Polyethylene Functionalized with Antifouling Acriflavine via Maleic Anhydride Grafting

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I dedicate this thesis to my mother khadija (late) and father Hussain Akhtar and my siblings Asma Mubeen Areeba Yasmeen and mubasher Hussain and teachers for their huge support, encouragement and motivation.

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List of Abbreviations

MAH	Maleic anhydride
BPO	Benzoyl peroxide
MAH-g-LDPE	Maleic anhydride grafted low density polyethylene
UV	Ultra Violet
HDPE	High density polyethylene
LDPE	Low density polyethylene
ASTM	American society for testing and materials
FTIR	Fourier transform infrared spectroscopy
SEM	Scanning electron microscopy
DSC	Differential scanning calorimetry
MFI	Melt flow index
UTS	Universal Testing Strength
DMF	Dimethyl Form amide
TBT	Tributyltin moiety
AF	Acriflavine
DNA	Deoxyribonucleic acid
PEG	Polyethylene Glycol
IR	Infrared
CFU	Colony Forming Unit

Abstract

Fouling is among the most important global issue now a day that adversely effects many fields including food preservation, purification of water, marine industry, biomedical implants and many more. There is large number of techniques and material systems are employed to inhibit bacterial growth or to disinfect underline bio-films. These are used in a variety of forms including copolymers, coatings, polymer brushes and polymer grafting. In considering the importance of antifouling polymers, polyethylene functionalized with acriflavine via maleic anhydride grafting can be consider among the best antifouling material. This is because of the wide applications of polyethylene. In present study, low density polyethylene was grafted with maleic anhydride with variable concentrations and categorized as low medium and high grafted PE. The LDPE was taken in internal mixer and reacted with BPO and MAH by varying their concentration. Significant torque changed indicated the transformation of LDPE from solid to melt and functionalization. The MA grafted PE sample were further purified and unreacted polymer left was removed. The grafted copolymer was dissolved in DMF solvent and mixed with solution of acriflavine using trimethylamine as catalyst. This solution was then refluxed for 42 hours at 70^0C. To humidify dark red solution was obtained that was then freeze dried at -130^0C. Amine group of acriflavine found to react with maleic anhydride functional group as verified by FTIR. Two peaks of amide were obtained between 3000 and 3500 cm^{-1}. The prepared grafted copolymer were further characterized for its In addition mechanical testing indicated that upon MAH grafting, LDPE found to become more tough. The resulting modified functional antifouling LDPE grafted with acriflavine via MA was then tested for its antibacterial activity against *E. coli and S. aureus*.

Chapter-1

Introduction

1.1: Background

Biofouling is the deposition of microorganisms, plants, algae, or animals on wetted surfaces. These types of deposition are termed as epi biosis when surface of a host is specie and relationship between them might not be parasitic. Antifouling is the ability of specifically prepared or synthesized materials and biotic to reduce the biofouling by organisms on wetted surfaces. Biofouling occurs almost anywhere where water is present, it damages to a vast variety of materials and numerous industries including biomedical environment, water and energy [1]. In industries, bio-dispersants are been used to combat biofouling process. In some environments, organisms may be killed by using coatings and biocides. There are also some nontoxic mechanical strategies which prevent organisms from attachment on the surface that include selection of a material and coatings containing a slippery surface, generation of an ultra-low fouling surface and the use of zwitterions and creation of nanoscale surface similar to the skin of sharks and dolphins that provides poor anchoring points. In the food industries, the problems of biofilms and biofouling are important. The microbes may actively adhered or passively attached on the surface, multiplying but not in all cases there is also a bio transfer potential, it is the ability of microorganisms adhered on equipment surfaces before and after cleaning processes to contaminate products during processing. Outcomes would be product spoilage, or, if pathogenic microorganisms are adhered, food borne diseases, that impact upon food quality and safety. In addition, for the food processor safe cost effective and proper functioning of equipment is important. Deposition of marine

fouling organisms on surfaces such as ship hulls may cause a lot of problems, which include extra energy consumption, high cost of maintenance, and increased level of corrosion. Marine antifouling is a very important problem now a day. Physical and biochemical advancement in the field of marine biofouling, it includes biofilm formation and macro-organism settlement[1, 2]. There are varieties of methods that are used as antifouling. Biocides are chemical substances it prevents the growth of microorganisms that are responsible for biofouling. The chemical substances which deter the microbial growth are attached or immersed into an anti-fouling coating, either through physical adsorption or through chemical modification of the surface. Biofouling basically occurs after formation of a biofilm on surfaces. The biofilm is a surface or medium onto which larger microorganisms attach themselves and growth take place. In marine environments, this growth usually occurs with barnacle attachment on the surface.

The biocides prevent the attachment of microorganisms that create the initial biofilms such as bacteria. When these are dead, are not able to spread and can be easily from the surface. Biocide and anti-fouling agent which is used most commonly is the tributyltin moiety (TBT). It deters to both microorganisms and aquatic macro organisms. To control biological growth biocides are added to pool water, drinking water, and liquid lines etc. TBT and other tin-based anti-fouling substances that were used on marine vessels causes environmental hazardous. TBT is very hazardous to marine organisms, e.g. oysters and molluscs. Very minute quantity of tributyltin moiety (TBT) is even cause of defective growth in the oyster. Alternatively, chlorine based anti-fouling solutions were used. But such type of coatings was also harmful to marine life. Copper compounds are used in paints, and can be used as metal sheeting but there is still doubts as to the safety of copper to marine life. Biological extracts

of secreted metabolites and enzymes are used to serve as antifoulants. Physical methods such as modification of surface topography, hydrophobic properties, and charge potential are also used to prevent and reduce biofouling. The physical antifouling technologies are the most effective antifouling, because they have broad and have level of toxicity close to zero[1].

1.2: Low Density Polyethylene

LDPE has branched chains that are attached to the main polymer backbone. Because of its branched structure, it does not pack tightly and have amorphous structure and low density. It is resistant to chemical, impact and moisture. It has high thermal expansion and have impact resistant from -40 to 90°C. LDPE have a density range of 0.910-0.940 g/cm^3 and have a very high degree of short and long chain branching and chains do not pack together into a crystal structure. Therefore, have less intermolecular attraction as instantaneous dipole induce dipole interaction is least. It has lower tensile strength and increased ductility. It is created by addition polymerization and has unique flow properties.

Physical properties Value

Tensile strength	0.20-0.40 N/mm^2
Impact strength	no break
Thermal co-efficient of expansion	100-220$\times 10^6$
Melting point	110 ^{0}C
Glass transition temperature	-125 ^{0}C

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1.4: Acriflavine

Acriflavine also named Acriflavinium chloride is an antiseptic dye. It is extracted from coal tar. Hydrochloride in the structure and the base neutral acriflavine that is less irritating both are odourless, reddish brown powders.
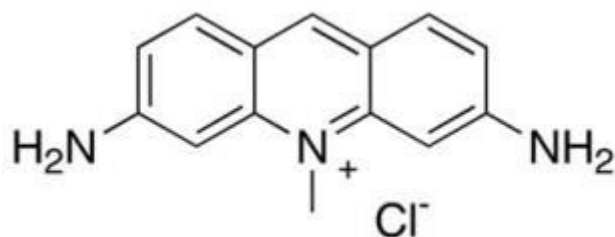
Acriflavine is also an antimicrobial dye its structurally is related to acridine. The acridine is a slow-acting antiseptic. These are bacterio static against Gram-positive bacteria, but less effective against Gram-negative bacteria, and are ineffective against spores. Their antibacterial activity is increased in alkaline media and is not decreased by tissue fluids. Acriflavine is also used in the treatment of infected wounds and for skin disinfection. However, very long treatment can delay wound healing. It is also used for the treatment of many infectious diseases like gonorrhoea, intestinal infections, diphtheria, pneumonia, cholera and infected wounds etc. It also has anticancer activity and is being used in AIDS treatments. Acriflavine is derived from acridine and were firstly synthesized in 1912 were used during the First World War, as topical antibacterial against sleeping sickness. It is soluble in water and insoluble in organic solvents such as ether etc. Systematic IUPAC name of acriflavine is 3, 6-diamino-10-methylacridine-10-ium chloride and molecular mass of Acriflavine (AF) is 259.73 g mol^{-1}.

Physical state	orange to brown powder
Melting point	260 ^{0}C
Specific gravity	250 g/l
Solubility	soluble in water
Stability	stable at room temperature

1.4: Scope of Study

The adverse effect of antifouling is already discussed. The main idea was to develop the functional antifouling system. From the literature review it was concluded that the antifouling system are effective against certain type of micro-organisms like certain species of bacteria. Certain bacterial colonies are very harmful in drinking water, a large portion of our society is not entertained with pure and clean water by drinking contaminated water a lot of water born disease spreads in human being like cholera etc. These are very dangerous diseases if remain unchecked and micro-organisms also cause a vast spoilage of food everywhere as well as cause major food borne diseases in human being and preservation of food is also a difficult task due to growth of these micro-organisms.

Similarly, there are biofouling related issues in marine industry. Algae and other micro-organism are involved in bio corrosion and cause pitting and deterioration of the ship surfaces as well as it can cause extra fuel consumption and energy loses. Bio-organisms also create a lot of problems in industry of medicines e.g. dental and orthopaedic implant etc. There are large number of systems that are worst affected by bio-fouling and corrosion. The main idea of the author is to synthesize a functional antifouling polymer which would be nontoxic and economical.

1.5: The present approach

The present work is focussed on the development of the functional antifouling polymer and studied its antibacterial properties. Polymers are most widely used materials now a day worldwide. These are the cheapest materials with wide range of properties. There are large number polymers are known which are being used as antifouling. Some of the examples of such types of polymers are functionalized polyethylene, polyethylene oxide, polyacrylamide, polyglycerol polystyrene etc. In recent research work, low density polyethylene was grafted with maleic anhydride and the acriflavine functional group (antibacterial and as well as anticancer) were attached to the grafted polymer and a new antibacterial group were synthesized. A total of 10 samples of polyethylene and maleic anhydride were prepared.

The amount of maleic anhydride was varied in each sample. Out of these ten samples the one with highest concentration of maleic anhydride grafted onto polyethylene were selected for further reactions. The grafted polyethylene was purified by dissolving it and then precipitation in acetone. Grafted polymer was collected by filtration, washed many times with acetone, and dried in the form of powder. The acriflavine group were attached to the grafted polyethylene by refluxing at $70^{o}C$ for 42 hours. As acriflavine group have excellence antibacterial properties. After attachment to the grafted polyethylene it imparts the antifouling properties to the polymer as well. The antifouling polymer was tested for *E.coli and S.aureus* bacteria. The agar diffusion method, well diffusion method and shake flask method proved the excellence antibacterial properties of polymer. The functionalized antifouling polymer have tendency to be used as antifouling agent.

The main research objectives are following:

Part I

Grafting of Maleic Anhydride onto the main chain of LDPE using BPO as Initiator in lab scale internal mixer by adding density and processing conditions and recipie preparation in lab scale internal mixer software and providing such a temperature at which maleic anhydride graft on LDPE.

Part II

Attachment of acriflavine on functionalized LDPE for its application as antifouling polymer.

Chapter-2

Literature Review

Low-density polyethylene mechanical properties can be improved by using filler such as calcium carbonate. For materials to fracture at elongation filler compatibility with a matrix is not enough. To increase compatibility 5 or 10% of compatibilizers can be introducing such as butadiene modified with maleic anhydride and triblock copolymers of styrene. By direct grafting of maleic anhydride onto low-density polyethylene[3] good results can also obtained.

Maleic anhydride has good releasing properties and can be grafted onto low density polyethylene by many methods. One of the methods used is extrusion process / batch mixing in a batch mixer[4]. Direct melt-compounding is simpler than other techniques and requires less capital investment because it's environmentally friendly, and the most popular commercially[5].In this method, several conditions such as temperature, mixing speed ,mixing time, screw design and rotor enhance final properties of product. However, each parameter has an individual effect and complex interactions with other parameters, so optimisation is required[6].

