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Research Article

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Antimicrobial screening and thermoanalytical studies of newly synthesized copolymer derived from p-hydroxybenzoic acid, and thiosemicarbazide

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ABSTRACT

The Copolymer (p-HBTF) was synthesized by condensation of p-hydroxybenzoic acid and thiosemicarbazide with formaldehyde in the presence of 2M HCL as a catalyst at 126 ± 2 ^{0}C for 5 hrs. with molar proportion of reactants. The copolymer (p-HBTF) was characterized by elemental analysis, FT-IR, UV-Visible ^{1}H-NMR Spectroscopy. The thermal decomposition behavior of copolymer was studied by using TGA in static nitrogen atmosphere at a heating rate of 10^{0}C/min. Freeman Carroll and Sharp-Wentworth methods were used to calculate the thermal activation energy (Ea), the order of reaction (n), entropy Change (ΔS), free energy change (ΔF), apperent entropy change (ΔS), and frequency factor (Z). The thermal activation energy determined with the help of these method was in good agreement with each other. The copolymer possesses antimicrobial activity for certain bacteria such as S. subtilis, , E.coli, S. typhi .

Keywords: Synthesis, condensation, antimicrobial screening, thermogravimetric analysis; decomposition, resins.

INTRODUCTION

There is a noteworthy demand to synthesize eco-friendly polymers having some biological activities like antifungal and antibacterial The invasion of polymers by fungi, bacteria and other organism is manifested by loss of mechanical properties, surface degradation, discoloration, staining and other deteriorations [1]. Polymers are used as biocidial agents in recent times. By incorporating biologically active organic moieties into the polymer backbone, the activities can be introduced. In terms of their biological activity, these polymers are more effective than their monomers. Such polymers are known for their biocidial activity against some bacterial, fungal and viral strains. Thermogravimetric analysis has been widely used to investigate the decomposition characteristics of polymeric matter . Copolymers can be used as high energy material, ion-exchanger, semiconductors, antioxidants, fire proofing agent, optical storage data, binders, molding materials *etc*. [2]. Copolymers were applied in various fields of research as ion-exchangers, high thermal resistance materials, and electrical appliances [3, 4]. The study of thermal behaviour of copolymers in air at different temperature provides information about the nature of species produced at various temperatures due to degradation. Copolymers having good thermal stability and catalytic activity have enhanced the development of polymeric materials. Copolymer resins are derived from 2,4-dihydroxypropiophenone, biuret and formaldehyde in hydrochloric acid as catalyst and studied their thermal degradation [5, 6]. Suhas Thattle et al. (2005) reported on the role of polymeric systems that can deliver drugs directly to the intended site of action and enhance antibacterial efficacy [7]. Recently, Rajakumar et al. (2005; 2006) synthesized dentritic architectures using 4, 4′-dihydroxy bis (arylidene) cyclopentanones and studied their antibacterial activity by disc diffusion method [7]. Terpolymers of salicylic acid, thiourea with trioxane and *p*-hydroxybenzoic acid, thiourea with trioxane

have been reported in the literature [8, 11]. Resins synthesized by condensation of mixtures of phenol or hydroxybenzoic acid with formaldehyde and various amines have also been reported [12]. Thiosemicarbazones and the corresponding metal complexes are widely known as having a large range of biological applications, such as antiviral, antimalarial, antifungal, etc (West et al. 1991, El- Sawaf et al. 1997). Some thiazole, thiosemicarbazole, imidazole, benzothiazole derivatives (Muthusubramanian et al. 2001, Chevica et al. 2003, Tolkova et al. 2001, Shaha et al. 2002, Paramashivappa et al. 2003) and isatin (Pandeya and Sriram 1998, Sarangapani and Reddy 1994, El-Sawi et al. 1998, Aanandhi et al. 2008) are mentioned in literature to show antimicrobial (Bartlett et al. 1992, Sunel et al. 2001, Basu et al. 2002, Koci et al., 2002) antifungal (Gbadamassi et al. 1988), antihelmitic (Hazelton et al., 1995) pesticide and herbicidal properties [13].

