An-Najah National University

Faculty of Graduate Studies

Monomers Design Strategy to Create Curcumin Based Polymers with Demanding Functionality

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Dedication

To my dear parents, sisters, brothers,

teachers and friends with love and respect.

Acknowledgments

First of all, I am grateful to The Almighty Allah for helping me to complete this thesis, Praise and thanks to Allah.

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الإقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

Monomers Design Strategy to Create Curcumin Based Polymers with Demanding Functionality

أقر بأن ما اشتملت عليه هذه الرسالة انما هو نتاج جهدي الخاص، باستثناء ما تمت الاشاره اليه حيثما ورد، وان هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أيّ درجة أو لقب علميّ لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher`s own work and has not been submitted from any where else, for any other degree or qualification.

Student`s name: **اسم الطالب: سناء محمد فياض صقر**

Signature: **التوقيع:**

Date: **التاريخ: 10/12/2017**

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List of abbreviations

DSC	Differential Scanning Calorimetry
DTG	Differential Thermogravimetric Analysis
MS	Mass Spectrometry
UV	Ultraviolet-Visible Spectrophotometry
FT-IR	Fourier-Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
S	Semin (Ω^{-1})
PPy	Polypyrrole
PAn	Polyaniline
PEDOT	Poly(3,4-ethylenedioxythiophene)
LUMO	Lowest Unoccupied Molecular Orbital
HOMO	Highest Occupied Molecular Orbital
DNA	Deoxyribonucleic Acid
HPV	Human Papilloma Virus
HIV-1LTR	Human Immunodeficiency Virus-1LongTerminal Repeat
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
DMAc	Dimethylacetamide
DMSO	Dimethylsulfoxide
E.coli	Escherechia coli
S.aureus	Staphylococcus aureus
K.pneumoniae	Klebsiella pneumoniae
MW	Molecular Weight

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Abstract

Several curcumin based polymers, poly(curcumin-co-phenylenediamine), were synthesized and their conductivities and antimicrobial activities were evaluated. A new polymer synthetic technology was used to synthesize the polymers. In this technology, curcumin was reacted with the diazonium salt of p-phenylenediamine to produce a polymer backbone that is completely conjugated from head to tail. The reaction was carried in a one pot process. The polymerization process was carried in two different solvents dimethylacetamide and water. The prepared polymers were characterized by various techniques such as DSC, DTG, MS, UV, FT-IR, ^{13}C and ^{1}H NMR spectroscopy.

The prepared polymers were evaluated for conductivity; and no conductivity was observed. Several modifications were carried out to enhance polymer conductivity. Polymer cross-linking was carried out using p-diaminobenzene. Derivatization was done by adding a heterocyclic ring to the polymer backbone was also performed. Cross-linking with transition metals iron and copper was carried out. Modified polymers were also evaluated for conductivity, and no conductivity was observed. The lack of conductivity could be related to several reasons such as: the equipments used

for making the pellet for conductivity analysis was not adequate; conductivity meter used could be unsuitable for this type of polymer; the doping process was not efficient and impurities could be present in the polymer.

The antibacterial activities of the polymers against four different bacteria strains (*Escherechia coli*, *Staphylococcus aureus* strain 1, *Staphylococcus aureus* strain 2 and *Klebsiella pneumoniae*) were evaluated. The results showed that the polymers have a good to excellent antibacterial potency especially against *Escherechia coli* and *Staphylococcus aureus* strain 2, and polymers with antimicrobial activities are unique and rare.

Chapter One

Introduction

Synthetic polymers have been widely used in every field of human activity during last decades. These substances are usually petroleum-based and regarded as non-degradable [1]. On the other hand, natural based polymers are biodegradable [2] and can be promising candidates to meet different requirements. Natural-based polymers are made from natural raw materials by chemical methods. Some examples are polylactic acid, polyhydroxyalkanoate, and starch-based plastic [3].

In this study, new natural polymers based on curcumin were prepared and their conductivity and anti-bacterial activity were examined.

1.1 Types of conducting polymers

Conducting polymers are two types: electrolyte and electronics. Electrolytes are ionically conducting polymers. Electronically conducting polymers include polymers with conjugated double bonds and a blend of conducting materials and the insulating polymers [4].

The first conducting polymer was discovered in 1977 by Alan G. MacDiarmid, Hideki Shirakawa, and Alan J. Heeger. It was found that, conductivity of polyacetylene after doping with electron-withdrawing AsF_5 increased nine fold (Fig 1.1), reaching 10^3 S/cm [5,6].

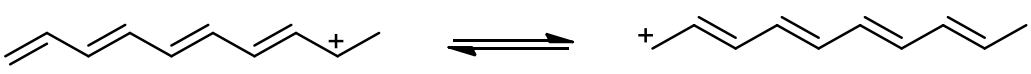


Fig 1.1: Resonance structure of doped Polyacetylene showing the charge transport across the polymer chain.

After the discovery of polyacetylene, a series of conducting polymers, including polypyrrole (PPy), polyaniline (PAn), and polythiophene (PTh) (Fig 1.2) were discovered (Table1.1). These polymers and their derivatives showed a combination of good properties, price, and ease of treatment and synthesis. The discovery of these polymers greatly promoted the area of conducting polymers by exploitation of their properties such as conductivity, catalytic, electrochromic, redox, sensor and other properties to various practical needs [7-10]. Later, it was realized that the conductivity of almost all conjugated polymers can reach the order of $10^{-3} – 10^{3}$ S/cm after doping [11].

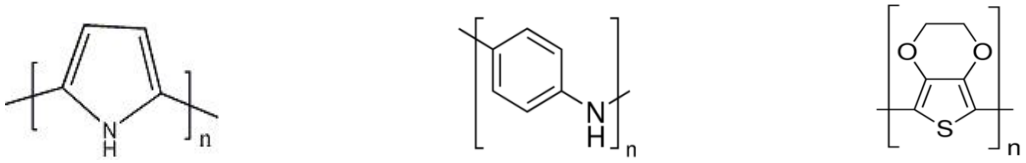
Polypyrrole (PPy) is one of the most stable and environmentally-friendly conducting polymers and the most studied one [4,12-17]. The first polypyrrole film was prepared in 1979 with a conductivity of 100 S/cm by electrochemical polymerization on Pt electrode in acetonitrile solution [4,18]. In 1994, an optically transparent polypyrrole thin films was synthesized and studied for mammalian cell culture [19].

Polypyrrole was easily synthesized at room temperature in large quantities and using water or one of other wide range of solvents. Furthermore, it can be easily modified to become suitable for biomedical applications [20-25]. Also, PPy is considered "smart" biomaterial as a result of its stimulus responsive properties [12,26,27]. It showed good conductivity under physiological conditions, and excellent chemical stability in water and air [11,20,21,26].

To become useful in applications, PPy must be transformed into a processable form because it is very difficult to be used alone as a structural material [16,23,26-30].

Polyaniline (PAn) is another important conducting polymer, which has an extensive range of applications as a result of its excellent environmental, thermal and chemical stability, ease of synthesis, reduced processing cost and conductivity, which can be as high as 10 to 10^3 S/cm [31-33]. On the other hand, the strong affinity of PAn for water has promoted many groups to investigate the compatibility of PAn with water soluble polymers like poly vinyl alcohol (PVA). But the poor processibility of PAn due to insolubility and brittleness limits it's commercial applications [31].

A third important conducting polymer is poly(3,4 ethylenedioxythiophene) (PEDOT), which is a derivative of polythiophene (PTh). A PEDOT has a dioxyalkylene bridging group across the 3- and 4-positions of its heterocyclic ring. This group improved its properties by lowering its band gap, oxidation and reduction potential. As a result of that, PEDOT has a good environmental, chemical and electrical stability, and a better thermal stability and conductivity than PPy [34-36]. currently, PEDOT is used in biosensing and bioengineering applications, e.g. in nerve grafts, heart muscle patches and neural electrodes [28,34,35,37].



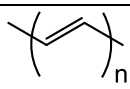
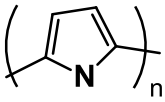
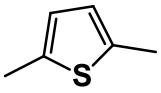
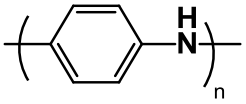
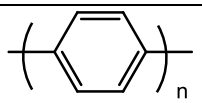
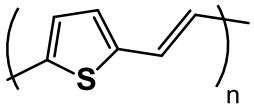
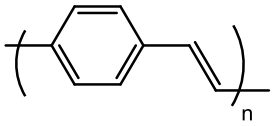
PPy PAn PEDOT

Fig 1.2: Structures of PPy,Pan and PEDOT

1.2 Properties of conducting polymers

Conjugated polymers have an extended system of alternating π-electrons along the polymers backbones on which the charge can be delocalized [27]. The carbon atoms in the conjugated system has three p-bonds and a nonbonded p atomic orbital which overlap with a p-orbitals of the nearest carbon atoms, and this leads to the formation of n-states delocalized over the full length of the polymer chain [38]. The huge number of atomic orbitals leads to a huge number of molecular orbitals, which form a band of energies. The conjugated polymers form conduction band (antibonding, π*) from the lowest unoccupied molecular orbitals (LUMOs) and filled valence band (bonding, π-band) formed by the highest occupied molecular orbitals (HOMOs). The bandgap (energy difference between the two bands), depends on the molecular structure of the repeat unit of the polymer. Because there are no partially filled bands and the band gap is usually not close to zero, the neutral conjugated polymers are usually semiconductors. LUMO and HOMO energy levels can be measured by cyclic voltammetry [4,39,40,41]. Oxidation of the conjugated system (done by chemical or electrochemical p-doping) introduces holes onto the system; reduction (n-doping) adds electrons to the system. Doping, causes the mobility of either holes or electrons to increase dramatically, thus increasing the electrical conductivity of the system causing it to become conductors.

Table (1.1): Repeat units of important conducting polymers [42].

Polymer	Structure	Conductivity (S/cm)	Bandgap
Polyacetylene		10^3-1.7X10^5	1.5
Polypyrrole		10^2-1.7X10^5	3.1
Polythiophene		10-1.7X10^3	2.0
Polyaniline		0-200	3.2
Poly(p-phenylene)		10^2-10^3	3.0
Poly(thiophenevinylene)		40	1.6
Ploy(p-phenylenevinylene)		3-5X10^3	2.5

Conjugated polymers have a number of advantages compared to inorganic semiconductors. They combine properties of both metals and conventional polymers, they are able to conduct charge, have great electrical and optical properties. In addition, they are easy to synthesize and flexible in processing [43]. Ease to tune their chemical, electrical and physical properties for specific needs of their application. For instance, reagent such as antibodies, enzymes and other biological moieties could be incorporated in the polymer chains [20,43-45].

Conducting polymers have unlimited number of applications. For instance, undoped polymers are used as semiconductors in electronic devices such as

light emitting diodes (LED) [46], transistors (FET) [47] nonlinear optical (NLO) devices, solar cells, chemical, biochemical and thermal sensors. The, doped polymers are used as electrostatic dissipation materials, electromagnetic shielding materials and electronic conductors. The switching between neutral and charged states makes the conducting polymers suitable for use in the electrochromic devices, battery electrodes and biosensors.

Other properties of conducting polymers make them considered as promising materials for the electrodes and the active layers are high electrical conductivity, transparency, flexibility, film forming ability, environmental stability and ease of synthesis [4].

Other application that was reported by Heeger et al. (in 1999) is in solar cells [4, 48]. Because they can be deposited on flexible substrates at low cost, they are desirable semiconductors for photovoltaic cells [49].

Polypyrrole conducting polymers, also used in many applications other than being conducting, including corrosion protection, fuel cells, biosensors, computer displays, drug delivery systems, and as a biomaterial in neural tissue engineering [22,27-29,50-53].

Recently, a new class of electroactive biomaterials for tissue engineering has been developed. This class of biomaterials are a part of a new generation known as ‘‘smart’’ biomaterials that allow the direct delivery of electrical, electrochemical and electromechanical stimulation to cells [54,55]. With an electrical stimulation, conductive polymers can modify cell adhesion, DNA

synthesis and protein secretion of electrically responsive cells, such as bone, nerve, muscle and cardiac cells [15,56-63].

1.3 Polymer doping

The conjugated system usually has a semiconducting characteristic, the conductivity of undoped conjugated polymers is in the range of 10^{-9}-10^{-6} S/cm [4].

The conductivity could be stimulated by doping. After doping, the electrical conductivity of the material increases by several orders of magnitude, and the highest conductivity recorded in the literature is 10^{5} S/cm for polyacetylene film [4,6,55,64].

A dopant, (a doping agent), is a material that is added to the conducting polymer at very low concentrations to alter the electrical or optical properties of the polymer. Based on the molecular size of dopants, they classified into large dopants, such as sodium polystyrene sulfonate, and small dopants, such as Cl^{-}. These two types affect the doped polymer differently [27,28,30]. Large dopants will affect the properties of material more dramatically. They can increase the density, they are more incorporated into the polymer and will not leave out with the application of an electrical stimulus or with time, thus giving the polymer grater electrochemical stability. On the other hand, small dopants with electrical stimulation can leave and re-enter the polymer, forming the basis of the conductive polymers drug release applications. Also, this allows controlling of the physical properties of the polymer through switching between doping and dedoping [27,30,65,66].

A particular dopant cannot provide all expected properties. For instance, a small one such as HCl is suitable if a polyaniline with high conductivity and high crystallinity is desired, and the organic aliphatic acid with long chain length like lauric acid is effective if a polyaniline with high solubility is desired [67].

The doping process can be carried out during or after the synthesis of the conductive polymer. It can be done in several ways: chemical, electrochemical, charge injection and photo-doping [55,68,69]. Chemical and electrochemical methods are the most convenient [55,69,70].

The type, concentration and synthesis methods of the dopant are the most factors influencing the conductivity of polymers [27,28,71]. The choice of dopant depends on the characteristics of the polymer including conductivity which can be further increased by choosing a different dopant. Type of dopant might have an effect on structural properties of polymer, such as volume, porosity and color [20,27,28,72]. For example, n-dodecyl benzene sulfonic acid (DBSA), when used as a protonating agent of polyaniline, it renders the polymer soluble in nonpolar solvents as a result of long hydrocarbon tail introduced with the dopant to the polymer matrix [73]. Another example, polypyrrole that is doped with hyaluronic acid is rougher and more brittle than that is doped with sodium polystyrene sulfonate [74].

On the other hand, there is a proportional relationship between the conductivity of the doped polymer and the amount of dopant used [75]. For instance, the conductivity of polypyrrole which is doped with chondroitin sulphate increases as the concentration of chondroitin sulphate increases

[76]. Also, studies on doping with various concentrations showed that conductivity of polyaniline increases and become more weakly dependent to temperature as the doping level is raised [77].

1.4 Synthesis of conducting polymers

Conductive polymers are synthesized by two methods: electrochemical and chemical [29,45]. With the electrochemical methods a conducting polymer with a thickness of 20 nm can be produced, where as powders or very thick films are typically produced with chemical polymerization. Electrochemical synthesis is limited to those systems in which the monomer can be oxidized in the presence of potential to form reactive radical ion intermediates for polymerization, but all conducting polymers can be synthesized chemically [27].

1.4.1 Electrochemical polymerization

The electrochemical polymerization can be performed in an electrochemical cell with a three electrode system: working electrode, auxiliary electrode and reference electrode [78]. This method occurs by applying an electrical current through electrodes placed into a solution containing the solvent, the monomer and the doping agent [79-81]. The electrical current causes the monomer oxidizes and deposit on the positively charged working electrode, forming insoluble polymer chains (Fig 1.3) [82].

