



# DIELECTRIC PROPERTIES OF AQUEOUS SOLUTION OF LIPASE USING TIME DOMAIN TECHNIQUE

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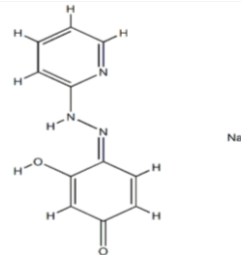
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**Abstract:** In this paper represents the study of static dielectric constant of aqueous solution of Lipase for various concentrations. The time domain reflectometry techniques used for the study in the frequency range from 10MHz to 30GHz. The static dielectric constant ( $\epsilon_0$ ), relaxation time ( $\tau$ ), static permittivity ( $\epsilon_\infty$ ) and hydration number ( $V_{hyd}$ ) calculated at 25°C. The hydration numbers were also determined from the static dielectric constant. Lipases are an important group of enzymes with various applications in the chemical detergent biocatalytic of pharmaceutical, food industry, making the fine chemical, esters, agrochemicals, leather industry and paper industry use to biosensor, cosmetics and bioremediation.

**Keywords:** Aqueous solution of Lipase, Dielectric constant, Relaxation time, Hydration number.

## I. INTRODUCTION

Lipases are produced by plants, animals and microorganism including bacteria and fungi. Lipase are a class of enzymes which catalyze the hydrolysis of long-chain triacylglycerol's and fatty acids. Lipases are produced by plants, animals and microorganism including bacteria and fungi. Biological Processes are very important stage of optimization to increase and improve the efficiency of cost. The aim to use of lipases in effluents with high lipid content and hydrolyzetriacyl glyceride, reducing of suspended lipids and solids. The catalyzing reactions of lipases are highly efficient in both aqueous and non-aqueous media because the high stability in extremes of pH, temperature and organic solvents. Lipases enzymes observation to bulk enzymes and high-value of production. The lipases are used as biocatalysts with modification of their physiochemical factors such as stability, specificity, pH and temperature. Lipases are used in biotechnological applications such that textile and dairy industries, detergent industry, biodiesel and oil processing and production of surfactants they have been obtained from microorganisms. The lipases are an important role in processing of polyunsaturated fatty acids, a food colorant, g-linoleic acid, methyl ketones of flavor molecules characteristic of blue cheese. In 19<sup>th</sup> century of the industrial revolution activities that greatly contributed to environmental depletion. Only a few lipase-producing bacteria were characterized, but research was intensified, became enzymatically active in organic solvents. The long chain triacylglycerol are the normal substrate for lipases. Lipases and esterase are very useful for biocatalyst. They are hydrolyses on carboxyl ester bonds. Lipases are useful for all commercial extracellular isolated from different bacterial species including pseudomonas and Bacillus. Among the pseudomonas species, pseudomonas aeruginosa, pseudomonas fluorescence and pseudomonas cepacia are the lipases of major producer. The important of lipases enzymes, the study was aimed to the bacterial lipases isolates to the higher activities such as natural and alkaline pH. The microbial lipases are detergent industry growth of industry to innovative key factors of lipases with chlorine bleach. Bacterial Lipases are lipoprotein however few extracellular bacterial lipases are glycoprotein. The molecular structure is shown in figure 1. Lipases are serine hydrolyses that catalyze the synthesis as well as the hydrolysis of long- chain triacylglycerol's. Lipases are enzymes that have the capacity to hydrolyze of fats into fatty acids and glycerol's. Lipases are enzymes that aid in the digestion, transport and processing of dietary lipid (fats and oils). The long chain triacylglycerol are the normal substrate for lipases. Bacterial lipases are glycoproteins in general, but certain extracellular bacterial lipases are lipoprotein. The lipases are used as biocatalysts with modification of their physiochemical factors such as stability, specificity, pH and temperature.