One of important factors is processing temperature, to produce better mixing it's to be set in the optimum range. Processing temperature be kept as low as possible, however for low melt flow index (MFI) polymers, temperature should be high without degrading the polymer. Processing temperature has strong influences on cooling rate, thermal history and melts temperature. It also affects the molecular orientation morphology of final product, and crystallization in polymers like PE because the number of nuclei has been affected by the melting temperature, the the

size of spherulites, thickness of lamellae, as well as degree of crystallinity. Residence time also influences properties because of its effect on polymer diffusion and particle dispersion. It is also found that exfoliation of nano-particles in a polymer matrix has been increased by screw having long residence time [7]. Polymer diffusion into nano particle galleries was due to better dispersion of nano-composites. It can be concluded that the better the final properties can be obtained from by increasing the time for diffusion. At high shear conditions, the morphology was exfoliated at longer times in a twin screw extruder and intercalated at short residence times. However, due to its dependence on screw rotation residence time cannot be controlled in an extruder. When screw speed increases, shear stress increases residence time decreases [8].

At higher screw speed, due to an increase in shear thinning behaviour of the polymer clay platelets were better dispersed. Shear stress had a more significant effect on delamination of nano particles than residence time, exfoliation and it is due to shear stress is the controlling. A useful factor of an internal mixer is that residence time is a controllable parameter, independent of rotation, exfoliation may be achieved both through shear stress and diffusion [9]. In an internal batch mixer, the rotors have also different functions. Due to the flow of molten material in between the rotor and chamber wall Shear stress is generated [10, 11].

The Roller rotor is commonly used for testing viscous properties, processing thermoplastics and shear stress analysis and cross linking reactions. The Banbury rotor is commonly used for compounding processing elastomers. The Sigma rotor is used for processing low shear materials and testing, such as powder, food, and flours. For the Sigma stresses and shear rates range from low to high. Fill factor is another

crucial factor in an internal batch mixer that influences properties by generating torsion and viscosity of final product [12].

The optimum fill factor using Banbury rotor is 60% to 80% in an internal mixer. The optimum fill factor using tangential rotor was at 70% in an internal mixer. Chaotic mixing produces better dispersion than conventional mixers. In chaotic mixing, a part of fluid is folded and stretched before to its previous location. Nano-composite samples had better dispersion made with chaotic mixing than those produced with additional turbine mixing elements with a high shear intensity screw [13, 14].

2.1: Fouling of Polymers

It is the deposition of unwanted substances on wetted surfaces to harm the function and as well as to deteriorate the surface. The fouling materials may be of two types, it can be living organisms (biofouling) or a non-living (inorganic or organic). Fouling differ from other surface-growth processes, "in that it takes place on a surface of a system performing a useful and defined function, and the fouling process interferes with this function and deteriorate it". The major types of fouling are discussed here and the most important is biofouling as it imparts a major contribution in fouling of the system [15, 16].

Organic Fouling

The deposition of naturally occurring organic material to membrane surface is the main cause of severe fouling of membrane surfaces.

Inorganic Fouling

It is due to the deposition of inorganic substances on the surfaces for example scaling. It is the common type of fouling and is associated with salts such as calcium carbonate ($CaCO_3$) present in water. Reverse soluble salts become less solute as the temperature rises and thus accumulates on the heat exchanger surface [1].

Particle Fouling

It is due to deposition of unwanted particles on the surface. For example, mud clay, etc.[1, 2, 15].

2.2: Bio Fouling

When water-born, bacteria coagulate in maximun numbers then there is on the surface of pipes formation of a film etc. This "biofilm" contain mainly water, suspended solids, bacteria, yeasts, corrosion products, algae etc. Biofilm may cause the contamination in the quality of water by bio corrosion and biofouling. The corrosion occur by this biofilm may cause damage, ranging from deterioration of pharmaceutical or microelectronic products, to decrease efficiency of heat corrosion of stainless steel, exchangers, and destruction of mineral substances. Many bacteria are *plank tonic*, so they can float in water. However, the large numbers of the bacteria causing problems are *sessile* in nature means unable to move remains attached to a surface. When bacteria get attach to a surface they went through a lot of changes, which are the excretion of a slimy material, biofilm. Industrial process water is not safe to use. Biofilm composition may vary per flow conditions in a water system, for example, a non-laminar flow produces slimy and homogenous slimy biofilms, which are harder to "inactivate" than produced by non-turbulent flows. Also, the effectiveness of an antifouling agent or biocide depends on the age of the

biofilm and its physical and chemical structure. The industrial water systems used to decrease both water consumption and water discharge by recirculation[17, 18].

As results the quantity of dissolved and suspended substances promotes growth of waterborne micro-organisms. The deposition of unwanted organic materials on any surface either man-made or natural is referred to as biofouling with subsequent macro fouling of the system and induced corrosion[19, 20].

2.3: Mechanism of Biofouling

When a material is in seawater, it is rapidly covered by unwanted fouling substances. Its growth occurs by a complex phenomenon. In marine environments, over 400 organisms cause the fouling problems. The fouling organisms are divided into microorganisms (biofilm) and macro-fouling according to their sizes. There are main four stages of succession of fouling organisms explained below.

- The first stage is the deposition of inorganic and organic macromolecules onto the surface after immersion: the primary film formation occurs.

- The microbial cells are transported to the surface, and bacteria immobilized on the surface.

- In the third event, the bacterial attachment to the substance is firmed through the production of extra-cellular polymer, formation of a microbial film on the surface of substrate.

- The fourth stage is the development of a more complicated community in the presence of multicellular species, micro-algae, debris, sediments on the surface.

- The last stage is the adherence of larger marine invertebrates such as barnacles, mussels, macro algae[18].

2.4: Preventing Techniques against Biofouling

Biofouling causes expensive lose each year. There are various methods which are used to control microbial activity. It includes the development of various antifouling substances and materials. Some of the techniques are as follow.

2.4.1: Biocides

A biocide is defined as a chemical substance or microorganism used to destroy harmful organism by chemical or biological method. The most appropriate definition of biocides is a group of poisonous substances which includes preservatives, insecticides, disinfectants, and pesticides to control the growth of organisms that are dangerous to human or animal health or those that are dangerous to natural or manufactured products.

2.4.2: Nontoxic Antifouling Coatings

Current antifouling methods focus on green and non-toxic processes. Fouling-release illustrates the force required to remove an organism that is attached to a substrate. Antifouling paints have the prime importance for combating biofouling in the marine industry. Biocides such as tributyltin (TBT) were used to combat antifouling and were the active components of antifouling paints until they improved hazardous to marine environment. The use of TBT-based paints was dangerous to non-target species and the surrounding environment[21].The ideal alternative of TBT is an environmentally neutral coating with both antifouling and fouling-release properties[1, 18, 22].

2.4.3: Antifouling Polymers

Antimicrobial agent-bound polymers show their antifouling activities by releasing the active agents through hydrolysis slowly, but some polymers are antimicrobial by themselves without attachment of any functional group. There are some advantages over low molecular weight agents because they are more stable against volatilization, dissolution, and diffusion. The active agents that released from polymers may be dangerous to the environment. In this type of polymers, the bioactive functional groups are attached to the polymers in a permanent way that is through chemical bonding [23]. However, polymers with active agents are attached to the polymer backbones via bonds that are hydrolysable can be used industrially if the releasing of biocidal agents are not very toxic and the protection time required is not very long[24].

There are several methods in preparing antimicrobial polymers with the attachment of bioactive agents of low molecular weights. One of the methods is the attachment, active biocidal agent to a backbone of the carrier polymer. Tani et al. prepared active hydrophilic–hydrophobic model to show the hydrolysis of bound agents in a polymer matrix. According to the model, the extent hydrophilicity of antimicrobial polymers is important in order to control and release of bound agents from the polymer matrix since the hydrophilic groups of the polymers can facilitate the growth of the free active agents from the polymer. Further, the hydrophilicity can affect interactions between the polymers and micro-organisms[1].

Antimicrobial polymers provide an antimicrobial method for fighting pathogens and are more important in industrial and academic research. Antimicrobial exhibits either passive or active action, polymer material containing either bound or

leaching antimicrobials are prepared[25].There are vast applications of these antimicrobial polymers in the food, medical, and textile industries. There are no bioactive repeating units; the biocidal polymers are embodied by antimicrobial site of the entire macromolecule. Many biocidal polymers consist of cationic biocides, such as tertiary sulfonium, quaternary ammonium, phosphonium, andguanidinium[26]. Microbes usually at the outer membrane of the cell have negative charge. Cationic polymers thus destabilized the cell surface and its lead to the bacterial death. The cationic polymers antimicrobial activity can be related to the charge density of cationic groups[2]. Besides the chemical compositions including the antifouling functional groups, the activity of an antifouling surface also largely depends on physical properties of the polymer layer such as grafting density, polymer film thickness, and uniformity.

With increasing film thickness of the antifouling polymer protein adsorption generally decreases, in smaller-polymer-molecular-weight range but more gradually at higher molecular weight. The recent works also show the presence of optimal intermediate film thickness with reduced protein deposition for some antifouling polymers[27]. Too thin or too thick polymer brushes both lead to large protein adsorption. If the film is too thin, surface hydration is not sufficient to resist protein deposition; if the polymer film is very thick, long polymer chains may self-condense through inter- or intra-molecular hydrogen bonding or electrostatic interactions, which results the reduced polymer–water interactions (weaker surface hydration) and increased protein deposition. Film thickness of the polymer grafts usually be controlled by manipulation of polymerization conditions such as the polymerization time, catalyst ratio, solvent composition, or ratio of monomer to the sacrificial initiator[1, 28].

2.4.4: Energy Methods

In this method energy is used to kill micro-organisms. Energy is used to kill the microbes in very short time. Some of these methods are as follow

- Laser irradiation

- Plasma pulse technology

- Ultrasonic transducers

- High energy acoustic pulses down pipes[28]

2.5: Bio Fouling Organisms

In marine environment, there are 4000 microbes approximately that are the main cause of fouling. The difference between them is adhesion mechanism and adhesion composition. Bio fouling organisms usually falls into two categories micro-fouling organisms which includes bacteria, fungi and algae etc. and macro-fouling organism which includes invertebrates etc[29].

2.6: Bacteria

Bacteria are species of very small size. These are prokaryotic micro-organisms. Bacterial cells are usually anucleus, and mostly are with no organelles and a membrane surrounds them. Mostly contain a cell wall. They also contain DNA, and their biochemistry is similar to other living organisms. Bacteria are the oldest and simplest organisms. And they live independently.

All bacteria are so small that by using a microscope they can only be visible. Bacteria consist of one cell, so they are a *unicellular organism*. They are the single-celled and simplest organisms on Earth. There are a number of extremophiles which used to live in extreme habitats. Most bacteria used to live in the ground or in water,

but mostly live inside or on the skin of other organisms, including humans. There are approximately ten times as many bacterial cells as human cells in our bodies. Some bacteria are harmful, but others are useful to human body and aids in performing many activities like digesting food. Some are used in factories, to make cheese and yogurt. Pathogenic bacteria, are harmful, enter the human body from the water air, or food. Once inside, these bacteria attack specific cells in our respiratory system, digestive tract etc. There they also begin to reproduce and spread while using body's nutrients and food to get energy which help them to reproduce[2].

2.7: Types of Bacteria

Bacteria are characterized into two categories according to their cell wall.

1) Gram positive bacteria
2) Gram negative bacteria[28]

The Gram stain is widely used to classify and characterize bacteria. This method permits microbiologists to differentiate bacteria into two large classes Gram-negative and Gram-positive bacteria. Chemical structure differences of the bacterial cell walls fundamentally divide bacteria into these two classes. When performing the Gram stain, bacteria observed to can retain the crystal violet dye and staining purple are referred to as Gram-positive. On the other hand, bacteria are Gram-negative if they are unable to hold the crystal violet dye and stain pink/red upon the addition of a counter stain (commonly safranin dye). The counter stain will leave the Gram-positive bacteria unchanged and they will therefore remain purple from the crystal violet dye [2].