In 1979, A. F. Diaz prepared polypyrrole as a film with 100 S/cm conductivity by electrochemical oxidation of pyrrole in acetonitrile [83].

In this method, the properties of the synthesized film are affected by the solvent, the doping agent, the electrode system and the temperature [84-87].

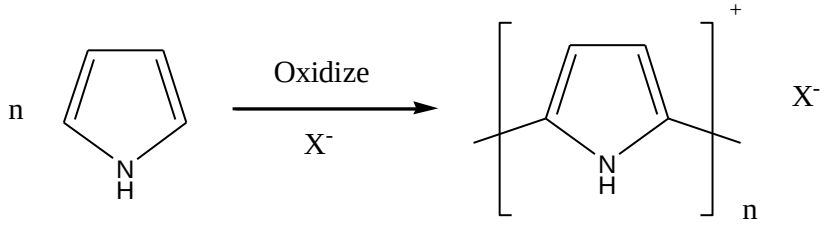


Fig 1.3: Electrochemical polymerization of polypyrrole

1.4.2 Chemical polymerization

Chemical polymerization is a simple and fast process with no need for special instruments [88]. In this method, the monomer solution is mixed with an oxidizing agent to form the polymer [89,90].

For example, polypyrrole was prepared by the polymerization of pyrrole with $FeCl_3$ as an oxidizing agent in an aqueous solution and dodecyl-benzene sulfonic acid was used as dopant (Fig 1.4). The conductivity of the compressed pellets of the polypyrrole powder was obtained equals 43.18 S/cm [4,91]. In 1986, a typical chemical polymerization method was reported for the preparation of polyaniline in a strongly acidic aqueous solution with $(NH_4)_2S_2O_8$ as oxidant [92].

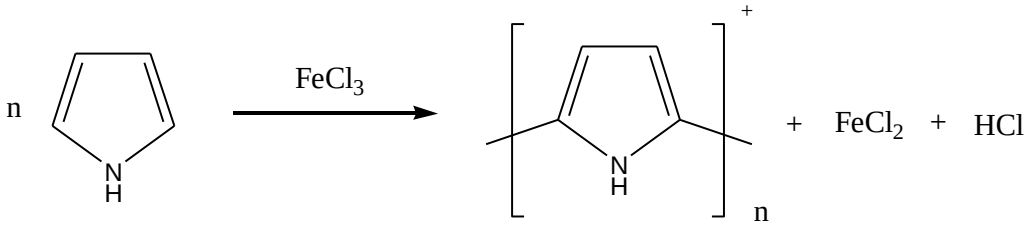


Fig 1.4: Chemical polymerization of polypyrrole

With this method all types of conductive polymers could be prepared, including some novel conducting polymers that cannot be prepared with the electrochemical method [27]. But the chemical method has many disadvantages, for example, the conductivity of the polymers has always been lower than their electrochemically prepared counterparts [93]. Additionally, the conductivity of the chemically synthesized polymers is highly sensitive to the oxidant, the relative concentration of the reagents, the choice and purity of the solvent, stirring rate, temperature, reaction time, etc., making reliable and repeatable chemical synthesis is a difficult thing to do [93-96].

1.5 Aims of the Study

The primary goal of this project is to prepare polymers derived from natural product. And the specific objectives for this proposal are:

1. Synthesize a bifunctional material to be used as a monomer for making a polymeric chain containing a conjugated sp^2-hybridized carbon (conjugated double bonds).
2. Develop a chemical method for co-polymerizing natural curcumin with the bifunctional material under mild conditions to form the polymer.
3. Develop a technology for enhancing polymer conductivity such as cross-linking with organic and inorganic crosslinkers.
4. Characterize the new polymers by various spectroscopic techniques.
5. Evaluate the antibacterial activity of the curcumin based polymers.

1.6 Curcumin

As shown in the objectives part, curcumin will be used to synthesize a novel biomaterial that possesses demanding functionalities such as electrically conducting and antimicrobial properties. Curcumin, (E,E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, also known as turmeric yellow, is a natural yellow pigment derived from the roots of curcuma plants, which is an Indian spice that belongs to the ginger family, e.g. C. tinctoria, C. xanthorriza and C. domestica, and is known since several hundred years [97,98].

Curcumin is yellow crystalline powder, a hydrophobic molecule and a weak Bronsted acid with three labile protons. It is practically insoluble in water and ether but soluble in ethanol, dimethylsulfoxide, acetonitrile, ethyl acetate, acetone and chloroform, with a melting point of 183ºC; its molecular formula is $C_{21}H_{20}O_6$ and molecular weight 368.37 g/mol [99].

Fig 1.5: Curcumin

Curcumin was first isolated in 1815, then, Vogel Jr. obtained a pure preparation of curcumin in 1842, but never report its molecular structure formula. In 1910 and 1913, Milobedzka and Lampe confirmed its chemical structure and synthesis, respectively [97,100]. Furthermore, during 1990s, Aggarwal and co-workers reported its potential anticancer effect, and after that, the speed of curcumin research has grown rapidly [101].

Besides curcumin, turmeric contains other chemical constituents known as the curcuminoids, e.g. demethoxycurcumin, bisdemethoxycurcumin, and the recently identified cyclocurcumin [102]. The structures of curcumin constituents are shown in Fig 1.6 [103].

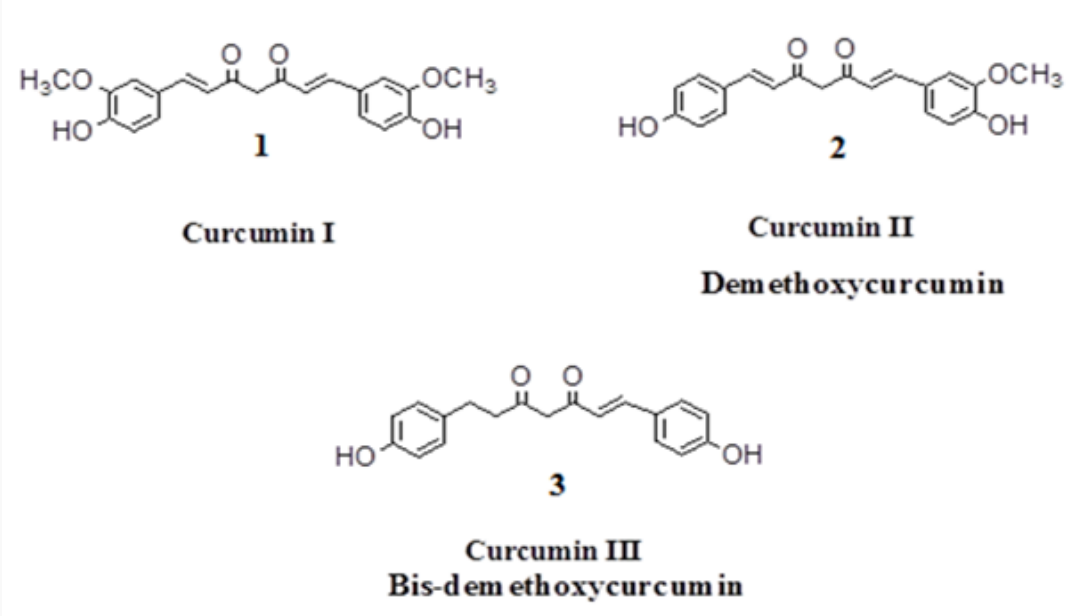


Fig 1.6: Structures of various curcumins

1.6.1 Extraction of Curcumin

The most common method for separating curcumin from turmeric is solvent extraction followed by column chromatography. Several organic solvents (polar and non-polar) have been used, but the preferred one is ethanol [99]. Other methods have also been employed, such as Soxhlet extraction, microwave and ultrasonic extraction [99,104-106].

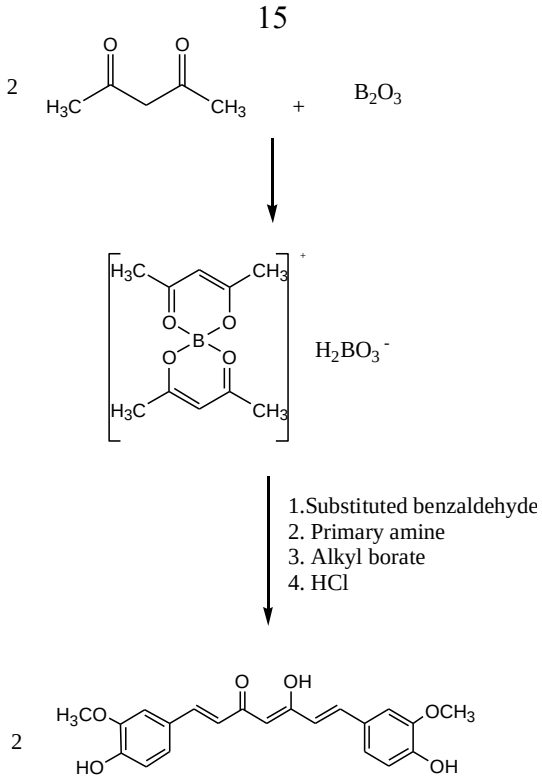
1.6.2 Synthesis of Curcumin

In 1918, the first paper on synthesis of curcumin was reported by Lampe. His method involved five steps starting with carbomethoxyferuloyl chloride and ethyl acetoacetate [107].

Later a simple method for the synthesis of curcumin in high yields was reported by Pabon using acetyl acetone and substituted aromatic aldehydes in the presence of boron trioxide, trialkyl borate and n-butylamine as shown in Scheme 1.1 [108].

Other method was proposed by Rao and Sudheer. They used trifluoroboronite and produced stable curcuminoid trifluorboronites that can be hydrolyzed in aqueous methanol (at pH 5.8) to get curcumin [109].

In all these methods the main step is the reaction of 2,4-diketoneswith suitably substituted aromatic aldehydes. The diketone is complexed with boron to prevent participation of the diketone in knoevenagel condensations and the boron complex dissociates into curcumin under slightly acidic conditions. Polar aprotic solvents and anhydrous conditions are suitable for these reactions. Amines are used as catalysts to provide the necessary basicity to deprotonate the alkyl groups of the diketone, and alkyl borates are used as scavengers to remove the water produced during the condensation reaction because the water can react with the diketone complex, thereby reducing the yield of curcumin [99].



Scheme 1.1: Synthesis of curcumin by the general method proposedby Pabon

1.6.3 Reactivity of curcumin

One diketone moiety and two phenolic groups are the reactive functional groups in curcumin [110]. Substantial chemical reactions associated with the biological activity of curcumin are reversible and irreversible nucleophilic addition (Michael reaction) reactions, the hydrogen donation reactions leading to oxidation of curcumin, degradation, hydrolysis and enzymatic reactions [99].

More than 30 different proteins have been found to interact with curcumin directly to modulate their activity, including thioredoxin reductase [111], DNA polymerase [112], lipoxygenase [113], focal adhesion kinase [114], protein kinase (PK) [115], and tubulin [116]. It has also been shown that curcumin can bind to certain divalent metal ions such as Cu and Fe [117].

1.6.4 Uses of curcumin

It is used as a food coloring agent and in traditional Indian medicine for treatment of various diseases that include biliary disorders, anorexia, cough, diabetic wounds, hepatic disorder, rheumatism, blood purification and rheumatoid arthritis [118-120]. Traditional Chinese medicine practitioners regularly use turmeric for treating diseases that associated with abdominal pain [121].

A hydroxyl group at the para-position is most susceptible for the expression of biological activity, based on structure-activity relationship studies [122]. As a result, curcumin known to have several pharmacological activities including potent antioxidant, anti-inflammatory, and antiviral activities [123-126], as well as anticancer activities against different forms of cancer, e.g., cervical cancer caused by HPV [127-129]. In 2016, Anushree Tripathi and Krishna Misra designed curcumin analogues/congeners against breast cancer stem cells [130]. Also, other studies have shown that curcumin represents a hopeful approach for delaying or preventing the progression of Alzheimer's disease [131-134], because it acts as free radical remover [135,136], and has been identified as inhibitor of HIV-1LTR directed gene expression and viral replication, besides its ability to block HIV replication by inhibiting HIV-integrase and protease [137].

The low cost, proven therapeutic efficacy, pharmacological safety and multiple targeting potential make curcumin a promising agent for prevention and treatment of various human diseases [97].

Beside bio-pharmaceutical activities of curcumin, it also exhibits strong fluorescence as biocompatible probe for bio-imaging [138]. Garcial-Alloza et al used curcumin as fluorescent agent for monitoring the structural changes of amyloid deposits in alzheimer treatment [139].

Moreover, analytical chemists have been utilizing the unique absorption spectroscopic properties of curcumin to identify and quantitatively estimate trace elements, e.g., estimation of boron, as a red colored product [140].

1.7 Antibacterial Activity

Currently, the probability for “superbugs” which are resistant to all antibacterial agents becoming more of a reality, especially when bacteria becoming capable of invading the whole human body. Therefore, humanity is in great need to develop new antibacterial agents.

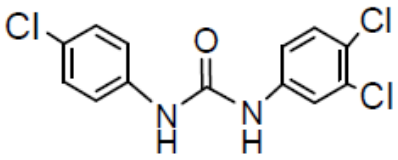
1.7.1 Antibacterial Agents

Bacteria were first identified in the 1670s by van Leeuwenhoek, following his invention of the microscope. The relationship between bacteria and diseases gradually set up in the nineteenth century. Since then, several effective antibacterial agents were developed [141]. Among these the sulfa drugs that were developed by Paul Ehrlich in 1910 [142,143]. The golden age of antibiotic agent and antibacterial agent has started after the penicillin G was developed by Sir Alexander Fleming in 1928[143].

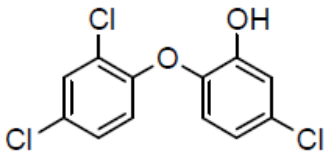
An antibacterial agent is a compound or substance that kills or slows down the growth of bacteria and they are used to sterilize surfaces and eliminate

potentially harmful bacteria [144]. As a result, they have found large number of applications in house hold products such as detergents, household cleaners and health and skin care products. Now, antibacterial agents are in bathrooms and bedrooms products and plastic food storage containers [145,146].

Antibacterial agents were divided according to their speed of action [145,146]: The first one contains those that act rapidly to destroy bacteria, but quickly disappear by evaporation or breakdown and do not leave any active residue behind. Chlorine, peroxides and alcohols are examples of this type. The second category consists of compounds that leave long-acting residues on the surface to be sterilized such as triclosan and triclocarban (Fig1.7).



Triclosan



Triclocarban

Fig 1.7: Structures of Triclosan and Triclocarban

1.7.2 Natural Products with Antibacterial Activities

Examples on natural products that exhibit antibacterial activities are: Alkaloids, Oregano oil, Colloidal Silver, Flavonoids , Curcumin,… etc.

Alkaloids are nitrogen containing cyclic compounds that produced by plants for protection from insects, also they exhibit a variety of bioactivities [147]. Berberine (Fig 1.8), as an example of isoquinoline alkaloids, is currently used clinically as antimicrobial agent [148].

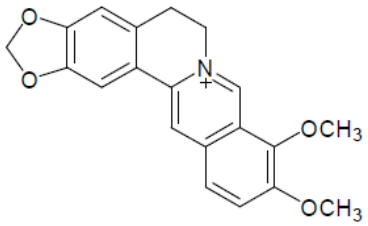


Fig 1.8: Structure of Berberine

Oregano oil is another potential natural anti-bacterial agent. Recent studies have shown that carvacrol (Fig 1.9), which is one of the oregano components, treats bacterial infections very efficiently. The phenolic hydroxyl group of Carvacrol is essential for action against the Food-Borne Pathogen *Bacillus cereus*, and carvacrol may be an effective therapy to drug-resistant bacteria [149].