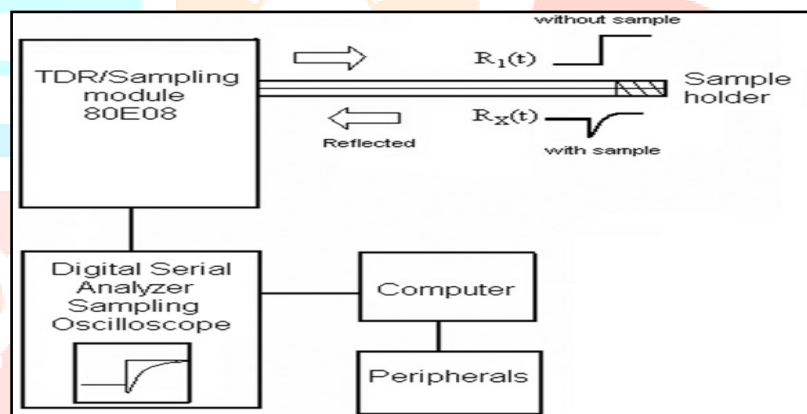


**Figure 1:** Molecular structure of Lipase

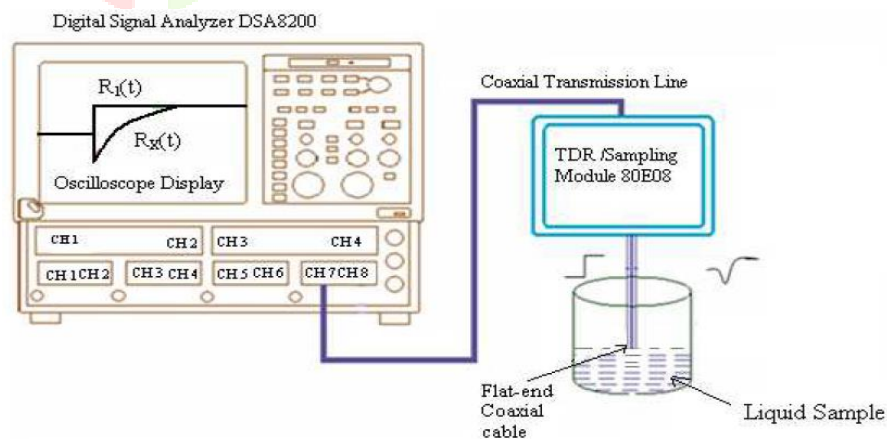
Lipases are used in variety of industries, including food, pharmaceutical fine chemical, oil chemical, biodiesel and industrial detergent. Tray Bioreactors are made up of a chamber and a tray. In this case, the controller air (Cabin air, cabin temperature and cabin humidity) is distributed in various trays. They have a wide range of applications in the industries of fat and oil processing, food industry, detergents, pulp and paper industry, tea processing, cosmetics and biosensors [1].

## II. EXPERIMENTAL METHOD

Lipase was purchased from Sigma Aldrich Chem. Ltd. and used without further purification. Solutions were prepared using potassium phosphate buffer in deionized water with electric conductivity lower than  $18.3 \text{ ms}^{-1}$  was obtained from an ultra-pure water distiller (Millipore, MILLI-Q Lab). Solutions of 0.5 and 1mM at variable pH range was prepared using monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and dibasic potassium phosphate ( $\text{K}_2\text{HPO}_4$ ). pH was recorded using Systronics digital (802) pH meter. The dielectric constants and relaxation time of various mixtures of solutions was measured by TDR, the Tektronix model No. DSA8200 digital serial analyzer sampling mainframe along with the sampling module 80E08. TDR dielectric measurement systems consist of step generator, which is produce fast rising pulse of the order of picoseconds. A train of suitable fast rising pulses is applied to a transmission line usually a co-axial line with characteristic impedance  $50\Omega$ . A co-axial line is connected to sampling device (sample holder), the systematic block diagram of the experimental set up for TDR is shown in figure 2. A suitable fast rising pulse  $R_1(t)$  is applied to a transmission line and incident to the sample under study and its reflected part  $R_X(t)$  from the sample solution in the sample holder is shown in figure 3. [2, 3]



**Figure 2:** Systematic block diagram of time domain reflectometry



**Figure 2:** b) Experimental Setup of Time Domain Reflectometry Technique.

### III. RESULT AND DISCUSSION

The complex permittivity spectra of Lipase 0.5 and 1.0 mM. It is seen from the real part of permittivity decreases with increase in frequency. The complex permittivity spectra were fitted to Havriliak–Negami equation using least square fit method [5].

