

## **Dielectric properties -Gate way to antibacterial assay: A case study of low density polyethylene/chitosan composite films**

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### **ABSTRACT**

Dielectric properties of low density polyethylene/chitosan composite films have been correlated with its antibacterial activity. The films were molecular-tailored by using palm oil as a plasticizer in an internal mixer. Maleic anhydride and dicumyl peroxide have been used as a coupling agent and free radical initiator respectively. The dielectric properties of the composite films have been studied as a function of chitosan loading, plasticizer and applied frequency. The dielectric constant of the samples was increased by increase in the amount of chitosan in the polythene matrix. The antibacterial properties of the composite films have been studied and found that a distinct inhibitory zone against *Escherichia coli* and *Staphylococcus aureus* has been developed and this zone was increased with chitosan loading. The low molecular weight chitosan based polythene films showed significant improvement in dielectric and antibacterial properties when compared to the native chitosan based films. From all these studies it has been observed that the antibacterial properties of the developed films were complementary with their dielectric properties. This study invokes the possibility of using dielectric properties of composites as a measure of their antibacterial properties.

Key words: chitosan; dielectric properties; antimicrobial activity; FESEM

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## INTRODUCTION

Antimicrobial food packaging has been an area of interest for many years, because it can enhance the safety and quality measures of food items.<sup>1-4</sup> It is one of the promising steps to prevent both contamination of pathogens and growth of spoilage microorganisms on the surface of food products.<sup>5, 6</sup> Simple and low cost method of producing eco-friendly and antimicrobial film is to incorporate an antimicrobial component into a suitable matrix. Cellulose and chitosan are commonly used for the synthesis of biopolymer composites, which are effective carriers of antimicrobial agents.<sup>7, 8</sup> Among these biopolymers, chitosan exhibits inherent antimicrobial properties.<sup>9, 10</sup> It does not require a carrier, and can also be used as a coating or can be cast into polymer films with good strength, barrier properties and biocompatibility.

The poly cationic nature of chitosan interferes with the negatively charged cell membrane of pathogens causes membrane leakages, which prevent the microbial growth by prohibiting their DNA transformation and metabolic activity.<sup>11</sup> A systematic investigation based on the dielectric properties of the polymeric composite films can provide the extent of antimicrobial activity of the same. Dielectric properties of various polymeric systems have been investigated by many researchers.<sup>12-14</sup> In all these reports, it has been found that the dielectric properties of polymeric materials depend on chemical structure, crystallinity, morphological features and presence of fillers or other additives. The dielectric constant of a material is found to increase with the increase in effective dipole moment.<sup>14</sup> It is reported that the introduction of polar components into polyethylene matrix could increase its dielectric constant and dielectric loss.<sup>15</sup> In heterogeneous polymer blends, the dielectric constant of the polymers is influenced by interfacial effects which are due to the polarization arising from difference in conductivities of the two phases.<sup>15</sup> The volume resistivity of various polymeric systems upon the incorporation of fillers has also been studied by researchers.<sup>16, 17</sup> Chitosan can be proposed for making bio-composite films for food packaging because of its good mechanical, oxygen barrier properties and antimicrobial activities. The antibacterial and antifungal activity of chitosan, arising from its polycationic nature, is well known for a variety of bacteria and fungi.<sup>18, 19</sup>

Low density polyethylene (LDPE) is a low cost material with good processability, excellent electrical insulation properties, good chemical resistance, high flexibility and low water vapor permeability.<sup>20, 21</sup> LDPE-Chitosan composite films were developed in this work is by using palm oil as a plasticizer. This plasticizer is a renewable resource, biodegradable, non toxic and it can act as a more effective coupling agent. The morphology, structural analysis, mechanical and thermal properties of the developed films were investigated earlier and the results were already reported by

our research group.<sup>22</sup> It has been found that the mechanical, thermal and biodegradation properties of plasticized films were improved when compared to the unplasticized composites. The optimum value of tensile strength and elastic modulus of the samples were found 11.77 MPa and 86.36 MPa respectively at 10 wt. % chitosan loading in LDPE matrix.<sup>22</sup>

The current work is the first attempt to correlate the dielectric properties and antibacterial properties of these bio-composite films. The observations will be promising in accessing the extent of antibacterial activity by dielectric property measurement in bio-composite films for food packaging applications.

## **EXPERIMENTAL PROCEDURE**

### **Materials**

The film grade LDPE (0.923 g/cm<sup>3</sup> density, 6.0 g/ 10 min Melt Index and 87.22 °C softening point) was supplied by Reliance Industries Limited, Mumbai, India. Powdered chitosan was obtained from India Sea Foods, Kochi, Kerala, India, with degree of de-acetylation 80.2 % and molecular weight 3.0200×10<sup>5</sup> g/mol. The chitosan dried at 100 °C for 5 hours prior to the mixing. Refined grade palm oil (0.924 g/cm<sup>3</sup> density) has been provided by Parisons Pvt. Ltd. KINFRA Park, Malappuram, Kerala, India. All other reagents such as dicumyl peroxide (DCP) and maleic anhydride (MA) were of analytical grade and obtained from Sigma-Aldrich (India).

