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Poly (para-acid phenol-D-Glucose): resin for the removal of fecal coliforms and *Escherichia coli*

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ABSTRACT

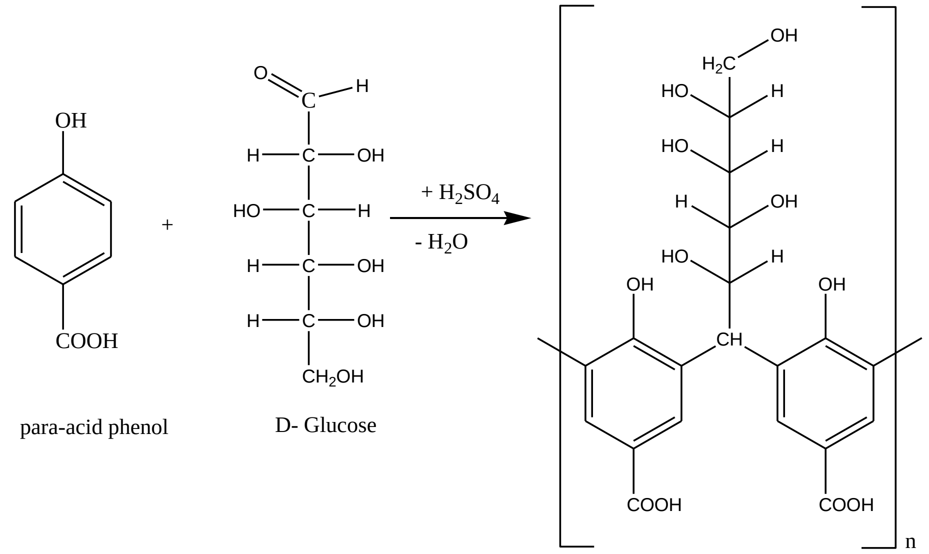
The present study was focused on the development of antimicrobial polymers to clean up the water of pathogenic bacteria. we describe a novel synthetic antibacterial phenolic polymer containing phenolic hydrooxy groups and acid group in para position, para acido phenol(pAP) with D-Glucose (DG) as monomers in the present of homogeneous catalyst (H_2SO_4) by polycondensation reaction to obtain apoly (para-acid phenol-D-Glucose) poly [(pAP-DG)] and at to increase the hydroxyls groups that have a large effect antibacterial; The polymer obtained were tested against Coliform bacteria have been used to evaluate the general quality of water (*Fecal coliforms*, thermotolerant coliform and *Escherichia coli*) bacterias .the antibacterial activity of the synthesis resin was tested by the contact method. The ability of this resin to remove bacteria from water was thus concluded to derive from the presence of the hydroxyl and acid groups. In general, the polymers showed good antimicrobial activity against the tested microorganisms; a possible mechanism of this polycondensation reaction is discussed based on the result of the^{1}H-RMN , ^{13}C-RMN, DSC and FT-IR.

Keywords: *antibacterial resins; poly (p-acid phenol-D-glucose); polycondensation; homogeneous catalyst.*

1. INTRODUCTION

The universe for polymer chemistry has developed to such a level that it now opens up immense possibilities for creativity chemists, we can now make new macromolecular substances that characterized by their molecular weight and structure .Current progress and recent developments of functional polymers dictated by the needs of the market are enormous. The application field of the effectiveness of functional materials to fulfill the new requirements of industrial worlds, space, medical, agriculture and the environment. The polymer demand has grown steadily for many years and then every day is enriched by new applications of specific macromolecular materials. Thermosetting phenolic resin a kind of ancient adhesive , which has been produced and used for nearly one hundred years [1-4].

