Synthesis of Phenolphthalein-formaldehyde resin and study of it's antibacterial activity

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Abstract

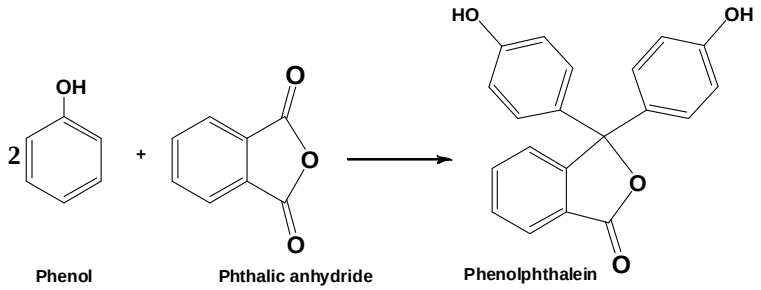
The Reaction of phenolphthalein with formaldehyde in the presence of Sodium hydroxide afforded phenolphthalein-formaldehyde (PPF), and the structure was confirmed by FT-IR spectrum study.

The *in vitro* antibacterial activity of the synthesized polymer was investigated and showed remarkable inhibition against both *Escherichia Coli* (Gram -) and *Staphylococcus aureus* (Gram +). The MIC (Minimal Inhibitory Concentration) values were determined based on Disc Diffusion Method. Allowing the possibility of using the polymer as water sterilizer.

Keywords: Phenolphthalein-formaldehyde, polymer, antibacterial activity.

Introduction

Phenolphthalein or 3,3-bis(4-hydroxyphenyl)phthalide [1], is a well known member of the family of acid-base indicators[2]. Phenolphthalein is odorless, white or faintly yellow-white powder [3]. It could be synthesized by a condensation reaction [4] from phenol and phthalic anhydride (Scheme 1).



Scheme 1

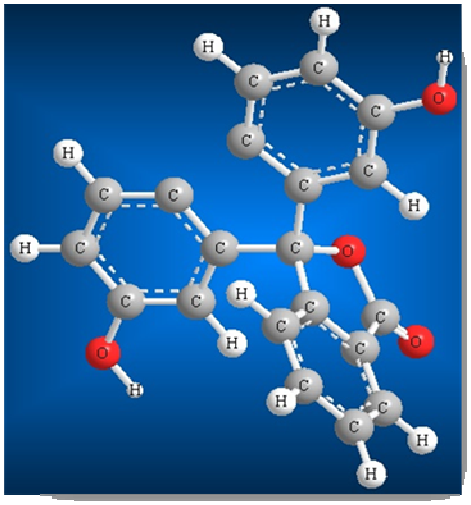
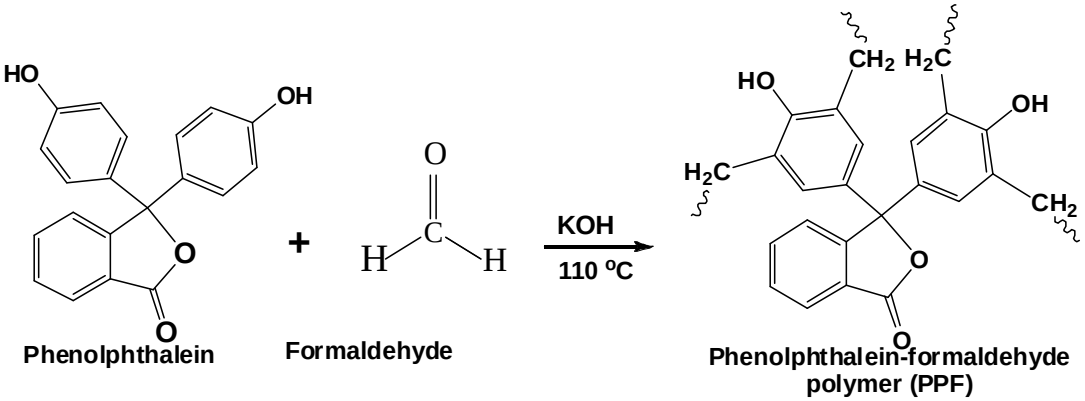