### **Sample preparation**

#### **Preparation of low-molecular weight chitosan (LWCS)**

The dried prawn chitosan flakes were added to 1 % (W/V) acetic acid solution in a reaction vessel, then allowed to dissolve and stirred for 30 min. The reaction mixture was precipitated with 50 % (W/V) NaOH. The resulting suspension was stirred at 3600 rpm and 160 °C for 5 hrs. Then the mixture was diluted with distilled water followed by centrifugation. Next, the suspension was transferred to a dialysis bag and dialyzed in running water for 2 hrs and then overnight in distilled water until a pH 7 were reached. The chitosan precipitate was rinsed with distilled water, freeze-dried and analysed. The de-acetylation degree of the resulting chitosan powder was assigned to 94.4% using Ultraviolet-Vis Spectrophotometry.

The de-acetylated chitosan was taken in 0.1M HCl in a reaction vessel and stirred for 30 min. Then 15% H<sub>2</sub>O<sub>2</sub> was poured in to the reaction mixture. The solution was heated at 65 °C stirred at 3600 rpm for 2 hrs and then filtered. To the filtrate 2M NaOH was added till pH become 7 and excess

ethanol added to precipitate the product. The chitosan precipitate was dialyzed in running water for 2 hrs and then overnight in distilled water. The dialyzed chitosan precipitate was freeze-dried and analysed. Molecular weight of the chitosan was measured by the viscometry method. In the present study it was found that molecular weight of the chitosan was reduced into  $1.473 \times 10^3$  g/mol from the initial value of  $3.0200 \times 10^5$  g/mol. The microscopic images of native chitosan and de-acetylated low molecular weight chitosan (LWCS) particles are shown in the Figure 1. The native chitosan is appeared as flake like structures but its de-polymerised sample appeared as in spherical shape with a considerable size reduction.

### Preparation of composite films

The compounds were prepared in a 'Thermo HAAKE PolyLab System', equipped with roller type rotors. The mixing was done at a rotor speed of 60 rpm and at a temperature of 140 °C. Initially LDPE was allowed to melt for 3 minutes. The DCP, MA and varying amounts of the bio-filler chitosan (0, 5, 10 and 15 wt. %, which are abbreviated as A1, A2, A3 and A4) were added in to the LDPE matrix within 2 minutes time intervals for each sample. Mixing was continued for another 4 minutes. The composites obtained from melt mixing were compressed using an electrically heated hydraulic press for 3 minutes at 150 °C under a pressure of 200 Kg/cm<sup>2</sup>. After pressing, the samples were allowed to cool at room temperature. The mechanism and role of dicumyl peroxide and maleic anhydride in the fabrication of composite films has been showed in Figure 2.

A second set of composite films (B1, B2, B3 and B4) containing fixed amounts palm oil, which added prior to the addition of the cross-linker, have been fabricated and tested. The amount of palm oil in the matrix has been optimized as 2.5 wt. %. Plasticized composite films based on the de-acetylated low molecular weight chitosan (LWCS) have also been prepared under the same formulations and conditions. The 5, 10 and 15 wt. % of de-acetylated low molecular weight chitosan composite films were abbreviated as nB2, nB3, and nB4 respectively. The formulation of the fabricated composite films has been given in Table 1.

### Morphology

The morphology was investigated by field emission scanning electron microscopy (FESEM) JEOL JSM-820, Hitachi SU6600 FESEM. The samples were sputter coated with gold to avoid subsequent charging before analysis by FESEM.

## Dielectric properties

Dielectric measurements were carried out at room temperature using an impedance analyzer. The impedance was measured with HOIKY 3532-50 LCR HI-Tester that has been interfaced to a computer, by applying 'ac' signal across the film with blocking silver electrode. This bridge has been set to measure the impedance and phase angle from 42 Hz to 5 MHz. The dielectric constant and 'ac' conductivity were calculated from the measured data by knowing the dimension of the film.<sup>23</sup>

## Antimicrobial activities

The testing of antimicrobial activity of the composite films was carried out using the agar diffusion method according to Pranoto et al.<sup>24</sup> The composite films were cut into 10 mm diameter discs and then placed on Mueller Hinton agar plates (Merck, Darmstadt, Germany). These had been previously seeded with 0.1 ml of inoculums containing tested bacteria. The plates were then incubated at 37 °C for 24 hours. Observation on the diameter of the inhibitory zone surrounding film discs and contact area of composites with agar surface were made. Experiments were done in triplicate.

