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RESEARCH ARTICLE

Electrochemical synthesis, characterization and antimicrobial activity of nano-obelisks poly methoxy phenol

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Abstract

A facile method is proposed to prepare poly methoxy phenol P (o-MP) by the electrochemical polymerization technique. Effects of different reaction parameters such as temperature, monomer and acid concentrations on the growth of the polymer are studied by using cyclic voltammetry. The kinetic studies indicate that the orders of the reaction are 1.3 and 1.17 for monomer and acid concentrations, respectively. The apparent activation energy is calculated to be 37.2 kJ/mol. In addition, the fabricated polymer is characterized and investigated by 1H-NMR, TGA, IR-UV spectroscopy, XRD, SEM and elemental analysis. Moreover, the mechanism of the reaction is proposed and discussed.The synthesized polymer was evaluated for their in vitro antimicrobial activity against Streptococcus pneumoniae (RCMB 010010) , Enterococcus faecalis (RCMB 010068) and Staphylococcus aureus (RCMB 010028) as a Gram positive bacteria, and against Salmonella typhimurium (RCMB 010072) and Escherichi coli (RCMB 010052) as Gram negative bacteria and against Aspergillus fumigates (RCMB 02568) as fungi. Result showed that the polymer exhibited fantastic antimicrobial activity.

INTRODUCTION

Catechol and its derivatives are organic pollutants encountered in the environment; they coexist as isomers with high toxicity in environmental samples due to low degradability even at low concentration [1]. They have been included in the list of priority pollutants to be monitored in the aquatic environment by international bodies such as US Environmental Protection Agency (EPA) [2]. Several technologies are used for recovering or removing these pollutants. One of the most promising technologies to treat these pollutants is the formation of useful polymers by using the electropolymerization technique as discussed by many authors [3-9]. Ortho methoxy phenol (guaiacol) is a derivative of the catechols and synthesized by methylation of catechol. The synthesis and characterization of poly methoxy phenol (polyguaiacol) by cyclic voltammetry have a unique interest in academic and research applications because of its contributions in many industrial fields such as in adhesives and composites [10]. In addition, they are used in many applications such as biosensors, mediator for the redox of NAD/NADH, novel cell-targeting, and pH sensitive carrier for delivery of the anticancer drug –bortezomib [BTZ] against cancer cell [11]. Also, an immunostimulating activity of polycatechol copolymers is detected [12]. These applications are facing a controllability problem on some properties of the fabricated polymethoxyphenol such as molecular weight, degree of cross linking and crystalinity [13].Generally polyphenols have an antimicrobial activity. The application of this type of polymers with antibacterial activities will be a major step toward a healthier living. On the other hand, though hundreds of thousands of polymeric compounds have been prepared, few of them were of visible antimicrobial

activities. JayaSanthi R et al [14] were reported that antibacterial activities of the poly m- aminophenol and its nano compound could be considered to be included as biomaterials in biological media .The antibacterial activity is in accordance with the literature which shows that this
Polymer containing phenol derivatives with one, two, or three hydroxyl groups exhibited good antibacterial activities [15]. One of the most promising options to control these properties is the using of the electrochemical method in the preparation of this type of polymers. The electrochemical process is distinguished by its feasibility, controllability and reducibility. In this reaction, the unstable phenoxy radicals generated at the anode are electrophiles capable of reacting with either the starting phenol or another radical by C–C and/or C–O coupling giving dimers [16-26]. The dimer may be further oxidized to create oligomers and then polymers. Formation of the insoluble polyphenol results in deactivation of electrode surface.
On the other hand, a few numbers of studies are concerned with the kinetic analysis of this electropolymerization reaction. So this attracts our interest to study the kinetics and optimize the conditions for the electrochemical oxidation of orthomethoxy phenol (o-MP) in aqueous H_2SO_4as a medium and electrolyte. The kinetic studies for this reaction are performed to calculate the orders of reaction with respect to monomer concentration, acid concentration and thermodynamic activation parameters such as enthalpy (ΔH^*), entropy (ΔS^*) and activation energy (Ea) for electro-oxidation of OMP. In addition, various techniques are used to characterize the formed polymer such as thermo gravimetric analysis (TGA), Proton nuclear magnetic resonance (^{1}HNMR), and UV-IR spectroscopy. In addition, scanning electron microscopy (SEM) and X-ray diffraction (XRD) are used to study the surface morphologies and compositions of P(o-MP) formed at different electrolytic composition, current density, and electrode configuration. Also we studied the antimicrobial activity of this polymer against Streptococcus pneumoniae(RCMB 010010) , Enterococcusfaecalis(RCMB 010068) and Staphylococcus aureus (RCMB 010028) as a Gram positive bacteria, and against Salmonella typhimurium (RCMB 010072) and Escherichi col (RCMB 010052) as Gram negative bacteria also against Aspergillus fumigates (RCMB 02568) as fungi.

1. Experimental details

2.1. Materials

O-methoxyphenol, sulfuric acid solution 98% (Merck). All solutions are prepared by using freshly double-distilled water

1.2. Electrodes

The working electrode (WE) is a platinum sheet with dimensions of 1cm length and 0.5 cm width.The auxiliary (counter) electrode (CE) is a platinum foil with the same dimensions as the WE. A saturated calomel electrode (SCE) was used as a reference electrode. Electrochemical experiments are performed using the Potentiostat / GalvanostatWenking PGS 95.

1.3. Characterization of the electro-prepared polymers

UV-VIS absorption spectra of the prepared polymer sample is measured using Shimadzu UV spectrophotometer (M160 PC) at room temperature in the range 200-400 nm using acetone as a solvent and reference. IR measurements are carried out using shimadzu FTIR-340 Jasco spectrophotometer (Japan) by KBr pellets disk technique.^{1}H-NMR measurements are carried out using a Varian EM 360 L, 60-MHz NMR spectrometer. NMR signals of the electropolymerized samples are recorded in dimethylsulphoxide (DMSO) using tetramethylsilane as internal standard. TGA of the obtained polymer is performed using a Shimadzu DT-30 thermal analyzer (Shimadzu, Kyoto, Japan). The weight loss is measured from ambient temperature up to 600 ºC, at the rate of 20 ºC min^{-1} and nitrogen 50cc min^{-1} to determine the degradation rate of the polymer. Elemental analysis is carried out in the micro-analytical center at Cairo University (Cairo, Egypt) by oxygen flask combustion and dosimat E415 titrator (Metrohm).