Phenolic resins [5,6] are among the oldest of industrial polymer that are still employed to date due to some of their superior properties , such as dimensional stability and chemical resistance, as well as their low cost of manufacture, phenolic resins are widely use in preparation of composite materials [7]. Phenolic polymers were found in many industrial applications due to its high dimensional stability, low cost, high resistant to chemicals, good thermal stability and heat resistant properties [8-10]. The use of polymers and resins for the treatment of industrial effluent in general and which have antibacterial activity in particular are subject to a number of research ciampa and his colleagues have intensively studied the biological activity of many resins polymer incorporating salicylic acid as well as their components Polymer structure and properties have been studied and near complete monomer conversions, quantitative polymer yields and high molecular weights have been reported [11]. Polyphenols are commercially produced as novolacs and resoles by condensing phenol with formaldehyde at different molar ratios depending on the type of polymer desired [12]. Random pre-polymers of phenol/formaldehyde are prepared by reacting phenol on the ortho and para positions in the boxing ring with bifunctional formaldehyde, where the bases catalyzed reaction produce a mixture of methylol phenols where the composition of the mixture can be varied by altering the phenol to formaldehyde ratio Inorganic catalysts such as copper halides are known to catalyze phenol polymerization in the presence of molecular oxygen some phenolic compounds (resins with phenol derivatives containing one, two, or three hydroxyls groups) are bactericides, were also brought back to the antibactériennes activities due to the groups of hydroxyls [13]. Considerable efforts have been made in phenolic resins production to replace the petroleum-based phenol titally or partially with lignocellulosic biomass, an abundant renewable resource. For instance, lignin was successfully used to replace phenol in production of novolac- and resol-type phenolic resins [14-17]. Given the ubiquity of the use of polymers all polymers development aspects can compete with those in place provide an outlet of size by agro resources. Moreover, the introduction of sugar into the structure brings new properties to polymers, which make them attractive for specific applications of higher added value, and could accommodate higher production costs. Glucose was successfully applied to replace the toxic formaldehyde to synthesize novel Novolac type phenolic resins, catalyzed by strong acid (H_2SO_4) at high temperature (120-150°C) the condensation polymerization could proceed readily with solutions of phenol and glucose at varying molar ratios (1:0.4 to 1:0.9) through the Friedel-Crafts reaction mechanism. We present a new approach to synthesis apoly [(pAP-DG)] by cationic polymerization using H2SO4 as a catalyst (Scheme 1)



Scheme 1. Schematic representation of the synthesis of copolymer [(pAP-DG)].

2. EXPERIMENTAL SECTION

2.1. Materials.

Phenol, p-acid phenol and D-Glucose (99%) were purchased from Aldrich (Paris, France) purified by fractional distillation under reduced pressure.

THF, DMSO, acetone, methanol a solution of sodium hydroxide, sulfuric acid (98%) and distilled water was used in preparation of solutions were used without any purification.

2.2.Polymer characterization.

The polymers were characterized by IR spectra of the resins were scanned on SHIMADZU spectrometer using KBr pellets at wave numbers from 400-4000 cm^{-1} fourier transform infrared FT-IR spectroscopy.

The spectra of nuclear magnetic resonance of proton (^{1}H-NMR) and carbon (^{13}C-NMR) was recorded in a 300 MHzBruker spectrometer, in deuterium acetone solution under ambient temperature using tetra methyl silane (TMS) as internal standard in these cases.

Thermal analysis (DSC) of poly(pAP-DG) was conducted on a differential scanning calorimetry apparatus (200PC NETZCH, 204F1PHONIX240-12-0110-L DSC) the measurements were carried out with a heating rate of 10°C /min under 50 cm^3/min nitrogen flow.

2.3. Polymerization procedure.

In a necked flask equipped with a reflux condenser and a thermometer were introduced (0.1 mole p-acid phenol), and (0.05 mol) of glucose, with 0.01 g initiator under a certain reaction time. the temperature is controlled at 130 °C. stirring the mixture for 1h reflux (oil bath). The polymerization of p-acid phenol and D-Glucose was carried out in a homogeneous system, using H_2SO_4 as a catalyst, the mixture were copolymerized by bulk polymerization, After 2 hours, recovering the contents of the flask and was added 1.7 ml of sodium hydroxide solution (1M) for neutralization, then heated to remove water. The reaction scheme is schown in scheme 1. The resulted polymer was dissolved in acetone, precipitated in THF, and then air dried at room temperature. The purification procedure was repeated several time in order to obtain a highly purified polymer to reached a higher yield of 80%. the precipitates were characterized by FT-IR spectroscopy, ^{1}H-NMR, ^{13}C-NMR and differential scanning calorimetry (DSC) to confirm the structure of polymer.