## RESULTS AND DISCUSSION

### Dielectric properties

Dielectric measurements were performed on the samples for studying the molecular behavior of polymeric materials, which is very important in the area of effective packaging. The charge interaction between the film surface and microbial organism will decide the antimicrobial properties of the packing materials.<sup>25, 26</sup> The dielectric constant, ' $\epsilon_r$ ' can be determined from capacitance by using the equation (1).

$$\epsilon_r = \frac{CL}{\epsilon_0 A} \quad (1)$$

Where ' $C$ ' is the capacitance, ' $L$ ' the sample thickness, ' $\epsilon_0$ ' the permittivity of air ( $8.85 \times 10^{-12} \text{ Fm}^{-1}$ ) and ' $A$ ' is the area of cross section of samples. The dielectric constant of a composite has contributions from interfacial, orientation, atomic and electronic polarizations of component molecules.<sup>23</sup> The interfacial polarization occurs in a composite due to the differences in the conductivities or the polarizations of the matrix and fillers.<sup>27</sup> When polymers containing polar groups are placed in an electric field, polarization occurs due to the orientation of dipoles. The orientation and interfacial polarization of a composite will mainly depend upon the concentration of fillers.<sup>27</sup>

Figures 3 show the variation of dielectric constant and loss with applied frequency for various unplasticized composite films. In Figure 3, A1, A2, A3 and A4 represents bio-composites with 0, 5, 10 and 15 wt. % of chitosan respectively. It can be seen from the Figure 3 that the pure LDPE exhibited a lowest value of dielectric constant ( $\epsilon_r$ ). This is due to the absence of permanent dipoles in the polythene matrix. The ' $\epsilon_r$ ' values increased with chitosan loading in composites, since dipole increases as the weight percentage of filler increases in the matrix. This observed increment in ' $\epsilon_r$ ' had been attributed to the increment in orientation and interfacial polarizations resulting from the presence of polar  $\text{NH}_3^+$  ions in chitosan. It can also be seen from Figures 3 that dielectric constant decreased with increase in frequency. The complete orientation of molecules required more time to reach the equilibrium static field value compared to the electronic and atomic polarizations. Therefore as frequency increased, the rotational motion of the polar molecules of dielectric is not sufficiently rapid for attain of equilibrium with the field.<sup>28</sup> Figure 4 shows the dielectric constant of the plasticized composite films and it follows the same trend as in the case of unplasticized films. It is found that there is an overall increase in the dielectric constant for plasticized samples compared the unplasticized samples.

Plasticizer molecules are relatively small in size as compared to that of polymer matrix molecule. It can easily penetrate into the polymer matrices, causing an interaction between plasticizer molecule and polymer chain. This may reduce the cohesive forces operating between the polymer chains resulting in an increase in chain segmental mobility. When dipoles are directly attached to the chain, their dipole moment will depend on the segmental motion of the polymeric chain.<sup>23</sup> Hence the incorporation of palm oil plasticizer in LDPE/chitosan composites brings out reduction in rigidity, which increases segmental mobility and enhances the value of dielectric constant. The scanning electron micrographs of the unplasticized and plasticized composite films with 15 wt. % chitosan loading are shown in the Figures 5(a) and 5(b) respectively. It is observed that relatively uniform filler dispersion in the case of plasticized composite film (b) as compared to its unplasticized congener (a). Due to the presence of palm oil plasticizer polythene matrix become more flexible and thus chitosan particles can distribute uniformly when compared to the rigid unplasticized matrix. Thus morphological study of the developed composites was also support the dielectric properties.

The electrical conductivity ( $\sigma$ ) of composite films was measured by using the following equation.

$$\sigma = \omega \epsilon_0 \epsilon_r \tan \delta \quad (2)$$

Where ' $\omega$ ' is the angular frequency which is equal to  $2\pi\gamma$ , ' $\gamma$ ' the applied frequency and ' $\tan\delta$ ' is the dissipation factor. Figure 6 showed the dependence of ' $\sigma$ ' electrical conductivity on chitosan

loading at different frequencies. LDPE has excellent insulation property with a larger band gap, lower concentration of free charge carriers and small carrier mobility. Therefore, in the case of LDPE we can ignore the effects of electrical conductivity ' $\sigma$ '. It is observed that on increasing chitosan concentration and frequency, the 'ac' conductivity is also increased. This is due to the increment in number of polar groups incorporated by chitosan loading. On chitosan loading, the fillers are more tightly packed and pressed against each other, resulting in the reduction of internal contact resistance with increased crystallinity. Thus, the net resistance (internal contact resistance and resistance of the fillers) is decreased with increase in chitosan loading level. When the contact resistance becomes very much lower, the aggregate resistance is contributed to the net resistance at lower and higher frequencies.<sup>29, 30</sup> Thus as chitosan loading increases, due to better conductive network the composite systems crossed percolation threshold and thus increases the conductivity.

The study of volume resistivity ( $\rho$ ) of an insulating material is important because the most desirable property of an insulator is its ability to resist the leakage of electric current. It is the reciprocal of electrical conductivity.

Figure 7 shows the plot of volume resistivity of unplasticized films as a function of logarithm of frequency at different loadings of chitosan. The volume resistivity decreases with increase in frequency and filler concentration. Since chitosan is hydrophilic in nature, they absorb moisture which contributes to the increased polarization resulting in a decreased volume resistivity with an increment in filler loading. It is well known that in any polymeric material, most of the charge transfer is through the crystalline regions, and the amorphous (non-crystalline) ones allow the static charge transfer due to the presence of moisture.<sup>31, 32</sup> As suggested earlier, with the incorporation of chitosan, the polarity of the system increased also due to the increased crystallinity. This increases in the orientation polarization as well as the interfacial polarization results in the increase in dielectric constant and a decrease in resistivity.