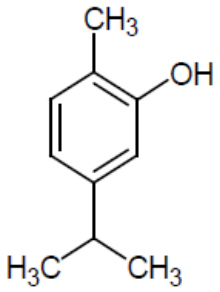


Fig 1.9: Structure of Carvacrol

Colloidal Silver, also called “Natural Antibiotic”, is another example. Colloidal chemistry is the science that converts minerals and metals into micro particles, that remain suspended without forming an ionic or dissolved solution, to be used by our living cells.

Silver, which suspended in a distilled water solution, is a universal, powerful and natural antimicrobial agent that kills parasites, mold, fungi, viruses and bacteria. Furthermore, Colloidal Silver is a relatively odorless, tasteless and

harmless liquid. Colloidal silver has been found to be highly effective in the prevention and treatment of infections and diseases (including Staph and AIDS) because it has strong germicidal action [150,151].

Flavonoids; polyphenol antioxidants that are isolated from many medicinal plants such as *Galium fissurense* Ehrend. & Schonb. -Tem. (Rubiaceae) or from *Viscum album* L.(Loranthaceae) [152-154], have been reported to possess a variety of biological activities including antidiabetic, anticarcinogenic, antiviral, anti-inflammatory, antioxidant and antimicrobial activities [154-156].

1.7.3 Antibacterial Activity of Polymers

The design and synthesis of antimicrobial polymers have obtained increasing attention by the scientific community as a safe and effective strategy to combat multidrug-resistant microbes. Polymers with antimicrobial activity are non-volatile, chemically stable and do not penetrate through the skin [157,158]. Antimicrobial activity of conjugated polymers was first reported in 2005 by Seshadri and Bhat. They deposited PPy and PAn on cotton fabrics by in situ chemical oxidative polymerization at cold temperature. The percentage reductions for PPy-coated fabrics were 59% against E. coli and 65% against S. aureus, and for PAn-coated fabrics were 85% against E. coli and ~95% against S. aureus [159,160].

1.7.4 Testing the Antibacterial Activities

Testing antimicrobial properties means testing the microorganisms' capability to survive under the effect of a given antimicrobial, at a particular concentration and for a certain time period [161]. Generally, antimicrobial susceptibility testing methods are divided into many types based on the principle applied in each method [162]. These methods are listed in Table 1.2.

Table 1.2: Types of Antimicrobial susceptibility testing methods

Diffusion	Dilution	Diffusion &Dilution
Stokes method. Kirby-Bauer method.	Minimum Inhibitory Concentration i) Broth dilution. ii) Agar dilution.	E-Test method.

In this study, we are concerned about Kirby-Bauer method and Broth dilution method.

The Kirby-Bauer disk diffusion method (or the agar diffusion test) can measure antimicrobial capability with solid media that obviously show areas of growth inhibition [163]. After a chosen period of incubation, the area of bacteria growth is noticed, and the zone of inhibition around the material tested is measured.

In the broth dilution method, sequent dilutions of the antimicrobial under test are performed and distributed in equal amounts in tubes of a standardized suspension of indicator organism [162]. After incubation, they evaluated for bacterial growth. The lowest concentration of a certain antimicrobial needed to inhibit bacterial growth is called the minimum inhibitory concentration (MIC). Nowadays, this method is performed in microplates [164]. In

addition to the determination of the MIC, the minimum bactericidal concentration (MBC) of the antimicrobial can be determined, which is the minimum concentration of antimicrobial need to kill most (>99.9%) of the viable organisms after incubation during a certain period.

Chapter Two

Materials and Methods

2.1 General Experimental

All reagents were purchased from Aldrich Chemical Company, and used as received unless otherwise specified. All prepared compounds were characterized by melting point and IR spectroscopy, but the major compound was characterized by ^{1}H NMR, ^{13}C NMR, UV, DSC, DTG, IR and melting point.

Nuclear Magnetic Resonance spectra were recorded on Varian Gemini 2000, 400 MHz instrument (Spain). All ^{1}H NMR experiments were reported in δ units, parts per million (ppm) downfield from tetramethylsilane ($Si(Me)_4$), and all ^{13}C NMR spectra were reported in ppm relative to deuterochloroform (77.0 ppm).

Infrared (IR) spectra were recorded using FTIR Spectrum 820 PC FT-IR (Shimadzu, USA) equipped with a Universal Attenuated Total Reflectance (UATR). The following parameters were used: resolution 4 cm^{-1}, spectral range 225-4000 cm^{-1}, number of scans 128.

Thermal analysis was performed using Pyris1TGA (PerkinElmer, USA). Thermograms were recorded between 37 and 600ºC with a heating rate of 10ºC/min in a flow of N_2 at 20 ml/min. The Pyris Analysis software was used to calculate the first derivatives of thermograms (DTG), as well as, estimate the percent weight loss and the decomposition temperatures.

UV spectrum was recorded using UV-spectrophotometer (Shimaszu-1700).

MS/MS analysis was carried out using Thermo LTQ Orbitrap XL mass spectroscopy. The LTQ Orbitrap XL instrument was operated in data-dependent mode to automatically switch between full scan MS and MS/MS acquisition. Instrument control was through Thermo Tune Plus and Xcalibur software (Thermo Fisher Scientific). Full scan MS spectra (from *m*/*z* 300–2000) were acquired in the Orbitrap analyzer and resolution in the Orbitrap system was set to *r* = 60,000. The standard mass spectrometric conditions for all experiments were: spray voltage, 35.0 kV; no sheath and auxiliary gas flow and heated capillary temperature, 250°C.

Schiff bases' melting points were taken on a Stuart Melting point apparatus SMP-3.

2.2 Preparation of the curcumin based polymer

2.2.1 Preparation of curcumin in organic medium

In a 200-ml flask, a 10% solution of NaOH in water was prepared by dissolving sodium hydroxide (3.0 g) in 27 ml water. A 5.4 ml of the NaOH solution were added to another flask (250 ml), to this flask curcumin (0.368 g, 0.01 mol) was added, and followed with 10.0 ml of dimethylacetamide solution. The mixture was stirred until a clear solution was obtained, then it was placed in an ice-salt water bath.

2.2.2 Preparation of Diazonium salt from 1,4-diaminbenzene

In a small beaker, $NaNO_2$ (0.28 g, 0.004 mol) was dissolved in 2 ml of water. A 1,4-diaminobenzene (0.12 g, 0.001 mol) was slowly added to a flask

containing HCl solution (2.4 ml of HCl in 4.5 ml of water). The produced mixture was stirred until 1,4-diaminobenzene completely dissolved. The flask containing 1,4-diaminobenzene solution was cooled in an ice-salt bath (-5 to -10 °C) (some 1,4-diaminobenzene may precipitate out upon cooling). While keeping the solution at -5 to -10 °C, the sodium nitrite solution was slowly added (previously prepared). The mixture was well-stirred during addition and when the addition was completed, the mixture was stirred for another 2 - 3 minutes to ensure complete reaction. The slightly turbid pale grey solution was formed and indicated for the formation of benzenediazonium salt solution. The reaction equation of making the diazonium salt is shown in Eq.3.1.

2.2.3 Polymerization of curcumin and the benzene diazonium salt

The alkaline solution of curcumin was slowly added to the benzene diazonium salt solution (about 5 min). During the addition brick red precipitate was formed. The reaction mixture was kept in a salt-ice water bath during the addition. When the addition was completed, the mixture was stirred while in the cooling bath for 30 minutes to ensure that the reaction goes to completion. After that, cold water was added to the mixture until the precipitation of the product was stopped. The precipitate was collected by suction filtration. Then the solid product was washed on the Büchner funnel with a small amount of cold water, dried with the suction turning on for a few minutes and allowed to dry at room temperature. Finally, the brown

product (5) was washed with 1% HCl solution for 2 hr, then it was filtered and dried at room temperature.

The product weight was about 0.42 g (percent yield = 95%), the melting point of the polymer was heated up to 350°C and did not melt.

2.2.4 An aqueous solution of curcumin

In a 200-ml flask, a 10% solution on NaOH in water was prepared by dissolving sodium hydroxide (3.0 g) in 27 ml water. A 5.4 ml of the NaOH solution were added to another flask (250 ml), and curcumin (0.368 g, 0.01 mol) was added to it. The mixture was stirred until a clear solution was obtained, then it was placed in an ice-salt water bath.

The prepared curcumin solution in alkali medium was added to the benzene diazonium slat as shown in section 2.2.3 to produce a material with a black color (6) with a percent yield of about 90.5%. The melting point of the polymer was heated up to 350°C and didn't melt.

2.3 Cross linking of curcumin based polymer (7)

A 1.0 g of prepared polymer (6) was dissolved in Ethanol (50 ml) in a round bottom flask, and 0.2 g of 1,4-diaminobenzene was added to it. The mixture was stirred until a clear solution was obtained. Then 6 drops of concentrated sulfuric acid was added to the reaction mixture in the flask and the solution was refluxed with stirring for about 1 hr. The reaction mixture was cooled down and transferred to a beaker contains 200 ml of water. The brown solid product was collected by suction filtration, washed with water, then with

diluted solution of sodium carbonate, and then with water. The brown solid was dried in an oven at 80 °C. The product weight was 0.097 g (% yield = 42.1%). The polymer did not melt at 350°C.

2.4 Preparation of polymer from cross-linked curcumin

2.4.1 Crosslinked curcumin (8) was prepared as follows:

Curcumin (1.0 g, 2.8 mmol) was dissolved in ethanol in around bottom flask, then 1,4-diaminobenzene compound (0.3 g, 3.0 mmol) was added to it, followed with 10 drops of concentrated sulfuric acid. The produced solution was refluxed for 2.5 hours with stirring. Then, ethanol was removed under vacuum and the orange to brown residue was washed sequentially with distilled water, diluted solution of sodium bicarbonate and water. The produced crude solid was re-crystallized from methanol-water (7:3 by volume), to shiny reddish brown solid.

2.4.2 Preparation of diazonium salt

In a small beaker a $NaNO_2$ (0.28 g, 0.004 mol) was dissolved in12.5 ml of water. A 1,4-diaminobenzene (0.12 g, 0.001 mol) was slowly added to a flask containing HCl solution (15 ml of HCl in 28 ml of water). The produced mixture was stirred until 1,4-diaminobenzene completely dissolved. The flask containing 1,4-diaminobenzene solution was cooled in an ice-salt bath (-5 to -10 °C) (some 1,4-diaminobenzene may precipitate out upon cooling). While keeping the solution at -5 to -10 °C, the sodium nitrite solution was slowly added (prepared as shown prevously). The mixture was well-stirred

during addition and when the addition was completed, the mixture was stirred for another 2-3 minutes to ensure complete reaction. The slightly turbid pale grey solution was formed and indicated for the formation of benzenediazonium salt solution.

2.4.3 Synthesis of Poly(curcumin-co-p-phenylenediamine) (9)

A solution of crosslinked curcumin (1.0 g, 0.001mol) and NaOH(aq) (14.7 ml ,10%) was slowly added dropwise within 5 min to the benzenediazonium salt solution (prepared in step 2.4.2 above) placed in an ice-slat bath . A large mass of brick red precipitate was formed during addition. The reaction mixture was stirred efficiently. When the addition was completed, the mixture was stirred and cooled for 30 minutes to ensure the reaction goes to completion. After that, the mixture was leaved without moving to complete precipitation of the product. The product was heated upto 350 ^{o}C and did not melt.

2.5 Preparation of polymer (12) from 4-((1*E*)-2-(5-(4-hydroxy-3-methoxystyryl)-1-phenyl-1*H*-pyrazol-3-yl)vinyl)-2-methoxyphenol

To a round bottom flask equipped with a magnetic stirring bar and a condenser was added 0.5 g of curcumin (1.5 mmol), to it was added ethanol (20 ml), the mixture was stirred until a clear solution was obtained. Then, 0.25 g of phenyl hydrazine hydrochloride (1.5 mmol) was added, followed with 10 drops of concentrated sulfuric acid. The produced solution was refluxed for 2 hr, then it was cooled down. Ethanol was evaporated and the

green solid residue (11) was washed with 20 ml 1% Na_2CO_3, filtered and washed with water and dried at room temperature. The reaction is shown in Eq. 3.4, page 54.

The diazonium salt solution was prepared as described in section 2.2.2.

A 0.453 g of pyrazol (11) was dissolved in 5.4 ml of 10 wt.% aqueous solution of sodium hydroxide. The resulted solution was added slowly to the benzenediazonium salt solution through 5 minutes and a large amount of brick red precipitate was formed during addition. The reaction mixture was stirred efficiently and cooled in an ice-salt water bath during the addition. When the addition was completed, the mixture was stirred and cooled for 30 minutes to ensure the reaction goes to completion.

After that, cold water was added to the mixture until the precipitation was stopped and the mixture was filtered by suction filtration. Then the solid product was washed on the Büchner funnel with a small amount of cold water, dried with the suction turning on for a few minutes and allowed to dry at room temperature. Finally, the black product (12) was stirred in a solution of 1% HCl solution for 2 hr to remove residual un-reacted amine, then it was washed with water, filtered and dried at room temperature. The prdocut did not melt by heating over a 350°C.

2.6 Preparation of Copper complex (13) from polymer (6)

A 0.2 g of the polymer (6) was dissolved in 5.0 ml of ethanol in a 50 ml round bottom flask and 0.1 g of copper acetate dissolved in 1.0 ml of water was added to it. The produced solution was mixed and refluxed with stirring

for 2 hours. Then, the condenser was removed and the reaction mixture was boiled until the volume is reduced by 50% and cooled to room temperature, the brown precipitate (13) was collected by suction filtration, washed with water and dried at room temperature.

Preparation of Iron complex (14) form polymer (6)

Iron (II) chloride ($FeCl_2 \cdot 6 H_2O$) (0.1 g) was dissolved in 1.0 ml of distilled water. A solution of the polymer (6) (0.2 g) in ethanol (5.0 ml) was added over a period of 10 minutes with stirring. To the resulting mixture, sodium acetate solution (0.11g in 1 ml of water) was added over 5 minutes. The reaction was heated to 80˚C for 15 minutes, followed by cooling in an ice bath. Finally, the brown precipitate (14) was filtered using Buchner filtration, washed with cold distilled water and dried at room temperature.

2.8 Hygroscopic properties of the curcumin based polymer

A sample from the curcumin based polymer (5) (0.2004 g) was placed on the dry watch glass and the weight of watch glass with polymer was recorded. After 6 days, the mass of watch glass with polymer was measured again and the difference between two weights was recorded. The mass of polymer was decreased by 0.0006 g. The results indicate that the polymer is not hygroscopic.

2.9 Solubility of the polymers in Ethanol

The solubility of all seven prepared polymers was tested in ethanol. None of them was soluble as shown in Table 2.1 below.

Table 2.1: Solubility of prepared polymers in Ethanol

Polymer	Solubility
5	Insoluble
6	Insoluble
7	Partially Soluble
9	Insoluble
12	Insoluble
13	Insoluble
14	Insoluble

2.10 Doping of the polymer

The doping process was carried out by suspending 1.0 g of the polymer in a 0.1 M of HCl-Ethanol solution and the mixture was stirred overnight. Then Ethanol was evaporated, and the product was dried in an oven at 80 ^{o}C.

The above experiment was repeated with 0.25 M and 0.5 M of HCl-Ethanol solution, and the conductivity of the doped polymers was measured.