2.8: Comparison between Gram-Negative and Gram-Positive Bacteria

Before delving into the specifics about their differences, it is interesting to first note their similarities. For instance, both types of bacteria possess peptidoglycan-rich cell walls that are responsible for providing a variety of rigid cell shapes specific to the species. Peptidoglycan is defined as a polymer with a glycan backbone made from alternating residues of N-acetylmuramic acid and Nacetyl glucosamine. Peptide chains attached to the N-acetylmuramic acid moiety will exhibit a high degree of cross-linking between these chains in Gram-positive bacteria and partial cross linking in Gram-negative bacteria. In terms of cell wall thickness, Gram-positive bacteria (20 to 50 nm) are much thicker than Gram-negative bacteria (5 to 10 nm). Another important feature attributed to Gram-positive bacteria is the presence of teichoic acids and lipoteichoic acids. While these are largely absenting in Gram-negative bacteria, they are largely embedded throughout the peptidoglycan cell wall matrix. Teichoic acids are polyol phosphate polymers that are covalently linked to peptidoglycan and exhibit a prominent negative charge. Lipoteichoic acids are polymers consisting of amphiphilic glycol phosphates and lipophilic glycol lipid that completely penetrate through the cell wall and are firmly anchored in the cytoplasmic membrane. The most distinguishing factor between Gram-positive and Gram-negative bacteria is the presence of an outer membrane structure beyond the peptide glycan layer in Gram negative bacteria. Lipoprotein molecules act as reinforcing connectors between the membrane structure and peptide glycan wall through respective non-covalent and covalent interactions. A main constituent of the outer membrane of Gram-negative bacteria is lipo polysaccharide. It is essentially a polysaccharide core with carbohydrate chains attached and is equipped with a lipid A

anchor. Variations in the sugar composition making up the polysaccharide help the Gram-negative bacteria achieve serologic specificity and therefore reveal the root cause of their pathogenesis[30].
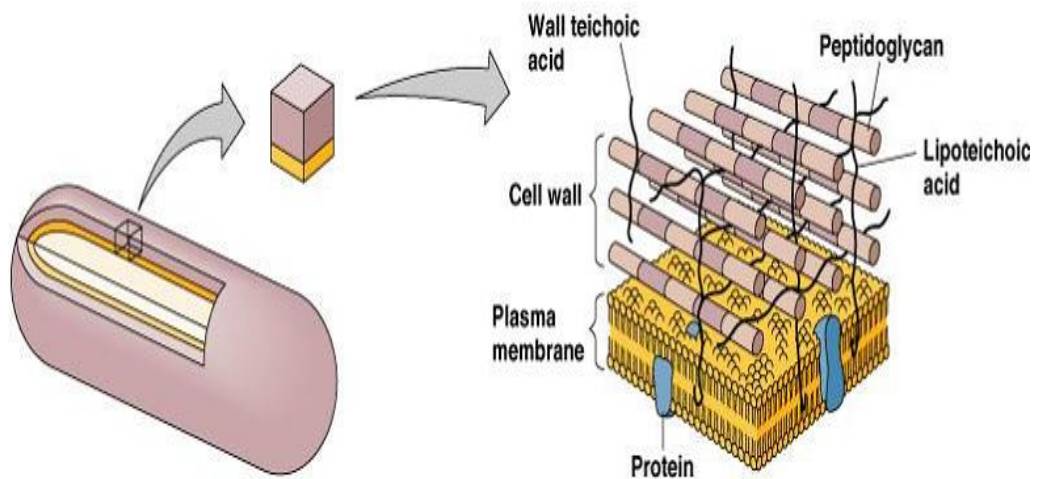

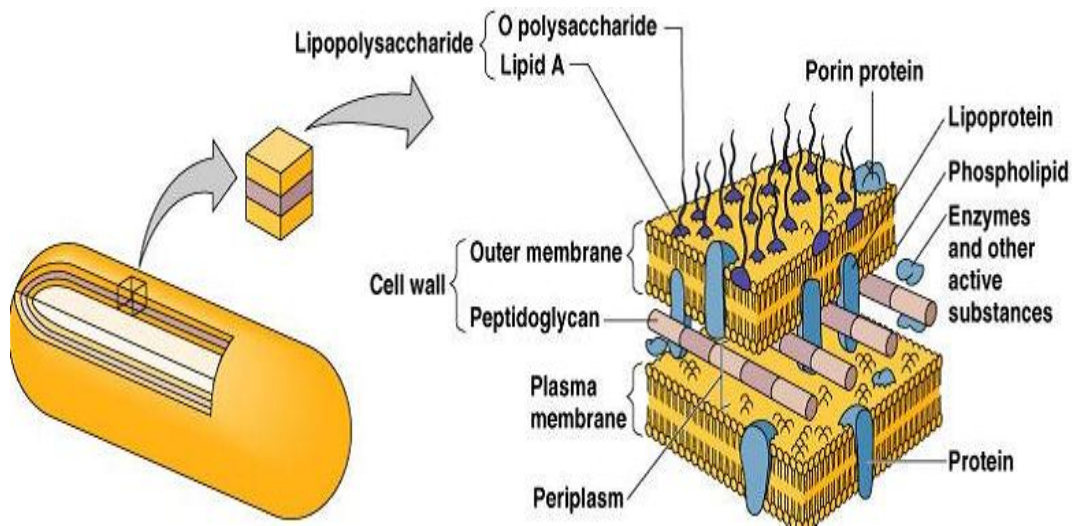*Figure 1: Illustration of Gram-positive cell wall structure[30].*

Figure 2:Illustration of Gram-negative cell wall structure [30].

2.9: Antifouling Polymer Systems

Limiting interaction of cells, proteins, and microorganisms with material substrate is critical. These interactions can cause a lot of problems for device efficacy and safety. Thus, a research continues to focus on the development of versatile, nontoxic, and cheaper methods to render surfaces resistant to fouling by proteins, cells, and bacteria. A common method to prevent cellular and proteinaceous fouling is to immobilize the antifouling polymers on biomaterial surfaces. There are several polymer classes that are being used as antifouling including polyacrylates, oligosaccharides, and polyethylene glycol (PEG) etc. The general physical and chemical properties that make surfaces antifouling are not known very well. However, Merrill as well as Whiteside's and co-workers proposed that non-fouling surfaces should be electrically neutral, hydrophilic, and as well as possess hydrogen bond acceptors but not hydrogen bond donors. Exceptions to these rules are observed, many polymers with antifouling properties possess most of these characteristics[1].

2.10: Factors Effecting Antifouling Properties of Polymers Systems

The hydrophilic groups of polymers enable the association with the water around them to form at the polymer surface a tightly surface-bound water layer. This layer provides a strong barrier to prevent approach of proteins from being deposited on the surface. The surfaces having coating of these polymer brushes enable high resistance to protein deposition from 100% blood plasma and serum and whole blood, prevents cell and bacterial adhesion, and do not induce capsule formation for healing of skin wounds. Polyacrylates and polyacrylamide are incorporated or copolymerized with other polymers to form antifouling and antimicrobial hydro gels.

Intermolecular interactions between proteins, environment and materials are the determinants for the macroscopic antifouling of the materials or polymers at an atomic level. The interfacial water behaviours of polymer surfaces reveal the relationship between surface hydration, molecular structure and antifouling activity.

Chapter-3

Experimental Methods

3.1: Grafting of Low Density Polyeythlene with Malic Anhyride

3.1.1: Materials and Methods

Polyethylene was used as purchased without further treatment. Density of LDPE was 0.901 g/cm^3. Melt flow index was 0.2 g/10min. Maleic anhydride was purchased from sigma Aldrich. It was in white powder form and was used as purchased without further treatment. Benzoyl peroxide was in cream that was diluted. Acetone was commercial grade, used as solvent for MAH and BPO. Prior to grafting of LDPE, differential scanning calorimeter (DSC) of 5-7 mg LDPE sample was done to check its melting temperature. Melting Temperature (T_m) was 110°C.

3.1.2: Grafting of LDPE with MAH by Reactive Extrusion

The ten-different samples were prepared to graft MAH on LDPE. Five solutions were prepared by varying concentration of MAH and five by varying BPO.

Table 1: Samples Details for Grafting of MAH on LDPE

Samples Names	MAH (phr)	BPO (phr)	Grafting %	Samples Names	MAH (phr)	BPO (phr)	Grafting %
PE-M1	0.05	0.5	0.6	PE-B1	0.14	0.1	0.2
PE-M2	0.09	0.5	2.01	PE-B2	0.14	0.2	1.1
PE-M3	0.13	0.5	5.01	PE-B3	0.14	0.3	2.5
PE-M4	0.17	0.5	2.2	PE-B4	0.14	0.4	3.8
PE-M5	0.21	0.5	2.1	PE-B5	0.14	0.5	5.5

*Figure 3: HAAKE Poly Lab OS internal engineering mixer system.*

For the functionalization of LDPE, MAH was grafted on LDPE chain using BPO as initiator. MAH and BPO were dissolved in acetone and LDPE pellets were added. After volatilizing the acetone, BPO and MAH adhered onto the pellets homogenously. Functionalization of LDPE was carried out by reactive extrusion process in HAAKE Poly Lab OS internal engineering mixer system displayed in figure 3. Internal engineering mixer has two counter rotating triangle shaped rotors having rotational speed ratio 1.25:1 (left to right). Recipe was prepared in internal mixer software HAAKE Poly soft by adding density and processing conditions of materials.

These samples were then extrudate by using Batch mixer / extruder. The temperature of the equipment was set $160^{o}C$ by using display buttons. The equipment was preheated for almost 5 minutes to stabilize the temperature. The sample to be melt grafted was added step by step. As the heavy plungers moves on the sample mixes and high temperature of $160^{o}C$ and rotation of the screw of 60 rpm and

maximum time of 10 minutes allows the maleic anhydride to graft on low density polyethylene and extrudate in irregular form was extracted. [29, 32-34]

A complete reaction mechanism during the grafting of MAH on LDPE by using BPO as initiator is detailed below.
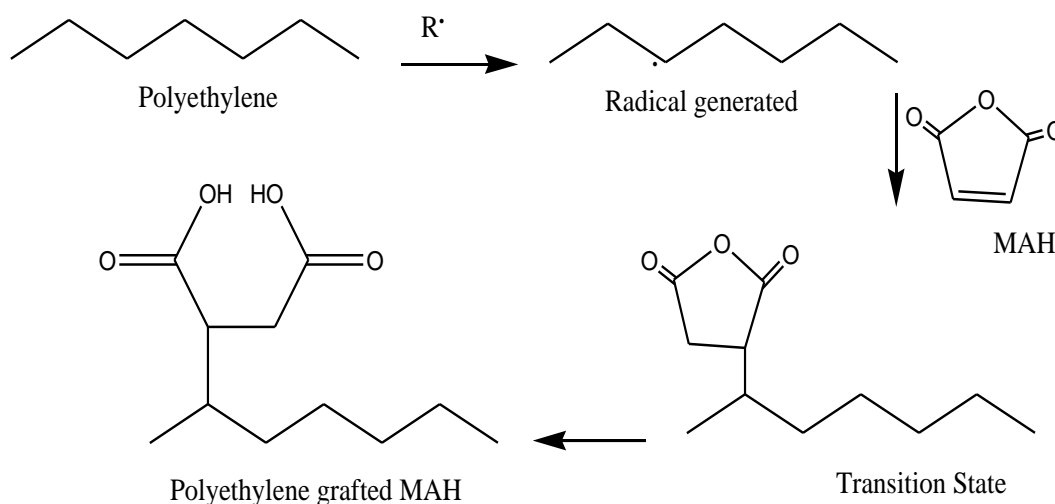
3.2: Characterization of Grafted LDPE

During the grafting of maleic anhydride on LDPE by reactive extrusion process, data obtained from internal mixer software was analysed to study the rheological behaviour of grafted LDPE. After reactive extrusion process, formulations that obtained were characterized by FTIR, MFI and DSC to confirm this reaction.

3.2.1: Torque evolution in Internal Mixer

Inside the internal mixer, external sensors are used to maintain a stable environment inside the reaction chamber. Torque was obtained by transducer to gather data about the viscosity of LDPE during reactive extrusion process. This mixer works at defined speed (shear rate) and time, and materials behaviour is recorded as torque. Rotors' rpm is defined, if there is any viscosity change inside

material, system will gain more energy to maintain speed of rotors, this will generate signal recorded by transducer. In mixing chamber temperature is controlled by independent heating and cooling zones. When there is friction due to mixing inside the chamber, heat will generate and cause a change in materials temperature. This change in material's temperature is recorded as measuring signal.

3.2.2: Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) FTIR Bruker Alpha spectrometer, spectrum was done to confirm the grafting of maleic anhydride functional group on LDPE chains. The spectrum was recorded by Bruker Alpha instrument. Spectrum was studied in FTIR Essentials software.

3.2.3: Thermal Analysis by Differential Scanning Calorimetry (DSC)

Effect of grafting maleic anhydride on the thermal properties (Tm) of LDPE was analysed by Differential Scanning Calorimetry. Grafted samples were characterized in a Perkin Elmer differential scanning calorimeter (DSC), by heating 5-8 mg of sample at 10°C/min under nitrogen (N_2) atmosphere from ambient temperature to 140°C.