Figure 8 shows dielectric constant of the 15 wt. % chitosan (B4) and its de-acetylated low molecular weight (nB4) based composite films. From the Figure 8 it has been found that de-acetylated low molecular weight chitosan based system showed significant increment in the dielectric constant when compared to its high molecular weight counterpart. In the case of de-acetylated low molecular weight chitosan particles, total number of polar hydroxyl groups and  $\text{NH}_3^+$  ions in the polymer chain get increased and lead to an increased dipole in LDPE/de-acetylated low molecular weight chitosan composite films. The size of filler particles were also plays a crucial role in the dielectric properties of composite films.<sup>33, 34</sup> The size of the LWCS particles were smaller than the high molecular weight counterpart and it is better revealed from the FESEM images (Figure 9), where chitosan particles have spherical form and dispersed uniformly in the polythene matrix. The dielectric

constant increases linearly with decrease in particle size of the filler. This is due to the smaller particles with higher surface area can provide better particle to particle contact. Due to the close packing of filler, the polarization arising from the dipole-dipole interaction will be enhanced. The addition of de-acetylated low molecular weight chitosan particles enhances the cationic charge density in the bio-composites as revealed by the dielectric study. This positive charge density enables these composite films to interact with negatively charged microbial cell membranes, polymers, macromolecules and even with certain polyanions upon contact in aqueous environment. So the significant charge interaction of the LDPE/de-acetylated low molecular weight chitosan films can be exploited for the antibacterial food packaging applications.

### Antimicrobial activity

The dielectric studies of the plasticized and unplasticized composite films, revealed the important role of amino groups of chitosan which can furnish  $\text{NH}_3^+$  ions into the LDPE matrix. These positive ions can interact with the negatively charged cell walls of the bacteria and hinder the growth of the same.<sup>35</sup>

The optical photographs in Figures 10 (a) and (b) shows the typical antimicrobial test results of the plasticized composite films (B1, B2, B3 and B4) against *Escherichia coli* and *Staphylococcus aureus* respectively. The listed inhibitory activities were estimated from the area measurement of clear inhibition zones surrounding the film discs in the agar plates. It can be seen from the figures that there was a negligibly small inhibition zones surrounding the pure LDPE film (B1). From the antimicrobial activity result of B1 it is clear that the additives plasticizer, MA and DCP have not much significant role in the antibacterial activity of the developed composite films. But in the case of chitosan loaded composites distinct inhibitory zones have been developed by surrounding the films. This inhibitory action is due to the antibacterial property of chitosan present in the film. The antibacterial properties of chitosan were attributed to the electrostatic interaction between electronegative microbial surface and positively charged chitosan. This charge interaction leads to the damage of microbial cell walls and the leakage of the intracellular constituents, which leads to the binding of chitosan molecules with DNA and the inhibition of mRNA synthesis in microbial organisms.<sup>36-38</sup> The experimental inhibition area for the various plasticized composite films against *Escherichia coli* are shown in Figure 11. It can be seen from the Figure 11 that the inhibition zone diameter increases with increase in chitosan loading. This is due to the charge density ( $\text{NH}_3^+$ ) increase with the increase in the chitosan content that is also observed in dielectric studies. The significant microbial cell wall inhibition taking place with the chitosan loads in composite



systems.<sup>39,40</sup> But in the case of pure LDPE film such charge density cannot be seen and hence resulted in a least inhibitory zone diameter. It is seen that from the limited number of microorganism tested; the inhibitory activity of LDPE/chitosan composite film is restricted to gram-positive and gram-negative species.

Figure 12 shows the antibacterial test results of B4 and nB4 films against *Escherichia coli*. The LDPE/de-acetylated low molecular weight chitosan composite films exhibited distinct and larger inhibitory zone diameter against microbial organism than its high molecular weight counterpart. This is because of the higher polycationic nature of the developed de-acetylated low molecular weight chitosan based films, it was revealed by the dielectric study (Figure 8). The antimicrobial activity of the tested samples is in accordance with their dielectric behaviour. This correlation will be helpful for the assessment of antibacterial activity of the bio-composite films with polar fillers without a biological test. So the dielectric property measurement can be taken as a crucial tool for ensuring the antimicrobial activity of the eco-friendly food packaging films.