Scanning electron microscopy (SEM) analysis is carried out on the as-prepared polymer film deposited on Pt-working electrode surface using a JSM-T20 Electron Probe Microanalyzer (JEOL, Tokyo, Japan). The X-ray diffraction analysis (XRD) (Philips 1976 Model 1390, Netherlands) is operated under the following conditions that are kept constant for all the analysis processes
: X-ray tube, Cu; scan speed 8 deg min^{-1}; current:30 mA; voltage 40 kV; and preset time 10 s.

2.4 Antimicrobial measurements

The disks of Whatman filter paper were prepared with standard size (50 mm diameter) and kept into 10 screw capped wide mouthed containers for sterilization. These bottles are kept into hot air oven at a temperature of 150°C. Then, the standard sterilized filter paper disks impregnated with a solution of the test compound in DMSO (1 mg/mL) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard conditions of 106 CFU/mL (Colony Forming U/mL) and 104 CFU/mL were used for antibacterial and antifungal

assay, respectively. Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. The utilized test organisms were Streptococcus pneumoniae(S. pneumoniae (RCMB 010010)) and Bacillissubtilis(B. subtilis (RCMB 010067)) as examples of Gram positive bacteria and Escherichia coli (E. coli (RCMB 010052)) as example of Gram negative bacteria. They were also evaluated for their in vitro antifungal potential against Aspergillusfumigatus(A. fumigatus (RCMB 02568)), Geotricumcandidum (G. candidum (RCMB 05097)) and Candida albicans(C. albicans (RCMB 05031)). Ampicillin, gentamicin and Amphotericin B were used as reference drugs against Gram positive bacteria, Gram negative bacteria and fungi, respectively. DMSO alone was used as control at the same forementioned concentration and during this, there was no visible change in bacterial growth. The plates were incubated at 37°C for 24 h for bacteria and 48 h for fungi. The derivatives that showed significant growth inhibition zones using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

2.5. Minimal inhibitory concentration (MIC) measurement:

MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

The microdilution susceptibility test in Muller–Hinton Broth (Oxoid) andSabouraud Liquid Medium (Oxoid) were used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, were prepared in DMSO. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions of the broth containing about 106 CFU/mL of test bacteria was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37°C for 24 h for antibacterial activity and at 37°C for 48 h for antifungal activity in a humid chamber. At the end of the incubation period, the minimal inhibitory concentrations (MICs) values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMSO and uninoculated media were investigated parallel to the test compounds under the same conditions.

2. Results and discussion

3.1. Electropolymerization of o-Methoxy Phenol

Electropolymerization of o-MP on platinum electrode, from aqueous solution containing 0.6 M H_2SO_4 at 303 K in the absence and presence of monomer, is studied by cyclic voltammetry at potential between -366 and +1600 mV(vs. SCE) with a scan rate of 30 mVs^{-1}. The obtained voltammograms in absence and presence of monomer are represented in Figure 1. The voltammogram in the absence of monomer, Figure 1 (a), exhibits an oxidation peak (I) which developed at -200 mV vs. SCE.due to is the hydrogen adsorption on Pt electrode [27]. While two oxidation peaks appear at-200 and 1080 mV and one reduction peak (II') appears at +225 mV (vs. SCE) in presence of monomer. The second oxidation peak (II) corresponds to the oxidation of monomer to give a phenoxy radical which adsorbed on Pt-electrode [20]. These adsorbed radicals are considered as initiator radicals to form the polymer. While thecathodic one is attributed to the reduction of the formed polymer.

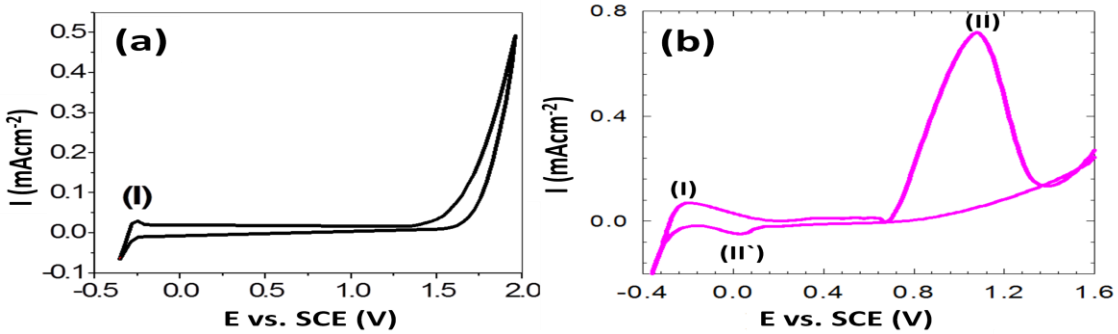

Fig. 1.Cyclic voltammograms of solution containing 0.6 H_2SO_4 at 303K with scan rate 30mVs^{-1} (a) in absence of monomer and (b) in presence of monomer.

3.1.1. Effect of scan rate

Effect of scan rate on the cyclic voltammograms of o-MP is shown in Figure 2. Figure 2(a) shows the cyclic voltammograms of electropolymerization of o-MP on Pt electrode from solution containing 0.6M H_2SO_4 and 0.09M of monomer at 303 K for scan rate from 10 to 50 mVs^{-1}. As shown in the figure, the peak current density (I_p) increases as the scan rate increases.This behavior may be ascribed to the depletion of the species in the vicinity of the Pt- surface when enough potential is applied to the Pt- surface causing oxidation of species in the solution. As a result, a concentration gradient (dC/dx) appears in the solution and the peak current (I_p) is proportional to the gradient slope (dC/dx). As the scan rate increases, the gradient increases and consequently the current (I_p) increases.

The linear dependency of the anodic peak current density (I_p) on the square root of scan rate ($V^{1/2}$) is shown in Figure 2 (b) and represented by the linear regression equation Eq. (1):

$$I_{p(II)}\,(mA) = 11.33\ V^{1/2}\,(mV\ s^{-1}\ )^{1/2} - 28.05 \qquad (1)$$

From the above equation, we note that correlation coefficient r = 0.94 < 1. This linear relation suggests that the electro-formation of P (o-MP) may be described by a partially diffusion - controlled process (diffusion of reacting species to the polymer film/solution interface) [28]. I.e., the process is not completely diffusion - controlled but it is exactly a partially diffusion- controlled. It seems that, initially the electro-formation of radical cations is controlled by charge transfer. When the polymer film becomes thick, the diffusion of reactant inside the film will be the slowest step and the process changes to diffusion transfer. In addition, the intercept is negative, -28.05, which could be attributed to the decrease of the active area of the working electrode during the positive scan or the increase of the covered area of working electrode by the adhered polymer layer [29].Using the values of I_p and scan rate, V (Vs^{-1}), the diffusion coefficient can be calculated using Randless and Sevick equation [30,31]:

$$I_p\,(II) = 0.4463\ n\ F\ A\ C\ (\ n\ F\ V\ D\ /\ R\ T\ )^{1/2} \qquad (2)$$

Where n is the number of exchanged electrons in the reaction, F is Faraday's constant (96485 C mol), A is the electrode area in cm^2, C is the bulk concentration, D is the analyst diffusing coefficient in cm^2s^{-1}, R is the universal gas constant (8.134 $Jmol^{-1}K^{-1}$), and T is the absolute temperature in K. The calculated values of D at 0.6 M H_2SO_4 and 303 K with scan rate from 10 to 50 mV s^{-1} are shown in Table 1. The values of D are seen to be slightly changed within the same order over the range of sweep rates, which again shows that the oxidation process is diffusion-controlled [32].

