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Synthesis and characterization of antimicrobial hyperbranched polyesters

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ABSTRACT

Hyperbranched polyesters are a group of materials within the family of dendritic polymers (dendrimers and hyperbranched polymers) that have enjoyed increased attention in recent years. Hyperbranched polymers have a large number of end groups, which allows them to be tailored for different applications. Hyperbranched, aromatic polyester of third generation was synthesized in the molten state from 2,2-bis(hydroxymethyl)propionic acid (repeating unit of AB_x type) and Phloroglucinol as core molecule using acid catalysis. Endcapping reactions were performed using selected heterocyclic phosphonyl dichlorides (example: indole and imidazole) to form an antimicrobial polymers (IN-HBPE and IM-HBPE). Formations of the compounds were identified by different spectral studies viz., FT-IR, ^{1}H NMR and ^{13}C NMR . ^{13}C NMR provides information about degree of branching of these polymers. Biological studies reveal that IN-HBPE and IM-HBPE has small activity than that of HBPE.

KEYWORDS

Hyperbranched polyester;
Heteroaromatics;
Spectral studies;
Biological Studies.

INTRODUCTION

Dentritic macromolecules are attractive scaffolds due to their well defined and unique macromolecular structure. These applications extended in various fields like catalysis, medicinal chemistry, magnetic resonance imaging, combinatorial chemistry, light harvesting, emission and amplification function[1-4]. Like dendrimers, hyperbranched polymers are built from AB_x type monomers. Because of the similarity in branching, hyperbranched polymers and dendrimers have many common features such as improved solubility compared to that of linear polymer.[5-8] Since, the pioneering work are well defined by Vogtle[9], Tomalia[10,11] and Newkome[12] in dendrimers and hyperbranched polymers has been increasing at an amazing rate.

The properties of hyperbranched polymers are indeed strongly determined by the nature of their terminal groups[13-16]. For example, solubility, which is mainly depends on end group structure, may be regulated by the partial or total chemical modification of the ''terminal'' groups[17]. Due to the irregularities of hyperbranched polymers in their molecular architecture, these functional groups can be located not only in the periphery, as in perfect dendrimers, but also inside the globular branched structure; even the terminal functional groups of dendritic macromolecules can fold back to the inside[18]. Nevertheless, several authors could show that nearly

all functional groups were accessible for small reagents[19,20], which easily allows a quite total modification of functionalized hyperbranched polymers.

Microbial infection remains one of the most serious complications in several areas, particularly in medical devices, drugs, health care and hygienic applications, water purification systems, hospital and dental surgery equipment, textiles, food packaging, and food storage. Antimicrobials gain interest from both academic and industrial research due to their potential to provide quality as well as safety benefits to many materials. However, low molecular weight antimicrobial agents suffer from many disadvantages, such as toxicity to the environment and short-term antimicrobial ability. Antimicrobial functional groups and heterocyclic agents can be introduced into polymer molecules may compensate to the above problem. The use of antimicrobial polymers offer promise for minimizing the environmental problems, residual toxicity of the agents, increasing their efficiency and selectivity, and prolonging the lifetime of the antimicrobial agents[21].

The synthesis of polymer which contains phosphorous and heteroaromatics in the terminal chain attracts the attentions of many researchers due to their peculiar characteristics viz., non-flammability, thermal stability, high melting points, appreciable biological activities[22-24]. Among the nitrogen containing heterocyclic compounds, six membered heterocyclic compounds are used in various applications as herbicides, pharmaceuticals and adhesives. Five membered heterocyclic compounds are used in electrical and pharmaceuticals applications[25]. Functional group modification of HBPE or introducing functional groups into the third generation of hyperbranched polyester has been believed to have basic significance with expanding its applications. Present investigation was devoted for partial endcapping of phosphorous containing N-heterocyclic compounds like indole, imidazole onto hyperbranched polyester matrix offers vast openings in many fields of applications.