The present paper deals with the synthesis, structural characterization of p-HBTF terpolymeric resin by various physicochemical studies and relative antibacterial activity against bacteria. Thermal analysis of the newly synthesized resin of p-Hydroxybenzoic acid, thiosemicarbazide with formaldehyde (F), by applying the Sharp-Wentworth and Freeman-Carroll methods. Energy of activation (*Ea)*, thermodynamic parameters viz. *Z*, □*S*, □*F*, *S**, and order of reaction/(*n)* were determined by applying Freeman-Carroll Method [14-15].

EXPERIMENTAL SECTION

Starting Materials

The entire chemical used in the synthesis of various new copolymer resins were procured from the market and were analar or Fluka or chemically pure grade. Whenever required they were further purified by standard methods like thin layer chromatography, reprecipitation and crystallization which are generally used for the analytical purification purpose.

Synthesis of p-HBTF copolymer resins:

The new copolymer resin p-HBTF was synthesized by condensing p-hydroxybenzoic acid (0.1 mol) and thiosemicarbazide (0.1 mol) with formaldehyde (0.2 mol) in a mol ratio of 1:1:2 in the presence of 2 M 200 ml HCl as a catalyst at 126 0 C ± 2 ^{0} C for 5h, in an oil bath with occasional shaking, to ensure thorough mixing. The separated copolymer was washed with hot water and methanol to remove unreacted starting materials and acid monomers. The properly washed resin was dried, powdered and then extracted with diethyl ether and then with petroleum ether to remove salicylic acid- thiosemicarbazide formaldehyde copolymer which might be present along with p-HBTF copolymer. The yellow color resinous product was immediately removed from the flask as soon as reaction period was over and then purified. The reaction and suggested structure of p-HBTF is shown in Fig. 1.

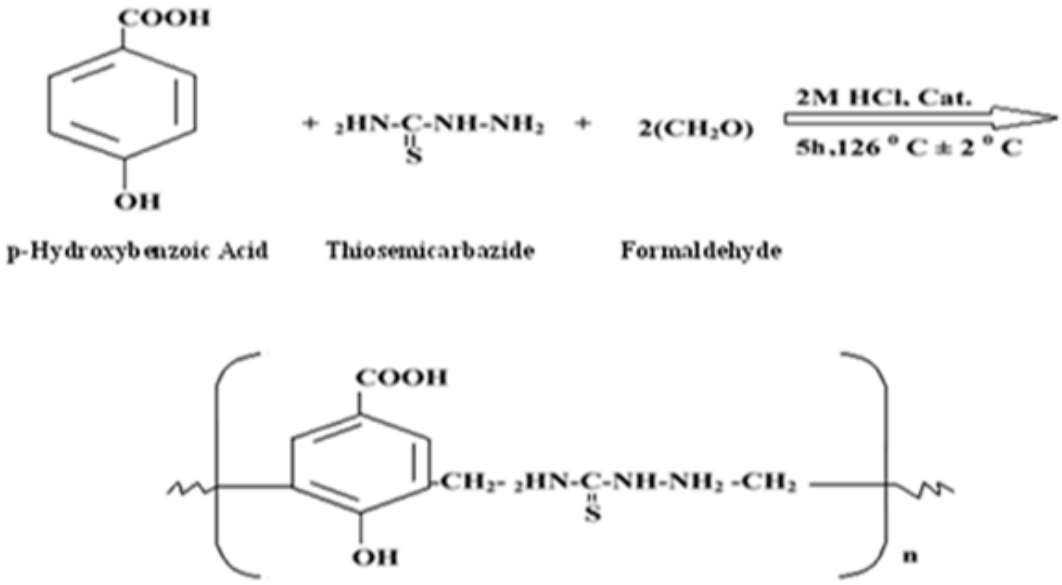

Fig.1: Synthesis of p-HBTF copolymer resin.

The copolymer was purified by dissolving in 10% aqueous sodium hydroxide solution, filtered and reprecipitated by gradual drop wise addition of ice cold 1:1 (v/v) concentrated hydrochloric acid / distilled water with constant and rapid stirring to avoid lump formation. The process of reprecipitation was repeated twice. The copolymer sample p-HBTF thus obtained was filtered, washed several times with hot water, dried in air, powdered and kept in vacuum desicator over silica gel.