Table 1.The calculated values of diffusion coefficient for different scan rates.

Scan rate(Vs^{-1}) o-MP→P (o-MP)	Diffusing coefficient, (m^2s^{-1})
0.010	1.59 x 10^{-9}
0.020	1.79 x 10^{-9}
0.030	3.31 x 10^{-9}
0.040	5.83 x 10^{-9}
0.050	6.42 x 10^{-9}

3.1.2. Effect of number of cycles

In order to examine the electrode stability, six repetitive cyclic voltammograms in a solution containing 0.09M of o-MP and 0.6 H_2SO_4 with scan rate 30mVs^{-1} at 303K are performed. As seen in Figure 2(c and d), the current involved in the oxidation of o-MP decreases gradually as the number of cycles increases. This inhibition process might be explained by the electrode fouling as a result of the formation of insulating polymeric products from the oxidation of monomer that block the electrode surface. Similar deactivation of different electrodes in the presence of aromatic organic substrates such as phenol and safrole has been reported in Literature [33-40].

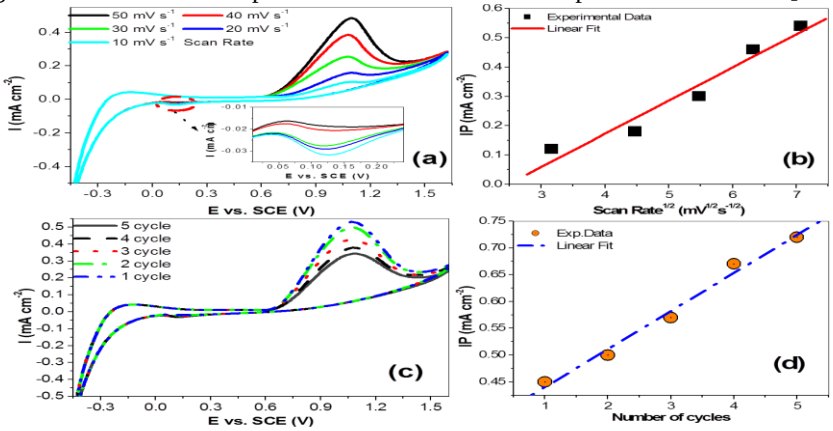

Fig. 2. (a) Effect of scan rate on electropolymerization of o-MP on Pt electrode from solution containing 0.6M H_2SO_4 at 303 K, (b) the variation of I_p(II)with the square root of scan rate, (c)cyclic voltammetry curves for the effect of repetitive cycles on the electropolymerization of 0.09M o-MP from solution containing 0.6 MH_2SO_4 at 303K with scan rate 30 mVs^{-1} on Pt electrode and (b) the variation of peak current, I_P, with the number of cycles.

2.2. Kinetic Studies

The electropolymerization kinetics are investigated by using aqueous solution containing (monomer concentration in the range between 0.05 and 0.09M where H_2SO_4 concentration in the range between 0.3 and 0.6 M at 303 K. The cyclic voltammogram for each monomeric system and the relation between the log I_p(II)vs. log [monomer conc.] or log I_p (II) vs. log [H_2SO_4 Conc.] are studied to obtain the order of the reaction.

2.2.1. Effect of Monomer Concentration on Electropolymerization Processes

Figure 3 (a) shows the cyclic voltammograms of o-MP at different concentrations (0.05 - 0.09M) in the presence of 0.6 M H_2SO_4 recorded at a scan rate of 30 mVs^{-1}. From the voltammograms, the oxidation peak of o-MP appears at an electrode potential of about 1.2V whereas the cathodic peak appears at 0.09V and attributes to the reduction of the polymer film of o-MP. The peak current of the oxidation peak increases as the guaiacol concentration increases to reach the maximum value and then decreases for o-MP concentration to be greater than 0.1M. This behavior may be attributed to the decrease in the activity of the Pt electrode at higher monomer concentrations or due to competition for the active sites on the electrode surface as a result of the formation of a large number of phenoxy radicals during the electropolymerization process. These radicals cause a faster deactivation to the electrode with the increase in o-MP concentration while some authors have observed that the fouling of the electrode by the phenolic oxidation products is more prominent at higher concentrations [27]. For different guaiacol concentrations, the anodic peak current density (I_p) is directly proportional to the concentration and a linear relation is obtained. The order of reaction is obtained from the slope of the linear relation between double logarithmic plot of I_p vs. monomer concentrations (C) to be 1.13, as shown in Figure 3(b).

2.2.2. Effect of H_2SO_4 concentration on the polymerization process

Experiments are performed to study the effect of H_2SO_4 concentration on the electropolymerization process. This effect is shown in Figure 3 (c) which represents cyclic voltammograms obtained from0.09 M of o-MP at a scan rate of 30 mVs^{-1} at constant temperature(303K) for different acid concentrations varied from 0.2 to 0.7M. The height of the peak current density increases with the increasing of acid concentration up to 0.6 M and then decreasing at 0.7M as a result of the degradation of the polymer film. This agrees well with the well known rule that the polymerization process is favored at low pH [27]. The order of reaction with respect to sulfuric acid concentration is found to be 1.17 from the slope of the linear relation between peak current and acid concentration, as shown in Figure3 (d).