EXPERIMENTAL

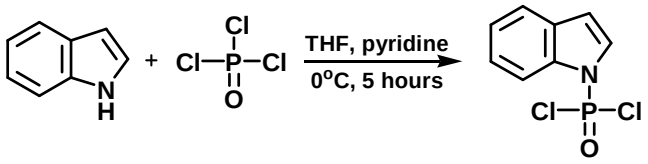
Materials and methods

Indole, imidazole, 2,2-Bis(hydroxymethyl) Propanoic acid (Bis-MPA) were purchased from Sigma-Aldrich. Phosphorus oxychloride, phloroglucinol, tetrahydrofuran (THF), dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO), acetone were purchased from sd-fine chemicals, Chennai. Thermonicolet-330 and JEOL model GSX400 instruments were used to record FT-IR and NMR studies respectively. Elementat Vario EL III Carlo Erba 1108 instrument was used for elemental analysis. Biological studies were made using agar well diffusion method. All the solvents and monomers were purified as per standard procedures prior to use.

Synthesis of 1H-indole-1-phosphonyl dichloride and 1H-imidazole-1-phosphonyl dichloride

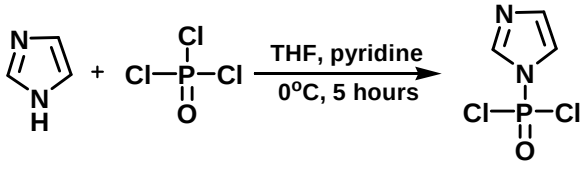
IN-PD and IM-PD was prepared as per our earlier report[26]. 1 mmol of purified indole (0.117 g) and 2 mmol of phosphorous oxychloride (0.153 g) were dissolved in dry THF added slowly with constant stirring for 15 minutes at 0^0C under nitrogen atmosphere in the presence of pyridine as catalyst in three necked round bottomed flask. The progress of the reaction was monitored by means of TLC. After completion of the reaction pyridine hydrochloride was filtered and evaporated the solvent to get 1H-indole-1-phosphonyl dichloride (IN-PD), (Scheme 1a). FT-IR (KBr): 1239.17 (P=O); 1008.77 (N-P); 484.67 (P-Cl) cm^{-1}; ^{1}H NMR ($CHCl_3$): 7.2-7.7 ppm (aromatic protons).

Similar procedure was adopted for the synthesis of 1H-Imidazole -1-phosphonyl dichloride (IM-PD) (Scheme 1b). FT-IR (KBr): 1250.21 (P=O); 1017.53 (N-P); 501.68 (P-Cl). ^{1}HNMR ($CHCl_3$): 7.3, 7.4, 7.8 ppm (aromatic protons).



Indole Phosphorus oxychloride IN-PD

Reaction Scheme 1a



Imidazole Phosphorus oxychloride IM-PD

Reaction Scheme 1b

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Synthesis of third generation (G_3) of hyperbranched polyester (HBPE)

The monomer (Bis-MPA), the core (phloroglucinol) and the catalyst (p-TSA) of different stoicometric ratios has been calculated as per our earlier finding for the formation of different generation viz., first (G_1) and second (G_2) and third(G_3)[27]. The third generation has been selected for further studies. This may be due the abundance of OH group for convenient end capping. The product description for HBPE and endcapped product was given in TABLE 1.