2.11 Conductivity Measurement

The conductivity of the curcumin based polymers (5,6) and its cross-linked derivative (7) was measured with and without doping by using Two Point Contacts technique.

The parallel plate capacitor style of the sample (pellet of the sample) was prepared. In order to measure the electrical conductivity, the samples were painted with silver paint and left to dry for 30 min. Thereafter, the samples

electrodes were connected in series to a Kiethley high resolution voltage source and a Kiethley 485 picoammeter. The picoammeter is capable of measuring low currents down to 10^{-14} A with the highest possible voltage value being 100 V and the maximum measurable resistance is 10^{16} **Ω**. To assure the accurate measurements, the Current-Voltage characteristics were recorded in the voltage range of -100-100 V in one volt steps. The average resistance (R) was calculated from the slopes of the I-V curves. The conductivity (σ) was determined from the rule: σ = L/RA, where A is the area of the sample and L is the distance between the electrodes. Results are shown in Table 2.2.

Table 2.2: Conductivity of polymers (5-7) with and without doping

Polymer	Doping with HCl (M)	Conductivity $(\Omega.m)^{-1}$
5	Without doping	$7.76493x10^{-08}$
6	Without doping	$2.3686x10^{-08}$
	0.1 M	$1.65143x10^{-08}$
	0.25 M	$1.85619x10^{-08}$
	0.5 M	$4.41805x10^{-08}$
7	Without doping	$2.23842x10^{-08}$
	0.2 M	$1.19592x10^{-08}$
	0.25 M	$2.11846x10^{-08}$
	0.5 M	$1.40112x10^{-08}$

2.12 Testing for Antibacterial Activity

2.12.1 Materials

Mueller-Hinton agar, Mueller-Hinton broth, 0.5 McFarland standard, normal saline and 5% Dimethyl sulfoxide (DMSO) solution.

2.12.2 Microorganisms used

Bacterial strains used in this study were *Escherechia coli*, *Staphylococcus aureus* strain 1, *Staphylococcus aureus* strain 2 and *Klebsiella pneumoniae.*

2.12.3 Testing the antibiotic sensitivity profile of bacteria used in this study

Three colonies of bacteria were transferred to sterile tubes each containing 5.0 ml of Mueller-Hinton broth. Then, Mueller-Hinton agar plates were inoculated by streaking bacterial swabs over the entire surface of the plates and allowed to dry at room temperature. The four types of bacteria were treated in the same manner. The antibiotic disks were sown in agar and the plates were incubated at 37˚C for 18 to 24 hours. Then zones of inhibition were measured in millimeters and the resulted response was classified to three types: resistant, intermediate and susceptible as shown in table 2.3.

The antibiotics that used in this test are: Tetracycline (TE), Kanamycin (K_{30}), Meropenem (MEM), Norfloxacin (NOR), Cefuroxime (CXM_{30}), Trimethoprim/Sulfamethoxazole (25 μg) (SXT_{25}), Amikacin (AK_{30}), Nalidixic acid (NA_{30}), Oxacillin (OX_1) and Cefoxitin (FOX_{30}).

Table 2.3: Antibiotic sensitivity profile of bacteria used in this study

Antibiotic	*E.coli*	*S.aureus*strain 1	*S. aureus* strain 2	*K.pneumoniae*
TE	S	S	S	R
K_{30}	S	R	R	R
MEM	S	R	R	R
NOR	S	S	S	I
CXM_{30}	S	S	S	R
SXT_{25}	S	S	S	R
AK_{30}	S	R	I	S
NA_{30}	S	R	R	I
OX_1	-	S	S	-
FOX_{30}	-	S	S	-

***R: Resistant, I: Intermediate, S: Susceptible**

2.12.4 Broth dilution method (Determination of MIC)

Three colonies of bacteria were transferred to sterile tubes each containing 5.0 ml of Mueller-Hinton broth and turbidity of the bacterial suspensions was adjusted to reach an optical density equivalent to a 0.5 McFarland standard to give a bacterial suspension of $1.5x10^8$ cfu/ml. (cfu: colony forming unit). Then, 5 µl of the previous solution were diluted in normal saline to give a bacterial suspension of $0.5x10^6$ cfu/ml and 2 µl of this solution were added to all wells that specified to this type of bacteria.

All wells in microplate, which used to measure MIC, contain equal volumes of basic solution (100 µl of Mueller-Hinton broth) and concentrations of cells (10^4 cfu/ml), but different concentrations of compounds. 100 µl from 200 µg/ml concentration of compound solution were added to the first row of microplate wells and two-fold serial dilutions were prepared from the compounds in the broth.

The microplates were incubated at 37°C for 18 to 24 hours and the lowest concentration of the compound that resulted in inhibition of bacterial growth was considered as the MIC. The results are shown in tables 2.4, 2.5, 2.6 and 2.7.

Table 2.4: MIC Determination of compounds (5-9,12-14) against *E. coli*

Conc. (µg/ml)	5	6	7	8	9	12	13	14
100	-	+	-	-	-	+	-	+
50	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+
12.5	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+
3.125	+	+	+	+	+	+	+	+
1.5625	+	+	+	+	+	+	+	+
0.78125	+	+	+	+	+	+	+	+
0.390625	+	+	+	+	+	+	+	+
0.1953125	+	+	+	+	+	+	+	+

*** Bacterial growth (+, -)**

Table 2.5: MIC Determination of compounds (5-9,12-14) against *S. aureus* strain 1

Conc. (µg/ml)	5	6	7	8	9	12	13	14
100	-	-	-	+	+	+	+	+
50	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+
12.5	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+
3.125	+	+	+	+	+	+	+	+
1.5625	+	+	+	+	+	+	+	+
0.78125	+	+	+	+	+	+	+	+
0.390625	+	+	+	+	+	+	+	+
0.1953125	+	+	+	+	+	+	+	+

* Bacterial growth (+, -)

Table 2.6: MIC Determination of compounds (5-9,12-14) against *S. aureus* strain 2

Conc. (µg/ml)	5	6	7	8	9	12	13	14
100	-	-	-	-	-	-	-	-
50	-	-	-	-	-	+	+	-
25	-	+	-	-	+	+	+	+
12.5	-	+	-	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+
3.125	+	+	+	+	+	+	+	+
1.5625	+	+	+	+	+	+	+	+
0.78125	+	+	+	+	+	+	+	+
0.390625	+	+	+	+	+	+	+	+
0.1953125	+	+	+	+	+	+	+	+

* Bacterial growth (+, -)

Table 2.7: MIC Determination of compounds (5-9,12-14) against *K.pneumoniae*

Conc. (µg/ml)	5	6	7	8	9	12	13	14
100	+	+	+	-	+	+	+	+
50	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+
12.5	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+
3.125	+	+	+	+	+	+	+	+
1.5625	+	+	+	+	+	+	+	+
0.78125	+	+	+	+	+	+	+	+
0.390625	+	+	+	+	+	+	+	+
0.1953125	+	+	+	+	+	+	+	+

*** Bacterial growth (+, -)**

2.12.5 Determination of MBC

Subcultures from the above dilutions, that inhibited the bacterial growth, were done on Muller-Hinton plates and incubated at 37°C for 18 to 24 hours. The lowest concentration that resulted in total inhibition of bacterial growth was considered the MBC. The results are shown in table 2.8.

Table 2.8: MIC and MBC Determination of compounds (5-9,12-14)

Compound	*E.coli* (μg/ml)		*S.aureus* strain 1 (μg/ml)		*S.aureus* strain 2 (μg/ml)		*K.pneumoniae* (μg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5	100	100	100	-	12.5	-	-	-
6	-		100	-	50	-	-	-
7	100	100	100	-	12.5	-	-	-
8	100	-	-	-	25	-	100	-
9	100	-	-	-	50	-	-	
12	-	-	-	-	100	-	-	
13	100	-	-	-	100	-	-	
14	-	-	-	-	50	-	-	

Chapter Three

Results and Discussion

Curcumin (1) is a multifunctional natural product with hydroxyl groups and carbonyl groups distributed throughout a complete conjugated structure of aromatic and aliphatic chain. The presence of the hydroxyl groups and the carbonyl groups makes excellent candidate for condensation and a nucleophilic substitution. Converting curcmin into a polymeric material with a complete conjugation from head to tail, and adding a heterocyclic moiety to the polymeric backbone could make the polymer a material with demand functionality.

Curcmin could be converted to a polymeric material by reacting it with a bifucntional monomer having substituent that react readily with the benzene ring of the curcumin. Monomer chosen for this purpose was the diazonium salt.

3.1 Preparation of the 1,4-bisdiazonium benzene salt monomer

Diazonium salt used in this works was 1,4-bisdiazonium benzene (3). Its structure is shown in Fig 3.1.

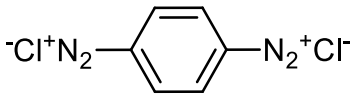
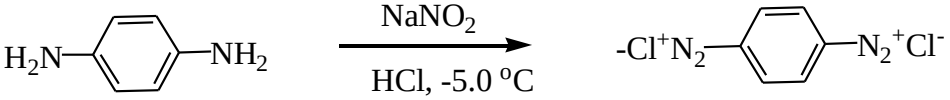


Fig 3.1: Molecular structure of 1,4-bisdiazonium benzene.

1,4-bisdiazonium benzene was prepared from reacting 1,4-diaminibenzene (2) with sodium nitrite in an aqueous HCl solution as shown in Eq. 3.1.

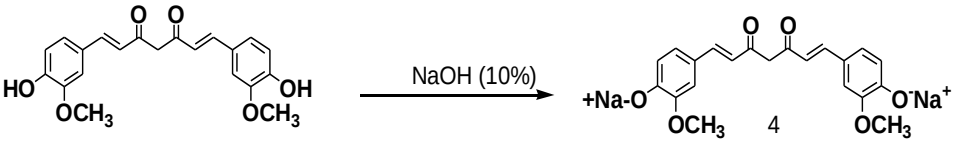


2 3 Diazonium salt **Eq. 3.1**

The diazonium monomer is known to under nucleophilic substitution with a variety of nucleophiles to new aromatic derivative with the loss of N_2.

3.2 Preparation of Curcumin diphenolate monomer

Curcumin has two phenolic units; they were converted to diphenolate by reacting it with sodium hydroxide as shown in Eq. 3.2. The conversion of curcumin to diphenolate was carried out in various solvents such as water, ethanol and N,N-dimethylacetamide (DMAc). Cucrmin was first dissolved or suspended in a solvent. Best results regarding yield and product quality was obtained using DMAc.



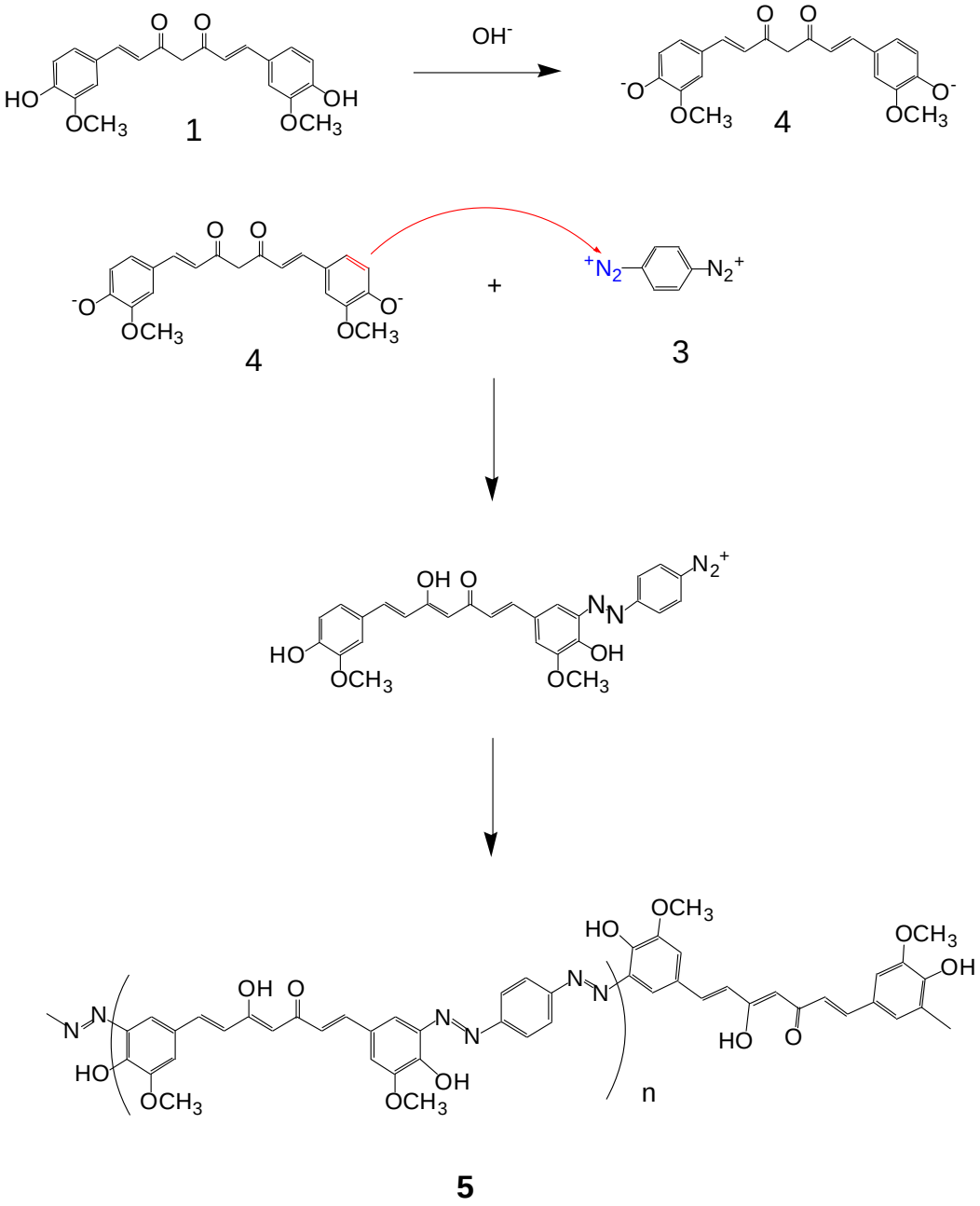
4 **Eq. 3.2**

sodium 4,4'-((1*E*,6*E*)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxyphenolate)

3.3 Preparation of curcumin based polymer

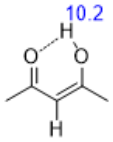
Curcumin based polymer was prepared by reacting the diazonium monomer (3) with curcumin diphenolate (4). The reaction is shown in scheme 3.1. The reaction occurs via diazo coupling as shown in scheme 3.1. The reaction as shown before was carried out in basic solution to convert OH in curcumin to phenoxide. Formation of phenoxide activates the benzene ring in curcumin for electrophilic aromatic substitution reaction.