3.2.4: Melt Flow Index (MFI)

To study the effect of grafting on the flow behaviour of LDPE, MFI of all grafted samples was measured at 180°C under the weight of 2.16kg in Noselab ATS Plastometer. The analysis was carried out 3 times for each reactive extruded samples[32].

3.3: Functionalization of MAH-g-LDPE with Acriflavine

3.1.1: Materials and Methods

Acriflavine was provided by Lahore pharma wise labs (PVT) Ltd and was in orange brown powder form and was purified with acetone. Strains of bacteria were provided by Atta-Ur-Rehman school of Applied Bio Sciences (ASAB), NUST Islamabad. DMF was obtained from sigma Aldrich. Other reagents were used without further purification and were AR grade.

The sample with highest degree of grafting was then selected for further reaction. The extrudate were in the form of irregular form. It was grind into fine powders and were purified by dissolving in acetone and providing some heat to the sample modified form of the sample were dissolved in solvent and unmodified polymer were precipitated. The dissolved polymer was then precipitated in acetone. Precipitate in its pure form was collected by filtration. Grafted polymer in its pure form was ready to use for further processing. Now, grafted polymer was dissolved into dimethyl formaldehyde. The complete dissolution of polymer in solvent was achieved after 24 hours at room temperature. Acriflavine was also dissolved in DMF. Complete dissolution was achieved after addition of some drops of water. Functionalization of acriflavine was achieved by the process of refluxing. Firstly, the solution of grafted polymer in DMF poured into three necked flasks which were the part of refluxing apparatus. The flask was placed directly on the hot plate. The temperature of hot plate was set to 70°C. After pouring of polymer solution in flask, solution of acriflavine in DMF was added drop wise into polymer solution. Three to four drops of trimethylamine were added into above prepared solution mixture as a catalyst. The reaction allowed accomplishing for 42 hours. The thick solution of red

brown colour was obtained. This solution was then freeze dried at -130 ^{0}C. The free dried sample was in evenly thin film form. Its colour was dark brown.

3.4 Characterizations of Functionalized LDPE

3.4.1: Mechanical Testing

Tensile testing of grafted polymer and functionalized polymer were performed by using universal testing machine (SHIMADZO AG-XPLUS 20KN) specifically designed for polymers. ASTM standard of D638-14 was used to determine the mechanical properties of rigid irregular shaped films. The focus was on the elongation break, elastic modulus and tensile properties. The length of the films was 10 cm and diameter 5 mm each. From each end, the gripped section was 20mm. The left-over length of 60mm was gauge length. The extension rate was 2mm/min. The polymer was gripped between two jaws of machine. The tightening and adjustment of equipment was done at moderate rate. The method and files was created in the software. When sample was gripped firmly between the jaws adjust the entire initial load and strain rate to zero before the start of the test [35].

3.4.2: Fourier transform infrared spectroscopy

ATR-FTIR technique was performed to get the useful actual spectrum of the prepared samples to check the attachment of antifouling functional group on the backbone of the grafted polymer. The spectrum was recorded by Bruker Alpha instrument. Spectrum was studied in FT-IR Essentials software.

3.4.3: Agar disk diffusion method

After the synthesis of functionalized polymer, the main and most important step was to study its antifouling properties. Two different types of bacterial strains were used

to study the antibacterial activity of polymer namely *E.coli* and *S.aureus*. The qualitative method was used to study the antibacterial properties. About 10 ml of each bacterial strain were put into two different flasks. Then about 2g of the synthesized polymer were added into each flask. All the samples were placed on shaker and put under constant vibration for 24 hours[36].The complete steps which were followed for agar disk diffusion method are as follow:

1. For one experiment, total 4 Petri dishes were required, one for the study of antibacterial properties of the antifouling polymer against *E.coli* and one for the study of properties of polymer against *S.aureus* and rest of the two were prepared to check the growth of bacterial strains in the absence of the polymer on the plate.

2. 1% agarose solution was prepared by using 1 g of agarose and dissolving it in 100 ml of BBM solution in a conical flask. For 4 Petri dishes 200 ml of solution were required and that was more than enough. The pH of agar solution was maintained between 7.2 and 7.4 before the solution to be autoclaved by using HCL and NaOH and measuring the pH by using pH meter. Agar solution and all the accessories needed in the experiment like Petri dishes, forceps, pipette, and bacterial spreader were autoclaved. Before operating the autoclave make sure the water is up to mark and exhaust of the machine is closed. If water is not up to mark adds distilled water up to the mark. The accessories like forceps, Petri dishes, and spreader should be wrapped in newspaper properly. The complete autoclaved cycle required 2 hours. The purpose of the autoclave was to sterilize the whole equipment before use. Agar solution dries quickly, try to

use it as it is autoclaved. Otherwise, it dried and it would be difficult to pore it.

3. The pouring of agar solution in the petri dishes, bacterial spreading and all other processes required in this method were done in bio safety cabin. Before pouring of the solution into the autoclaved plates, all the apparatus and plates were placed in safety cabin and switch on the UV light for 10 to 15 minutes and cabin were closed. Try to stay away from the cabin when UV light is on because this light is dangerous to the health. The purpose of the UV light was to kill all others contamination from the apparatus and from the cabin as well before working. The cabin should be as clean as possible because a minute quantity of a contamination may interfere in the experimentation by effecting the microbial growth. After 15 minutes, switch off the UV light. Absolute ethanol was sprayed in the cabin and cleans it with tissue paper. The exhaust fan of the safety cabin was also turned on in order to avoid any type of contamination present in atmosphere.

4. Burn the spirit lamb. Spirit lamp was burn during the whole process to avoid any type of contamination that may affect the growth of micro-organisms. Before pouring used the gloves and sprayed the absolute ethanol on gloves to avoid any contamination. Now pour the agar solution in Petri dishes, in a way that it completely fills the plate with 2mm thick layer. Pour all the Petri plates. Keep the plates slightly open for 5 to 10 minutes to dry the agar solution and formation of agar layers. The plates were then placed in incubator at 37^0C for 24 hours before further use.

5. Before use of the bacterial strains Luria broth were added in both strain and these strains were placed in a shaker or water bath for 24 hours before use.

Before spreading of bacterial strains on petri plates, dilution of some solvent was made which was non-antibacterial. In this experiment DMF was used. The polymer was dissolved into specific concentration of DMF in the bottle. The sterilized filter paper was placed into the dilution so that the specific amount of the polymers gets attached to these disks. This solution was placed in the freezer before being used.

6. The next process was application of bacterial strains on the petri dishes. It is important to have an appropriate quantity of bacterial strain in its respective solution so it can easily disperse homogenously across the plate. The bacterial solution was concentrated by centrifugation at 5000 rpm for 10 to 15 minutes. 5ml of each strain was used for spreading. Bacterial spreading was also performed in safety cabin by following the same protocol as were used for the pouring of the plates under extremely clean conditions. The two strains of bacteria were poured on the plates by using pipette. The spreader was used to spread the bacteria evenly across the plate. The whole process occurs under the spirit lamp.

7. When the spreading of specific types of strain was completed, spreader was cleaned by using Absolute ethanol and dried by spirit lamp, so that this present bacterial strain does not grow on second plate. When the bacterial spreading was completed the next step was to check the antibacterial properties of functionalized polymer. Paper disks of every polymer solution were placed on petri plate at specific place on petri dish. The control disk was also placed. The control disk is of solvent DMF, which needs not to be antibacterial to judge the antibacterial activity of the polymer. DMF and distilled water does not show any type of antibacterial activity. These plates

were then placed under fluorescent light for 2 to 3 days or even in some cases for a weak to check the antibacterial behaviour of the polymer.

8. After 2 to 3 days 'different zone of inhibition was observed for polymer, distilled water and DMF. The zone of inhibition was measured to check the antibacterial activity.

3.4.4: Well Diffusion Method

The same protocols were followed to prepare the agar plates. After pouring the plates were placed in fluorescence light for 24 hours. When plates were prepared the plates surfaces of agar was streaked by bacterial reference strains by cotton swab. Agar plate surface was then punched with a tip of micro-pipette borer with a size of 4 mm were formed and 50 μL of the sample was poured with the help of micropipette in the bore. The plates could standby for 30 min. The plates were then incubated at 37°C for 48 h in incubator[37].

Figure 4: demonstration of Well Diffusion Method

Chapter-4

Results and Discussion

4.1: Results and discussion of Grafting of Low Density Polyeythlene with Malic Anhyride

4.1.1: Torque Evolution of Grafted Polypropylene in Internal Mixer

Figure 5 and 6 shows the processing window formed from the data obtained by internal mixer during grafting of MAH on LDPE. Figure 7 and figure 8 are the evolution in torque by varying MAH and BPO concentration.

During the reactive extrusion process for grafting MAH on LDPE chains, in all grafted samples, after the initial high filling peaks that is because of the quick insertion of raw materials into the internal mixer. A high value of torque was visible during first 2 minutes due to friction caused by pellets inside the mixer and high viscosity. The recorded instantaneous torque τ was correlated with viscosity η of material in the reaction at temperature T for time t.[34]

$$\tau_{(t,T)} \propto \eta_{(t,T)} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots 12)$$