TABLE 1 : Product description

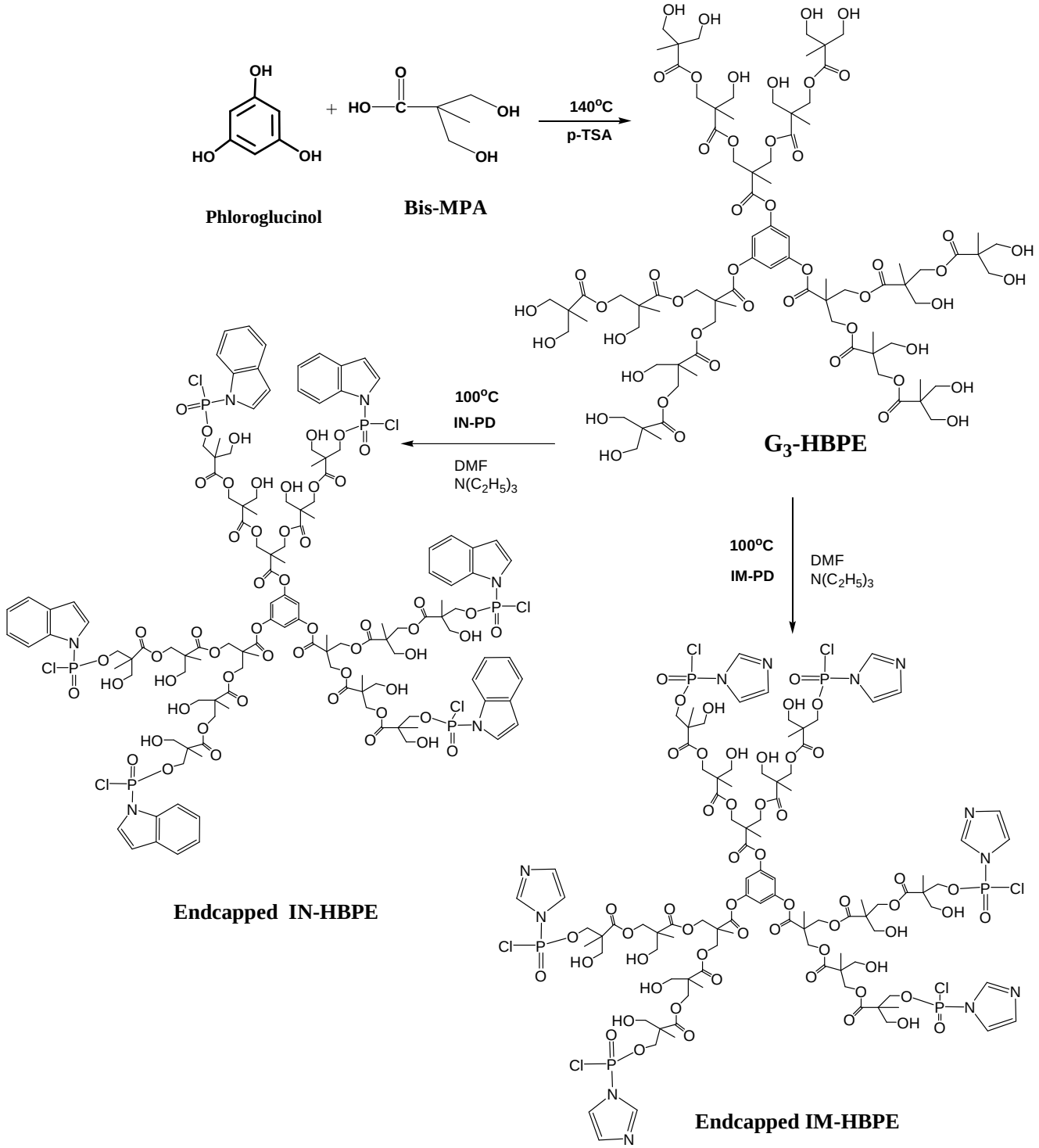
	HBPE	IN-PD	IN-HBPE	IM-PD	IM-HBPE
Yield(%)	85	88	85	88	80
Solvent	Acetone DMSO	DMF DMSO	Acetone DMSO	DMF DMSO	DMSO
Color	Red	Crimson Red	Crimson Red	Dark red	Light pink