3. RESULTS SECTION

The cationic polymerization of pAP with DG was examined in the presence of H_2SO_4 as a catalyst at 130 ◦C (Scheme 1).

3.1. Copolymerization of pAP with DG. We intended to prepare poly(pAP-DG) by cationic polymerization with 0.1 mole p-acid phenol and 0.05 mol of glucose, in the presence of 5 gutters 1% of sulfuric acid as a catalyst. The reaction was carried out in tubes sealed and immersed into an oil bath preheated at 130 ◦C.; Each tube contains a mixture of 0.1 mole p-acid phenol, 0.05 mol of glucose and an amount of 1% of sulfuric acid. The molar ratio of p-acid phenol to glucose [p-acid phenol] / [glucose] was kept constant in all tubes. the system is put under mechanical agitation during 3 h; when the mixture becomes viscous and at the end of reaction, we added thereafter the 1.7 ml of sodium hydroxide solution (1M) for neutralization, then heated to remove water. The polymer was dissolved with 20 ml of acetone and precipitated in tetrahydrofuran, the samples were filtered, and dried in vacuum. The precipitates were characterized by ^{1}H-NMR, ^{13}C-NMR, and Infrared spectropie (FT-IR) analysis. The presence of PpAP and PDG chains causes termination step and lead to the (pAP-DG) copolymers (see Scheme 1).

3.2. Determination of the pKa of the copolymer.

The aqueous solution was made by mixing 25mg resin in aqueous solution $NaNO_3$ (0.01M), stirred under magnetic stirring for 24h for déprotoneted the carboxylic groups of resin and the functions carboxylic are protonic with $HNO_3$0.01N finally the excess of the free protons in the solution was titrated by a standard solution of NaOH (0.01 M) at controlled temperature (25.0 ± 0.5 °C). The titration was initiated when the temperature stabilized. The solution is stirred and the pH is measured when equilibrium is reached which in our case occurs in a few seconds. The variation of pH with $V_{NaOH}$of polymer and its derivative curve (The Gran method) [18], depicted in Figure. 1 (a) and (b), the pKa corresponds to the volume of half-équivalanlance (V=62ml) is 4.28. which allow to determine the total concentration CA of reactive carboxylic sites of copolymer, CA was found to be $1{,}398.10^{-3}$ mole. The pH measurement were performed thanks to a pH meter type Inolab Multi Level combined with a glass electrode (Schott), preliminary calibrated by two buffer solutions (pH 7and 10).

The solution was placed in a thermo stated water bath whose temperature was regulated by a temperature controller. All solutions were thermostated at 25.0 ± 0.5 °C and contain high purity $NaNO_3$ (0.01 M) as supporting electrolyte.

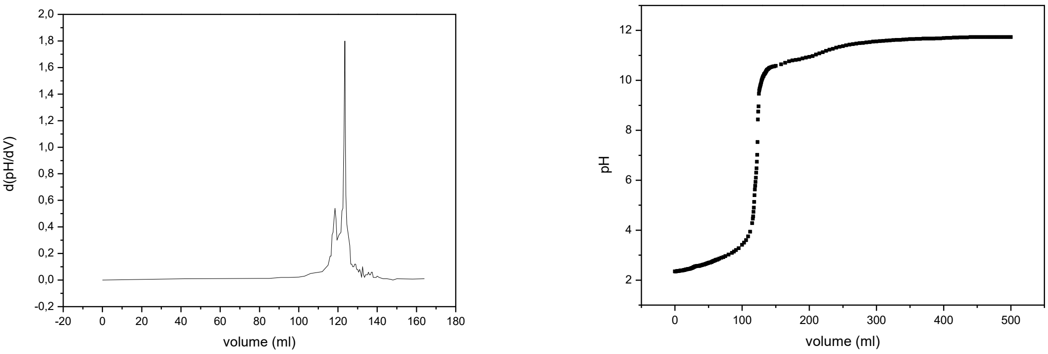


Figure .1. Plots of the experimental results for the pH titration for the polymer (pAP-DG) (a), the pH variation vs. the volume of NaOH (0.1 M) for the compound (pAP-DG) (b).