Scheme 3.1

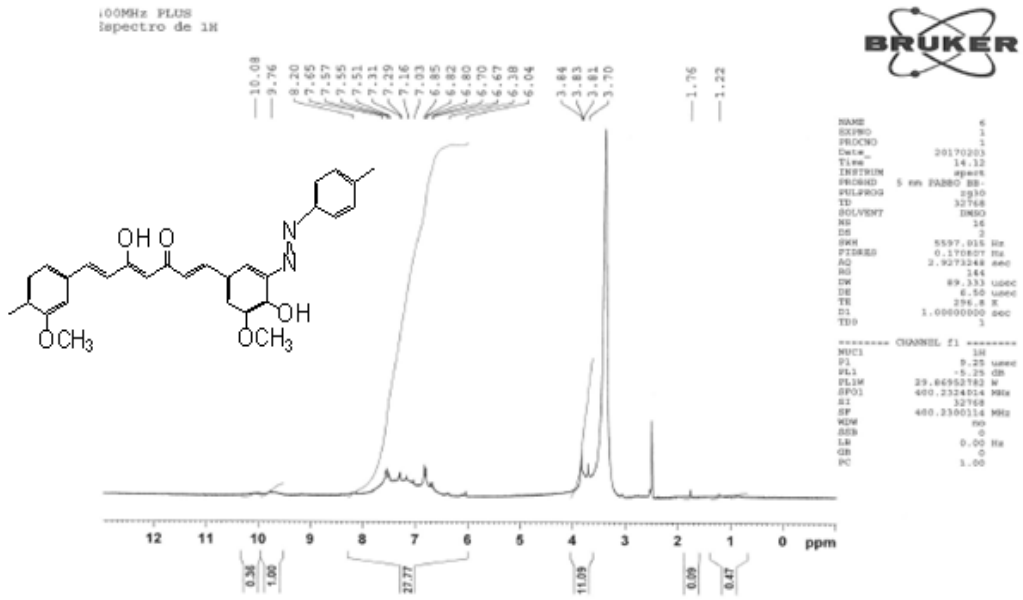


3.3.1 NMR analysis of the polymer (5)

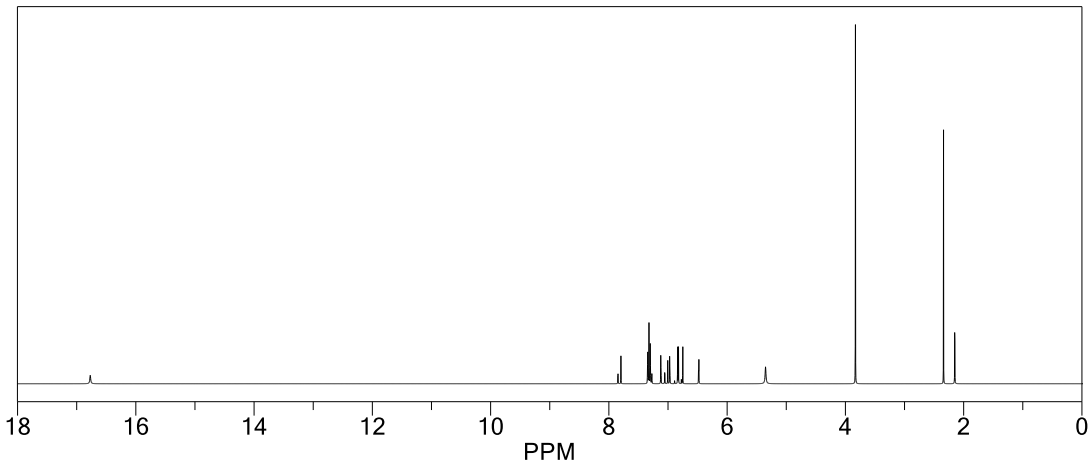
Proton NMR and C-13 were performed on the polymer. The obtained ^{1}H NMR spectrum is shown in Fig. 3.2. The NMR spectrum clearly shows aromatic protons (δ 6.7 to 7.8 m) and methoxy protons (δ 3.8). The obtained ^{1}H NMR is in agreement with that generated by chemdraw. The integration of the aromatic peaks is about 27.8 and that for the methoxy groups is about 11.1, the proton ratio in the aromatic region to that in the methoxy region is in agreement with the structure (12:6). The hydroxyl proton results due to resonance of curcumin β-diketone showed at a chemical shift of about δ10.2.



The polymer was also analyzed by ^{13}C NMR. The obtained spectrum is shown in Fig 3.3. The spectrum clearly shows the methoxy carbons at δ 56.1, the aromatic and vinylic carbons at δ 115 to 160 ppm and the carbonyl carbons at δ 183.1. The obtained ^{13}C NMR is in agreement with that generated by chemdraw shown in Fig 3.3b.

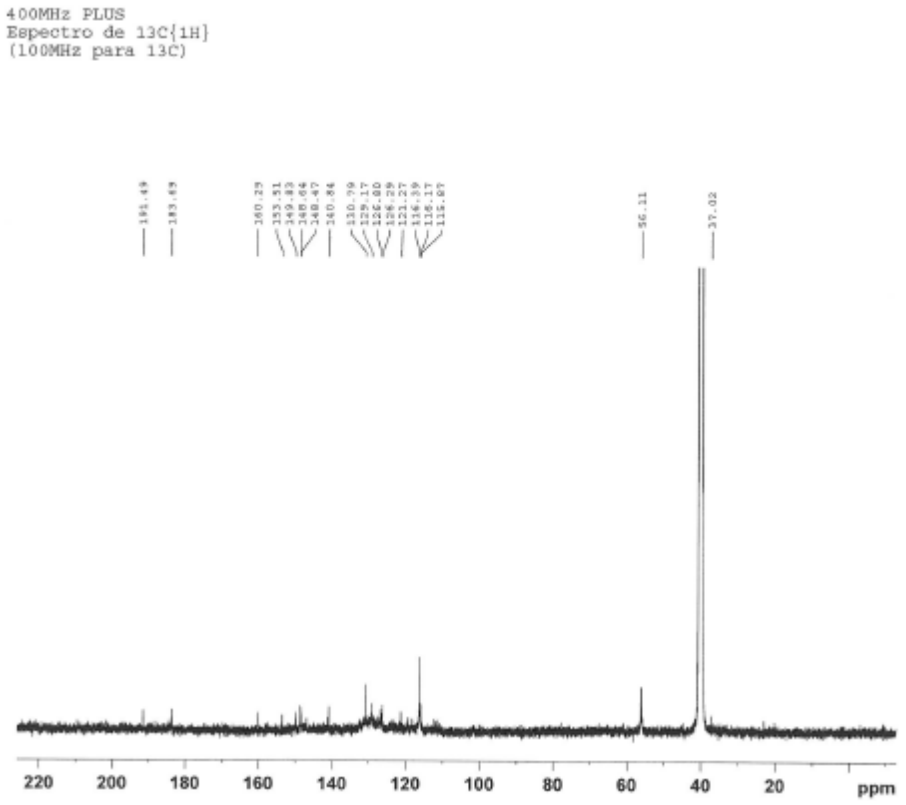


(a)

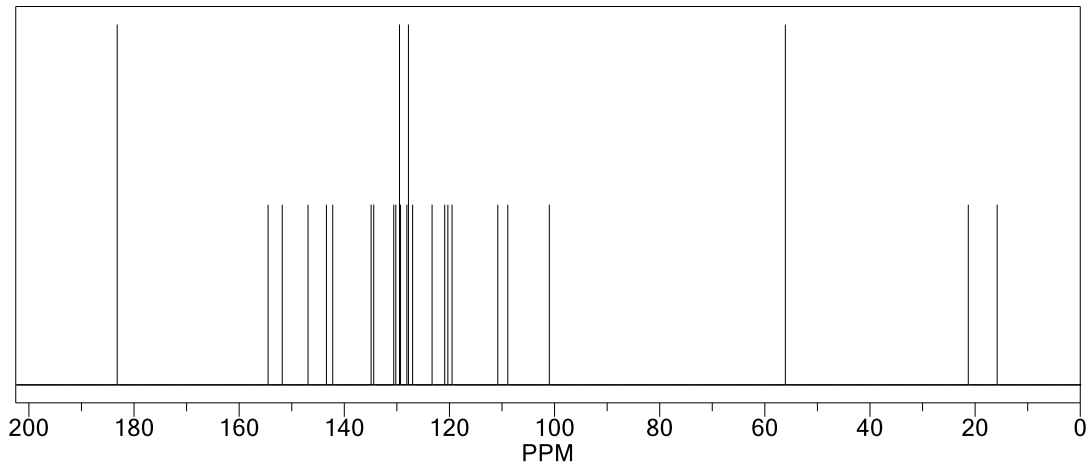


(b)

Fig 3.2: a) ^{1}H NMR of polymer repeat unit; b) NMR of polymer repeat unit predicted by Chemdraw.



(a)



(b)

Fig 3.3: a) ^{13}C NMR spectrum of the polymer. b) ^{13}C NMR spectrum of the polymer generated by the Chemdraw.

3.3.2 MS/MS analysis of poly(curcumin-co-p pheneylenediamine) (5).

A sample of polymer (5) was dissolved in DMSO (0.1 ppm) and injected in the MS/MS instrument. Obtained MS spectrum is shown in Fig 3.4. The spectrum showed a MW higher than 1922 g/mol. The instrument can only record a molar mass of up to 2000 g/mol. Figure 3.5, shows a selected part of the MS spectrum, it shows the repeat unit of the polymer with a molar mass of 473 g/mol.

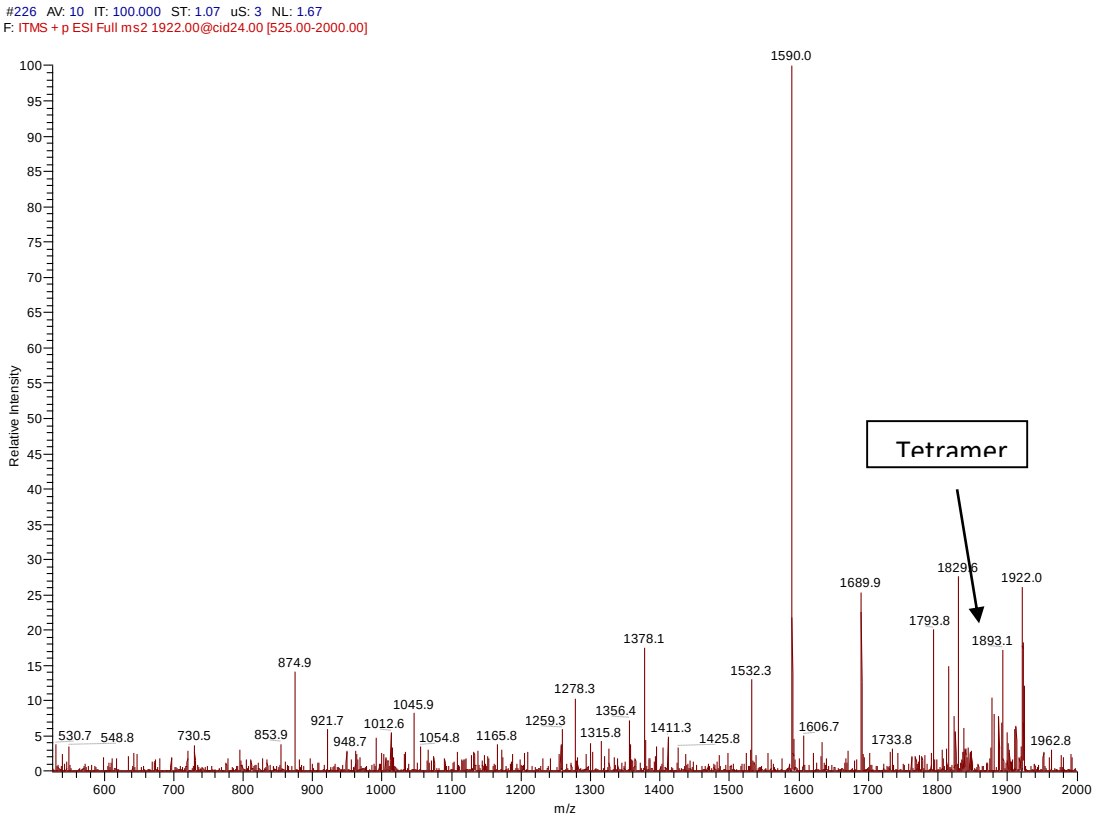


Fig 3.4: MS spectrum of polymer (5)

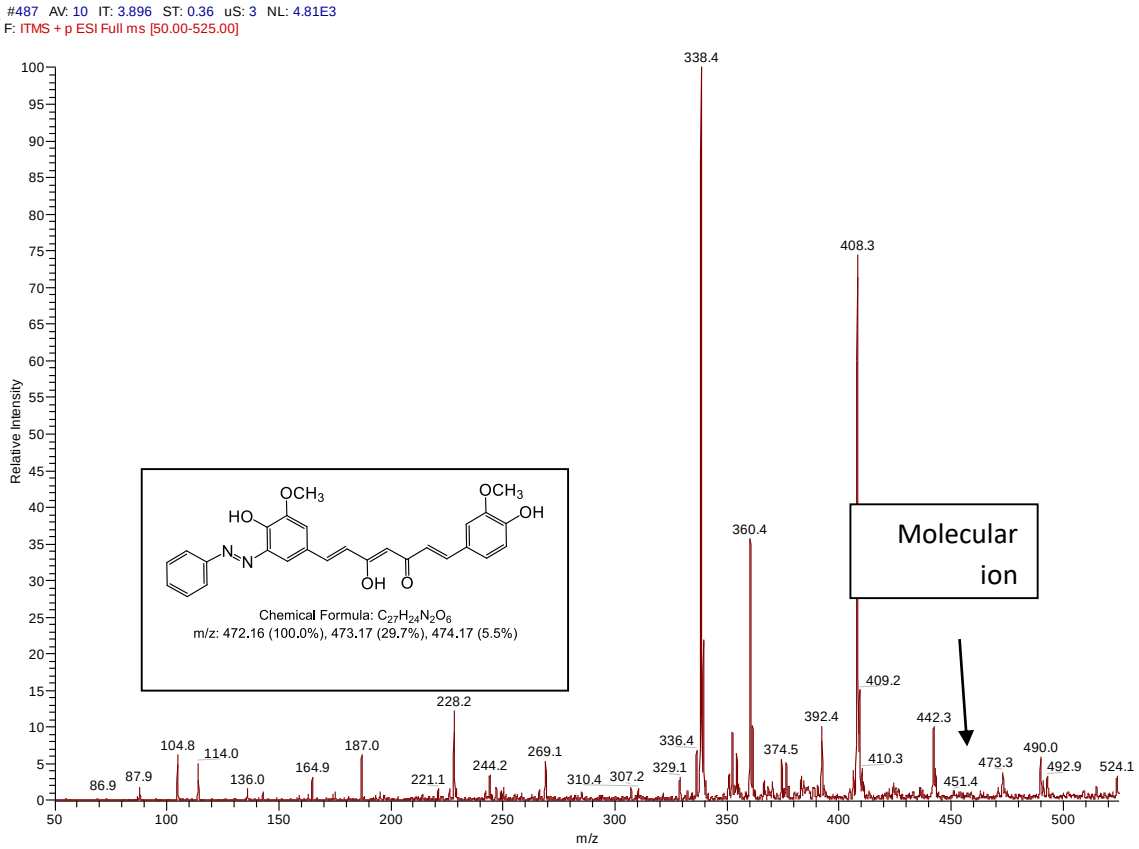


Fig 3.5: MS of polymer (5) shows the molar mass of the repeat unit

3.3.3 Spectrophotometric analysis of the polymer (5)

The synthesized polymer was analyzed by UV spectroscopy. The analysis was performed on a Spectrophotometer using a cell with 1 cm width. The UV scanning was performed in the range of λ 190 to 600 nm. The acquired UV spectrum is shown in Fig 3.6. The figure shows two bands with high intensity at 201 and 414 nm and third band with low intensity at 279 nm. The bands could be attributed to C=C of the aromatic ring, C=C of alkene, N=N of azo and C=O of ketones.