Bis–MPA (28.16 g, 0.21 mol), phloroglucinol (1.26g, 0.01 mol) and p-TSA (0.020 g, 0.18mmol) were mixed in a three-necked round bottom flask equipped with a nitrogen inlet and a drying tube. The RB flask was placed in an oil bath at 140°C with constant stirring for 10 hours under nitrogen atmosphere in order to remove the water molecules formed from the reaction mixture. The reaction was monitored by TLC and the formation of the red waxy product was purified by using column chromatography (Silica gel with petroleum ether/ethyl acetate as the gradient eluent). FT-IR (KBr): 1735.57 (C=O of ester); 3433.62 (OH); 2979.13 (aromatic C-H) (cm^{-1}); ^{13}C NMR (DMSO-d_6) d (ppm): 47.52-51.49 (quaternary carbon); 39.1-41.5 and 65.04, 66.35 (methylene carbon); 157-161.6 (phenyl); 18.1 (methyl carbon); 174.42, 175.68, 176.71 (ester carbonyl). Anal.Calcd for $C_{81}H_{126}O_{48}$: C, 52.09%; H, 6.8%; Found; C, 51.26%; H, 6.12%;

Synthesis of 1H-indole-1-phosphonyl dichloride end-capped HBPE (IN-HBPE)

1H-indole-1-phosphonyl dichloride (4.6g, 10 mmol) and HBPE (0.474 g, 1 mmol) were dissolved in dry DMF (50 ml) and stirred for 26 hours at 100°C in the presence of triethylamine (TEA) as acceptor of HCl. As the reaction proceeds, TEA hydrochloride precipitates from the reaction medium and its quantity corresponds to consume IN-PD. A precipitate of triethylamine hydrochloride was filtered off, and the solvent DMF was completely removed by vacuum distillation. Crimson red waxy IN-HBPE was obtained and dried in vacuum oven at 60°C. The final product was purified by column chromatography (Silica gel with petroleum ether/ethanol as the gradient eluent). The scheme of the reaction displayed in Scheme 2. FT-IR (KBr): 1260.11 (P=O), 1021.50 (N-P), 1734.94 (ester carbonyl), 3404.71 (O-H), 2966.27(aromatic, C-H), 1163.03 (P-O-C), 494.61(P-Cl) ^1H NMR (DMSO- d_6): 7.108, 7.128, 7.473, 7.493, 7.938, 8.925 ppm (aromatic protons), 3.424, 3.645, 3.794, 3.845, 4.096 ppm (methylene protons), 1.690, 1.209, 1.053 ppm (methyl protons). ^{13}C NMR (DMSO-d_6): 173, 174, 175 ppm (C=O, ester), 46.5-50.5 ppm (quaternary carbon), 39.1-40.3 and 64.09-64.24 ppm (methylene), 17.1, 17.2, 17.4 ppm (Methyl carbon).Anal. Calcd. for $C_{122}H_{156}O_{54}N_6Cl_6P_6$: C, 50.75%; H, 5.15%; N,2.75% Found; C, 50.45%; H, 5.23%; N, 2.59%.

Similar procedure was adopted for the synthesis of 1H-imidazole-1-phosphonyl dichloride endcapped HBPE (IM-HBPE) shown in scheme 2. The light pink waxy polymer was obtained and purified by using column chromatography (Silica gel with petroleum ether/ethanol as the gradient eluent). FT-IR (KBr): 1210.11 cm^{-1} (P=O), 1008.88 cm^{-1} (N-P), 1732.52 cm^{-1} (C=O of ester), 3417.38 cm^{-1} (O-H), 2961.66 cm^{-1} (aromatic, C-H), 1162.07 cm^{-1} (P-O-C). 503.33 (P-Cl) ^1H NMR (DMSO): 7.108, 7.128, 7.478, 7.498, 7.926, 8.955 ppm (aromatic protons), 3.626, 3.774, 3.812, 4.084 ppm (methylene protons), 1.752, 1.675, 1.140 ppm (methyl protons). ^{13}C NMR (DMSO-d_6): 135, 137, 138 ppm (C=O, ester), 45.5-50 ppm (quaternary carbon), 38.8-40.5 and 63.82- 64.7 ppm (methylene), 17.09 ppm (Methyl carbon).Anal.Calcd for $C_{99}H_{138}O_{54}N_{12}Cl_6P_5$: C, 43.10%; H, 5.04%; N, 6.09%. Found; C, 42.76%; H, 5.58%; N, 6.75%.