3.3. Antimicrobial Activity. The polymers obtained were tested against Coliform bacteria have been used to evaluate the general quality of water (*Fecal coliforms ,thermotolerant coliform* and *Escherichiacoli*) bacteria .the antibacterial activity of the syntheses resins was tested by the contact method [19]. The number of the bacteria colony was counted after the test tubes were incubated at 37°C for 24h under (200rpm) , the percent reduction of the bacteria (CTT, GT and SF)cells was calculated from the following formula using (pAP-DG) insoluble polymeric material. The percentage of bacterial reduction of the cells is calculated according to the following formula:% reduction =[(N_{Rb} - N_{Rt}) / N_{Rb}].100. Where N_{Rt} is the number of the bacterial cells counted after pouting the biocide (resin) and N_{Rb} is the number of the bacterial cells before pouting biocide (resin).

The number of bacteria cell per ml was calculated by multiplying the number of colonies by the dilution factor and the result is listed in table 1.

The *E.coli* cells disappeared completely (reduction 100%), but the number of *Fecal coliforms*, *thermotolerant coliform* cells was reduced only to 98.5% and 95.11% respectively after contact with (pAP-DG) for 24h.The bacteriocidal activity of poly(p-acid phenol -D-glucose) toward *E.coli* was much lower.

This study show clearly that the reduction of pathogenic bacteria is better with the resin which contains an aromatic, -COOH and –OH groups on its chain, which resin present also the qualities required as an antibacterial activity materials.

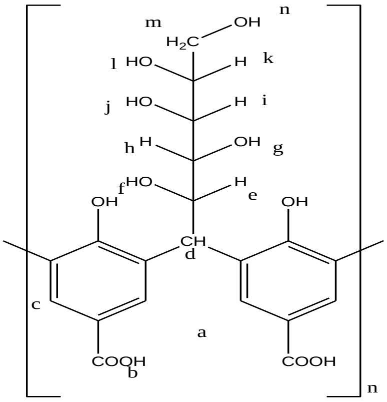
Table 1. Antibacterial activity of p APG resin (MIC 0.01g/ml).

		24h	48h	72h
GT		404	1248	1287
BlankSF		1536	5285	>
CTT		3392	>	>
GT		46 (88.6%)	61(95.11%)	202(84.3%)
(pAP-DG)SF		616 (59.9%)	79(98.5%)	00(100%)
CTT		1810 (46.6%)	00 (100%)	00(100%)

MIC minimum inhibitory concentration was determinate by agar dilution method

3.4. HNMR spectra. The spectrum of 1HNMR is among the spectroscopic techniques used to determine the structure of our product. The structure of the resulting copolymer was confirmed by ^{1}H-NMR, ^{13}C-NMR measurements and Infrared spectroscopy (IR). The assignments of peaks of copolymer are given in Tables 2 and 3. It is based on the carbon coupling proton. The important proton NMR peaks of poly(p -acid phenol-D-Glucose) was assigned , as shown in figure 2 new peak emerged at 1.42 ppm in this synthesis polymer represent methylene protons bridge , confirmed by chemical shift carbons shown in figure 3.

Table 2. ^1H -NMR Chemical shifts and signal assignation of poly(pAP-DG).