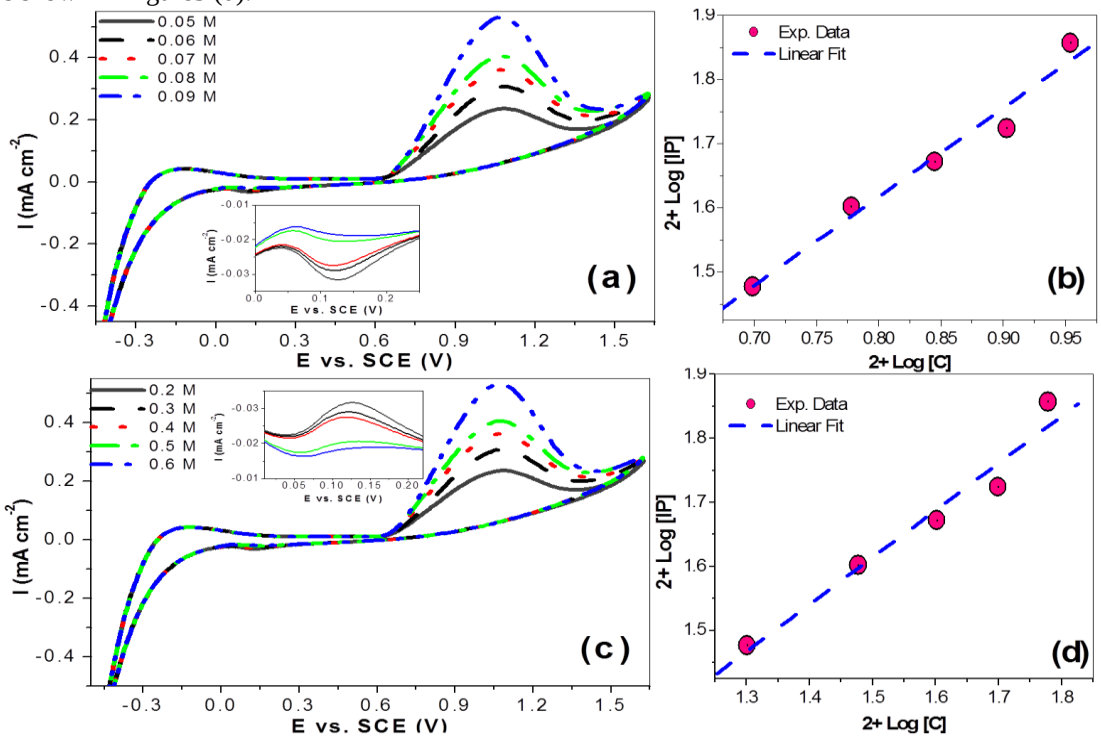

Fig. 3. (a) Effect of monomer concentration on the electropolymerization of o-MP from solution containing 0.6M H_2SO_4 at 303K, (b) double logarithmic plot of I_p vs. monomer concentration**,** (c) effect of H_2SO_4 concentration on the electro-polymerization of o-MP from solution containing 0.09M monomer at 303K, and (d) double logarithmic plot of I_p vs. acid concentration C.

2.2.3. Effect of temperature and calculation of thermodynamic parameters

The effect of temperature on the process is represented in Fig. 6. The potentiodynamic profiles of 0.09M o-MP in a solution containing 0.6 M H_2SO_4 at different temperatures are varied from 283 to 303 K as shown in Figure 4(a). As shown the reaction strongly depends on the temperature and I_p increases with the increasing of temperature. Reaction activation energy is calculated by plotting relation between logarithm of peak current and reciprocal of absolute temperature as shown in Figure 4(b). A straight line is obtained and the activation energy is calculated from the following equation: .

$$E_a = -2.303 \times 8.314 \times 10^{-3} \times \text{Slope} \quad (3)$$

The value of the activation energy is found to be 37.2 kJ/mole. In addition, the enthalpy ΔH* and entropy ΔS* for the electropolymerization reaction can be calculated from Eyring equation plot at different temperatures (Figure 4(c)) [41].This results in linear relationship with slope equal to ΔH*/2.303R and intercept equal to (R/N h) +Δ S*/2.303R, where N is Avogadro's number and h is Planck's constant. From the slope and intercept values, ΔH* and ΔS* for o-MP are calculated to be 50.82 kJ mol^{-1} and 424.7 JK^{-1} mol^{-1}, respectively.