Fig.1. The lactone structure of phenolphthalein

Phenolphthalein and derivatives are widely used in medical and biological fields. The general population is exposed to phenolphthalein through its common application as an over-the-counter drug, particularly as a laxative [5-7]. Phenolphthalein can be used as a reagent in indicator test of blood [8], as a tumor localizing agent[9], antimicrobial[10], antibacterial agents[11,12] and in the treatment of amyloid-associated diseases[13]. Additionally, it is used in separation and determination of mercury ion from a mixture of metal ions in solution[14]. Some new derivatives of phenolphthalein were studied as thymidylate synthase (TS) inhibitor, the important target in the anticancer chemotherapy[15]. It could be used in many electronic, optical applications[16-22]. Furthermore, it is also employed to prepare applicable copolymers use in various fields[23-25].

Polymeric phenolphthalein has been synthesized by the reaction of phenolphthalein with formaldehyde in an alkaline medium to produce phenolphthalein-formaldehyde polymer [26], as shown in scheme 2.



Scheme 2

The alkaline phenol-formaldehyde reaction is more versatile and related to the molar ratios, catalysts and reaction conditions applied than the production of novolaks[27].

Each free phenol molecule can undergo reaction at both *ortho* and *para* positions for the hydroxyl group, hence allowing the network to be obtained[28].

Phenolphthalein-formaldehyde showed a resistance to solubility in many polar and non polar solvents. Polymerization of PPF does not stop the property of being effective indicator, but it has a broader dynamic range from pH 8.0 to pH 12.5 than the free phenolphthalein (from pH 8.0 to pH 10.0) which let to be used in membrane for an optical pH sensor[29].

Results and Discussion

Chemistry

The preparation of phenolphthalein-formaldehyde (PPF) was based on the preparation procedure of resoles polymers[30]. The two phenolic rings of phenolphthalein could be purposive for the condensation reaction with formaldehyde molecules to form methylene bridges, thus phenol-formaldehyde polymer derivative. The yellowish-white polymeric phenolphthalein showed a high resistance against various polar and non-polar solvents, which demonstrated the rigidity of the product.

Methylene bridges play an important role to give the hard nature of polymer. The IR spectrum of PPF (Fig. 3) showed the C-H symmetrical stretching vibration band of methylene bridges at $\bar{\nu}$ 2881 cm^{-1}, with three twisting and wagging vibrational bands at $\bar{\nu}$ 1288 cm^{-1}, 1245 cm^{-1} and 1163 cm^{-1} and a weak band of rocking vibration at $\bar{\nu}$ 752 cm^{-1}. The OH stretching band of phenolic part was observed at $\bar{\nu}$ 3440 cm^{-1}. The intense band at $\bar{\nu}$ 1747 cm^{-1} was assigned to C=O stretching of lactone group. Table 1 showed the characteristic bands of PPF with their frequencies[31,32]