## CONCLUSIONS

The newly developed plasticized LDPE/chitosan film is promising as a food packing material with excellent antimicrobial activity. The dielectric properties such as dielectric constant, volume resistivity and conductivity of LDPE/chitosan composite films have been studied as a function of filler loading, frequency and plasticizer. The results were correlated with the antibacterial activity of the film. The maximum values of dielectric constant at the lower frequency region have been attributed to the interfacial polarization. An increment in dielectric constant of composites with filler loading was due to the increased orientation and interfacial polarizations. The addition of palm oil as a plasticizer increased the segmental motion of the polymeric chains and filler dispersion in the matrix, which further increased the dielectric constant of the composites. The volume resistivity of the composites was found to be decreased with chitosan loading. The conductivity of composites was also found to increase with increase in filler loading. From the study of antimicrobial activity, plasticized LDPE/chitosan composite film developed an inhibitory zone diameter against *Escherichia coli* and *Staphylococcus aureus*, which increases with the chitosan content in the films. This is due to the electrostatic interaction between the negatively charged microbial cell membrane and positively charged chitosan in the composites. The effect of molecular weight of chitosan on the dielectric and antimicrobial properties of the composites have been also studied in the present research work. It was found that the dielectric constant and the antibacterial properties of the samples increases with decrease in the molecular weight of chitosan. It is concluded that the antibacterial

properties of the developed films were complementary with their dielectric properties. This study highlights the possibility of using dielectric properties of composites as a measure of their antibacterial properties.

## ACKNOWLEDGEMENT

The authors are grateful to Kerala State Council for Science and Technology and Environment (616/2012/KSCSTE), Thiruvananthapuram, Kerala, India for awarding a project under thrust area. The authors also like to thank Mr. Gopi K. S. and Miss. Sreebha K., MRCMPU Ltd., MILMA, Kerala, India for the antimicrobial studies.

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**TABLE 1:** Formulation of the composite films

Sample	LDPE (gm)	Chitosan (Wt. %)	DCP (Wt. %)	MA (Wt. %)	Palm oil (Wt. %)
A1	100	-	0.5	2	-
A2	100	5	0.5	2	-
A3	100	10	0.5	2	-
A4	100	15	0.5	2	-

Figure 1: Scanning electron micrographs of pure chitosan (a) and low molecular weight chitosan (b) particles.

Figure 2: The mechanism of maleic anhydride grafting on LDPE by using dicumyl peroxide as a free radical initiator.

Figure 3: Effect of filler loading on dielectric constant as a function of frequency where (A1) pure LDPE, (A2) LDPE/5 wt. % chitosan, (A3) LDPE/10 wt. % chitosan and (A4) LDPE/15 wt. % chitosan composite films.

Figure 4: Effect of filler loading on the dielectric constant of the plasticized film as a function of frequency where (B1) pure LDPE, (B2) LDPE/5 wt. % chitosan, (B3) LDPE/10 wt. % chitosan and (B4) LDPE/15 wt. % chitosan composite films.

Figure 5: Scanning electron micrograph images of LDPE/15 wt. % chitosan composites (a) unplasticized and (b) plasticized films.

Figure 6: Effect of chitosan loading on conductivity of plasticized composites films at various Frequencies.

Figure 7: Effect of chitosan loading on volume resistivity as a function of frequency. Where A1, A2, A3 and A4 are the 0, 5, 10 and 15 wt. % of chitosan loaded unplasticized LDPE composite films.

Figure 8: Effect of molecular weight of chitosan on the dielectric constant of the composite films as a function of logarithm of frequency. Where B4 and nB4 are plasticized 15 wt. % chitosan and its de-acetylated low molecular weight chitosan based composites respectively.

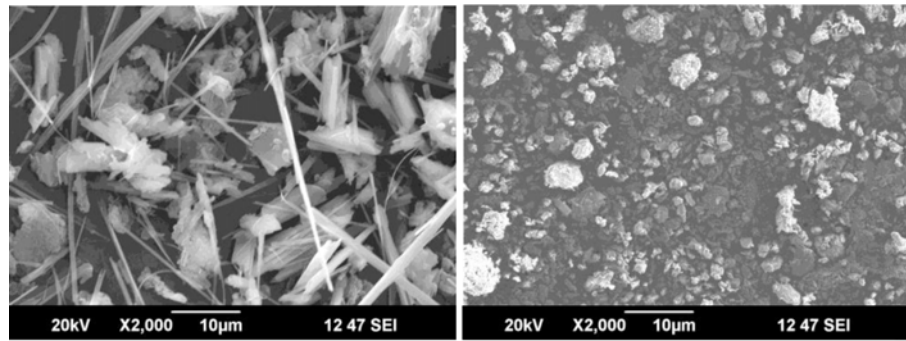
Figure 9: Scanning electron micrograph images of (a) LDPE/15 wt. % chitosan composites and (b) LDPE/15 wt. % de-acetylated low molecular weight chitosan composite films.

Figure 10: The results of antimicrobial test of the plasticized composite films against (a) *Escherichia coli* and (b) *Staphylococcus aureus*.

Figure 11: Effect of chitosan loading on inhibitory zone development against *Escherichia coli*.

Figure 12: The results of antibacterial tests of the B4 and nB4 films against *Escherichia coli*.