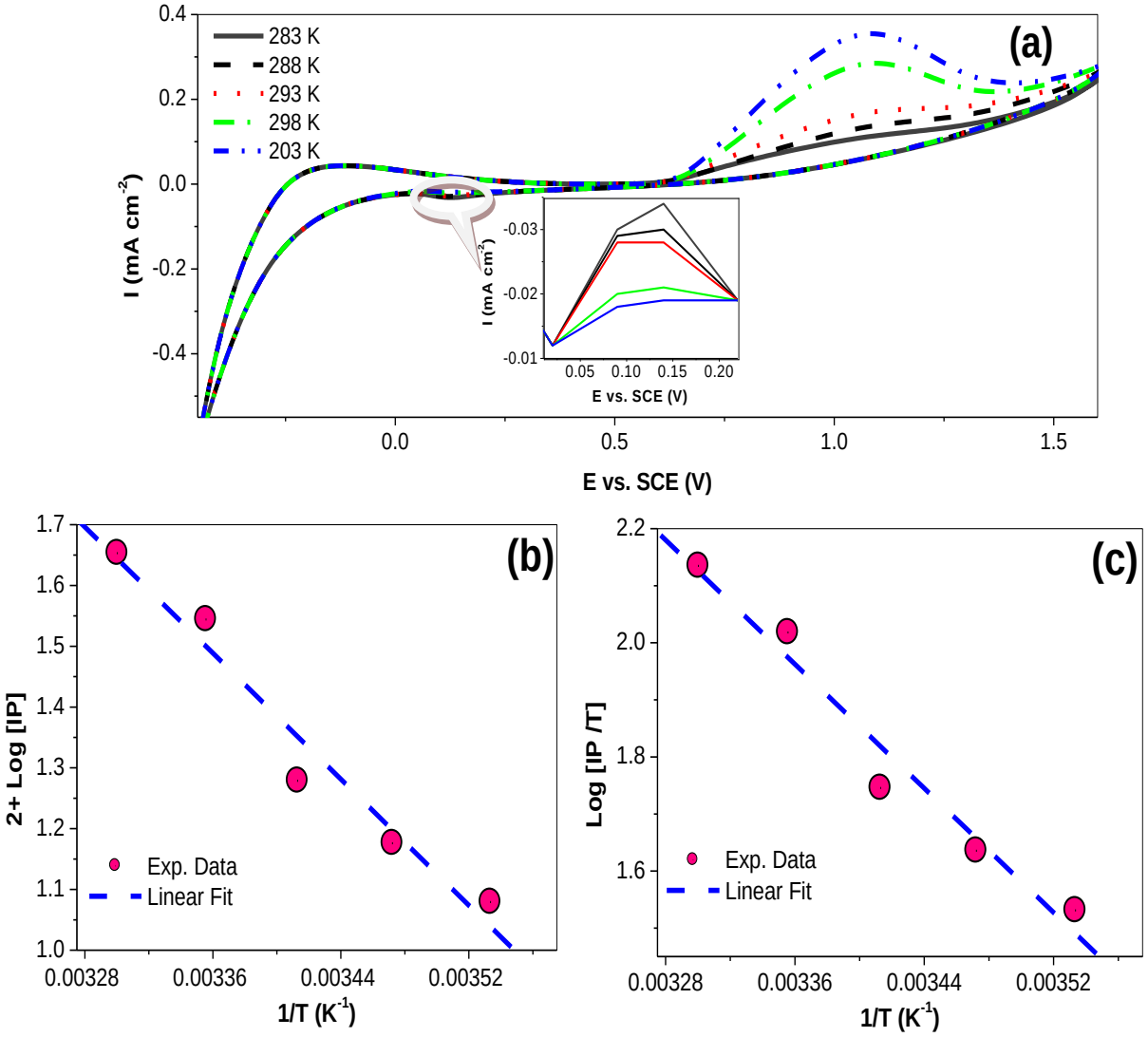

Fig.4. (a) Cyclic voltammetry curves for the effect of temperature on the electropolymerization of o-MP from solution containing 0.09M monomer with scan rate 30mVs^{-1} on Pt electrode, (b) Arrhenius plot and (c) Eyring equation plot.

2.3. Mechanisms of electropolymerization

The anodic oxidative polymerization of o-MP is preceded in different steps as follows:

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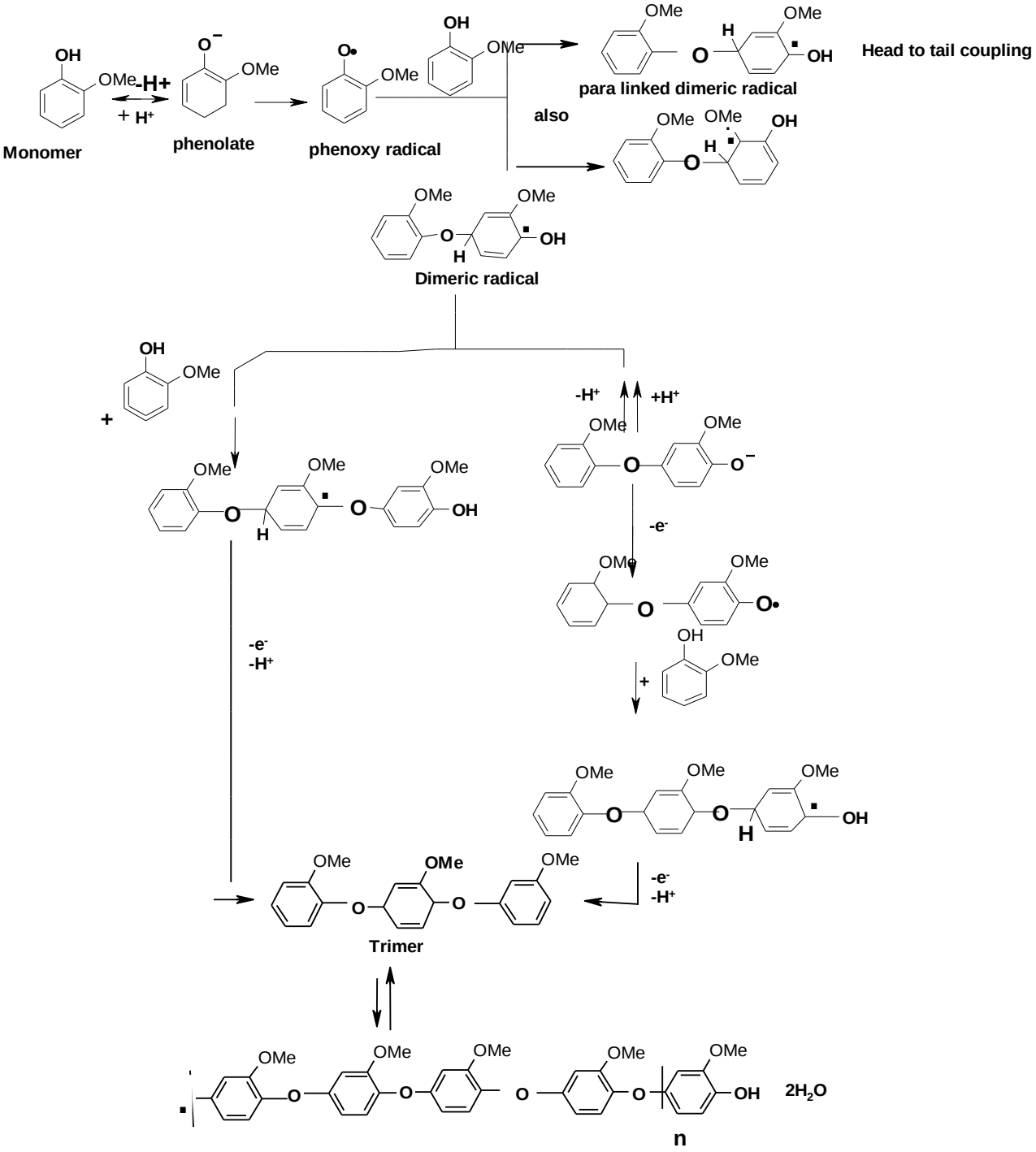

Where Me is CH_3 group.

Scheme 1. The mechanism of electropolymerization of o-MP.

2.4. Characterization of the prepared poly Guaiacol

2.5. Elemental analysis

Elemental analysis of the prepared P (o-MP) is carried out in the micro- analytical laboratory a Cairo University. The percentage of C, H and S for the investigated sample where the percentage of carbon in the sample is found to be 63.8% which is in a good agreement with the calculated value (64.1%), while the H content in the sample is found to be 5.2% which is in a good agreement with the calculated value (4.9%) and the sulfur content is traces in the polymer .The obtained elemental analysis is in a good agreement with the calculated data for the suggested structure in scheme1.

2.5.1. Infrared spectroscopic studies

The infrared spectra of o-MP monomer and the prepared homopolymer P (o-MP) are represented in Figure 5 ((a) and (b)). The IR absorption bands and their assignments are given in Table 2.

