of Urine for Diabetes Mellitus and Chronic Kidney Disease between 0.2 GHz and 50 GHz

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Abstract—This paper investigates the dielectric properties of urine among normal subjects, subjects with diabetes mellitus (DM) and subjects with chronic kidney disease (CKD) at microwave frequency between 0.2 GHz and 50 GHz. The measurements were conducted using open-ended coaxial probe at room temperature (25°C), 30°C and human body temperature (37°C). Statistical significant differences in dielectric properties were observed across temperatures among normal, DM and CKD subjects. Significant differences were reported across subject groups at 25°C, 30°C and 37°C respectively.

Keywords—dielectric properties, urine, diabetes mellitus, chronic kidney disease

II. Introduction

Recently, dielectric properties measurement is generating interest for clinical utility. Dielectric properties are categorised in terms of dielectric constant (ε') and dielectric loss factor (ε'')[ε' and ε'' measure polarization and heat dissipation of material respectively on the presence of electromagnetic field that affected by electric field. Generally, dielectric properties are affected by temperature, frequency, composition and orientation.

Previous studies had reported that the dielectric properties changed with tissue type [1, 2] and biological fluid [3-8].Gabriel, Gabriel [1] and Gabriel, Lau [2] reviewed frequency and temperature dependency of dielectric properties with tissues. They reported that the dielectric behaviors were changed due to different water content and blood infiltration rate within tissues [1, 2]. Dielectric properties were varied with different hematocrit, ionic salt or glucose level in blood [6, 9, 10]. Lonappan, Rindu [4] pointed out that the presence of glucose in urine (diabetes mellitus) caused increment of dielectric constant at frequency up to 5GHz.

Chronic kidney disease (CKD) is a disease that is presence of proteinuria followed by progressively decline of renal function (increment of serum creatinine level)[11]. Monitoring urinary protein is required as a standard care of diagnosis and prognostication of patients with CKD. Studies had investigated the dielectric properties of protein solution from animal hemoglobin [12, 13], bovine serum solution [14], whale myoglobin [15] and chicken lysozyme [16]. Nandi and Bagchi [15] found that the dielectric constant increased with concentration of whale myoglobin solution at low frequency while decreased at high frequency up to 1GHz at 20°C.

In this study, we aim to investigate dielectric properties of urine among normal subjects, subjects with diabetes mellitus (DM) and subjects with chronic kidney disease (CKD) at room temperature (25°C), 30°C and body temperature (37°C) respectively between microwave frequency of 0.2 GHz and 50 GHz. Statistical analysis will be conducted to determine the significant difference in dielectric properties across the temperatures and subject groups.

II. Methodology

A total of 51 subjects were recruited in this study.50ml random spot urine samples were collected from 20 normal subjects as the control group, 15 diabetes mellitus (DM) patients with glycosuria and 16 chronic kidney disease (CKD) patients with proteinuria. Medical ethics approval was obtained from the Institutional Ethics Review Committee, UNIMC, Malaysia before carrying out the study. All subjects were given of informed consent before the urine collection. Fresh urine samples were collected and stored in refrigerator at temperature of 4°C before measurement within 4 hours. No preservatives were added to the urine upon collection.

Open-ended coaxial slim probe with computer controlled network analyzer (PNA Agilent E8364C)) in frequency range between 0.2 to 50 GHz was used to measure the dielectric properties in terms of dielectric constant (ε') and dielectric loss factor (ε''). The network analyzer was calibrated with reference air, short circuit and deionized water before measurement. Refresh calibration type was set by using e-calibration as standard.

Urine samples were heated to room temperature (25°C) using water bath with a precision of ± 0.1°C and the samples were gently stirred. The dielectric probe was cleaned and sterilized using alcohol wipe before each measurement.

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