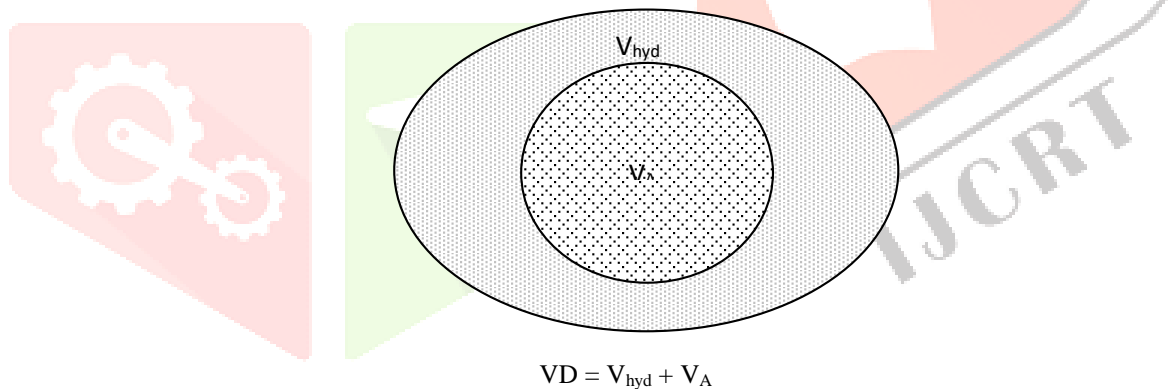
$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{(\varepsilon_0 - \varepsilon_\infty)}{[1 + (j\omega\tau)^{1-\alpha}]^\beta} - j \frac{\sigma}{\omega \varepsilon_v} \quad (1)$$

Where  $\varepsilon_0$  is the static dielectric constant,  $\varepsilon_\infty$  is the permittivity at high frequency,  $\tau$  is the relaxation time,  $\sigma$  is the dc conductivity of the solution, which is essentially due to free ions, and  $\varepsilon_v = 8.85 \times 10^{-12} \text{ Fm}^{-1}$  is the permittivity of vacuum.  $\alpha$  &  $\beta$  are the empirical parameters for the distribution of relaxation times with values between 0 and 1, with  $\varepsilon_0, \varepsilon_\infty, \tau, \alpha, \beta$  and  $\sigma$  as fitting parameters in equation 1. The Havriliak–Negami equation includes three relaxation models as limiting forms: the Cole–Cole ( $\beta = 1, 0 \leq \alpha \leq 1$ ), Cole - Davidson ( $\alpha = 0, 0 < \beta < 1$  and Debye ( $\alpha = 0, \beta = 1$ ) relaxation models [6, 7]. For the systems studied here, the values of  $\alpha$  and  $\beta$  are found to be 0 and 1, respectively, within experimental error. The resulting values of dielectric relaxation parameters for aqueous solution in Table 1.

**Table 1.** Dielectric fit parameters for Lipase in aqueous solutions at 25°C.

C (mM)	pH	$\varepsilon_0$	$\tau$ (ps)	$\sigma$ (mho m <sup>-1</sup> )	$\beta$
0.5	7.1	62.26(4)	8.69(3)	0.09252(5)	0.989(4)
0.5	7.3	68.68(6)	8.75(4)	0.09419(7)	0.991(4)
0.5	7.4	76.02(7)	8.92(4)	1.08471(6)	1.002(6)
1.0	7.1	79.63(8)	8.90(5)	1.8610(2)	1.006(8)
1.0	7.3	81.04(6)	8.87(4)	1.8808(2)	1.009(6)
1.0	7.4	87.13(9)	8.98(4)	1.8976(3)	1.010(9)

Note: The number of bracket denotes uncertainties in the last significant digits obtained the least squares fit method, e.g. 62.26(4) means  $62.26 \pm 0.04$ .



**Figure 3:** Model for the dielectric excluded volume (VD), the effective displacement volume (VA) of the Lipase and the volume of the hydrated water ( $V_{\text{hyd}}$ ).