2.5.2. Ultraviolet spectroscopic studies

The UV spectra of o-MP and its homopolymer P (o-MP) are shown in Figure 5 (c) and (d). Different absorption bands can be obsereved at λ_{max}= 212, 252 and 308 nm. These bands may be attributed to π- π* transition of benzene ring. But the UV spectrum of the polymer shows three absorption bands at λ_{max}= 208, 230 and 245 nm, which may be attributed to π- π* transition. Also an absorption band is observed at λ_{max}= 330 nm, which may be due to the conjugation of the aromatic polymeric chains and may participate in the conductivity value.

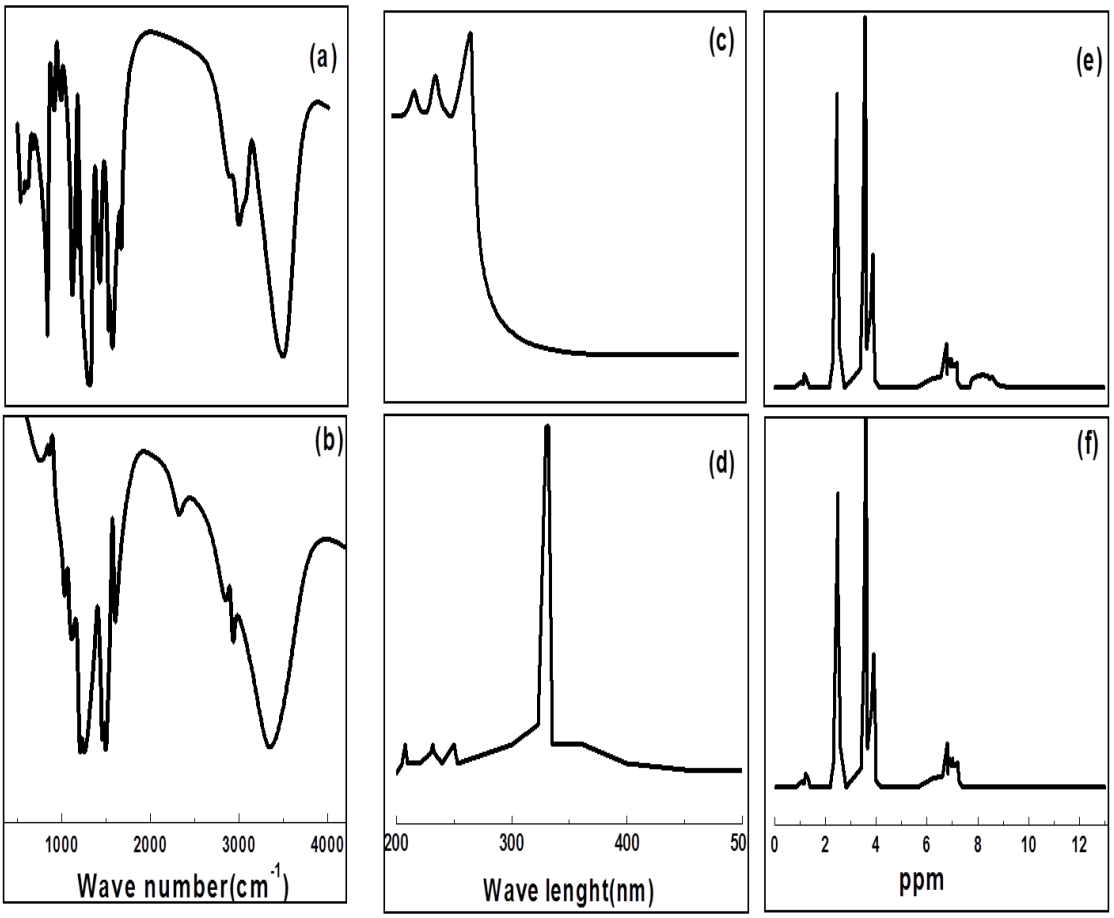

Fig.5. IR spectra of (a)o-MP and (b) p(o-MP); UV-Vis Spectra of (c) o-MP and (d) P (o-MP); ^{1}HNMR spectra of P (o-MP) in (e)DMSO and (f) DMSO+D_2O.

Table 2.IR adsorption bands and their assignments for o-MP and P (o-MP).

o-MP	P(o-MP)	Assignment
749[s]	---------	**out of plane bending for 1,2 di-substituted benzene ring**
836[m]	--------	
---------	**865[w]**	
--------	**821[w]**	**CH out of plane for tri substituted benzene ring**
1109[s]	**1116[m]**	**C-O streching vibration**
1029[s]	**1210[s]**	
	1258[s]	
1454[s]		
1503[s]	**1454[s]**	**Stretching vibration of C=C in benzene ring**
1662[s]	**1502[s]**	
	1600[s]	**Methoxy group**
2950	**2844[w]**	**Stretching vibration for CH aromatic**
3055[m]	**2936[w]**	
3509[s]	**3428[s]**	**Stretching vibration intermolecular hydrogen solvated OH group or end group OH of polymeric chain**

s: strong, w: weak, b: broad, m:medium.

2.5.3. ^{1}HNMR spectroscopic studies

Figure.5 shows the ^{1}HNMR spectra of the prepared P (o-MP) in (e) DMSO and (f) DMSO + D_2O. This figure shows one solvent signal at $\delta = 2.45$ ppm. The protons of benzene rings in the polymeric structures are appeared in the region from $\delta = 6.04$ to $\delta = 8$ ppm. The singlet signal appears at $\delta = 3.45$ ppm is attributed to OH protons for water of solvation. The singlet signal appears at $\delta = 9$ ppm is attributed to OH proton attached to benzene ring. The signals of different (OH) are disappeared when deuterated water is added to the investigated sample as shown in Figure 5(f).

2.5.4. Thermo gravimetric analysis (TGA)

Since the temperature plays an important role in the polymer formation, the temperature induced phase changes are important for the utility of this polymer invarious applications. The thermal behavior of the prepared P (o-MP) sample has been studied by thermo gravimetric analysis (TGA). The TGA-curve in Figure 6(a) showsthe following criterias, four stages occur during the thermo-analysis of the P (o-MP) sample. The first stage includes the loss of $2H_2O$ molecules in the temperature range between 25 ^{o}C and 200 ^{o}C and shows the sharp weight loss of about 6.4% which is in a good agreement with the calculated value (6.48%) and the literature for water release [30]. The second stage includes the loss of methanol, CH_3OH, in the temperature range between 200to 300 ^{o}C which is produced by decomposition of methoxy group. The weight loss for this step is found to be 6.1% which is in a good agreement with the calculated value (6.8%) and with what was found in the literature [42]. The third stage takes place in the range of temperature between 300 - 450 ^{o}C; this stage includes the loss of benzenoid unit. The weight loss for this step was found to be 21.3% which is in a good agreement with the calculated value (22%). The fourth stage in the range of temperature > 450 ^{o}C, the remaining part of the decomposed polymer is equal to 64.8% and this in a good agreement with the calculated value which equal to 65%.

