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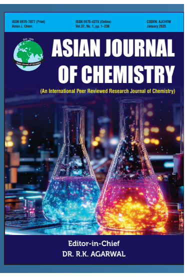

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Exploring the Antimicrobial and Anticancer Activities of Novel Copolyester Derived from Isophthaloyl Chloride and 4,4′-Biphenol

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The copolyester PBHI was prepared from isophthaloyl chloride, 4,4′-biphenol and 2,5-hexanediol by means of solution polycondensation procedure. The solubility and inherent viscosity were measured for the synthesized polyester. The structure, composition and distribution of the repeat sequence in the polyester was ascertained by means of UV-visible, infrared and ^{1}H NMR spectroscopy. The thermal stability and energy of activation for decomposition were probed by thermogravimetric analysis (TGA) and the transition temperatures in the melting of the polymer were scrutinized using differential scanning calorimetry (DSC) and the thermograms revealed a relatively higher melting temperature (T_m) and also displayed a two stage decomposition in the copolyester prepared. Wide angle X-ray diffraction (WAXD) and scanning electron microscopy (SEM) techniques were utilized to investigate the crystallinity and the morphology of the surface in the copolyester. The antimicrobial studies using well-diffusion method and *in vitro* cytotoxicity study using MTT method against HeLa cell line were carried out.

Keywords: 4,4′-Biphenol, Isophthaloyl chloride, Isophthalate, *In vitro* cytotoxicity.

INTRODUCTION

Renewable sources and biodegradable materials are imperative in reducing carbon footprint and are promptly becoming a benchmark which is being executed globally. Many researchers have thoroughly explored the field of bioplastics and have pinpointed the advantage of these macromolecules which are analogous in performance and properties compared to conventional polymeric materials [1,2]. Isophthalic acid and its derivatives have been documented as potential sources which can be obtained *via* cycloaddition reaction involving bioisoprene moieties with bioacrylic acid units and also by fermentation of biomass [3]. Recently, Quattrosoldi *et al.* [4] have reported a novel polycondensation procedure involving a bio-based glycol (1,4-butanediol) form which it is possible to create a homopolymer, specifically poly(butylene isophthalate), using only biobased materials. In the last decade, special emphasis has been devoted by researchers focusing on synthesizing diverse organic-based unsaturated polyesters derived from itaconic acid along with several aliphatic diols like glycerol, 1,4-butanediol and 1,6-hexandiol utilized as UV-curable coatings which exhibit superior solvent resistance coupled with thermal stability [5]. Recent literature on hexanediol based commercial unsaturated polyesters utilizing methacrylate derivatives as reactive diluents has triggered interest in the use of novel acid derivatives to prepare unique polyesters [6].

Wang *et al.* [7] have reported some bio-based crystalline and amorphous polyesters comprising of 2,5-thiophenedicarboxylic acid and numerous aliphatic diols derived from neopentyl moiety, 1,6-dihydroxy hexane and 1,10-dihydroxydecane with weight-average molar mass (M_w) greater than 1.8 × 10^4 g mol^{-1}. As the number of methylene units in the main chain and methyl groups in the side chain increased, the value of glass transition temperature (T_g) also increased. It has been revealed in the literature that the comparison between polyesters based on isophthaloyl and terephthaloyl chlorides involving hindered biphenols and hydroquinones comprising of bulky substituents has divulged that notable solubility and lesser T_g

values are observed in polyisophthalates [8]. Influence of rigid of mesogenic diols like 4,4′-dihydroxy biphenyl in the mesophase formation of thermotropic liquid crystalline copolyesters blended with rigid and flexible spacers has been documented by Wale *et al.* [9]. Amorphous aromatic copolyesters comprising of biphenyl moieties prepared from 1-(4-biphenylyl)-1,1-*bis*(4-hydroxyphenyl)ethane and isophthaloyl dichloride were found to possess inherent viscosity values ranging from 0.44-1.26 dL/g [10]. The influence on the molecular weight and yield of diphenolic acid and isophthaloyl chloride based copolyesters prepared *via* a novel synthesis involving polymeric catalysts containing pendant quaternary ammonium units has also been investigated [11].

Extensive research in the field of novel copolyesters consisting of isophthalate and terephthalate moieties has revealed that the copolyester were less likely to be perfectly crystallizable with involvement of greater number of isophthalate units [12]. According to Paek & Im [13], copolyesters containing butylene carbonate amalgamated with isophthalate units exhibited improved thermal stability, superior optical qualities and enhanced gas barrier characteristics when dimethyl isophthalate groups were added. Katoh *et al.* [14] described in detail the utility of ester-ester exchange reaction and polycondensation procedures at elevated temperatures to produce copolyesters comprising of isophthalate and poly(ε-caprolactone) moieties . Contemporary overview of the biochemical attributes coupled with biotechnological utilization of enzymes capable of degrading well known copolyesters are also reported in the literature to kindle the interest in the field of biodegradability as a sustainable alternative for removal of plastic wastes [15]. To explore this dynamic field of polyesters, a novel semi-aromatic random copolyester was synthesized employing polycondensation methodology comprising of isophthalate moieties with 4,4′-biphenol and 2,5-hexanediol was included in the backbone to investigate the properties of the copolyester, which was characterized by well-established analytical methods.