Thermoanalytical Study :
The non-isothermal thermogravimetric analysis was performed in air atmosphere with heating rate of 10 ^{0}C.min^{-1} from temperature range of 40 ^{0}C to 700 ^{0}C using Perkin Elmer Diamond TGA analyzer in argon environment. The thermograms were recorded at Sophisticated Instrumentation Centre for Applied Research and Testing (SICART), Vallabh Vidyanagar, Gujrat . The thermal stability of copolymer, based on the initial decomposition temperature, has also been used here to define their relative thermal stability, neglecting the degree of decomposition. A plot of percentage mass loss versus temperature is shown in the Fig. 4 for a representative p-HBTF copolymer. From the TG curves, the thermoanalytical data and the decomposition temperatures were determined for different stages. To obtain the relative thermal stability of the copolymer, the method described by Sharp-Wentworth and Freeman-Carroll adopted.

Antibacterial Screening :
p-HBTF copolymer have been synthesized and agar diffusion method was employed to study their antibacterial activity (Fig.8). Test bacterial pathogens used in this study includes B. Subtilis , E. Coli , S. Typhi The antibacterial screening of p-HBTF is analysed at BIOGENICS, Hubli (Karnataka). Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10^4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with Gentamycin. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zones were noted. Test samples were tested at different concentration to test their efficacy in inhibiting the growth of the human pathogens.

RESULTS AND DISCUSSION

The newly synthesized purified p-HBTF copolymer resin was found to be yellow in color. The copolymer is soluble in solvents such as DMF, DMSO and THF while insoluble in almost all other organic solvents. The melting point of p-HBTF copolymer resin is 186 ^{0}C and the yield of the copolymer resin was found to be 82 %.

FT-IR Spectra : A broad band appeared in the region 3500-3200 cm^{-1} may be assigned to the stretching vibrations of phenolic hydroxy (-OH) groups exhibiting intermolecular hydrogen bonding . The presence of -NH in thiosemicarbazide moiety may be assigned due to sharp band at 2800-3200 cm^{-1} . The sharp band displayed at 1685-1430 cm^{-1} may be due to the stretching vibrations of carbonyl group (C=O) of both as well as (C=S) moiety . The bands obtained at 1400 – 1200 cm^{-1} suggest the presence of methylene bridges in the polymer chain. The weak band appearing at 750 - 780 cm^{-1} is assigned to C – OH bond. 1, 2, 4, substitution of aromatic ring is recognized from the bands appearing at 1279, 1178, 1112, cm^{-1} respectively.

NMR Spectra:
Weak signal in the range of 8.00 ppm is attributed to phenolic -OH proton. The NMR spectra of p-HBTF copolymer resins show a weak multiplate signal (unsymmetrical pattern) in the region 6.8 (δ) ppm which is due aromatic protons. A medium singlet peak appeared at 3.7 (δ) ppm may be assigned to methyl protons of Ar-CH_2-NH group. Intense signal appeared in the region 2.5 (δ) ppm may be due to Ar–CH_2-NH .Triplet signal appeared in the region 3.35 (δ) ppm can be assigned to amido proton of –CH_2-NH-CO- linkage.