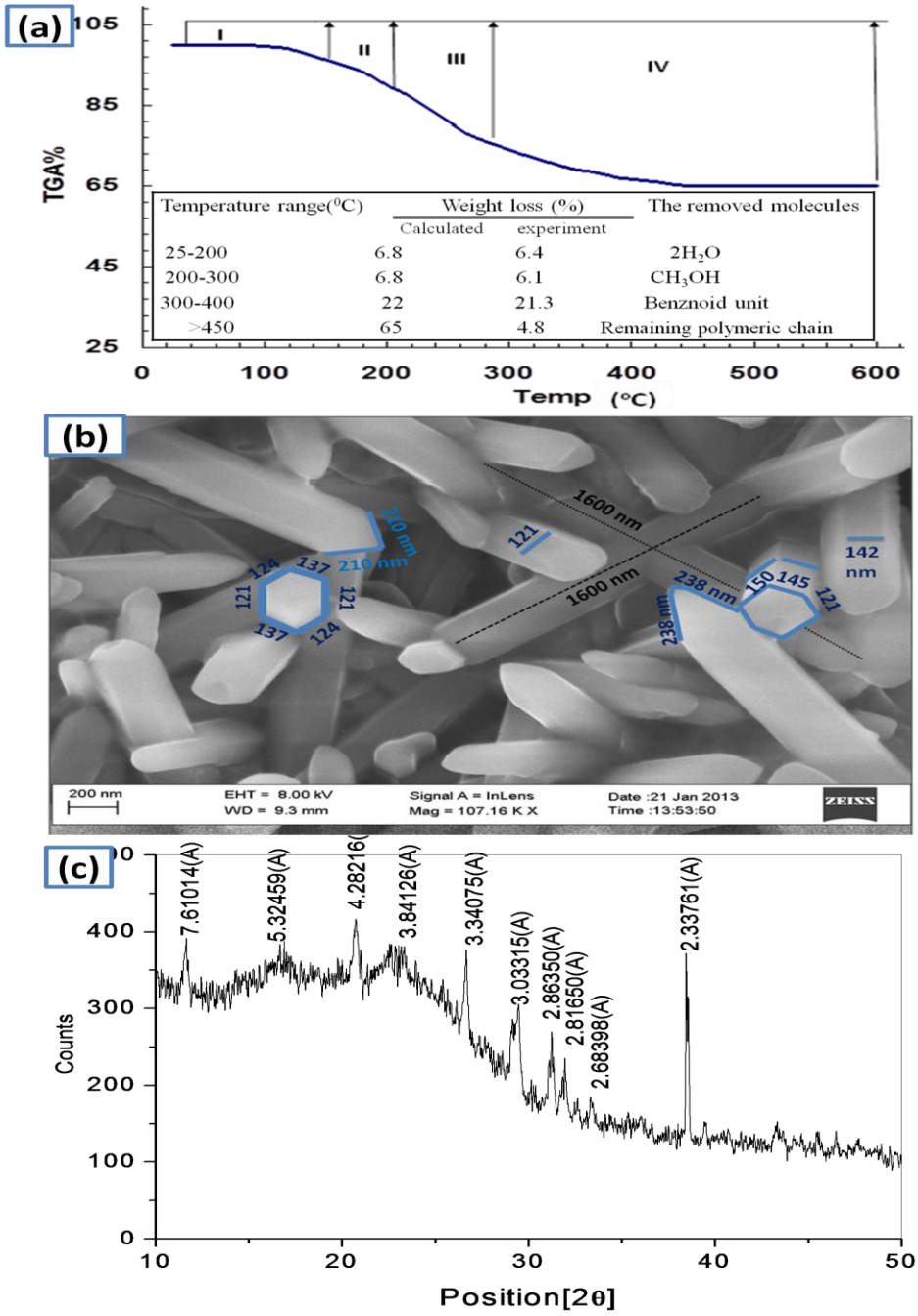

Fig. 6. (a) Thermo gravimetric analysis (TGA) of P (o-MP). (b) Top-view FE-SEM image and (c) XRD of P (o-MP) on Pt electrode prepared at the optimum conditions.

2.5.5. Surface morphology study

The surface morphology of the polymer obtained at the optimum conditions is examined by scanning electron microscopy. Figure 6(b) shows top- view FE-SEM image of the electropolymerized film on Pt electrode. As shown in this figure, obelisks have1 hexagonal cross sections and six rectangular sides that taper towards a pyramidal top are grown on pt electrode.The height of the obelisk is ~1600 nm and the pyramidal top height is ranged from 210 nm to 240 nm. In addition, the rectangular sides widths are ranged from 120 to 150 nm as indicated in the figure.

In order to check the chemical composition of the fabricated POMP, the sample is analyzed by XRD as shown in Figure6 (c). The XRD in Figure 6 (c) is in agreement with the P (o-MP) diffraction pattern. This is clearly a consequence of the configuration of prepared polymer on the Pt surface as already revealed in the SEM images in Figure 6(b). In addition, the XRD pattern in Figure 6(c) indicates that the crystal orientation of polymer is obelisks.

3.5. Antimicrobial activity

electro synthesized polymer p(o-MP), was evaluated for their in vitro antimicrobial activity against Streptococcus pneumoniae(RCMB 010010) , Enterococcusfaecalis(RCMB 010068) and Staphylococcus aureus (RCMB 010028) as a Gram positive bacteria, and against Salmonella typhimurium (RCMB 010072) and Escherichi col (RCMB 010052) as Gram negative bacteria and against Aspergillus fumigates (RCMB 02568) as fungi. Agar disk diffusion method was used for the determination of the antibacterial and antifungal activity. Ampicillin, Gentamicin and Amphotericin B were used as reference drugs against Gram positive bacteria, Gram negative bacteria and fungi, respectively

3.5.1. Antibacterial activity

P (o-MP) showed in vitro antibacterial activity against the tested bacteria. The result of antibacterial activity using inhibition zone method is listed in Table3. P (o-MP) showed a better antibacterial activity. Several mechanisms elucidating the antimicrobial activity of polymer have been postulated. The most acceptable mechanism is the interaction between partially positively charged and free radicals of polymer molecules and negatively charged microbial cell membrane. This electrostatic interaction results in two fold interferences: (1) by promoting changes in the properties of membrane wall permeability, thus provoke internal osmotic imbalances and consequently inhibit the growth of the microorganisms and (2) by the hydrolysis of the peptidoglycans in the microorganism wall, leading to the leakage of intracellular electrolytes such as potassium ions, and other low molecular weight proteinaceous constituents (e.g. protein, nucleic acid, glucose and lactatedehydrogenase) [43].