Figure 1



(a)

(b)

Figure 2

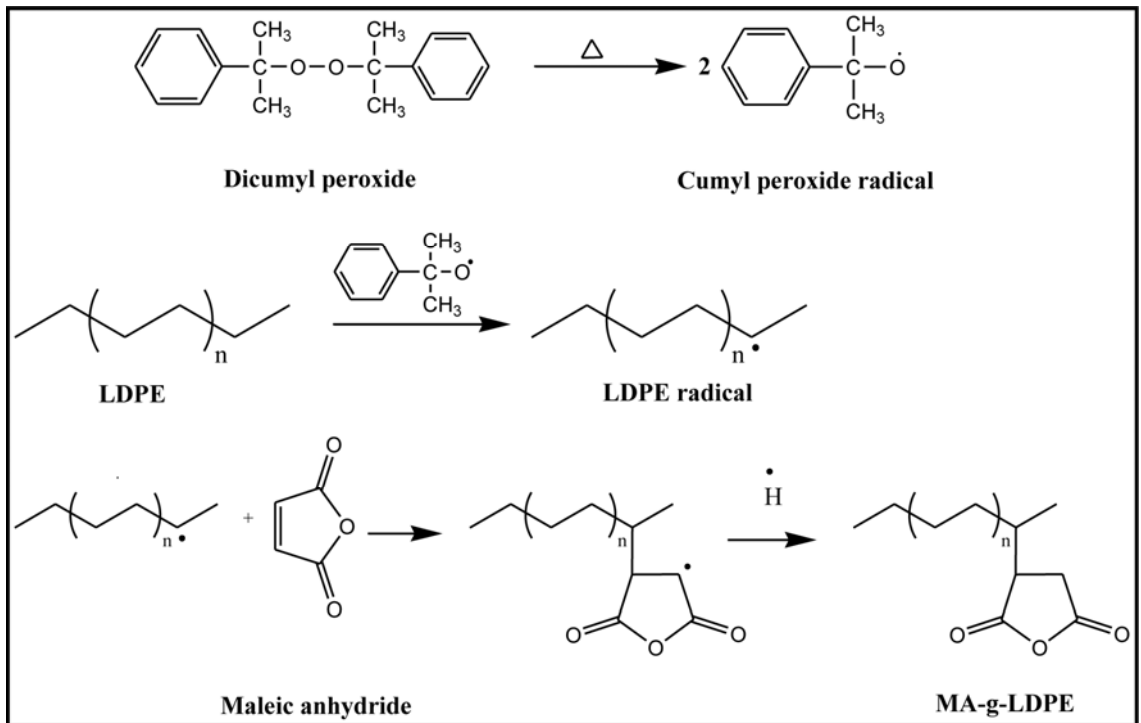


Figure 3

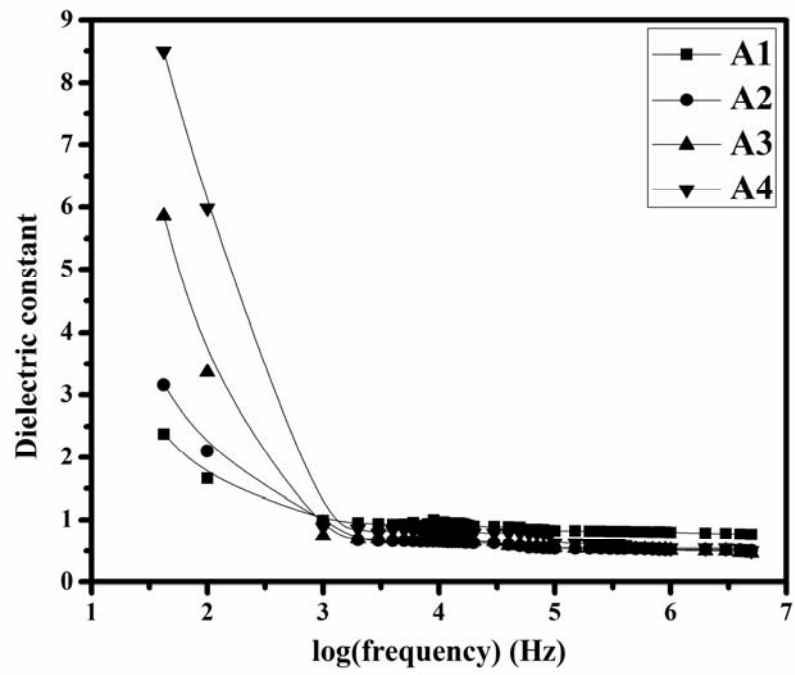


Figure 4

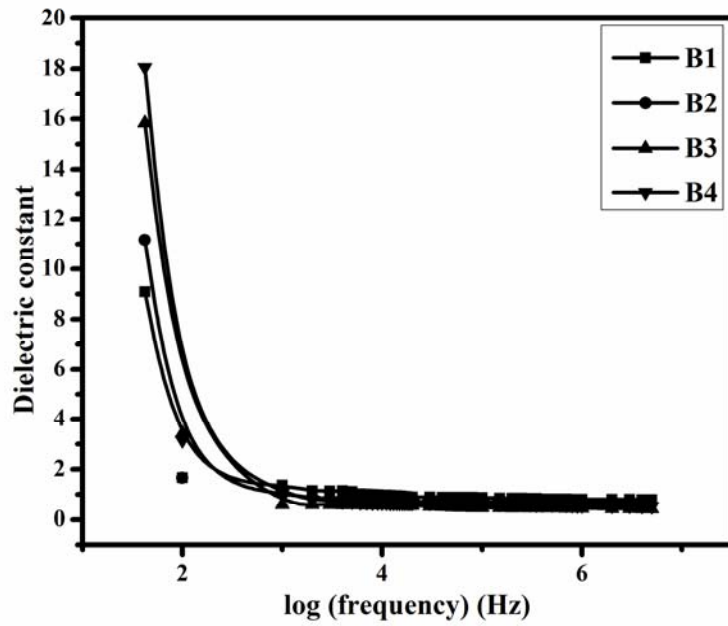




Figure 5