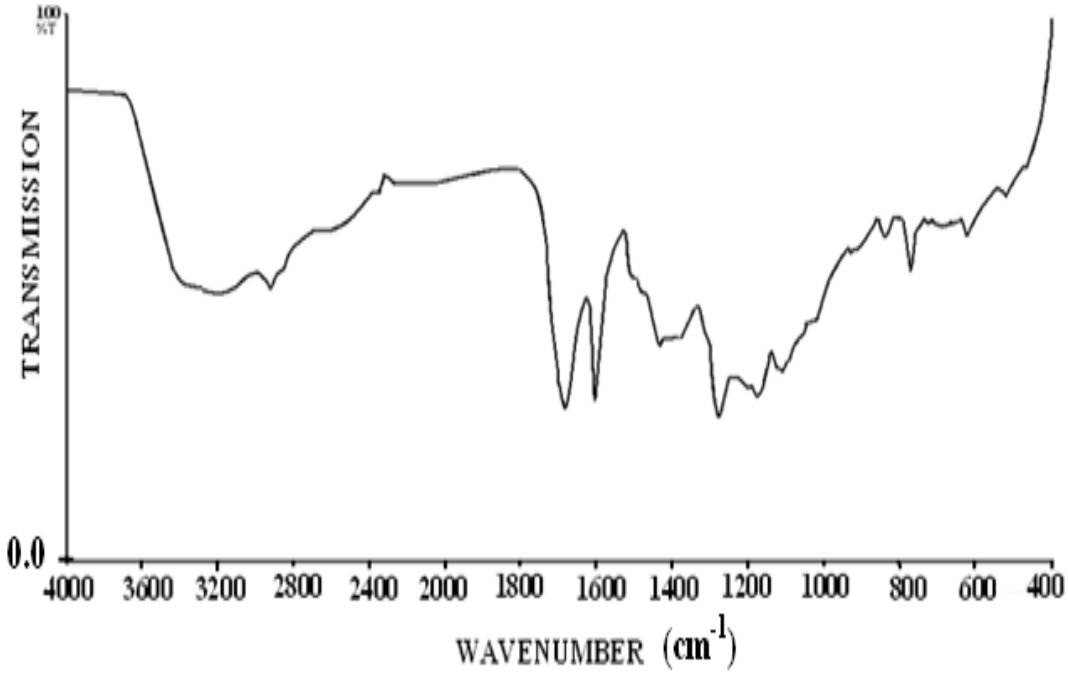

Fig.2: FT-IR Spectra of p-HBTF copolymer

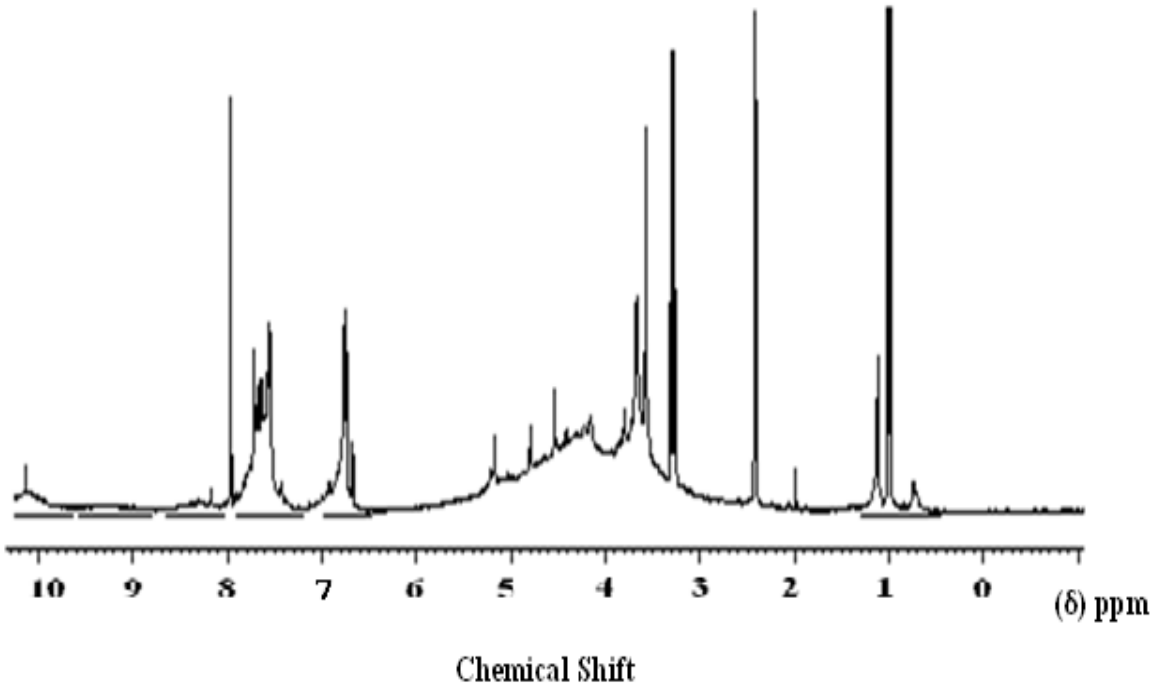

Fig.3: NMR Spectra of p-HBTF copolymer

Thermogravimetry :
The thermogravimetric data provide information regarding the thermal stability of a polymer. The thermograms were obtained by heating resin sample in air at 10^0 C/min. The results of percentage weight loss of the copolymer resins depicts three steps decomposition in the temperature range 140-600ºC and are furnished in Figure 4. The slow decomposition between 0-140ºC corresponds to 5.5 % loss which may be attributed to *loss of water molecule* against calculated 6.64% present per repeat unit of the polymer. The first step decomposition start from 140-250ºC which represents *loss of hydroxyl group and acid group* (32.67% found and 29.52% cal.). The second step decomposition start from 250-480ºC corresponding to 66.54% removal of *aromatic nucleus and methylene bridge* against calculated 68.26%. The third step decomposition side chain from 480-600ºC corresponding to removal of *Thiosemicarbazide moiety* (100.00% found and 100.00% cal.).

With the help of thermogravimetric data the thermal activation energies (Ea) and order of reaction (n) calculated. Also other thermodynamic parameters such as entropy change (DS), apparent entropy change (S*) and frequency factor (Z) are determined and reported in the *Table 1& 2*. To provide further evidence regarding the degradation system of analyzed compounds, we derived the TG curves by applying an analytical method proposed by Sharp-Wentworth and Freeman-Carroll. The 'average *Ea' calculated by Freeman-Carroll (22.97 KJ/mole) and 'average Ea' by Sharp*- Wentworth (22.78 KJ/mole) is nearly same.

Sharp -Wentworth method:
Using the equation derived by Sharp and Wentworth [1],

$$\log [(dc/dT)/ (1-c)] = \log (A/\beta) – [Ea/2.303R]. 1/T \text{ ……..}(1)$$

Where,
dc/dT = rate of change of fraction of weight with change in temperature
β = linear heating rate *dT/dt.*

By plotting the graph between (log dc/dt)/ (1-c) vs 1/T we obtained the straight line which give energy of activation (*Ea) from its slope. Where β is the conversion at time t, R is the gas constant* (8.314 Jmol-1K-1) and *T is the absolute temperature. The plots (figure 2) give the activation* energies at different stages of degradation reaction take place.

Freeman-Carroll method:
The straight-line equation derived by Freeman and Carroll , which is in the form of n

$$[\Delta \log (dw / dt)] / \Delta \log Wr = (-E / 2.303R) . \Delta (1/ T) / \Delta \log Wr + n \text{ ----------}(1)$$

Where,
dw/dt = rate of change of weight with time.
Wr = Wc-W
Wc = weight loss at completion of reaction.
W = fraction of weight loss at time t.
Ea = energy of activation.
n = order of reaction.

The plot between the terms [Δ log (dw/dt)] / Δ log Wr Vs Δ (1/T) / Δ log Wr gives a straight line from which slope we obtained energy of activation (*Ea) and intercept on Y-axis as order of* reaction (*n). The change in entropy (S), frequency factor (z), apparent entropy (S*) can also be* calculated by further calculations

Table. 1: Results of Thermogravimetric Analysis of p-HBTF copolymer

Coplymers	Half Decomposition Temp.T*K	Activation Energy (kJ/mol)	
		F.C	S.W
p-HBTF-I	673	22.97	22.78

Table.2: Kinetic Parameters of p-HBTF copolymer

Coplymers	Entropy Change -ΔS(J)	*Free Energy Change ΔF (kJ)*	*Frequency factor Z (S_{-1})*	Apparent Entropy Change (S*)	Order of reaction (n)
p-HBTF-I	-299.96	204.17	636	-23.82	0.95