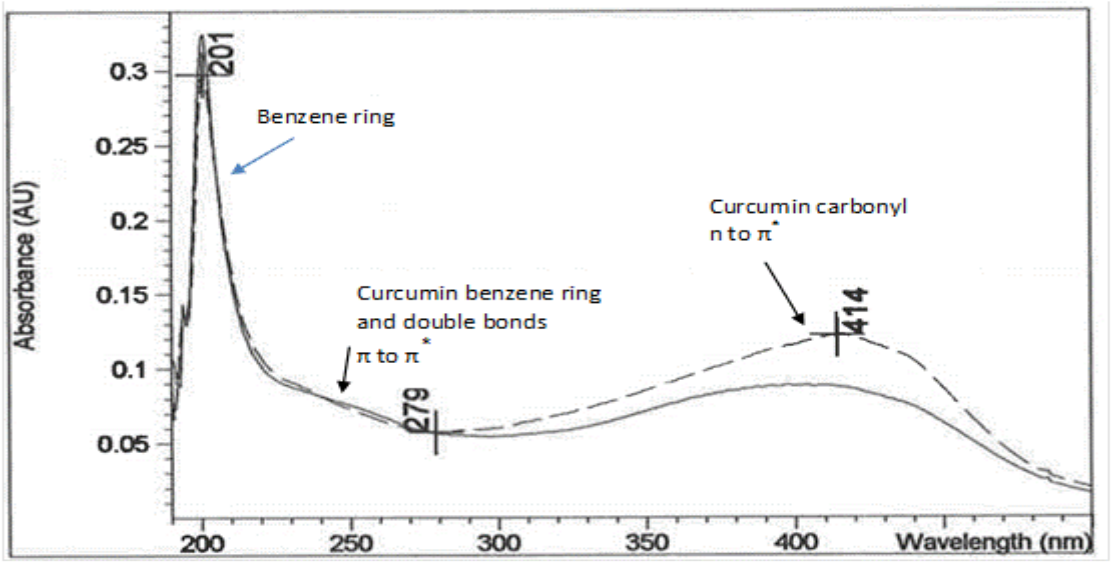


Fig. 3.6: UV spectrum of the polymer (5).

3.3.4 FT-IR analysis of curcumin based polymer (5)

The polymer was also analyzed by FT-IR. The acquired FT-IR spectrum is shown in Fig 3.7. The IR spectrum shows the following band: a strong band at 1550 cm^{-1} which correspond to a conjugated carbonyl, a medium band at 1511cm^{-1} which could be related to C=C cm^{-1} (aromatic), a 1120 cm^{-1} corresponds to C-O of alcohol and a 1272 cm^{-1} for the C-O of ether. A weak band appears at 3100 cm^{-1} which could be related to OH of phenol.

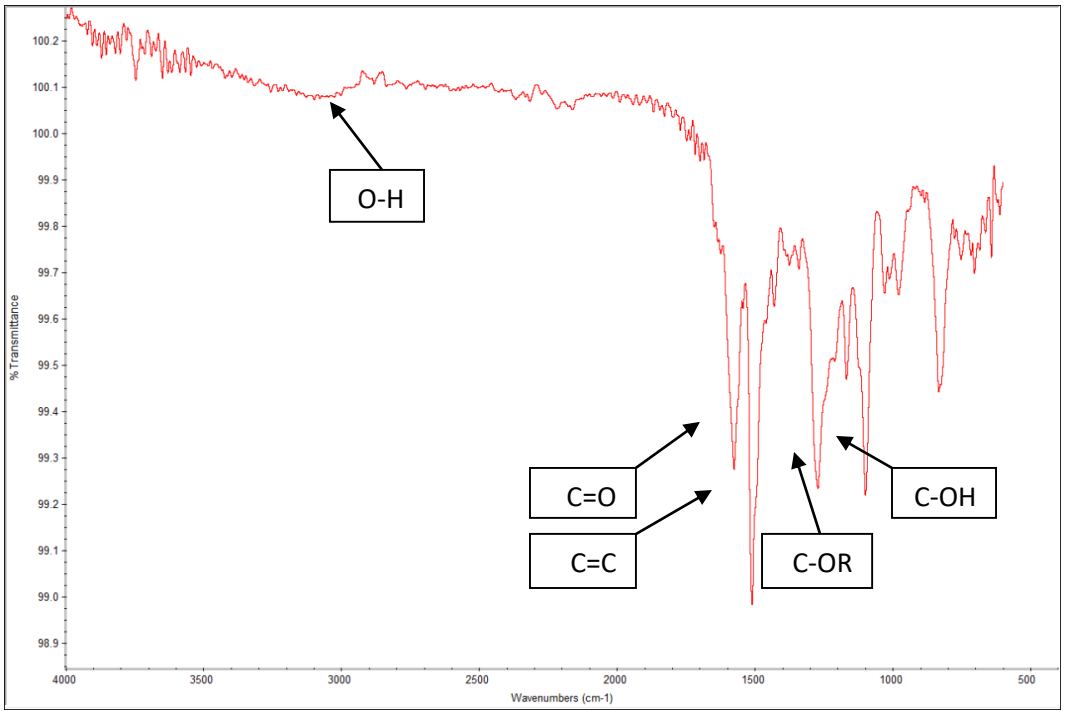


Fig 3.7: FT-IR of the curcumin based polymer (5)

3.3.5 Thermal analysis of the polymer (5)

The polymer was subjected to differential thermogravemetric analysis (DTG). The results are shown in Fig 3.8. The thermogram shows that, the polymer stables up to 500 ^{o}C, then starts to decompose. No glass transition temperature is shown in the thermogram, indicating that the polymer is highly crystalline.

Minimum weight loss was shown below 500 ^{o}C. The differential scanning calorimetry shown in Fig 3.9 shows a minor weight loss at about 380 ^{o}C which could be related to the pendant group (OH, OMe, and carbonyl) then major chain breaking occurred at about 500 ^{o}C.

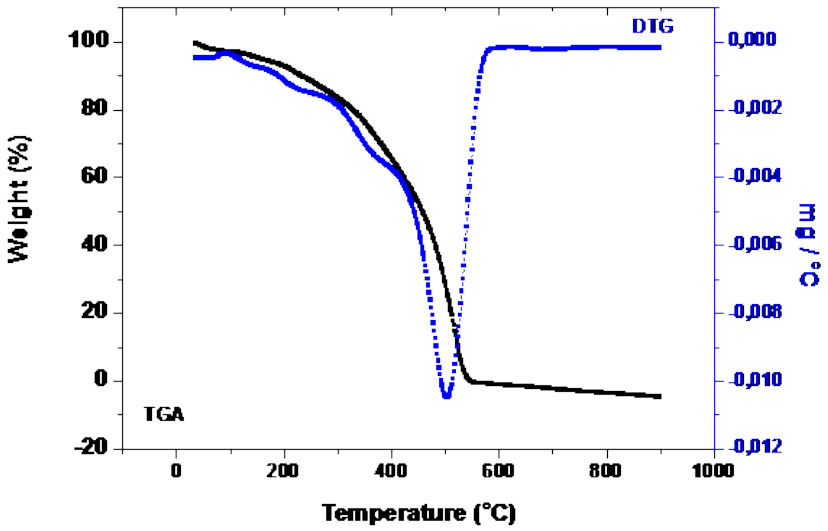


Fig 3.8: DTG thermogram of curcumin based polymer (5)

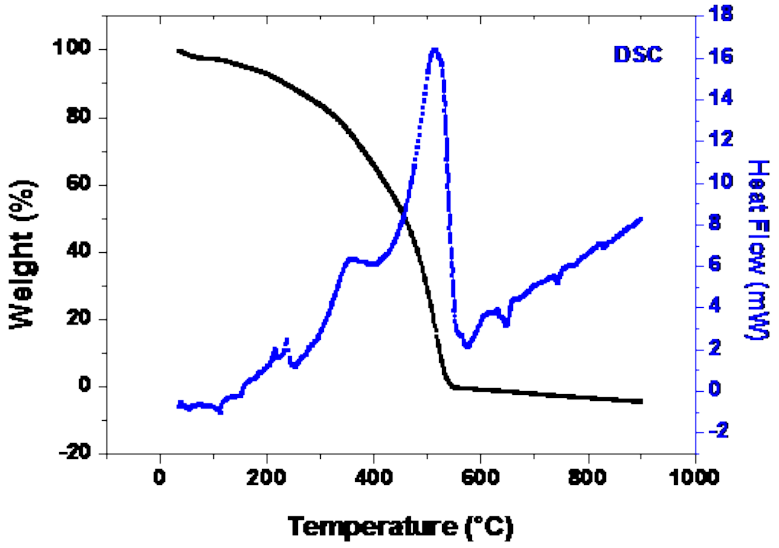


Fig 3.9: DSC thermogram of curcumin based polymer (5)

3.4 Polymer cross-linking

The polymer based curcumin (6) was cross-linked to increase number of conjugations and consequently enhances the conductivity. Polymer (6) was reacted with p-phenylene diamine (2) in ethanol. The reaction was catalyzed by sulfuric acid. Refluxing the mixture for 1 hr produced cross-linking

polymer shown in Fig 3.10. The new polymer (7) has imine functionality. The polymer shows complete conjugation. The structure of the cross-linked polymer (7) was confirmed by FT-IR.

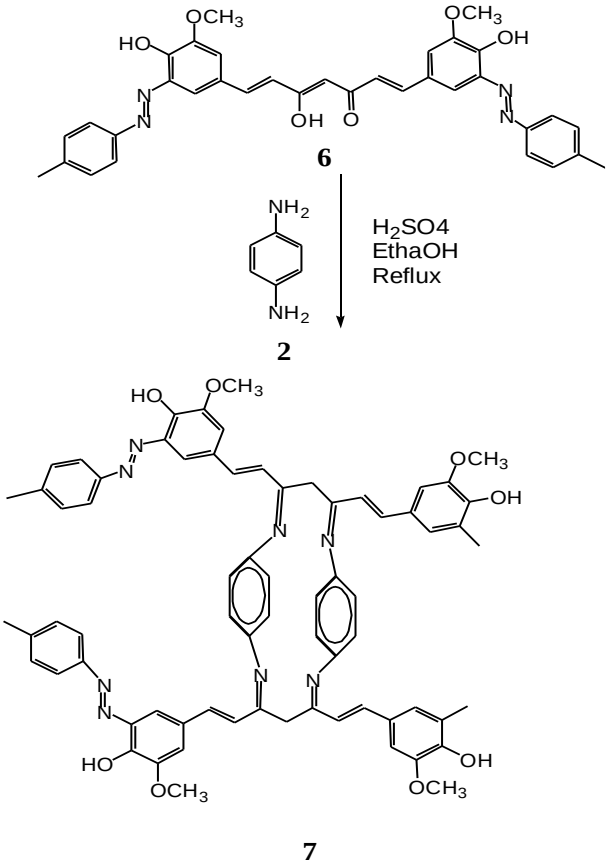


Fig 3.10: Preparation of cross-linked polymer

The aquired FT-IR spectrum is shown in Fig 3.11. The FT-IR spectrum clearly shows that, the carbonyl groups are reacted, since the carbonyl peak disappeared. The IR spectrum shows the following major bands: a weak band at 1541 corresponding to a conjugated C=N, a medium band at 1511 cm^{-1} which could be related to C=C cm^{-1} (aromatic).

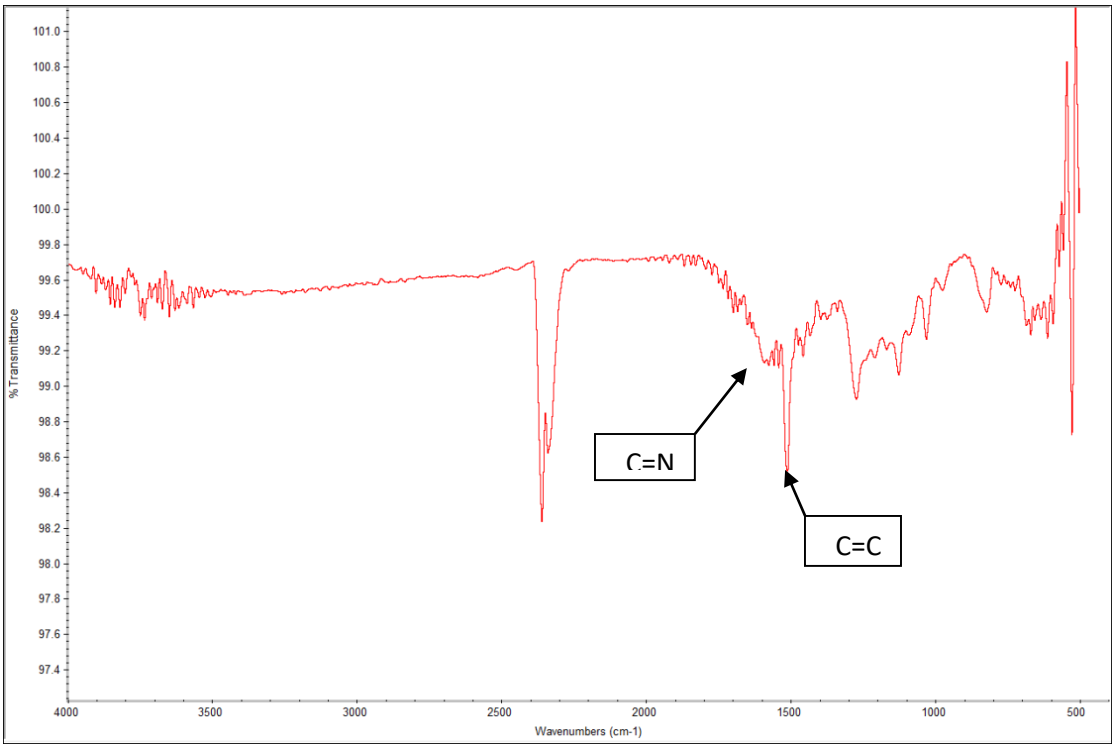


Fig 3.11: FT-IR of cross-linked polymer (7).

3.5 The second approach for curcumin based cross-linked polymer

In this approach curcumin was first cross-linked by reacting it with 2,4-diaminobenzene (2) in ethanol. The reaction was catalyzed by a couple of drops of sulfuric acid. Refluxing the reaction mixture for 2.5 hr produced the target cross-linked curcumin (8). The produce cross-linked curcumin (8) was analyzed by FT-IR. The aquired spectrum is shown in Fig 3.13. The IR spectrum shows the following major bands: a weak stretching band at 3055 cm^{-1} cross-pond to aromatic C-H, a weak band at 1544 cm^{-1} corresponding to a conjugated C=N, a medium band at 1513 cm^{-1} which could be related to C=C cm^{-1} (aromatic).