Reaction Scheme 2

BIOLOGICAL STUDIES

Source of microorganism

S. Paratyphi, *Bacillus subtilis* (ATCC 11778), *Kelbsilla*, *Escherichia coli (E.coli)* (ATCC 10412), *P. Vagaris*, S*taphylococcus aureus (S.aureus)* (ATCC 700699), were used as micro organisms for the present investigation.

Preparation of innoculum

The innoculum was prepared by innoculating a loop

of each test organism for 24 hours culture into a sterile nutrient broth and incubated at 37°C for 3 Hrs, till an optical density value of 0.3 was reached in polarimeter.

Disc diffusion method

The medium was sterilized by autoclaving at 121°C for 15 min, cooled to 45°C and then poured in 20 ml quantity of petri dishes. A loopful of overnight broth culture was spread evenly over whole plate with sterile cotton wool swab. The culture plates were dried in an incubator with the lid until its surface was free from visible moisture. Subsequently 5 mm diameter sterile discs (made from whatmann filter paper sterilized in UV lamp) are dipped in DMSO dissolved 1 ppm concentration of IN-HBPE and IM-HBPE, standard (penicillin) and control (DMSO) were placed on the surface of agar plates. The plates were left for one hour at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions of modified polymers. The plates were incubated at 37° C for 24 hours and observed for antibacterial activity. The diameter of the zones of inhibition was measured for the plates in which the zone of inhibition was observed. The average area of zone of inhibition was compared with that of standard.

RESULTS AND DISCUSSION

Synthesis of third generation HBPE

The synthetic procedure for various polymers outlined in scheme (1-2). Phosphorous containing indole and imidazole was first synthesized by condensation of indole or imidazole with $POCl_3$ at a molar ratio of 1:2 using pyridine as a catalyst. The mechanism of the formation of the product was described in our earlier findings[26]. Then third generation of aromatic HBPE can be prepared by using Phloroglucinol and Bis-MPA at a molar ratio of 1:21 using p-TSA as a catalyst. The chemical structure of HBPE was characterized by FT-IR, ^{13}C NMR and elemental analysis. From FT-IR, the strong absorption of ester C=O formed at 1735 cm^{-1} and disappears the acid C=O in Bis-MPA confirms formation HBPE. The FT-IR spectrum also shows a strong absorption peak at around 3433 cm^{-1} corresponding to the O-H stretching. Figure 1 expressed the ^{13}C NMR, the signals at 47.5-51.4 ppm indicates the existence of quaternary carbon. Because of methylene carbon attached with dendritic carbons and free OH, it appears in 39.1- 41.5 and 65.1-66.3 ppm region. Three carbonyl carbons formed at 174, 175 and 176 ppm used to identify the degree of branching for HBPE. The elemental analysis data found C, 51.26%; H, 6.12%; are in good agreement with that C, 52.09%; H, 6.8%; calculated for $C_{81}H_{126}O_{48}$: From this we have calculated the approximate theoretical molecular weight found from elemental analysis is 1867 g.