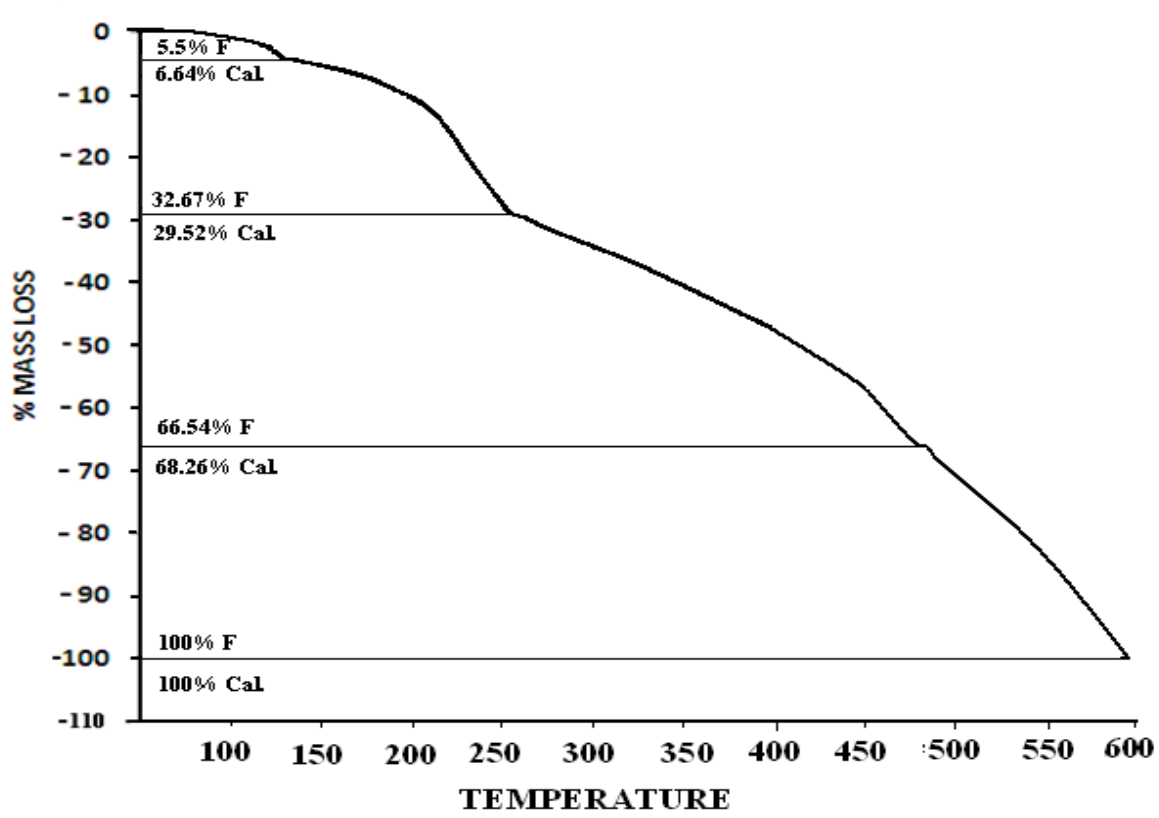

Fig. 4: Decomposition Pattern of p-HBTF copolymer Resin.