The value of torque gradually falls with time which is due to the structural changes in LDPE after grafting with MAH in the presence of BPO. It was observed that during the initial reaction time, torque was high that caused enhanced viscous dissipation. With passage of time fusion of LDPE pellets occurred, the chains started to break and molecular weight decreased in response to grafting MAH functional group on LDPE.
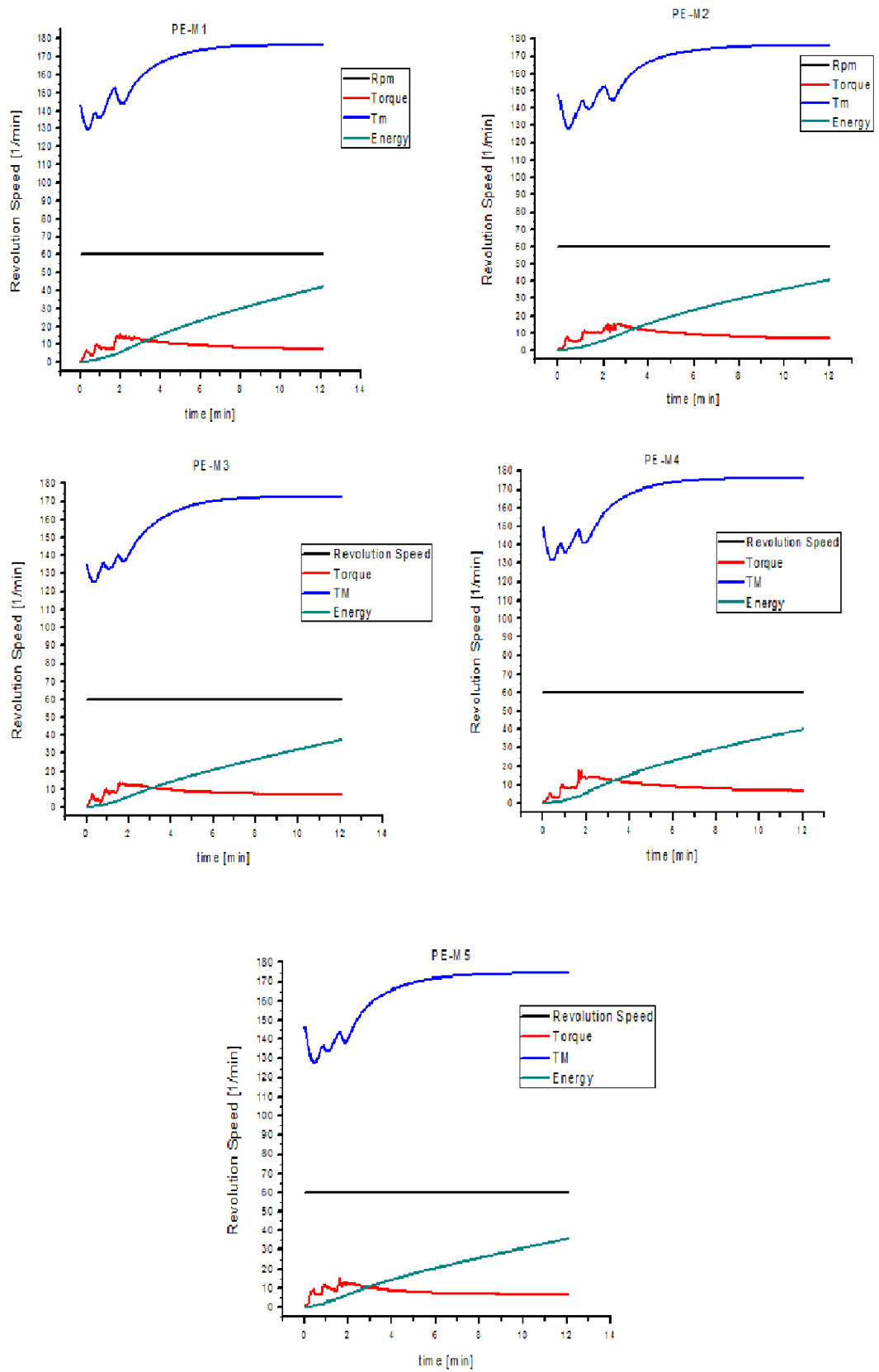
Figure 5: Processing window obtained from the data of internal mixer
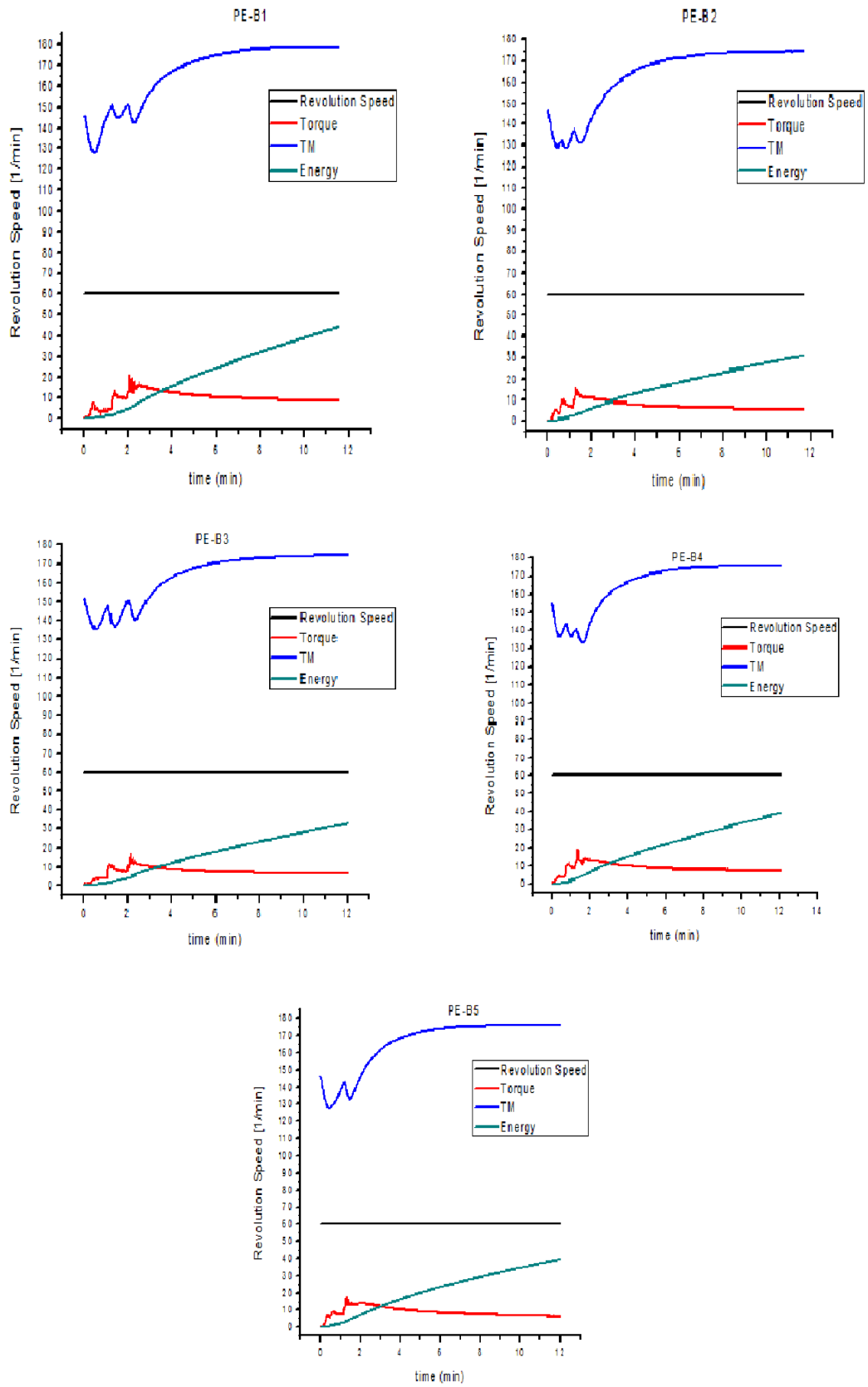
Figure 6: Processing window obtained from the data of internal mixer.

Owing to reduction in molecular weight after grafting, viscosity decreased by radical attack and hence there was clear slop change in torque value. It stabilized at the end of reaction after all particles completely melted.

Equilibrium was achieved between shear heating and constant chamber temperature that resulted in stable torque value as shown in figure 7 and 8.

It was observed that increase in the amount of MAH or BPO shortens the time require to reach steady state torque value, this can be analysed in figure 7 and 8 comparison chart. High value of BPO and MAH reduced chain length and chain entanglements that caused low viscosity [34, 38].
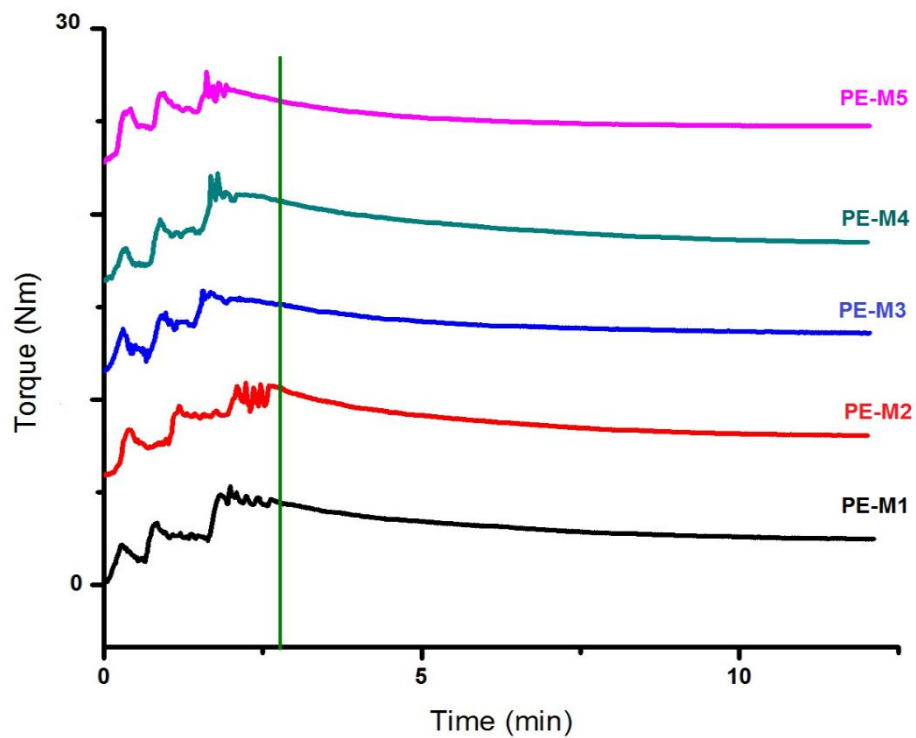
Figure 7: Torque Evolution by varying MAH concentration.
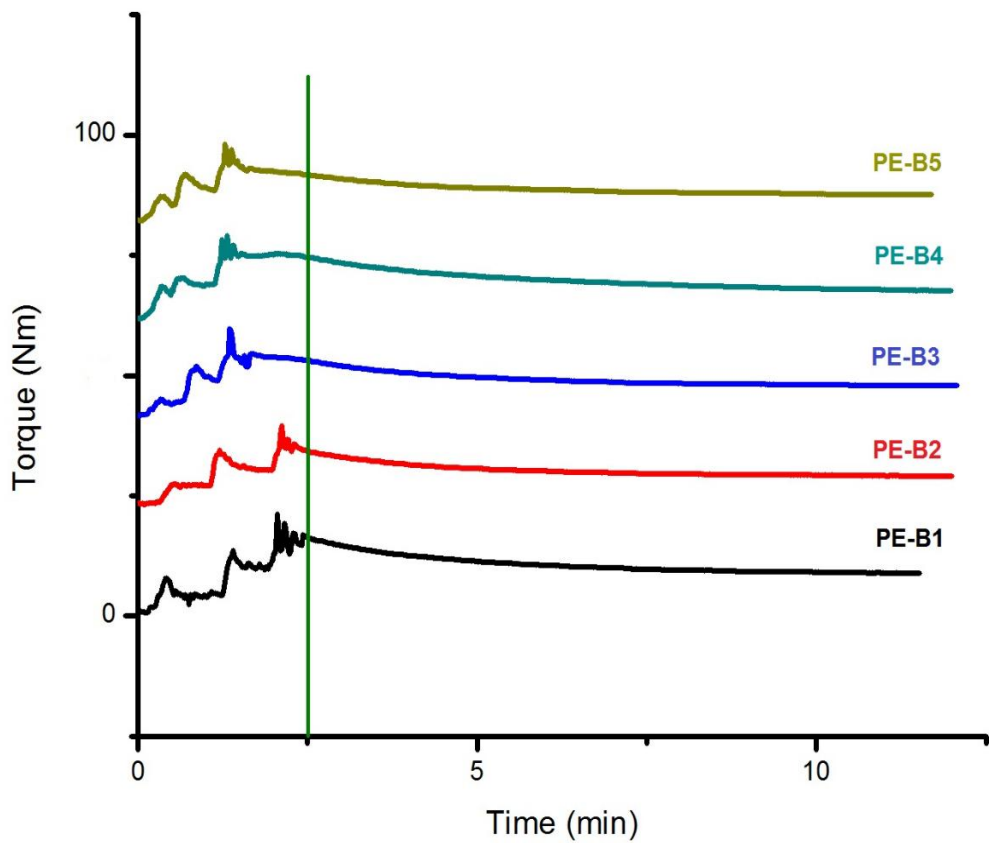
Figure 8: Torque evolution by varying BPO Concentration

4.1.2. Fourier Transform Infrared Spectroscopy (FTIR) of MAH-g-LDPE

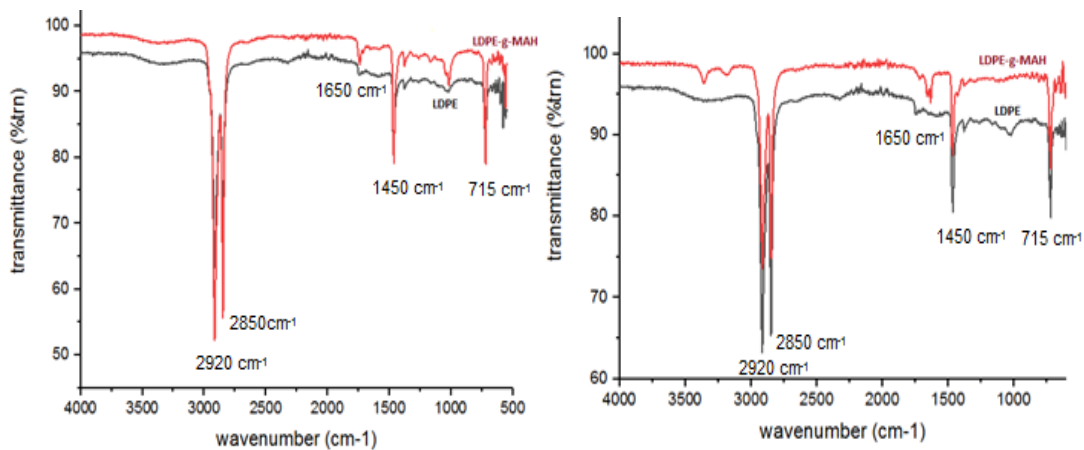FTIR spectra of grafted LDPE by varying MAH and BPO are shown in figure 9 and 10 respectively.