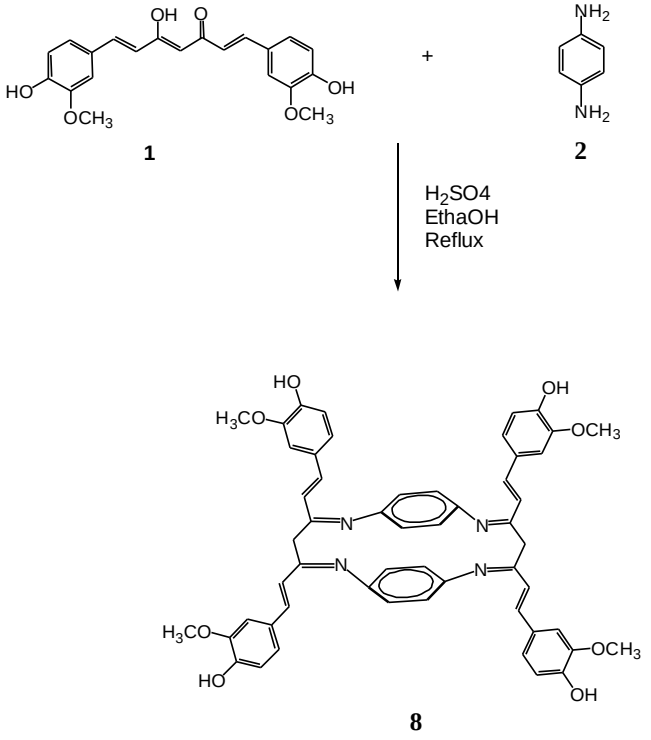
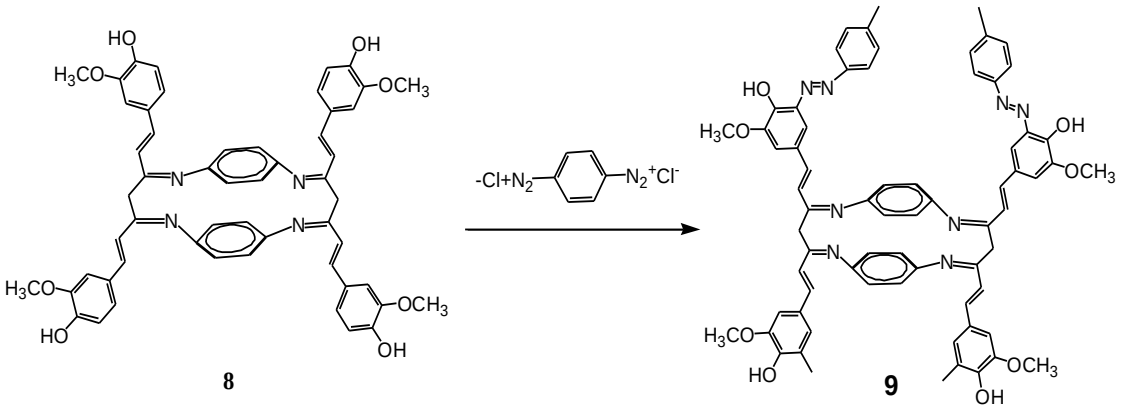


Fig 3.12: Preparation of cross-linked curcumin.

Compound (2) was converted to diazonium salt by reacting it with sodium nitrite in acid medium as shown before. The dizonium salt was then reacted with the cross-linked curcumin (8) as shown in Eq. 3.3 to produce the cross linked polymer number (9).



Eq. 3.3

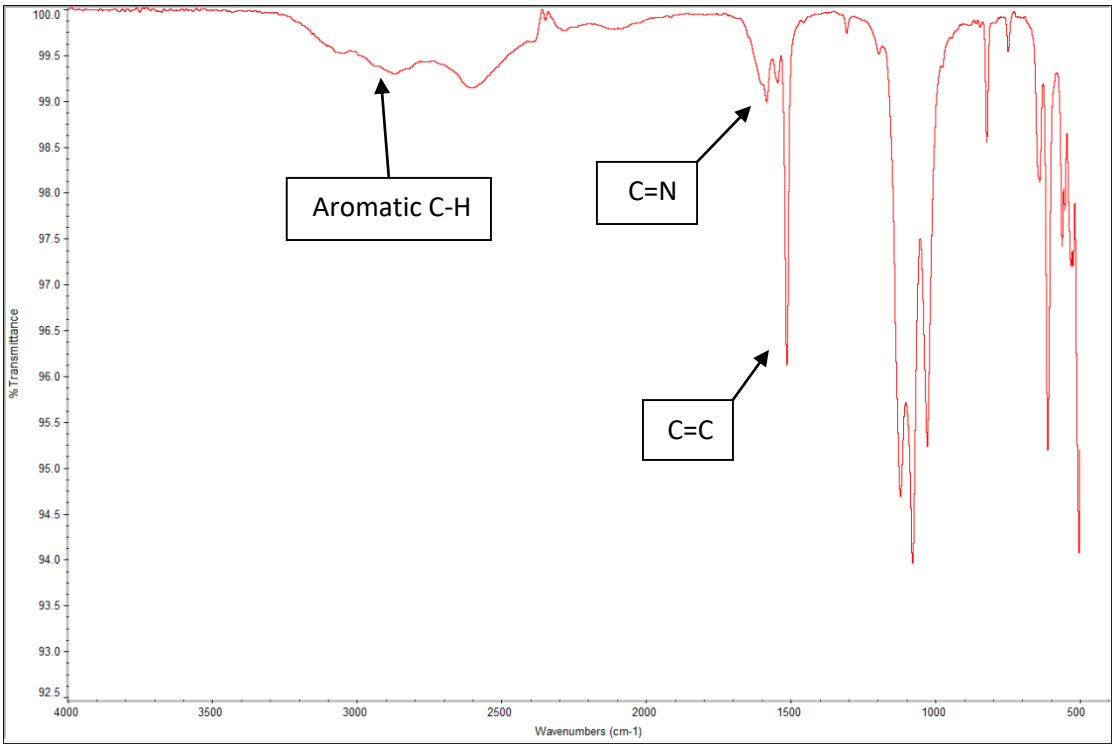


Fig 3.13: FT-IR spectrum of cross-linked curcumin (8)

3.6 Curcumin based polymer with pyrazole pendant group (12)

To increase number of conjugations and the rigidity of the polymer, a pyrazole group was added to curcumin then it was polymerized. Curcumin with pyrazole group was prepared by reacting phenylhydrazine (10) with curcumin in ethanol. The reaction was catalyzed by sulfuric acid. Refluxing the reaction mixture for about 2 hr produced the target derivatized curcumin (11). Compound (11) was analyzed by FT-IR. Obtained spectrum showed the following peaks: 767 cm^{-1} (C-N), 1201 cm^{-1} (C-O of ether), 1508 cm^{-1} (C=C), 1540 cm^{-1} (C=N) and 3734 cm^{-1} (O-H).

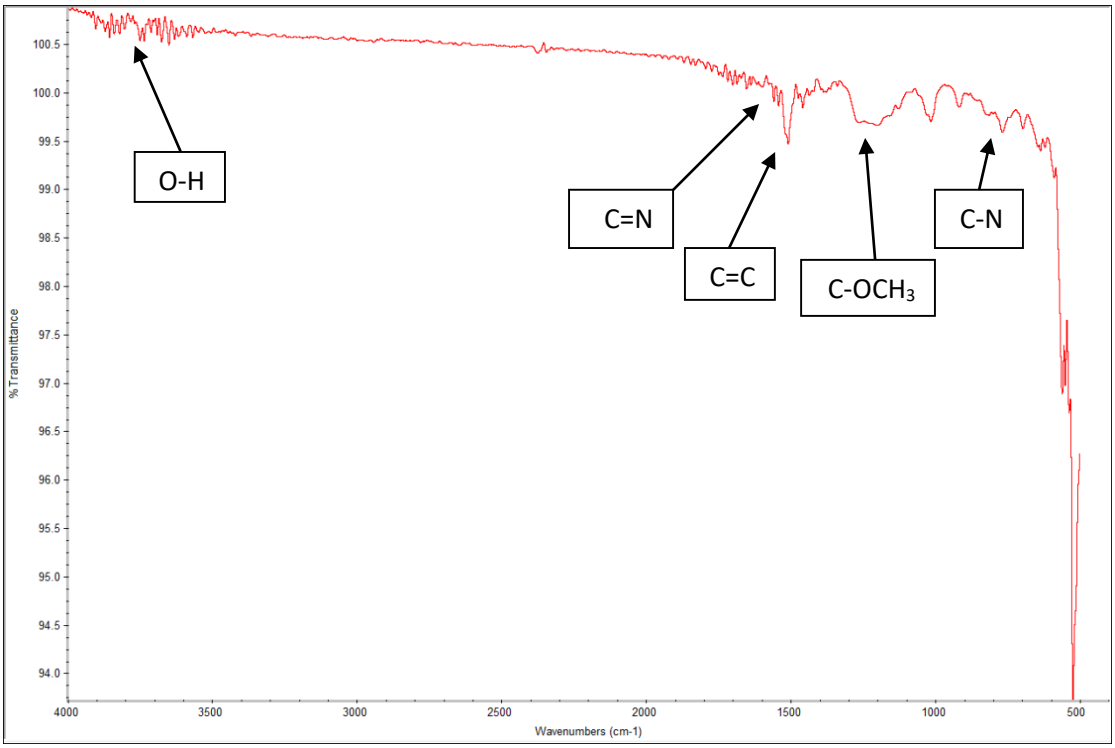
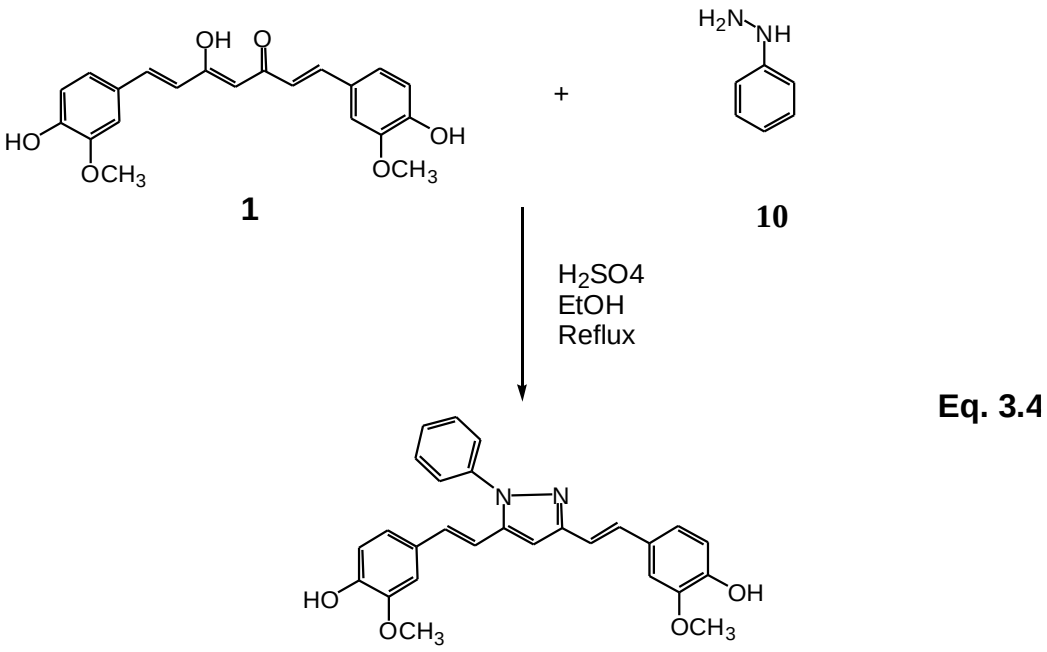


Fig 3.14: FT-IR spectrum of curcumin-pyrazol derivative (11**).**



Eq. 3.4

4,4'-((1*E*,1'*E*)-(1-phenyl-1*H*-pyrazole-3,5-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol)

11

Produced curcumin-pyrazol derivative (11) was then polymerized by reacting it with two equivalents of the diazonium salt of phenylene diamine to produce polymer (12) shown in Fig 3.15.

Compound 12 was analyzed by FT-IR shown in Fig 3.16. Obtained spectrum showed the following peaks: 3734 cm^{-1} (-C-OH), 1540 cm^{-1} (C=N), 1507 cm^{-1} (C=C), 1225 cm^{-1} of (–O-CH_3) and 765cm^{-1} (-C-N).

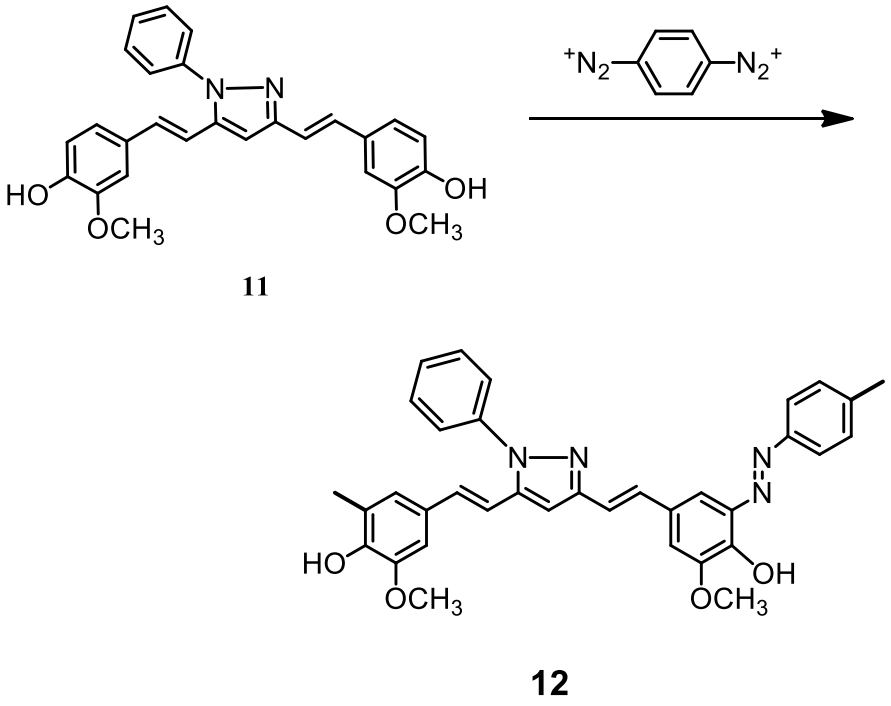


Fig 3.15: Synthesis of curcumin based polymer with pyrazole pendent group

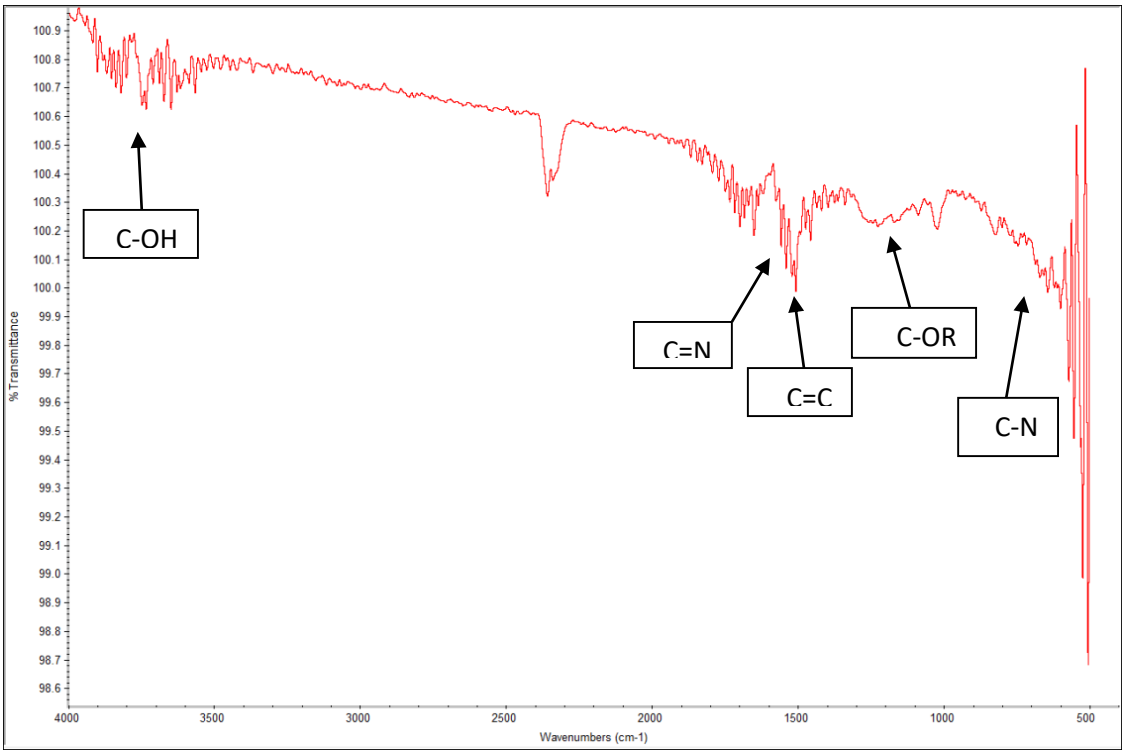


Fig 3.16: FT-IR spectrum of curcumin based polymer with pyrazole pendent group (12).