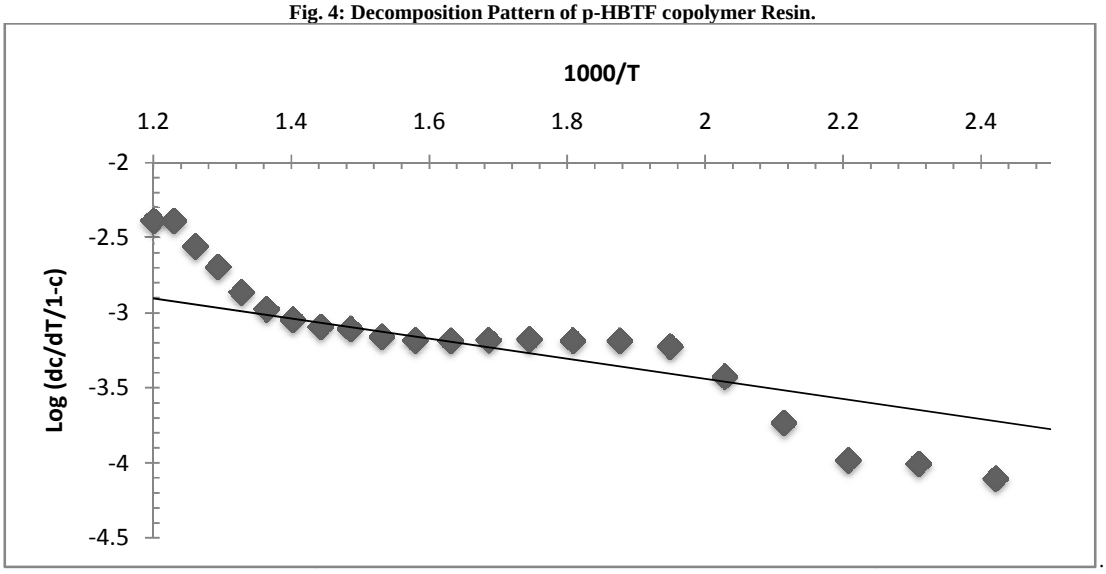

Fig. 5: Sharp-Wentworth plot of p-HBTF copolymer

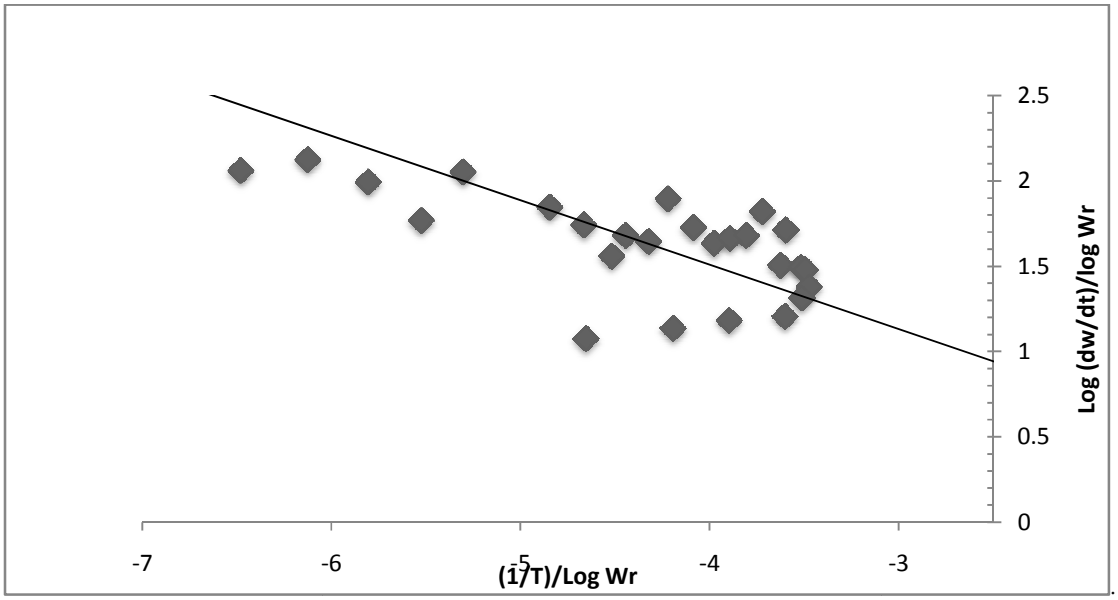

Fig. 6: Thermal activation energy plot of p-HBTF copolymer

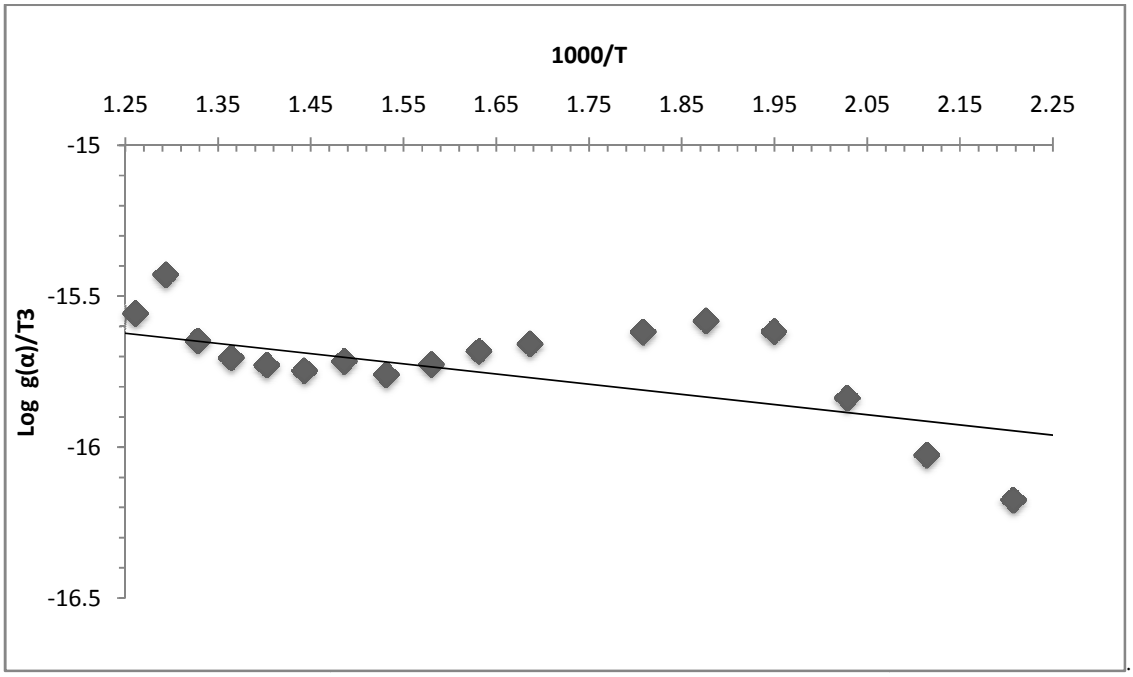

Fig. 7: Freeman-Carroll plot of p-HBTF copolymer

Antimicrobial Screening

Biological assay depends upon a comparison of the inhibition of growth of microorganism by measuring the concentration of the sample to be examined with the known concentration of standard antibiotic. For the antimicrobial analysis of p-HBTF copolymer the agar diffusion method was employed. During the course of time, the test solution diffuses and the growth of the inoculated microorganisms such as *B. subtilis* , E. coli, and S. typhi were found to be affected. The activity developed on the plate was measured by measuring the diameter of the inhibited zone in millimetres. The drug gentamycin was used as the standard for bacteria.

In the present work, p-HBTF copolymer were tested at different concentration to test their efficacy in inhibiting the growth of the human pathogens. The bacterial activity of p-HBTF was assayed against B. Subtilis, E. Coli , S. Typhi . The diameters for the zone of inhibitions at different concentration against the test bacteria are given in Table 3. The standard antibiotic disc (Gentamycine disc 5µg/disc) inhibited the growth of B. Subtilis by 8-25 mm E. Coli by 18-25 mm, and S. Typhi by 2-25 mm.