Structure	δ (ppm), intensity	Attribution
	(2,05-2,23,d)	H_m
	(1,42,d)	H_d
	(4,25,s)	H_a
	(6.7-6.93,s),	H_c aromatic
	(11,15, s)	H_b
	(3,61-3,66, m)	$H_{n, l, j, g, f}$
	(1,78-1,82, m)	$H_{e ,h, i, k}$

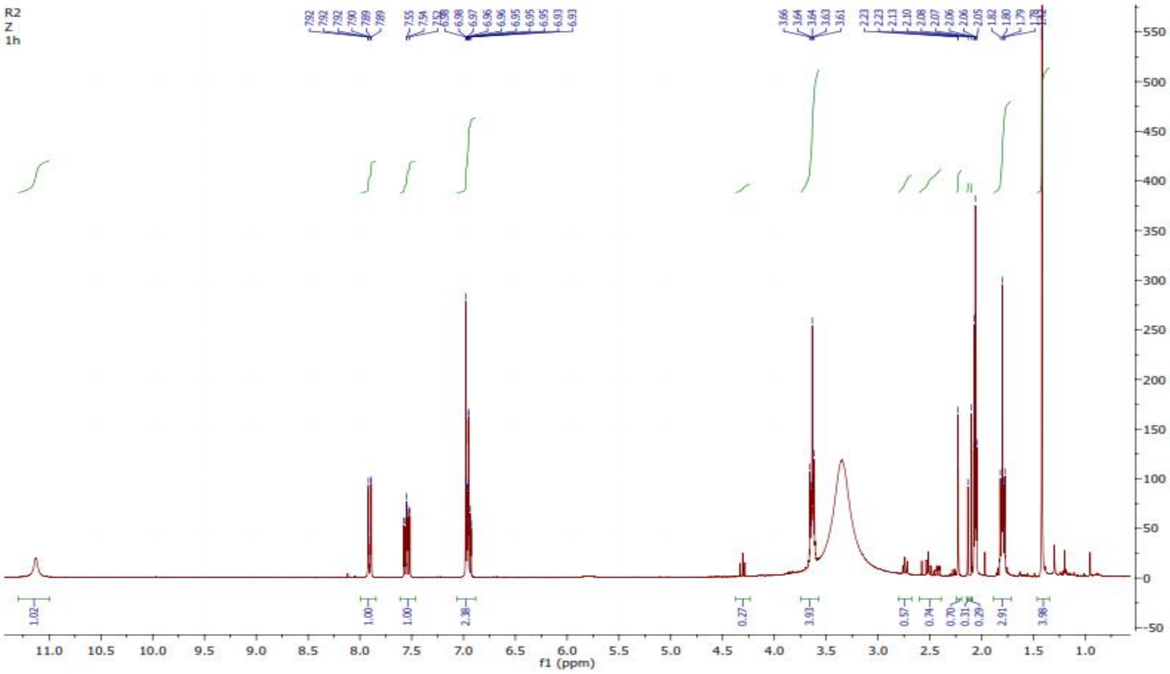
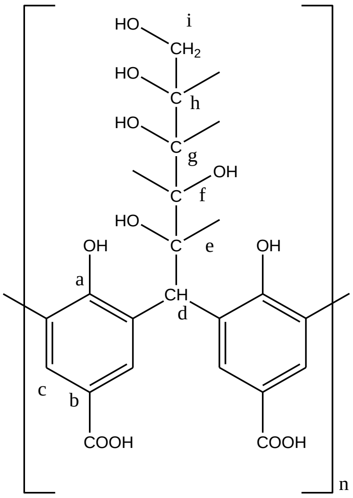


Figure 2. ^1H-RMN spectrum of the (pAP-DG) copolymer.

Table 3. ^{13}C- NMR Chemical shifts and signal assignation of (pAP-DG) copolymer.