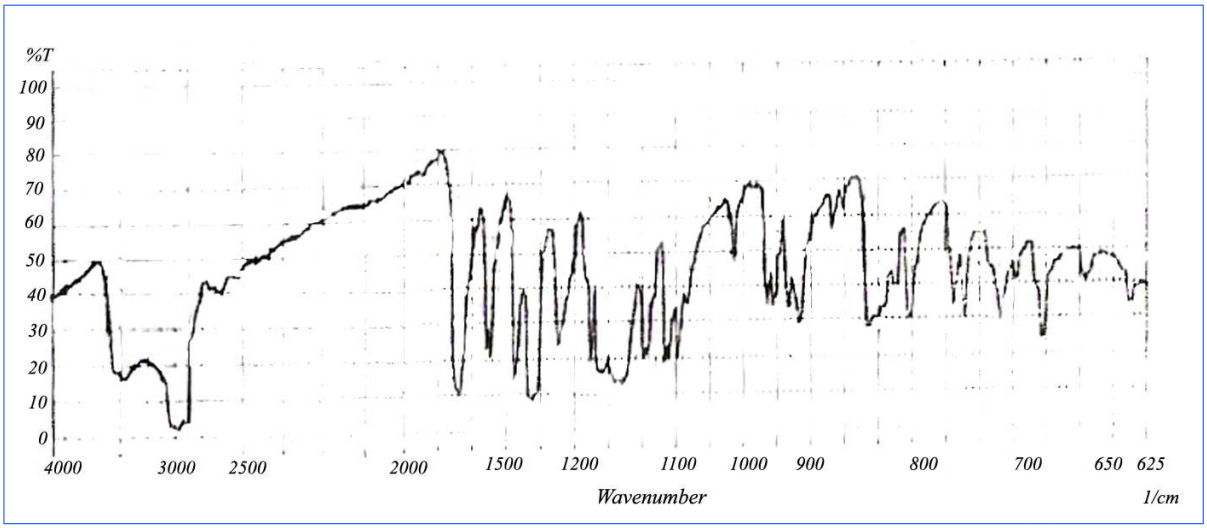


Fig. 2.FT-IR-Spectrum of phenolphthalein.

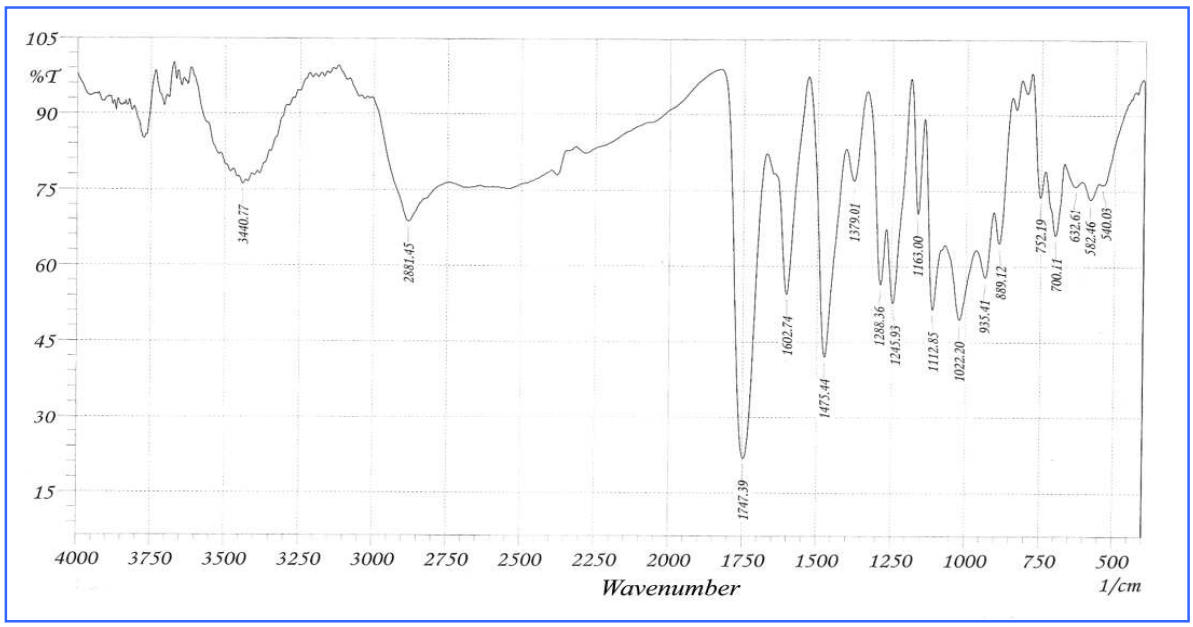


Fig. 3. FT-IR-Spectrum of phenolphthalein-formaldehyde polymer.

Table 1. Wave numbers of the characteristic bands of phenolphthalein-formaldehyde (PPF)

	O-H	C-H Methylene group	C-H Aromatic ring	C=O	C=C	C=O Lactone ring
Str. Vibration	3440	2881	-	1747	1602,1475	1112
Out-of-plane bending	6321	-	889	-	-	-
Rocking	-	752	-	-	-	-
Twisting and wagging	-	1288 1245 1163	-	-	-	-

Antibacterial Activity

Many chemicals can cause the death of micro-organisms but their modes and rates of action are extremely diverse. Chemical disinfectants may be bactericidal at certain concentrations but bacteriostatics[33] at high dilution.