PE-M1   **PE-M2**

PE-M3   **PE-M4**
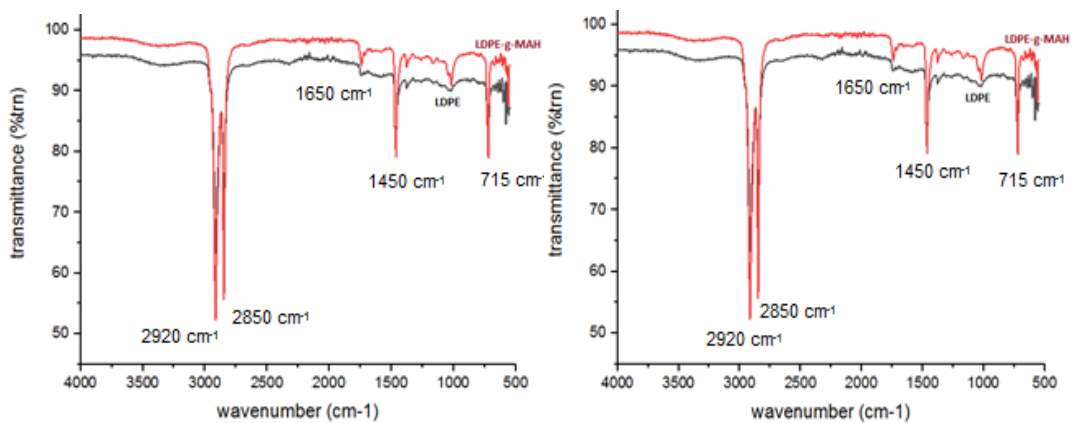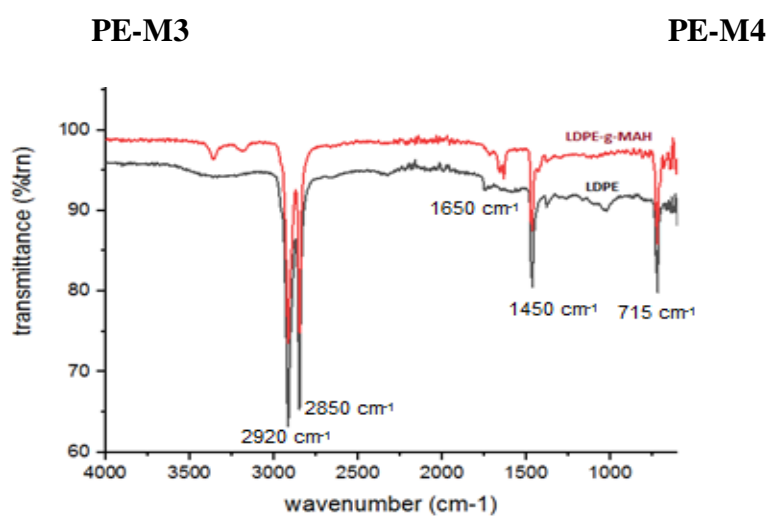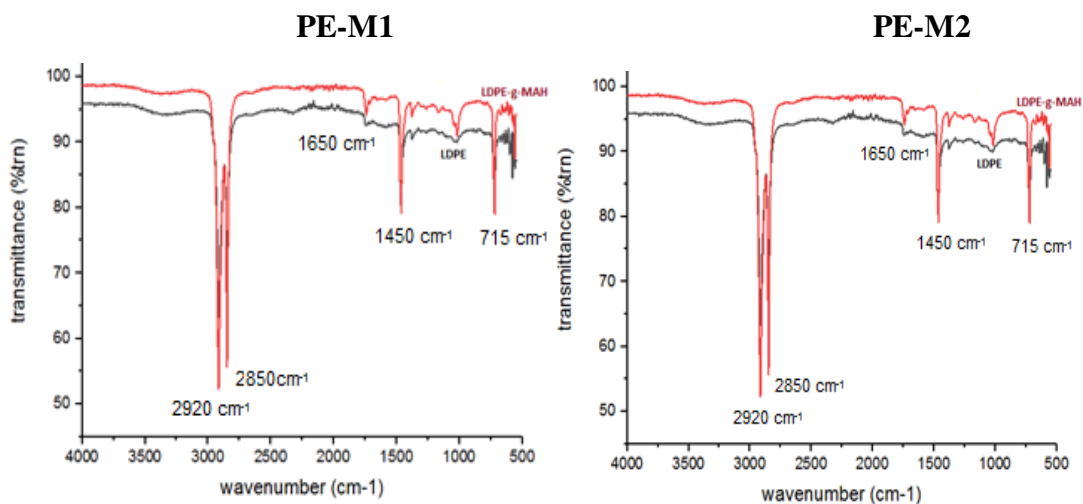
PE-M5

Figure 9: FTIR Spectra of MAH-g-LDPE by varying MAH concentration.
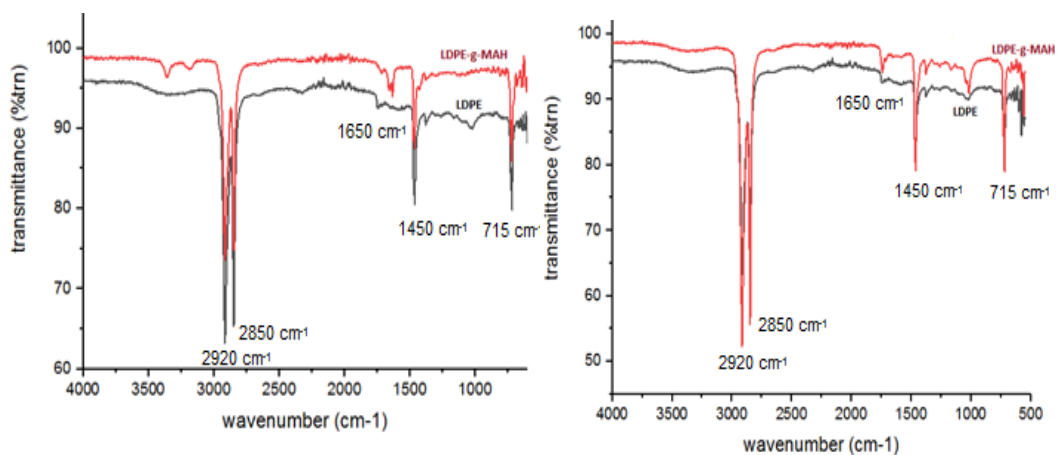
PE-B1   **PE-B2**

PE-B3

PE-B4
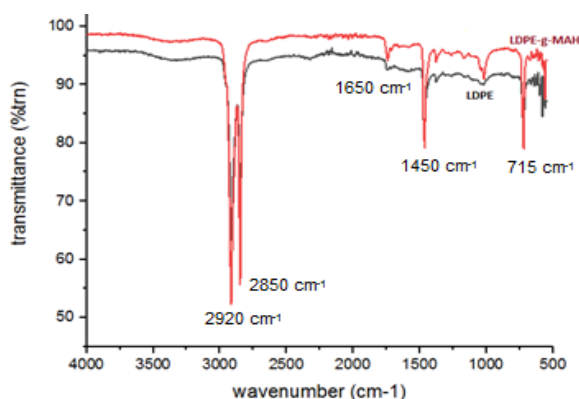
PE-B5

Figure 10: FTIR spectra of MAH-g-LDPE by varying BPO concentration.

In all spectra, pure and grafted, there are peaks at 2920 cm^{-1} for the asymmetric stretching of $-CH_2-$, at 2850 cm^{-1} for the symmetric stretching of $-CH_2-$, bending peak of $-CH_2-$ is at 1450 cm^{-1}. In all grafted samples, peaks appeared on 1650 cm^{-1} for carbonyl group (C=O) of five membered ring anhydride. These peaks confirmed the presence of MAH grafted LDPE chains along with pure LDPE. Peak at 715 cm^{-1} is due to C-H out of plane deformation. There was no peak clear peak shift visible in FTIR spectra after grafting [29, 39].
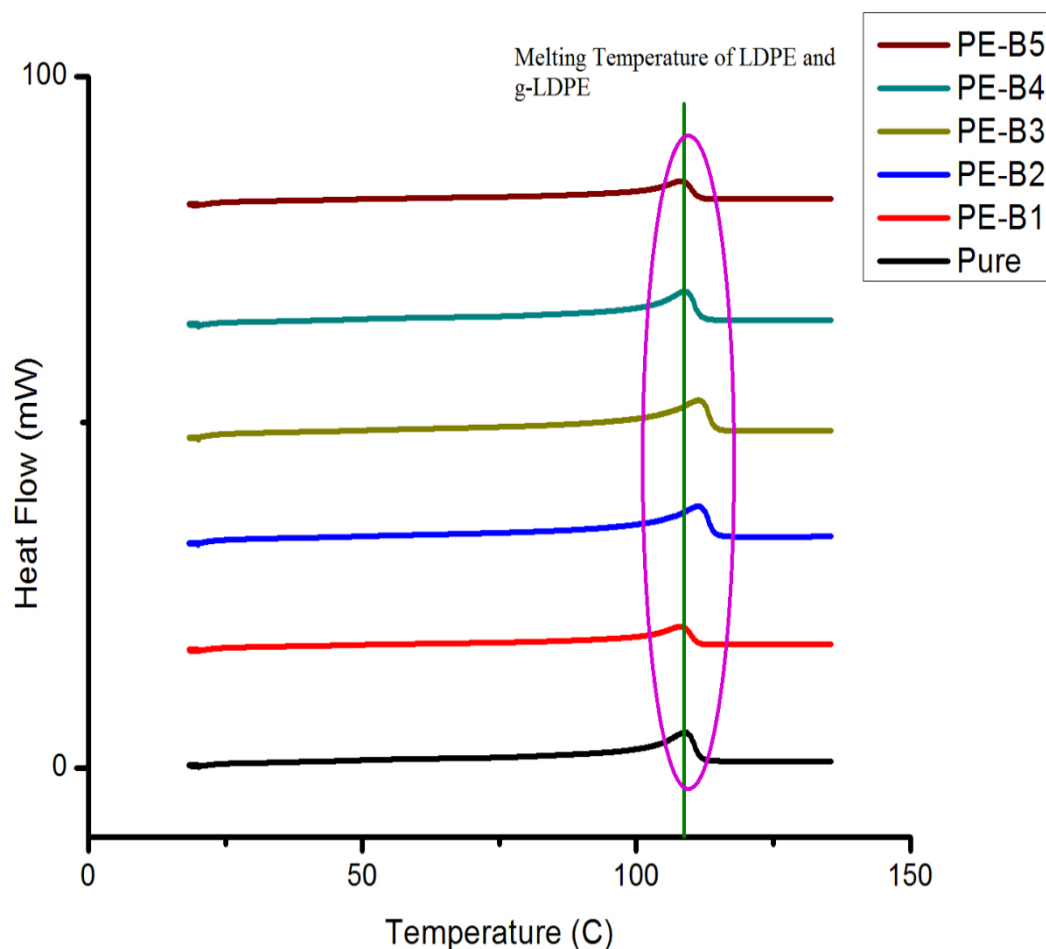
4.1.3: Differential Scanning Calorimetry (DSC)

Figure 11: DSC thermo grams of MAH-g-LDPE by varying BPO concentration

Thermal properties of functionalized LDPE that include melting temperature was analysed by DSC thermo grams. A comparison of all processed samples by reactive extrusion with varying MAH and BPO contents are shown in figure 11.Variation in melting temperature by different MAH and BPO contents for grafted LDPE are also exhibited in figure 12. Chain scission caused reduction in molecular weight and further reduced entanglements in chains[40].