Structure	Attribution	δ (ppm)
	C_a C_b C_c C_d $\underline{C}OOH$ $C_{e,f,g,h,i}$	205,32 135,86 119,06 25,25 130,35 28,16-29,81

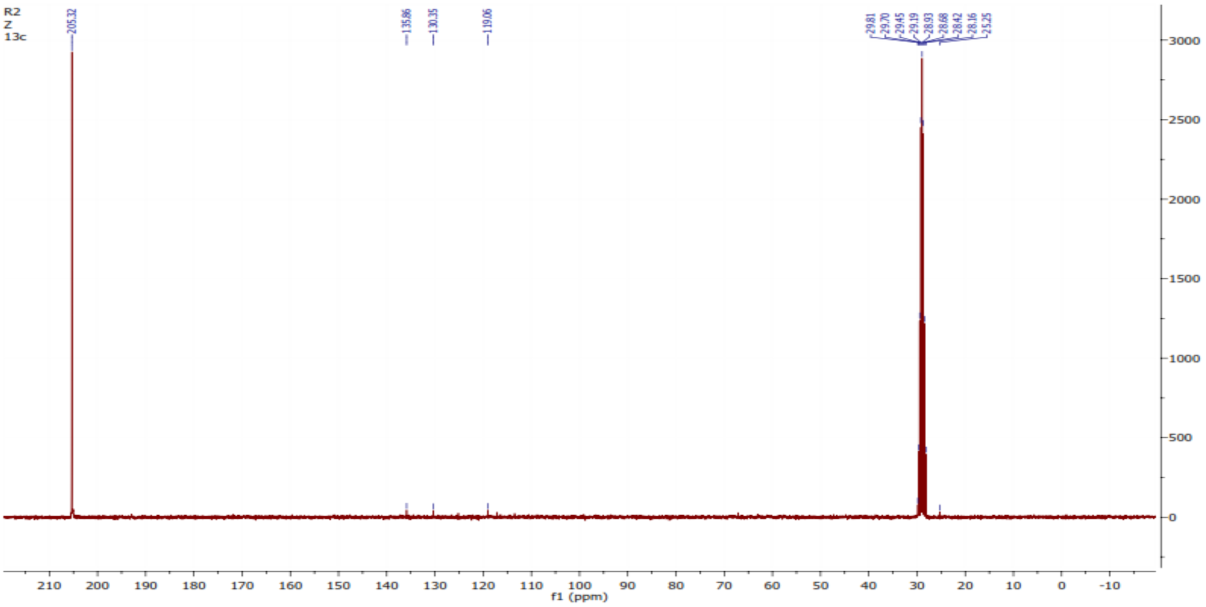


Figure 3.^{13}C- RMN spectrum of the (pAP-DG) copolymer.

3.5. Fourier Transform Infrared Spectroscopy.

The IR measurements of product are in a good agreement with poly(p-acid phenol-D-Glucose) structure. The polymers were characterized by IR spectra of the resins were scanned on SHIMADZU spectrometer using KBr pellets at wave numbers from 400-4000 cm^{-1} Fourier transform infrared spectroscopy(FT-IR) was investigated as a complementary technique to NMR due to its sensitivity. The infrared spectra of the synthesis resins, as prepared in the present investigation were dominated by the large OH stretch which encompasses signals related to the hydroxyl. IR spectrum in **Fig** 4. Shows absorption bands characteristic of the resin (pAP-DG), and their functions are shown in the following table (Table 4). The position of the absorption band around 1660.41 cm-1 corresponding to the stretching vibration of C = O indicates that the group C = O and conjugated double bond (aromatic ring) Broadband between 3065-3400 cm^{-1} corresponding to the OH function is attributable to the presence of carboxylic acid and alcohol.

The (–CHR–) bond of the bending vibration indicates the presence of (–CHR–) methylene bridg.

3.6. DSC analysis.

We took 9.5mg of resin, put in a capsule SETARAMDSC131 type of device, the test was done in the laboratory polymer ORAN University. The thermogram is present in figure 5. The thermal properties of the copolymers were investigated by differential scanning calorimetry (DSC). **Fig. 5** shows the results of the DSC measurements. Glass transition temperature Tg of the resulting copolymers was observed in the temperature range of 50-70 °C. The DSC analysis of (pAP-DG) copolymers, show two closely neighboring melting endotherms at temperatures between 160 and 210 °C, they may be interpreted as crystallites. This analysis indicates the semi-crystalline state of the resulted copolymer.