One of the most promising approaches to the regulation mechanism of a cell cycle is to find a suitable and specific inhibitor which blocks a specific event, such as DNA replication and cell division[34]. The majority of disinfectants are general poisons, their lethal action being the result of their capacity of coagulate, precipitate or otherwise denature both structural and essential enzymes of tissue and micro-organismal cells. All inhibitors can be classified under two main headings:

1. Inhibitors that interfere with the function of enzymes by the interaction at the enzyme active center (Type A).
2. Inhibitors that interfere with nucleic acid metabolism become incorporated as part of a normal replication activity (Type B).

There are few compounds, notably those that interfere with ribosome function, that may not fall into this general scheme. Some pathogenic organisms are water-born and when present in drinking water may infect susceptible hosts. Water-borne pathogenic organisms usually gain access to the water from animal and human excreta and sewage[33].

This paper reports the preparation of phenolphthalein-formaldehyde and its possible application in water sterilization by remarkably inhibiting the growth of *Escherichia Coli* (Gram

negative) and *Staphylococcus aureus* (Gram positive), with suggesting the inhibition mechanism**.**

PPF was screened against *Staphylococcus aureus* (Gram +) and *E. coli* (Gram -) bacteria. Unusual antibacterial action obtained in this study. The range of polymer concentration used in this study was located from 100 mg. to 5 mg. Minimal inhibitory concentration (MIC) of the polymer was determined based on the disc diffusion method. The polymer showed a remarkable inhibition for *S. aureus* bacteria at a concentration of 100 mg, with 70 mm of inhibition zone whereas the MIC of polymer against *S. aureus* bacteria was 33 mm of inhibition zone at 5 mg of polymer. Furthermore, the polymer exhibited a higher MIC of 61 mm inhibition zone against *E. coli* bacteria at 100 mg, whereas it showed an MIC of 31 mm inhibition zone against the same bacteria at 5 mg. of polymer. Table 2 showed the inhibition zones in mm against *S. aureus* and *E. coli* with their MIC values of the polymer.

Table 2. The diameters of inhibition zones for PPF against *E. coli* and *S. aureus*.

	E. Coli		***S. aureus***	
	(conc. mg)	inhibition zone (mm)	(conc. mg)	inhibition zone (mm)
1	100	61	100	70
2	75	59	75	70
3	50	45	50	58
4	25	41	25	40
5	10	47	10	44
6	5	31	5	33

To understand the mechanism of PPF as bacterial retardant, it should be taken into account that our new polymer carrying phenolic groups. Such groups can play an important role in the inhibition of bacteria. It is well known that phenols have the ability to work as disinfectants in addition to its potent toxicity by virtue of the protein denaturation. Proteins are precipitated by one to two percent of phenol, thus most vegetative cells of bacteria are killed by 1% phenol in 5-10 min. at 20^{o} C [33].

Otherwise, some antibiotics like tetracycline inhibit the protein-building by preventing amino-tRNA complex from entering to ribosome unit (30 S) which is related to (mRNA) in the bacterial cell. The functional group which is responsible for this process is the hydroxyl groups of tetracycline. This can lead us to believe that the hydroxyl groups in the synthesized polymer are presumably responsible for the effectiveness of the polymer as antibacterial candidate.

Some microbial parasites cannot use external sources of folic acid or folinic acid since these compounds are not able to penetrate through the cell wall, then they must be synthesized within the cell from PABA (*para*-aminobenzoic acid), petridine and glutamic acid[35]. The structural similarity of polymer by having carboxylic group which could make it a competitor of PABA to hinder the way leading to DNA synthesis. This can be valid even if the mechanism is not clear so far. Figures (4-13) showed the antibacterial activity of phenolphthalein-formaldehyde towards *E. coli* and *Staphylococcus aureus* with their MIC values.

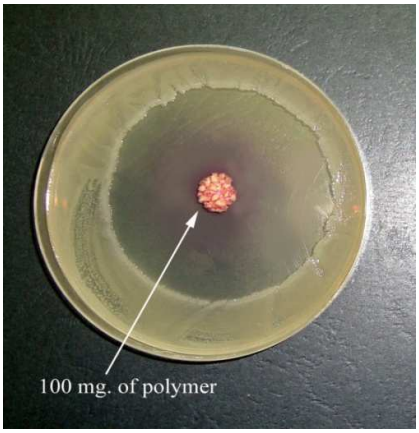


Fig.4. The inhibition zone by 100 mg of PPF against *E. coli*.