Variation in MAH at constant BPO and in BPO at constant MAH, first reduced melting temperature of processed samples and then a sudden rise was detected[41]. The fall in T_m at low concentrations of MAH and BPO was owing to

the chains breakage and branching on main chain[42, 43]. But at high amount of MAH and BPO complex molecular structures formed and melting temperature increased[40, 44].
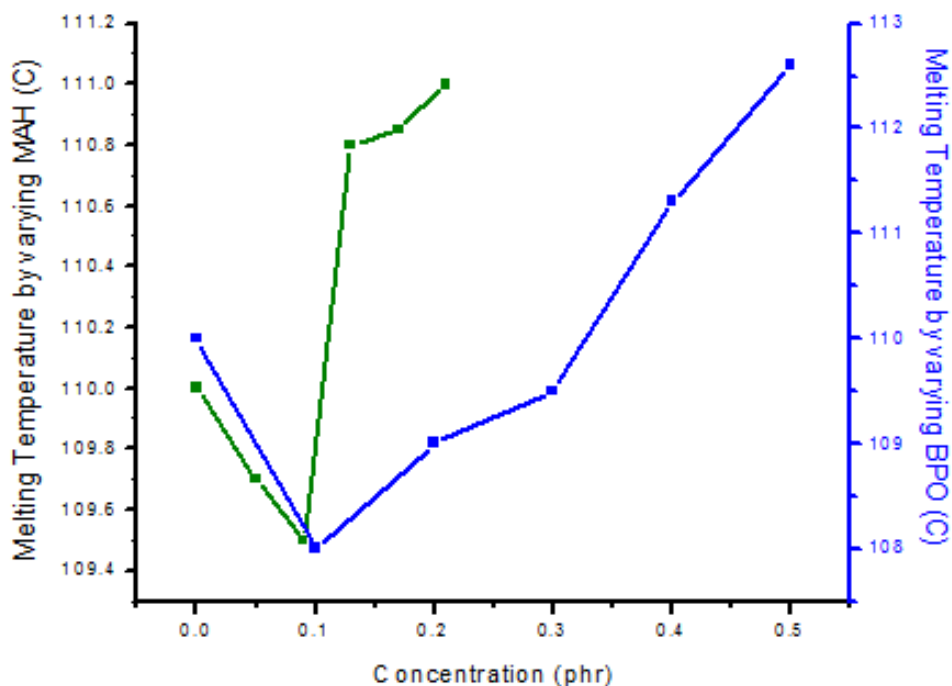

Figure 12: Variation in Melting Temperature by changing MAH and BPO concentration.

4.1.4: Melt flow index

MFI values for all functionalized low-density polyethylene samples are displayed. Figure 13 displays that by adding low content of MAH and BPO, MFI value raised remarkably. This was confirmed from literature[40, 45]. By adding MAH and BPO polymer chain scission occurred due to termination by chain transfer not by combination.

It can be inferring that combination reaction for termination is less probable than chain transfer. Shorter chains with low molecular weight causes high flow rate. But

as the amount of MAH and BPO increases further, complex molecules form this will lower its MFI values [40].
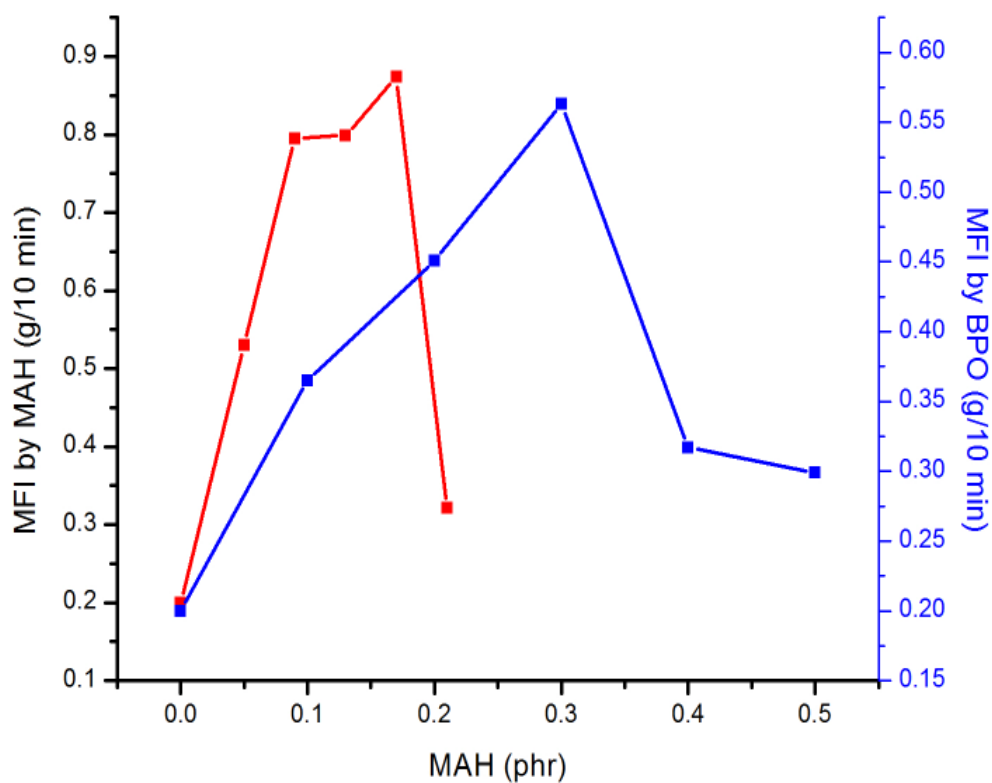

Figure 13: Melt Flow Index of functionalized samples by varying MAH and BPO.

4.2.2: Mechanical Testing

Mechanical testing was done to check the fracture behaviour of functionalization LDPE incomparison of pure LDPE. Testing was done according to the ASTM-d638. Stress strain curve of Functionalized LDPE in comparison of pure LDPE is displayed in figure 15. From these curves it can be deduced that pure LDPE has high ultimate tensile strength (UTS) that is 8 MPa in comparison of functionalized LDPE that have 5.8 MPa. This fall in UTS is due to the reduction in lengths of LDPE chains after functionalization. Functionalized LDPE showed huge necking till fracture and much ductile behaviour but pure LDPE has less ductility. This high necking is due to high

strain localize disproportionately in small area of the material. Chains in Functionalized LDPE showed ability to elongate maximum as the stress level increases. Yield Stress of Functionalized LDPE is also high in comparison of pure LDPE[46].
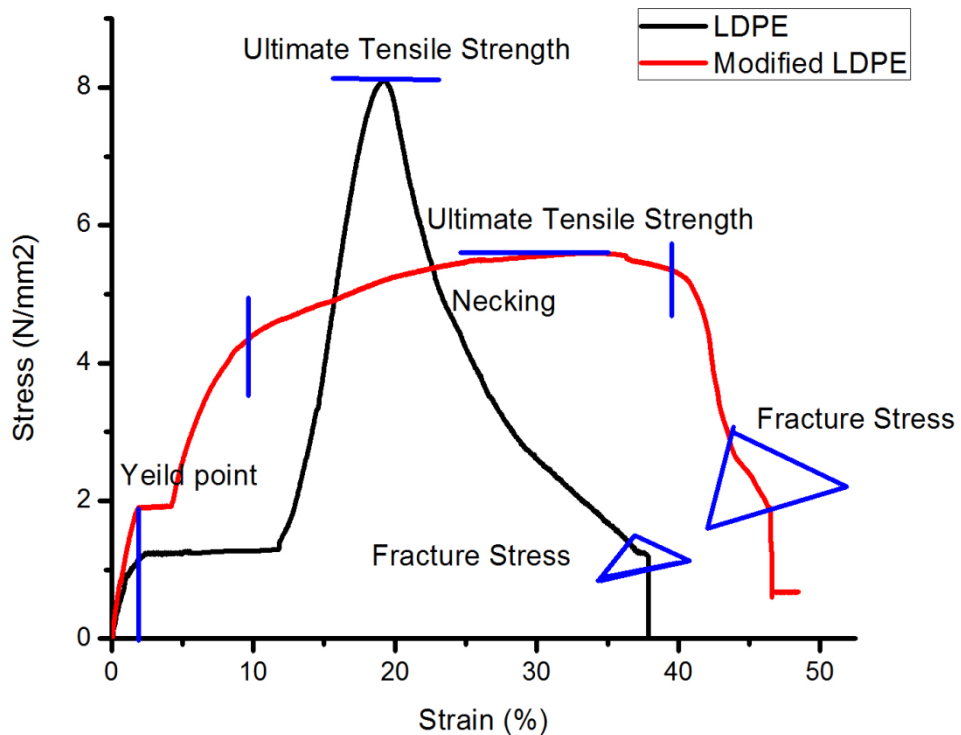

Figure 14: Stress Strain Curve of Functionalized LDPE in Comparison of pure LDPE.

Young's Modulus was calculated according to the gradient method from stress strain curves. Modulus of pure LDPE was 0.89 MPa and functionalized LDPE was 1.38 MPa. This was deduced from above calculations that functionalization on LDPE increases the inter chain bonds this leads towards an increase in the strength of the LDPE. Toughness of functionalized LDPE was also detected by integrating the stress strain curves using origin. Pure LDPE showed 114.63 $J.m^{-3}$ and toughness of functionalized LDPE was detected to be 209.02 $J.m^{-3}$. Functionalized LDPE absorbed more energy before fracture as compare to pure LDPE. By attaching

functional group with LDPE chains resist their movement that made them tough and absorb more force before going towards failure[47][48, 49].

4.2: Results and discussion of functionalized Low Density Polyeythlene with Malic Anhyride

4.2.1: Fourier Transform Infrared Spectroscopy (FTIR) of Functionalized LDPE

Acriflavine amine group reacts with functional group of maleic anhydride. Peaks of amide group at 3250 cm^{-1} and 3000 cm^{-1} appeared in the graph of FTIR. These new peaks showed the attachment of acriflavine group to maleic anhydride of low density of polyethylene.Comparison of grafted low density polyethylene and acriflavine functionalized low density polyethylene is displayed in figure 14.
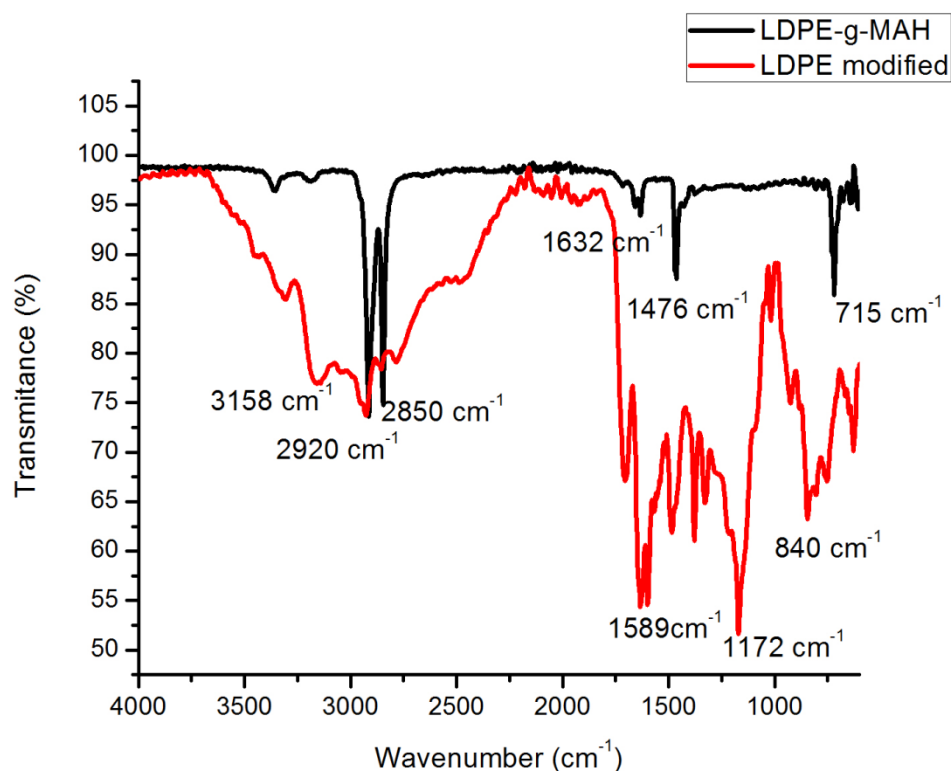

Figure 15: FTIR comparison of MAH-g-LDPE with functionalized Acriflavin LDPE.

There is a clear peak shift at 2920 and 2850 cm^{-1} of CH stretching and peak of CH bending that was at 1476 cm^{-1} in pure LDPE moves towards large wave number which confirm the presence of a chemical reaction during the functionalization.

4.2.3: Antibacterial Studies

4.2.3.1: Agar Disk Diffusion Method

The details and protocol is already discussed in the experimental section. There were two types of bacterial strains namely *E.coli* and *S.aureus*. Each bacterial strain was cultured on a separate plate. There were four petri plates (2 plates for each bacterial strain)[50].These two bacterial strains were not only tested for their growth on agar plate but also observed their growth in the presence of antibacterial polymer. The polymer was diluted in DMF and solution of different conc. Of polymer in DMF were prepared.

Table 2: Details of various samples prepared by dilution

Sample Name	Sample weight	Dilution description
S1	2g	Grafted PE were dissolved in DMF
S2	2g	5 % modified polymer in 20ml DMF soln.
S3	2g	10% modified polymer in 20ml DMF soln.
S4	2g	15% modified polymer in 20ml DMF soln.
S5	2g	20% modified polymer in 20ml DMF soln.