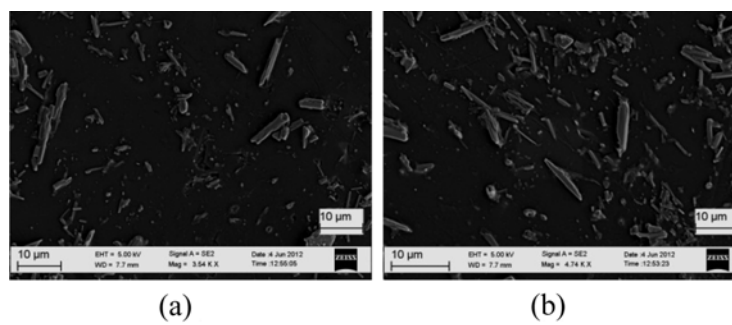


Figure 6

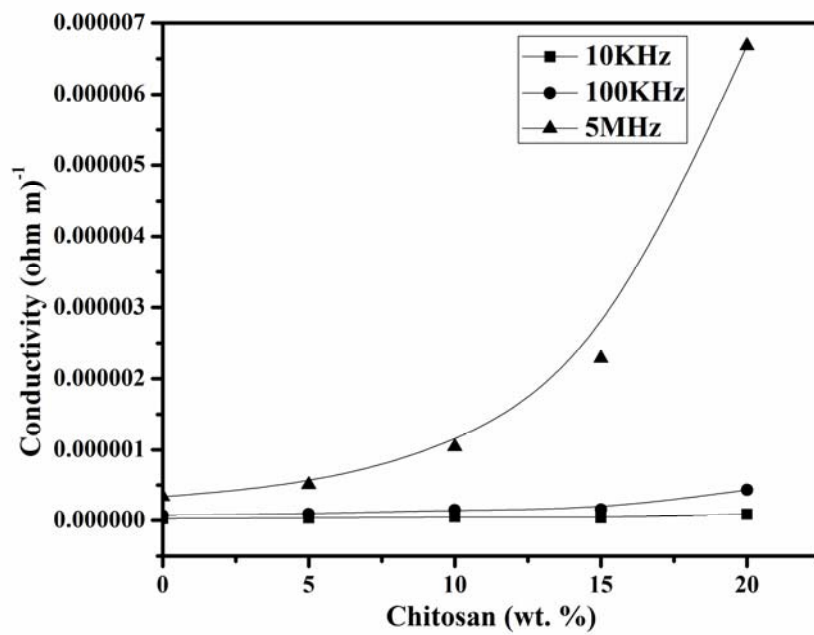


Figure 7

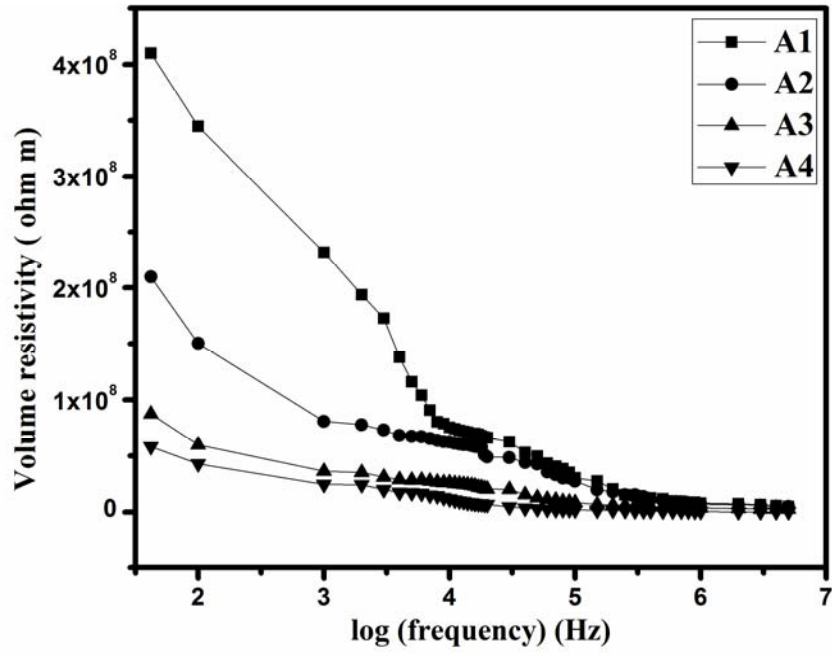
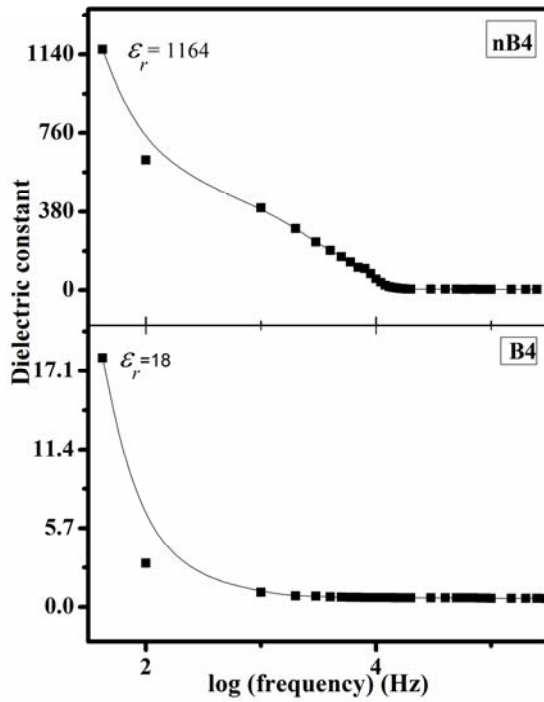