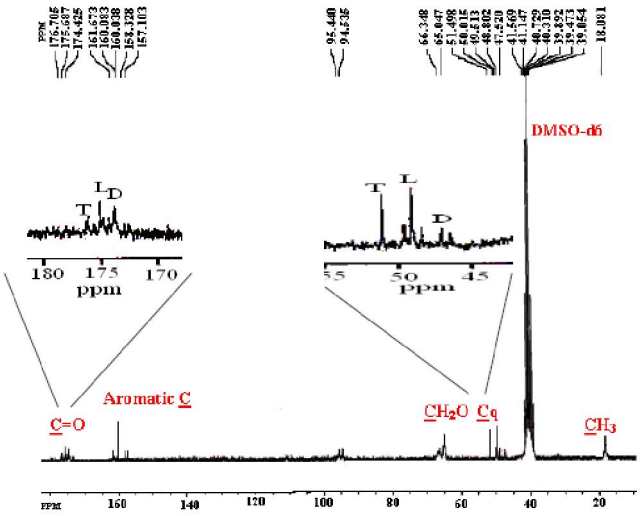


Figure 1 : ^{13}C-NMR of HBPE

Synthesis of IN-HBPE and IM-HBPE

End capping of HBPE was achieved by reacting of IN-PD and IM-PD with HBPE was shown in scheme 2. The formation of the end-capped products was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy. FT-IR spectrum reveals the strong absorption around 1260 cm^{-1} corresponds to the vibration with P=O group, P-O-C bond formed at 1163 cm^{-1} and C=O appears at 1734 cm^{-1}. Formation of P-O-C and existence of P-Cl bonds and OH bonds in IN-HBPE an IM-HBPE indicates partial endcapping was achieved. From the ^{1}H NMR spectrum, the signals at 7.108-8.925 ppm indicate the presence of aromatic protons. Methylene protons were appeared at 3.424- 4.096 ppm. Methyl protons appear in 1.053-1.690 ppm for IN-HBPE and 1.140-1.752 for IM-HBPE. OH protons observed in 2.279 ppm for IN-HBPE and 2.273 for IM-HBPE

shows partial endcapping was achieved with HBPE. The detailed assignment for ^{13}C NMR given in Figure 2-3. From this study, Tertiary carbon peak appears in the range of 45.5-50 ppm. Methyl carbon signal was observed in 17.1-17.2 ppm and methylene carbon showed in two regions as 39.1- 40.3 and 63.8-64.7 ppm. This range of signals also proves IN-PD and IM-PD functionalize partially with OH containing HBPE. Formation of three carbonyl carbons at 173,174 and 175 ppm confirms the tilting three different direction of branching of HBPE with IN-PD compounds. Due to the presence of electronegativity in imidazole moiety (contains two nitrogen present in the ring) for IM-HBPE, the ester carbonyl (C=O) peak was shifted to down field and therefore, three carbonyl carbons formed at 138, 140 and 144 ppm respectively, confirms branching of HBPE in IM-PD compounds. Similar to our conclusions, Yuxia kou etal., reported for this system[28]. Percentage of carbon, hydrogen and Nitrogen present in the endcapped polymers are shown in experimental section. From the results of the elemental analysis it is evident that the results of the chemical analysis are in good agreement with the calculated values of the respective polymers, i.e., on the basis of the empirical formula ($C_{122}H_{156}O_{54}N_6Cl_6P_6$), ($C_{99}H_{138}O_{54}N_{12}Cl_6P_5$) for the polymer IN-HBPE and IM-HBPE. On analyzing the reason for partial endcapping, OH groups in HBPE are not equal-reactive, and more precisely that terminal type hydroxyls are more reactive towards IN-PD and IM-PD than linear type hydroxyls. This has been found in the literature in terms of a screening effect that would be most important for inner linear hydroxyls[29-30].