Table 4. Different IR absorption bands of the synthesized poly(pAP-DG).

Chemical shift	Absorption Band	Nature
ν_{OH}	3200-3500 3241.75	Band d'OH OH acid Stretching vibration
ν_{CH}	3065.3	Aromatic
ν_{CH}	2859.92	Aliphatic
$\nu_{(C=O)}$	1660.41	Carboxylicacid
$\nu_{(C=C)}$	1611.23-1578.45	benzene ring
$d_{ip(OH)}$	1363.6	Phenolic
$\nu_{ip(C-O)}$	1232.4	Phenolic
$d_{ip(CH)}$	1103.2-1014.5	Aromatic
$d_{op(CH)}$	890.952	Para substituted
$d_{op(CH)}$	758.85	Ortho substituted

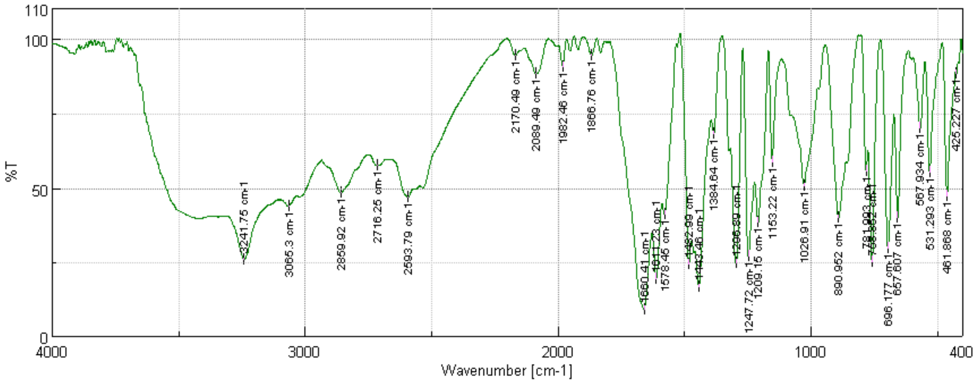
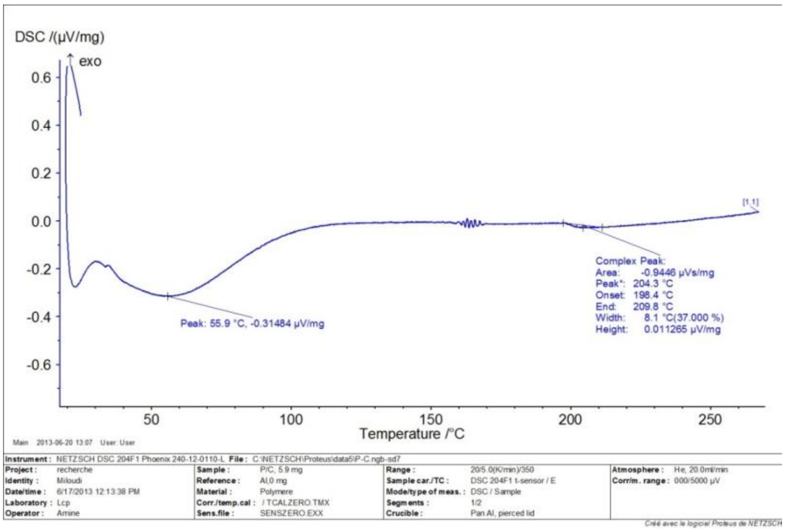


Figure 4. Infrared spectrum of poly(pAP-DG) in the region 400- 4000 cm$^{-1.}$



Instrument : NETZSCH DSC 204F1 Phoenix 240-12-0110-L **File :** C:\NETZSCH\Proteus\data5\P-C.ngb-sd7

Project :	recherche	**Sample :**	P/C, 5.9 mg	**Range :**	20/5.0(K/min)/350	**Atmosphere :**	He, 20.0ml/min
Identity :	Miloudi	**Reference :**	Al,0 mg	**Sample car./TC :**	DSC 204F1 t-sensor / E	**Corr/m. range :**	000/5000 µV
Date/time :	6/17/2013 12:13:38 PM	**Material :**	Polymere	**Mode/type of meas. :**	DSC / Sample		
Laboratory :	Lcp	**Corr./temp.cal :**	/ TCALZERO.TMX	**Segments :**	1/2		
Operator :	Amine	**Sens.file :**	SENSZERO.EXX	**Crucible :**	Pan Al, pierced lid		

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Figure 5. thermogramof the (pAP-DG) copolymer.