3.7 Polymer cross-linking with transition metals

In order to enhance the conductivity of the polymer it was complexed with Iron (II) chloride and copper (II) acetate. The complexation was carried out at 80 ^{o}C in Ethanol solution. A representative scheme showing polymer cross-linking with metal and possible structure of metal cross-linked polymer is shown in Fig 3.17.

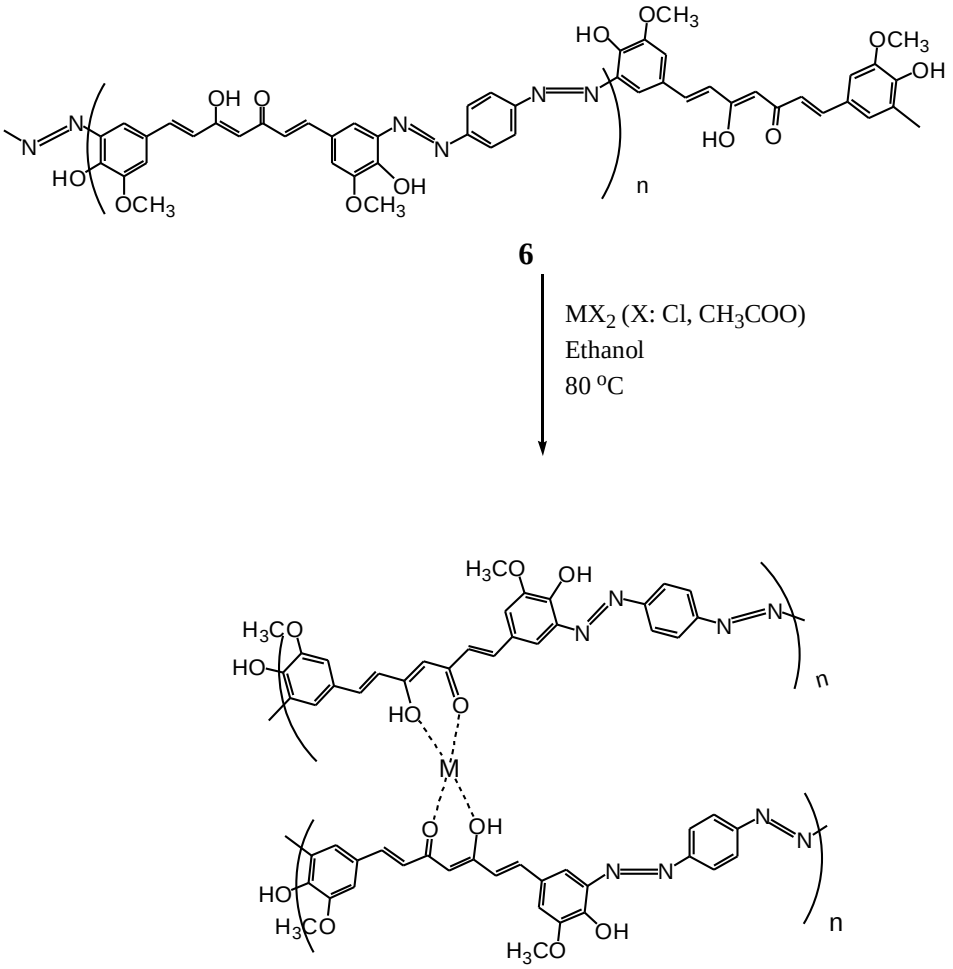


Fig 3.17: synthesis of curcumin based polymer cross-linking with metal, the figure is a representative structure of cross-linked polymer

The FT-IR spectra of cross-linked polymer with iron and copper are shown in Figs 3.18 and 3.19 respectively. Both IR spectra showed the following peaks: 1273 cm^{-1} (C-OCH_3), 1509 cm^{-1} (C=C), 3750 cm^{-1} (O-H) and the absence of the carbonyl, an indication the absence of the carbonyl group.

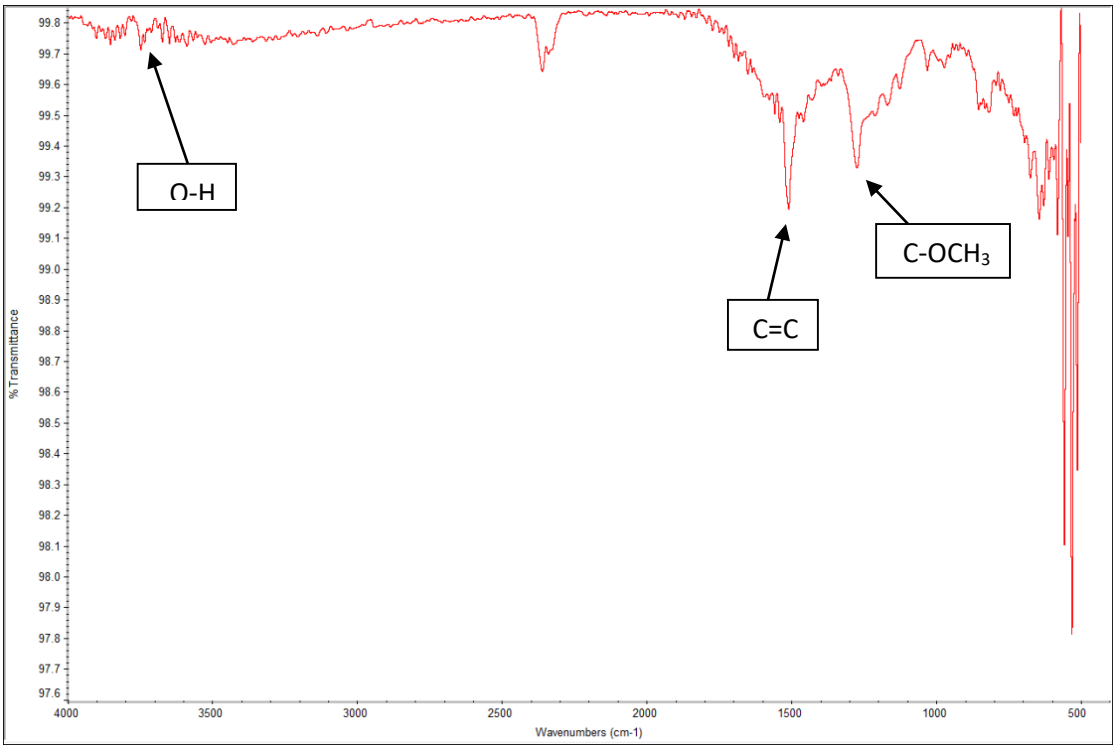


Fig 3.18: FT-IR of curcumin based polymer cross-linked with $FeCl_2$ (14)

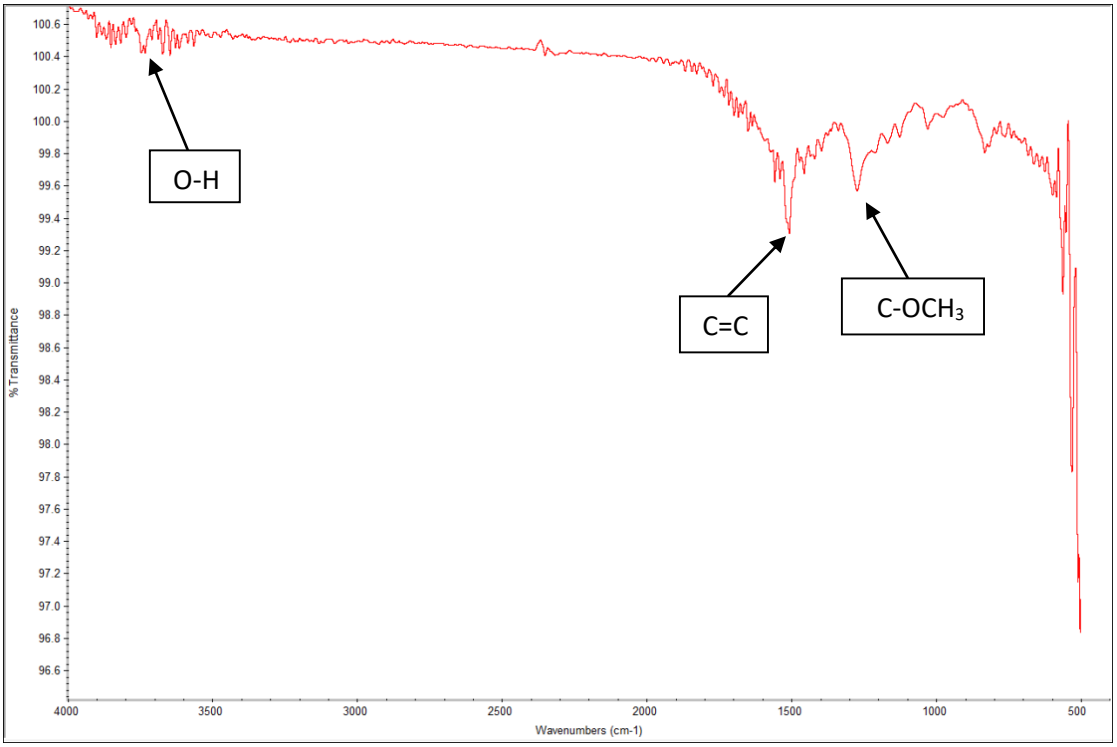


Fig 3.19: FT-IR of curcumin based polymer cross-linked with $Cu(CH_3COO)_2$ (13)

3.8 Evaluation of polymer conductivity

Two Point Contacts technique was used to measure the conductivity of polymers (5,6 and 7). Results showed in table (2.2) revealed that the three polymers are semiconductors and have conductivity about 10^{-8} S/m.

Doping process should enhance the charge transfer and increase the polymer conductivity, but low solubility of them may cause the doping process to fail. Since these polymers are conjugated from head to tail, it is expected to fully conductive. But the semiconductivity was observed could be due to the following reasons: the equipments used for making the pellet for conductivity analysis was not adequate, conductivity meter used could be unsuitable for this type of polymer, the doping process was not efficient, and impurities could be present in the polymer.

3.9 Antimicrobial activities of the prepared polymers

The *invitron* antimicrobial activity was performed on four types of bacteria strains: *E.coli*, *S.aureus* strain 1, *S.aureus* strain 2 and *K.pneumoniae* using the Broth dilution method. All strains were isolated from patients suffering from bacterial infections with the relevant bacteria.

3.9.1 Screening Results:

The curcumin based polymers (5-9,12-14) are subjected to minimum inhibition concentration (MIC) testing and the results are shown intable 2.8. Results revealed that the majority of the synthesized compounds showed varying degree of inhibition against the tested microorganisms.

All prepared curcumin based polymers have shown excellent potency

against *S. aureus* strain 2. The antibacterial potency of polymer (5) that prepared using DMAc is better than polymer (6) that prepared using water against gram positive and negative bacteria.

The cross-linked polymer (7) have the same antibacterial potency of polymer (5) and both have the best potency against *S. aureus* strain 2 (MIC of two polymers equal 12.5 μg/ml).

The cross-linked curcumin (8) is the only prepared compound that has an antibacterial potency against gram negative K.pneumoniae (MIC equals 100 μg/ml).

In comparison between the two prepared polymers that cross-linked with transition metals, we find that the polymer cross-linked with Cu (13) has more potency against gram negative *E.coli* but lower potency against gram positive*S. aureus* strain 2 than the polymer cross-linked with Fe (14).

In addition to the determination of MIC, the minimum bactericidal concentration (MBC) of the prepared compounds was determined and the results are shown in table 2.8.

The results indicate that the polymer (5) prepared using DMAc and the cross-linked polymer (7) are the only ones that give the MBC results. Both polymers have a bactericidal activity against *E.coli* at concentration 100 μg/ml and this means that they kill most (>99.9%) of the viable *E.coli* bacteria at this concentration, and the rest polymers are not have bactericidal activity at all.

Conclusion

1) Curcumin based polymer was prepared by a new polymerizable monomers under mild conditions.
2) Curcumin based polymers could be prepared in several solvents, such as water, Ethanol, and DMAc; and the best results was obtained using DMAc solvent.
3) The prepared polymer could be crosslinked with organic and inorganic crosslinkers.
4) Curcumin based polymer is a semiconducting polymer with conductivity about 10^{-08} S/m..
5) The prepared polymers have a good antibacterial potency especially against *E.Coli* and *S.aureus*.

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جامعة النجاح الوطنية

كلية الدراسات العليا

استراتيجية تصميم مونيمرات من الكركم مكونة لمبلمرات ذات قيمة عالية

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس- فلسطين.

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استراتيجية تصميم مونيمرات من الكركم مكونة لمبلمرات ذات قيمة عالية

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الملخص

العديد من المبلمرات المشتقة من الكركم تم تحضيرها وفحص موصليتها ونشاطها المضاد للبكتيريا. تم تحضير هذه المبلمرات باستخدام طريقة جديدة من خلال مفاعلة الكركم مع ملح الديازونيوم من مادة (p-phenylenediamine) في مذيبين مختلفين: ثنائي ميثيل أسيتاميد (DMAc) وماء، لإنتاج مبلمر يعتبر (completely conjugated). ثم تم تحليل المبلمرات الناتجة باستخدام تقنيات مختلفة، مثل: DSC, DTG, MS, UV, FT-IR, ^{13}C and ^{1}H NMR.

تم فحص موصلية هذه المبلمرات، ووجد أنها أشباه موصلات. ومن أجل تعزيز الموصلية لهذه المبلمرات تم التعديل عليها باستخدام عدة طرق. تم عمل (cross-linking) للمبلمر باستخدام مادة (p-diaminobenzene)، كما أجريت عملية اشتقاق للمبلمر بإضافة حلقة غير متجانسة إليه. بالإضافة إلى عمل (cross-linking) للمبلمرباستخدام المعادن الانتقالية: الحديد والنحاس. بعد ذلك تم فحص موصلية المبلمرات التي تم تعديلها، ولم يلاحظ أي تحسن على موصليتها. وقد يعزى السبب في ذلك إلى عدة عوامل مثل: عدم مناسبة المعدات المستخدمة لتجهيز العينات لفحص الموصلية، عدم مناسبة التقنية المستخدمة في قياس الموصلية لهذا النوع من المبلمرات، عدم فعالية عملية ال (doping)، أو احتمال وجود بعض الشوائب في تلك المبلمرات.

إضافة إلى ذلك تم اختبار النشاط المضاد للبكتيريا لهذه المبلمرات ضد أربعة أنواع مختلفة من البكتيريا (*Escherechia coli*, *Staphylococcus aureus* strain 1, *Staphylococcus aureus* strain 2 and *Klebsiella pneumoniae*). وأظهرت النتائج أن هذه المبلمرات لها فعالية جيدة في مقاومة النشاط البكتيري وخصوصا بكتيريا (*Escherechia coli*)، حيث أن المبلمرات المضادة للميكروبات تعتبر نادرة.