The images of petri dishes having *E.coli* bacterial strain tested against various diluted samples as shown in figure 16. The inhibition zones were formed around every filter paper disk except the controlled one. From the image, it can be analysed that grafted maleic anhydride functionalized with acriflavine is an antibacterial polymer. There is a direct relationship in amount of functional group and antibacterial performance.

Antibacterial character also depends upon the diffusion of agent, maleic anhydride present in the grafted polymer has good releasing properties.

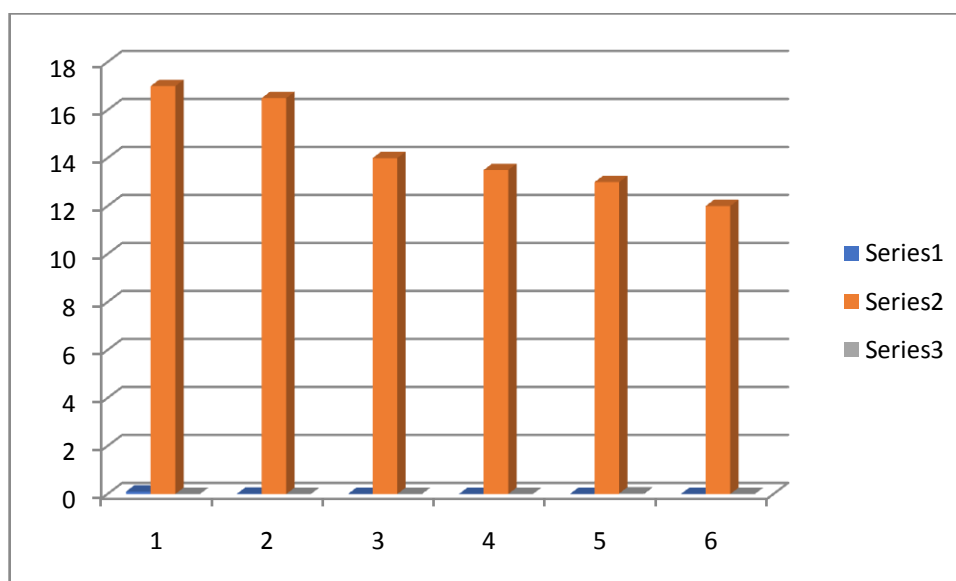*Figure 16: Photographic images of modified polymer against S.aureus.*

Figure 17 Antibacterial activity graph of S.aureus of different conc. of polymer against zone of inhibition.

As the amount of functionalized polymer increased, there is increase of inhibition of zone in petri plates. The paper disk diluted with the antibacterial polymer put on the petri plate immediately after bacterial spreading. One disk was also wetted with the

solvent DMF which were non-antifouling and called controlled paper disk. After 3 to 4 days the zone of inhibition that was formed were compared with the controlled disk as no zone of inhibition was formed by controlled disk[1].
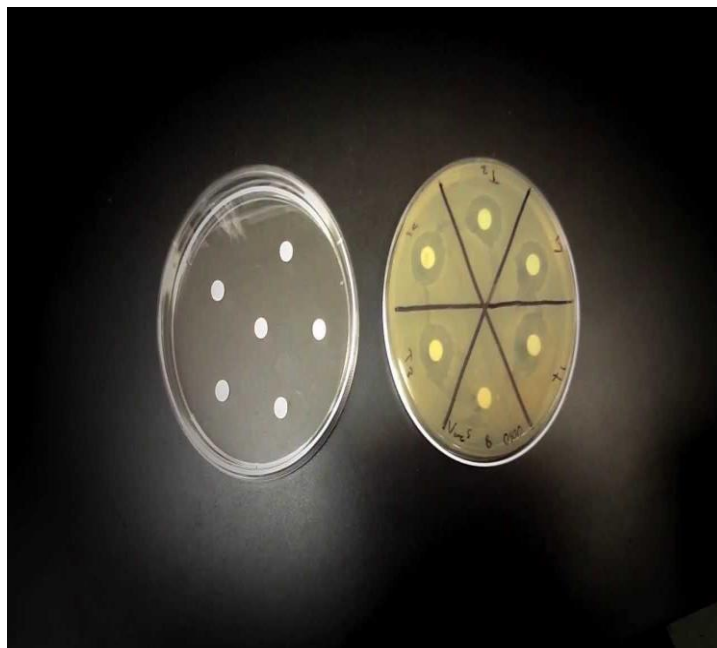

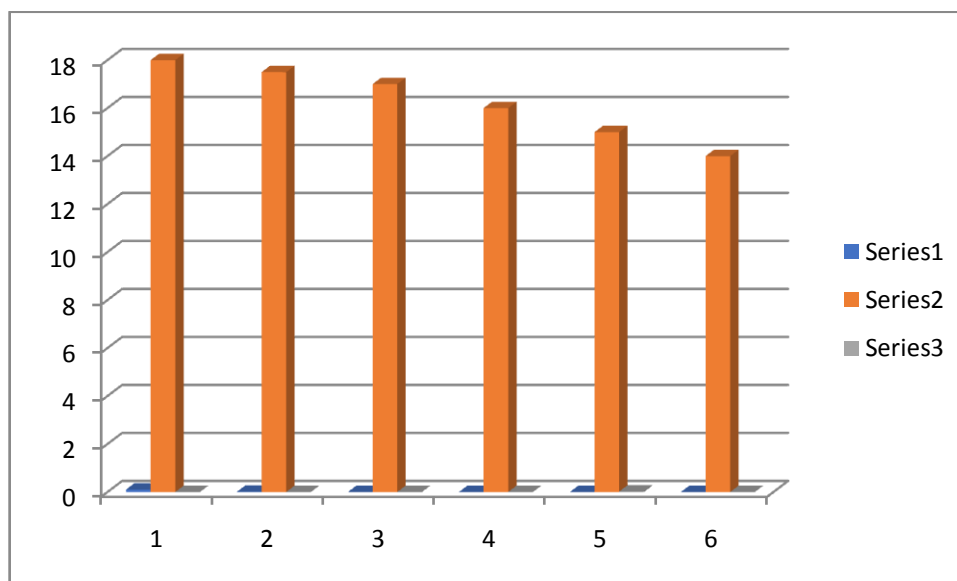*Figure 18: Inhibition zones formed by modified polymer against E.coli.*

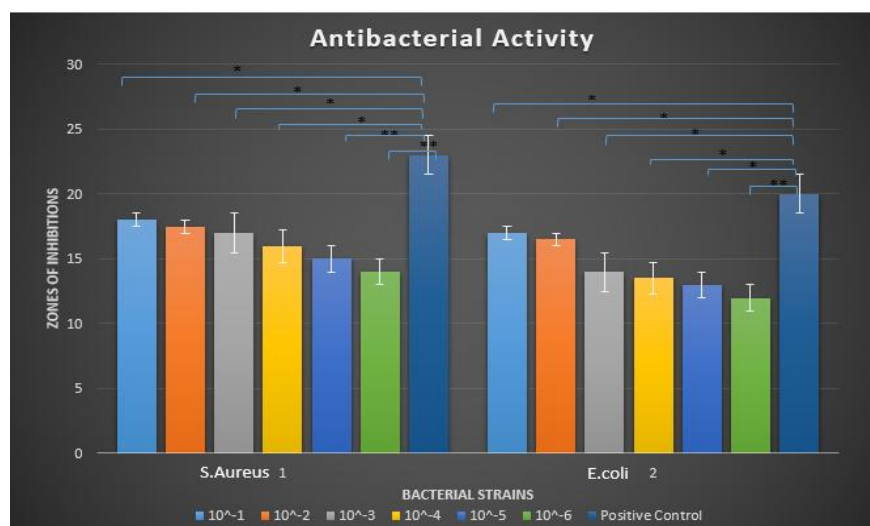*Figure 19: Antibacterial activity graph of E.coli zone of inhibition Vs different conc. of polymer.*

Fig 20 Comparison of zone of inhibition formed by E.coli and S.aureus by applying student T-test.

Error bars illustrates the standard deviation and asterisks (*) are showing p value. The asterisks (*), (**), are indicating significance difference (p ≤ 0.05), (p ≤ 0.01) respectively[51].
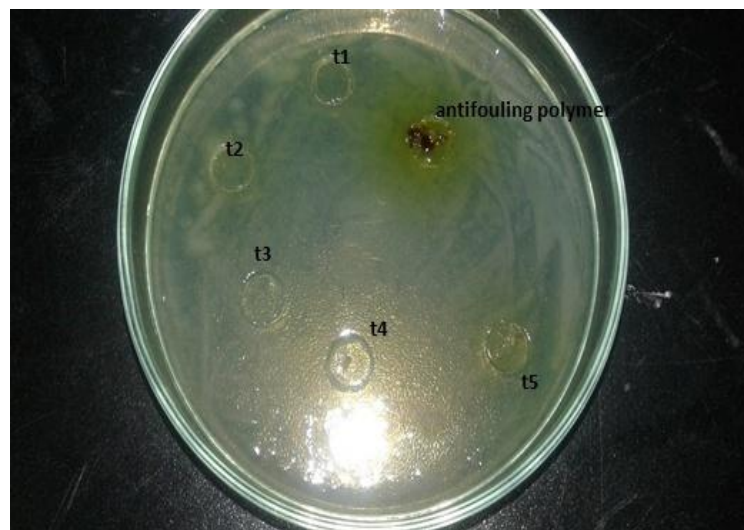
4.2.3.2: Well Diffusion Method

This method was used to check antibacterial activity of polymer. Antifouling polymer forms inhibitions zones around the well and inhibits the bacterial growth. Antibacterial activity of modified polymer was evaluated using well diffusion method on Mueller-Hinton agar. The inhibition zones were reported in millimetre (mm). *S.aureus* and *E.coli* were used as references for the antibacterial assay of modified polymer. Briefly, agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter=6mm) were filled with 50 µl of the test samples and incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was measured.
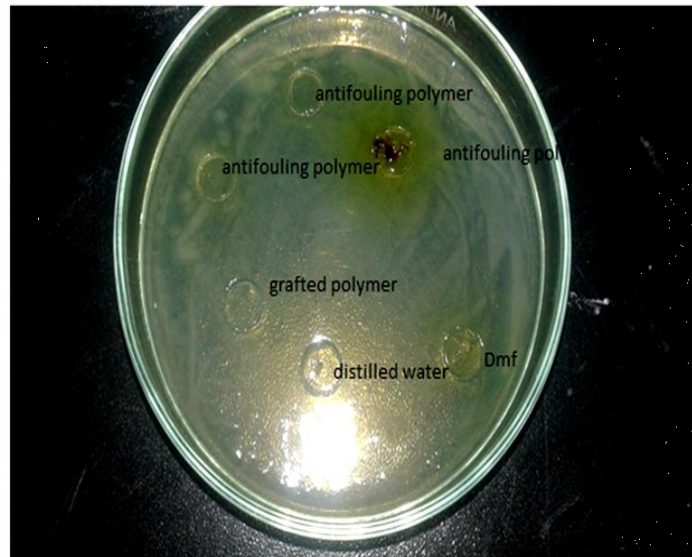
Figure 21: well diffusion method used against S.aureus by varying conc. of antifouling sample.

Figure 22: Well diffusion method used against E.coli by varying conc. of antifouling sample.

With the turbidity of 0.5 McFarland (equal to 1.5×10^8 colony-forming units (CFU)/ml) 18 to 24 hrs single colonies on agar plates were used to prepare the bacterial suspension. Turbidity of the bacterial suspension was measured at 600 nm. The DMF and distilled water was used as negative standards while modified polymer were used as positive standards.

Conclusions

- Low density polyethylene was melt grafted with maleic anhydride and copolymer was prepared.MAH has good releasing properties and it act as useful group to attach many antimicrobial mioties.

- The thermal properties using DSC were studied. Furthermore MFI of grafted copolymer were also studied.

- Mechanical properties tested through UTM showed reasonable properties of copolymer to be used in certain application.

- Acriflavine was attached to this grafted copolymer to make it as antibacterial copolymer. As acriflavine have good antiseptic properties.

- Acriflavine grafted to low-density polyethylene is excellent antibacterial agents as far as their susceptibility to inhibit bacterial growth is concerned.

- The agar diffusion method was used to check the inhibition of bacterial growth against *E.coli* and *S.aureus*. The polymer shows excellent antibacterial properties in testing.

- The prepared modified copolymer can also act as anticancer and can be tested.