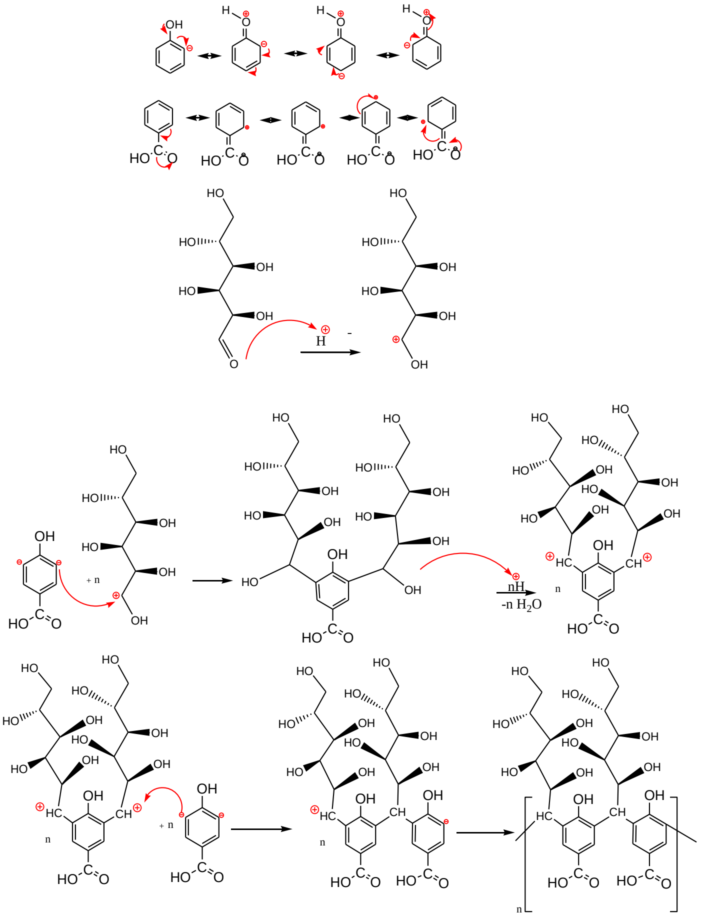

Figure 6. Thermogram of the (pAP-DG) copolymer.

4. CONCLUSIONS

It is now possible to prepare macromolecules with predominant structures, discrete molecular weights and with specific functional groups. Compounds with hydroxyls and acids handed groups are used widely as anti-bacterial new synthetic methods must include health and environmental point of views. Therefore, it has become primordial to decrease the amount of catalyze and reactif, The poly(p-acid phenol -D-glucose) phenolic polymer was successfully synthesized from acid p-phenol and D-glucose synthesized by condensation reaction .The modification of the polymer was confirmed by FT-IR and NMR spectroscopy . The synthesis polymer was seen to have good antibacterial properties and was found to have a novel and remarkable ability to remove bacteria the *E. coli* were completely removed (100% reduction) but *fecal coliforms, thermotolerantcoliform were* reduced only to (84.3%). The bactericidal activity of the poly (p-acid phenol -D-glucose) increased with increasing phenolic hydroxyl groups in the resin. In particular Poly (p-hydroxyphenol-D-glucose*)* had the highest antibacterial activity toward *E. coli*. This polymer with an acid, alcohol aromatic and aliphatic as simple structure can be used as material for waste water purification infected by pathogenic bacteria.

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