Since such mechanism is based on electrostatic interaction, it suggests that the greater the number of partially positive charges and free radicals, the higher will be the antimicrobial activity. So in p (o-MP) has a methoxy group (o-MP) which is a withdrawing electron group leading to increased polycationic character and has high sensitivity against all tested bacteria. Also the introduction of OH moieties onto aromatic ring increases their solubility both in organic and aqueous media and also increases their cationic centers and consequently the net positive charge was strengthened, leading to a better antibacterial activity [44]. Moreover, p(o-MP) showed higher antibacterial activity against the Gram positive bacteria than against the Gram negative bacteria. P (o-MP) caused inhibition zone diameter of Streptococcus pneumoniae(RCMB 010010) , Enterococcusfaecalis(RCMB 010068) and Staphylococcus aureus (RCMB 010028) of 22.2± 0.34, 18.9 ± 0.19 and 20.3± 0.67mm, respectively, corresponded to 19.6 ± 0.34 and 20.0 ± 0.34 mm of E. coli and Salmonella typhimurium (RCMB 010072) . This may be attributed to their different cell wall. The cell wall of Gram positive bacteria is fully composed of peptide polyglycogen. The peptidoglycan layer is composed of net works with plenty of pores, which allow foreign molecules to come into the cell without difficulty and allows more rapid absorption of ions into the cell. But the cell wall of Gram negative bacteria is made up of a thin membrane of peptide polyglycogen and an outer membrane constituted of lipopolysaccharide, lipoprotein and phospholipids. Because of the complicated bilayer cell structure, the outer membrane is a potential barrier against foreign molecules with high molecular weight. Therefore, p(o-MP) has different effects on the different kinds of bacteria. An additional evidence for the greater activity of different polymers and copolymers against Gram positive bacteria than that against Gram negative bacteria comes from their minimum inhibitory concentration (MIC) values. The MIC values of p (o-MP) against Streptococcus pneumoniae(RCMB 010010) , Enterococcusfaecalis(RCMB 010068) and Staphylococcus aureus (RCMB 010028) were 0.98,7.8 and3.9 Mg/mL, respectively, corresponded to 8Mg/mL against E. coli .these results were represented in Table3.

3.5.2. Antifungal activity

The antifungal activities of p(o-MP) against A. fumigatus (RCMB 02568) are shown in Table 3. The results show that this polymer had effective activities against the tested fungus with inhibitory indices ranging from21.3 ± 0.23 mm inhibition zone.and with MIC value, where it may be very effective in inhibiting spore germination, germ tube elongation and radial growth .the polymer may be diffuse inside hyphae interfering on the enzymes activity responsible for the fungus growth .

Table 3 Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms
using (1mg/ml) concentration of tested samples. Results are depicted in the following table:The test was done using the diffusion agar technique, Well diameter: 6.0 mm …… …… (100 µl Was tested),

Sample / Tested microorganisms	P(o-MP)	St.
FUNGI		Amphotericin B
Aspergillusfumigatus (RCMB 02568)	21.3± 0.23	23.7± 0.1
Gram Positive Bacteria:	Ampicillin	
Streptococcus pneumoniae(RCMB 010010)	22.2± 0.34	23.8± 0.2
Enterococcusfaecalis(RCMB 010068)	18.9 ± 0.19	20.3± 0.3
Staphylococcus aureus (RCMB 010028)	20.3± 0.67	28.3± 0.1
Gram negativeBacteria:	Gentamicin	
Escherichia coli (RCMB 010052)	19.6 ± 0.34	20.4 ± 0.6
Salmonella typhimurium (RCMB 010072)	20.0 ± 0.34	23.7 ± 0.7

Table4: Antimicrobial Activity as MICS (µg / ml) of tested samples against tested microorganisms:

Sample / miroorganism Tested microorganisms	P(0-MP)	Standard.
	Minimum inhibitory concentration	
FUNGI	AmphotericinB	
Aspergillusfumigatus (RCMB 02568)	1.95	0.24
Gram Positive Bacteria:	Ampicillin	
Streptococcus pneumoniae(RCMB 010010)	0.98	0.24
Enterococcusfaecalis(RCMB 010068)	7.81	3.9
Staphylococcus aureus (RCMB 010028)	3.9	0.03
Gram negativeBacteria:	Gentamicin	
Escherichia coli (RCMB 010052)	7.81	3.9
Salmonella typhimurium (RCMB 010072)	3.9	0.24

4. Conclusion

In conclusion, cyclic voltammetry is one of the promising tools for the electro oxidation of pollutants as phenol derivatives. The electropolymerization of o-MP is a notoriously complex process which depends on the monomer structure, the potential scan rate, the pH and the temperature. The optimum concentrations of acid and monomer are 0.6 and 0.09M, respectively. From the kinetic studies of the electropolymerization, the orders of the reaction with respect to acid and monomer concentrations are 1.17 and 1.3 respectively. In addition, the apparent activation energy, enthalpy and entropy are estimated to be 37.2 kJ/mol, 50.82 kJ/moland 424.7 JK^{-1} mol^{-1}, respectively. The morphology, structure and chemical composition of the obtained polymer are studied by SEM, XRD, IR, UV, TGA and elemental analysis. The electropolymerized film which was nano-obelisks with hexagonal cross sections six rectangular sides and tapering towards a pyramidal top are grown on pt electrode.The height of the obelisk is ~1600 nm and the pyramidal top height is ranged from 210 nm to 240 nm. In addition, the rectangular sides widths are ranged from 120 to 150 nm as indicated. Moreover, the mechanism of the process is proposed and discussed depending on a free radical polymerization.P(o-MP) was evaluated for their efficiency in vitro against some species of bacteria and fungi using agar diffusion technique. This polymer exhibited good antimicrobial potency with MIC values ranging from 0.98 to 7.81 _g/mL as antibacterial agents, and with 3.25–125 _g/mL as antifungal agents. Some o these derivatives displayed antimicrobial activities against sometested strains almost equivalent to the standard drugs Gentamycin,Ampicillin and Amphotericin B.

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