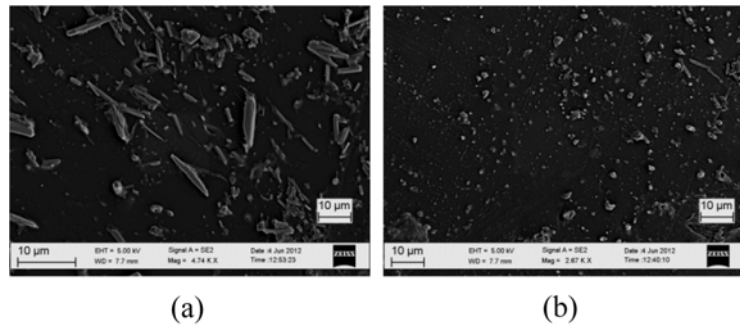


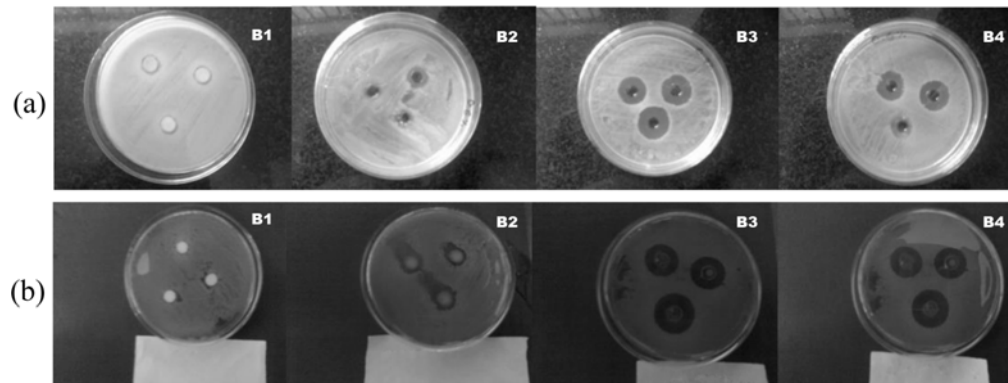
Figure 8



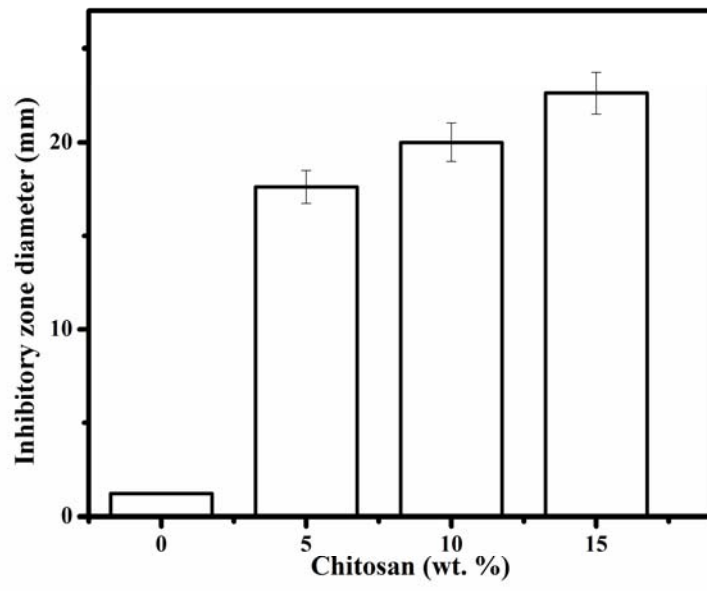
**Figure 9**



**Figure 10**



**Figure 11**



**Figure 12**