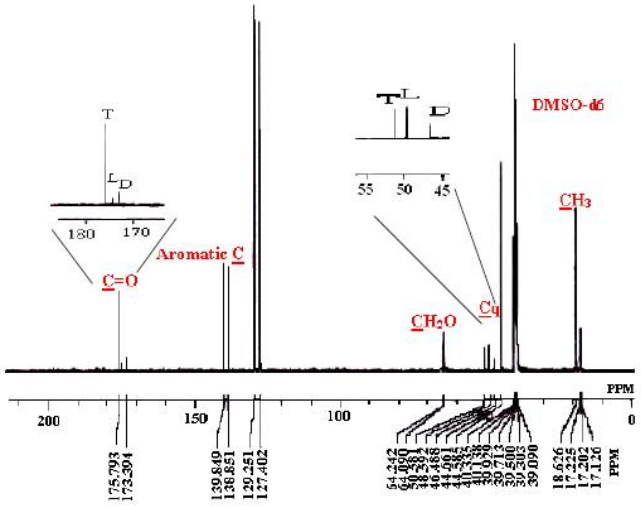


Figure 2: ^{13}C-NMR of IN-HBPE

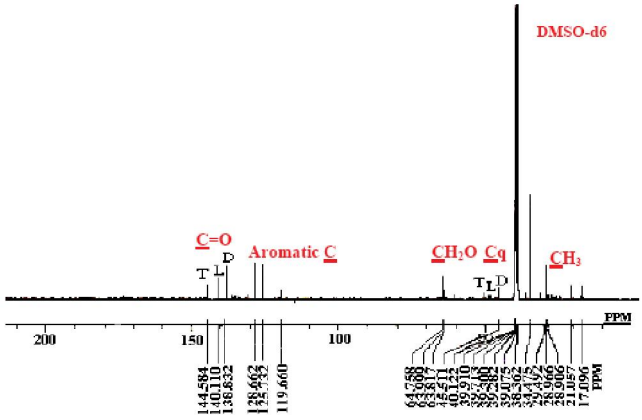


Figure 3: ^{13}C-NMR of IM- HBPE

DEGREE OF BRANCHING

The structural perfection of the hyperbranched polymers is usually characterized by the determination of their degree of branching (DB), which was determined either by Fretchet's equation[31]

DB (Fretchet) = (D +T) / (D+ T+L)

or Frey

DB (Frey) = 2D / 2D+L

Where D, T and L refer to the number of dentritic, terminal and linear units in the structure of the polymer, respectively. The integration of the peaks for the respective units dentritic (D), linear (L) and terminal (T) in the polymer can be distinguished by CH_3 protons signals in ^1H NMR and ^{13}C NMR using either the carbonyl peaks (C=O) [171–175 ppm], or that of the quaternary carbons(cq), [45–51 ppm]. These three particular areas of the NMR spectra are presented in Figure 1-3 and summarized in TABLE 2. The main difference lies in the fact that DB (Frechet) takes into account the linear propagation as a branching direction and thus overestimates DB for small molecules, whereas both definitions merge for higher molar masses. For C=O signals, the average values of $DB_{Frechet}$ ~ 0.55 (DB_{Frey} ~ 0.54) for HBPE was observed in our present investigation. After endcapping of HBPE with IN-PD and IM-PD, the DB was increased (shown in TABLE 2) and hence, DB for all polymer exhibits near to

hyperbranched structure rather than linear and dendrimer (According to the literature[32], DB closes to zero for linear, less than 1 for hyperbranched and 1.0 for dendrimer).

TABLE 2 : ^{13}C NMR and ^{1}H NMR analysis of HBPE and endcapped HBPE

Product	Signals	D		L		T		DB Frechet	DB Frey
		ppm	Unit	ppm	Unit	ppm	Unit		
HBPE	C=O(^{13}C)	174.4	0.165	175.6	0.280	176.7	0.18	0.55	0.541
	Cq (^{13}C)	47.5	0.195	49.5	0.4	51.4	0.525	0.64	0.49
IN-HBPE	C=O(^{13}C)	173.4	0.33	174	0.14	175	1.845	0.93	0.825
	Cq(^{13}C)	46.5	1.2	48.5	1.995	50.5	1.47	0.57`	0.54
	CH_3(^{1}H)	1.690	1.18	1.209	0.68	1.053	2.71	0.85	0.77
IM-HBPE	C=O(^{13}C)	138.8	0.42	140.1	0.255	144.5	0.28	0.80	0.76
	Cq(^{13}C)	45.5	0.13	48.8	0.135	50	0.18	0.69	0.65
	CH_3(^{1}H)	1.752	1.2	1.675	0.7	1.140	2.88	0.85	0.72

(DB closes to zero for linear, less than 1 for hyperbranched and 1.0 for dendrimer).