Therefore, the microwave dielectric excluded volume  $V_D$  is assumed to have a dielectric constant of  $\varepsilon_{\text{ex}} = \varepsilon_{\text{w}\infty} = 5.0$ . The effective permittivity of voids in bulk water may be modeled by electrostatic theory. We define the dielectric excluded volume fraction  $R_V$  as the total volume of the hydrated Lipase molecule ( $V_D$ ) per liter of the solution. The permittivity of the solution can be described by the Maxwell-Wagner formula [7, 14].

$$\varepsilon_s(\omega) = \varepsilon_w(\omega) \frac{2(1 - R_V)\varepsilon_w(\omega) + (1 + 2R_V)\varepsilon_{\text{ex}}}{(2 + R_V)\varepsilon_w(\omega) + (1 - R_V)\varepsilon_{\text{ex}}} \quad (2)$$

where  $R_V$  is the Volume fraction of solute particle and is as follows:

$$R_V = \frac{(\varepsilon_{w0} - \varepsilon_{s0})(2\varepsilon_{w0} + \varepsilon_{w\infty})}{(2\varepsilon_{w0} + \varepsilon_{s0})(\varepsilon_{w0} - \varepsilon_{w\infty})} \quad (3)$$

$R_V = 0$  if the dielectric constant of the voids is  $\varepsilon_{w0} (\varepsilon_{s0} \equiv \varepsilon_{w0})$  and  $R_V = 1$  when  $\varepsilon_{s0} = \varepsilon_{w\infty}$

and

$$V_D = \frac{R_V}{cN_0} \quad (4)$$

where,  $\epsilon_{w0}$  and  $\epsilon_{w\infty}$  are static and high frequency dielectric constant of pure water at 25°C,  $c$  is the concentration of Lipase and  $N_0$  is the Avogadro's constant. The excluded volume fractions are calculated according to equation (3) and plotted against concentration of CsCl in water solution as shown in figure 3.

The mass (in Kilograms) of free solvent per liter is

$$W_{fw} = (1 - R_V)d_0 \quad (5)$$

$$W_{fw} = (1 - R_V)d_0 \quad (6)$$

Where,  $d_0$  is the density of the water.

Therefore, total mass of water in 1 liter of the solution can be found as

$$W_w = d - 10^{-3}Mc \quad (7)$$

Where,  $M$  is the molecular weight (grams/mole) of the Lipase,  $d$  is the solution density (kilograms/liter) and  $c$  is the concentration (moles/liter). The difference between the total mass of water and that of the free water is the mass of hydrated water per liter of the solution. Therefore, the number of hydrated water molecules for each Lipase molecules is given by.

$$N_{hyd} = (W_w - W_{fw})/(0.018c) \quad (8)$$

The weight ratio of bound water mass to Lipase mass is

$$W_{hyd} = 18N_{hyd} / M \quad (9)$$

The hydration number is determined by the charge density at the ion surface, which progressively decreases. As a consequence the ion-water interaction is progressively weaker, with fewer water molecules bound by Coulomb forces in the Lipase solution. The estimated dielectric excluded volume per Lipase, number of hydration water molecules, and derived weight ratio are listed in table 2.

Table 2. Lipase hydration information extracted from the dielectric measurements at 25°C.

C (mM)	pH	Density (gcm <sup>-3</sup> )	VD (nm <sup>3</sup> )	W <sub>hyd</sub>	N <sub>hyd</sub>
0.5	7.1	1.0194	839.19	89	31312.738
0.5	7.3	1.0167	756.17	56	30372.568
0.5	7.4	1.0122	372.15	46	21232.596
1.0	7.1	1.0134	423.11	37	6489.356
1.0	7.3	1.0154	224.09	21	5689.289
1.0	7.4	1.0131	179.06	19	4897.569

#### IV. Conclusion

The dielectric dispersion of aqueous Lipase has been studied at various concentrations using Time Domain Reflectometry. The static dielectric constant, relaxation time and hydration number have been determined. The dielectric data reveal the formation of a solvation sheath of bound water molecules around the ions. As a consequence the ion-water interaction is progressively weaker, with fewer water molecules bounded by Coulomb forces in the Lipase solution.

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