Table 3. Antimicrobial activities of p-HBTF copolymer resin

Organism	0.0625 mg	0.125 mg	0.25 mg	0.5 mg	1.0 mg	2.0 mg	MIC mg
B. subtilis	-	-	-	-	-	6mm	2mm
E. coli (ETEC)	-	-	-	-	-	7mm	2mm
S.typhi	-	-	-	-	-	5mm	2mm

Fig.8. Antibacterial activity of p-HBTF copolymer

B. subtilis ***S. typhi*** ***E. coli (ETEC)***

Table.4. Antibacterial activity of standard antibiotic (Gentamycin)

Organism	25 µg	50µg	100µg	200µg	400µg	800µg	MIC µg
B. subtilis	8	10	15	19	22	25	25
E. coli (ETEC)	18	20	23	26	28	31	25
S. aureus	13	18	21	25	27	34	25
S. typhi	2	13	16	21	25	27	25

CONCLUSION

A copolymer, p-HBTF based on the condensation reaction of p-hydroxybenzoic acid thiosemicarbazide formaldehyde in the presence of acid catalyst was prepared. Low values of collision frequency factor (Z) may be concluded that the decomposition reaction of p-hydroxybenzoic acid, thiosemicarbazide , formaldehyde copolymer can be classified as 'slow reaction'. The decomposition reaction was started at higher temperature, indicating a copolymer p-HBTF is thermally stable at higher temperature. The results of present antimicrobial assay revealed that the p-HBTF copolymer showed inhibitory activity against all the tested pathogens, suggesting that the presence of thiosemicarbazide group may enhances antibacterial activity. As the p-HBTF content increases in the copolymer, the effectiveness of the copolymers to inhibit the growth of microorganism increases as expected.

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