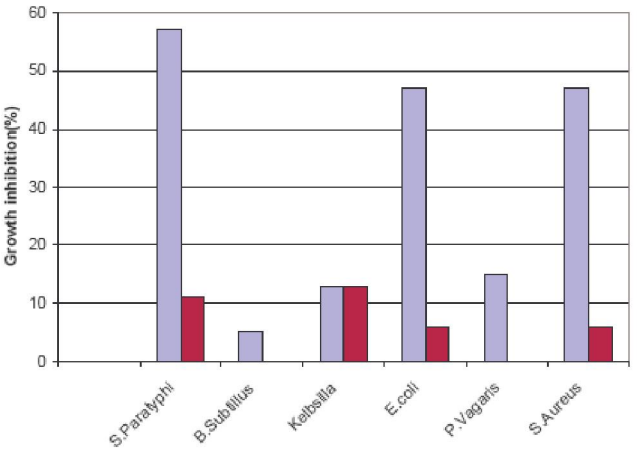
ANTIMICROBIAL ACTIVITY

The polyesters were tested for their antibacterial activity by disc-diffusion method using agar-agar gel medium. *S. Paratyphi, B.Subtillus, Kelbsilla, E.Coli, P. Vagaris and S. Aureus* were used as microorganism. TABLE 3 and Figure 4 indicated the bacterial studies of parent heterocycles and heterocyclic functionalized hyperbranched polyester respectively. This shows clearly that growth inhibition zones of indole and imidazole was higher than indole and imidazole functionalized hyperbranched polyesters. In general, hyperbranched polyesters are not bearing any antibacterial activity. On inclusion of indole and imidazole moiety onto HBPE by endcapping, the activity was slightly improved.

Our early reports reveal that incorporation of N-heterocyclic compound onto the polymer matrix enhances the activity for linear polymers. However, *E.coli*, and *B.Subtillus* has zero activity after incorporating parent indole and imidazole[26]. But present studies on hyperbranched polyester exhibits small activity on the addition of parent indole and imidazole. Hence, N-heterocyclic based hyperbranched polyester exhibits small activity changes than N-heterocyclic based linear polymer. Based on the obtained results from biological studies, the order of antibacterial activity was given as Indole > Imidazole > IN-HBPE >IM-HBPE. It was found that the endcapped HBPE has ability to inhibit the reproduction of gram positive and gram negative bacteria.

TABLE 3 : Antimicrobial activity data of heterocyclic compound and its polyesters

Name of the Organism	Zone of inhibition (mm)				
	Indole	Imidazole	In-HBPE	IM-HBPE	Standard Penicillin
S.Paratyphi	2.4	0.8	1.1	0.2	1.9
B.Subtillus	1.4	-	0.2	-	3.9
Kelbsilla	1.7	0.4	0.2	0.2	1.6
E.coli	2.0	1.6	0.9	0.1	1.9
P.Vagaris	1.8	0.8	0.2	-	1.4
S.Aureus	1.6	-	0.9	0.1	1.9



Bacteria growth inhibition by IN-HBPE ( ) and IM-HBPE ( )

Figure 4 : Bacterial growth inhibition

CONCLUSIONS

The following points summarize the conclusions of the present investigation:

1. Third generation of HBPE was synthesized by melt condensation method.
2. Endcapping of IN-HBPE, IM-HBPE was synthesized by using IN-PD and IM-PD with HBPE.
3. Formation of the compounds were confirmed by using, FT-IR, ^{1}H NMR and ^{13}C NMR studies, respectively.
4. Degree of branching confirms the formation of hyperbranched polyesters.
5. Compare to linear polymers, hyperbranched polymers holds better bacterial activity after the addition of heterocyclic compounds.
6. IN-HBPE showing improved antibacterial response compare to IM-HBPE specifically for the microorganisms like *S. Paratyphi, E.coli* and *S.Aureus*

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