Fig.5. The inhibition zone by 75 mg of PPF against *E. coli*.

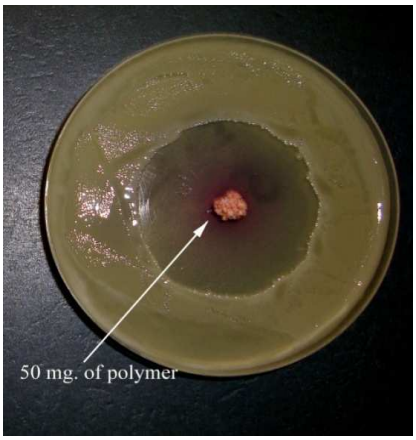


Fig. 6 The inhibition zone by 50 mg of PPF against *E. coli*.

Fig. 7. The inhibition zone by 25mg of PPF against *E. coli*.

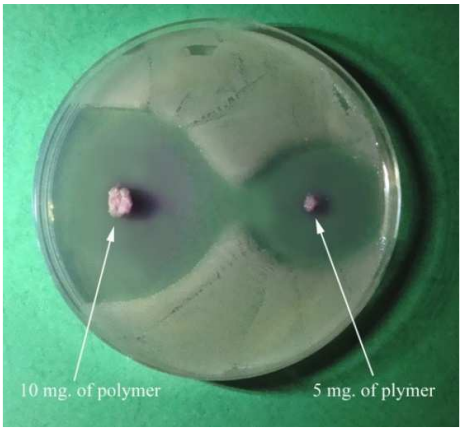


Fig.8The inhibition zone by 10 mg and 5 mg of PPF against *E. coli*.

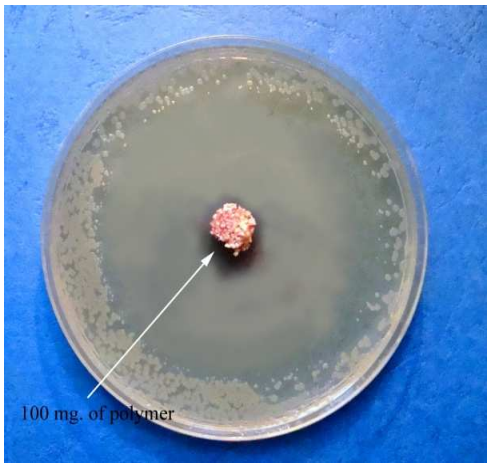


Fig. 9 The inhibition zone by 100 mg of PPF against *S. aureus*

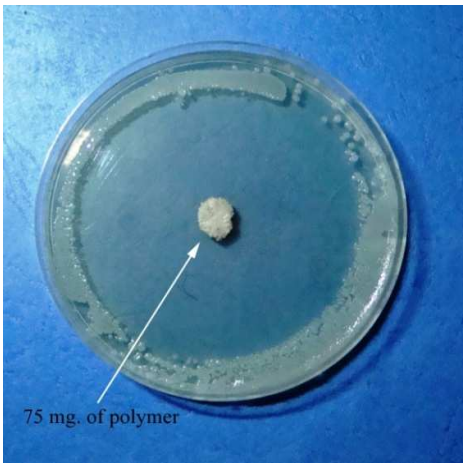


Fig. 10 The inhibition zone by 75 mg of PPF against *S. aureus*

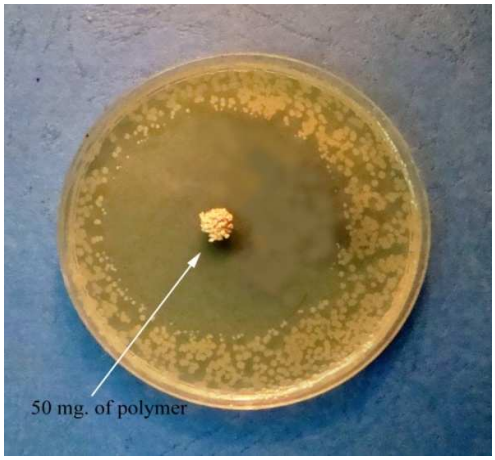


Fig. 11 The inhibition zone by 50 mg of PPF against *S. aureus*

Fig. 12 The inhibition zone for 25 mg of PPF against *S. aureus*

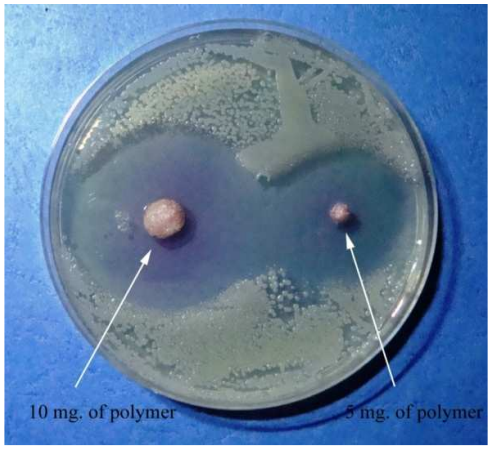


Fig. 13 The inhibition zone by 10 mg and 5 mg of PPF against *S. aureus*

Experimental section

Chemicals and Materials. IR spectrum was measured by FTIR-8400 (Shimadzu)- Department of Chemistry \College of Science\ University of Basrah \Iraq. Phenolphthalein was supported by B.D.H. Formalin (37%) was supported by Fluka. The media culture for bacterial growth was done, using Muller-Hinton Agar from Hi-Media (Mumbai-India)- Department Of Chemistry\College of Science\University Of Basrah\Iraq.

Preparation of phenolphthalein-formaldehyde polymer (PPF)

The preparation method of the new polymer is based on the general procedure of phenol-formaldehyde polymerization[30]. In a thoroughly cleaned three-neck round bottom flask with a thermometer, reflux condenser and mechanical stirrer, phenolphthalein (10.0 g, 0.03 mol), 37.2% formalin (54.5 g, 1.08 mol) and 1N KOH (30 ml) as condensation catalyst, were added. The mixture is heated to 110 ^{o}C for 3 h, with stirring. A solid yellowish white polymer is separated at the flask bottom with an upper aqueous layer. The polymer is separated after being neutralized by 10% H_3PO_4 to adjust the pH to 6-7, then washed with excess of distilled water. The product is dried and crushed to powder.

In vitro antibacterial activity

Procedure: Strains (*Staphylococcus aureus* ATCC25923 and *Escherichia Coli* ATCC25922) were obtained from the immunology lab. of Biology Department, College of Science, University of Basrah. Disc diffusion method[36] was used to determine the minimal inhibitory concentration (MIC). After preparing the medium according to the manufacturer instructions and it was poured to a depth of 4 mm (25 ml of medium) in flat-bottomed 9 cm Petri dishes on a level surface, dried, then the control inoculum was speared on the plates by using an inoculating loop. The polymer pressed into discs in serial of weights. The plates incubated at 37 ^{o}C for 24 h, then the diameter for each inhibition zone was measured by millimeter.

Acknowledgment

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الخلاصة:

من خلال تفاعل الفينولفثالين مع الفورمالديهايد وبوجود هيدروكسيد البوتاسيوم كعامل مساعد قاعدي تم تحضير فينولفثالين – فورمالديهايد , أثبتت صحة بوليمر الفينولفثالين– فورمالديهايد الناتج من خلال دراسة طيف الأشعة تحت الحمراء له. كما تم فحص الفعالية ضد البكتيرية مختبرياً للبوليمر المحضر, حيث أبدى البوليمر قابلية تثبيط قوية وواضحة تجاه كل من بكتريا الاشريكية القولونية (سالبة لصبغة غرام) وبكتريا المكورات العنقودية الذهبية (موجبة لصبغة غرام). كما حددت قيم التركيز المثبط الأدنى للبوليمر وبالاعتماد على طريقة الأقراص . مما يسمح بإمكانية استخدامه كمعقم للمياه.