EXPERIMENTAL

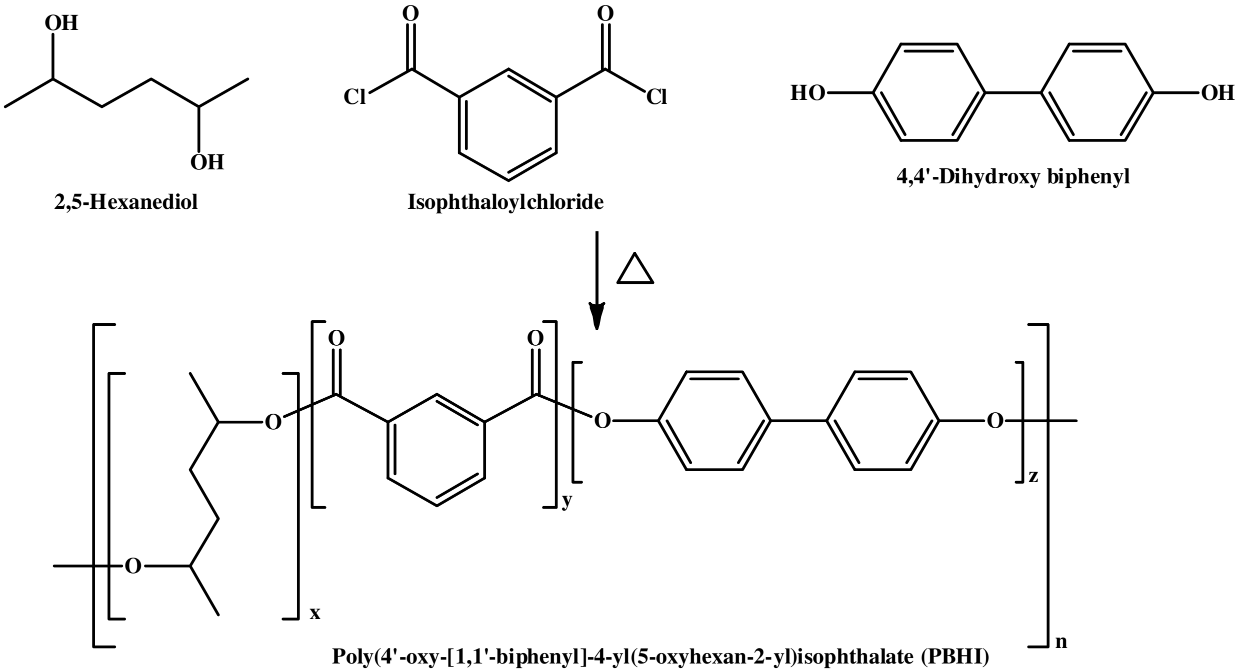
The organic solvents like acetone, chloroform, methanol, tetrahydrofuran, dimethyl formamide along with dimethyl sulfoxide were procured from Merck (Mumbai, India) and used as obtained. The chemicals such as 4,4′-biphenol and 2,5-hexanediol were purchased from Avra Synthesis Pvt. Ltd. Hyderabad and were used as received. The isophthaloyl chloride was obtained from Merck, Mumbai and was used as such. Analytical reagent grade 1,2-dichlorobenzene (Ranbaxy, India) was kept over anhydrous $CaCl_2$ for a day and filtered before use. The inherent viscosity value of given copolyester was measured in *o*-chlorophenol (0.1 g/dL) employing Ubbelohde viscometer at 30 ºC [16]. The UV-visible absorption spectrum of the polyester was investigated in acetone solvent in wavelength range from 200 to 800 nm on a UV-160A (Shimadzu) spectrophotometer. The characteristic functional groups in the polyester employing KBr pellet technique were confirmed from FT-IR spectrum (Alpha Spectrometer, Bruker) in the frequency range 4000-450 cm^{-1}. The ^1H NMR spectrum was recorded on a JEOL 500 spectrometer instrument at room temperature using deuterated chloroform [17]. Thermogravimetric analysis of around 15 mg sample of the copolyester warmed from 40 to 780 ºC was performed in a SDT Q600 thermal analyzer involving nitrogen environment with the heating rate around 20 ºC/min [18]. Differential scanning calorimetry using a simultaneous thermal analyzer NETZSCH STA 449 F3 Jupiter was also carried out under similar sample conditions. Wide angle X-ray scattering (WAXS) curve obtained emplying PANalytical X'Pert Pro multi-purpose X-ray diffractometer equipped with a Cu*K*α source 1.54 Å imaged in the 10º-80º (2θ) range. Surface morphology study was accomplished on a Tescan Vega 3 Microscope equipped with an accelerating voltage of 30 kV. Agar disc diffusion procedure on Sabouraud Dextrose agar and Mueller-Hinton Agar medium was carried out to ascertain the antibacterial efficacy of the copolyester. The impact of the copolyester sample on the growth of HeLa cell line (cervical carcinoma) was assessed using the MTT assay, which appraises cell proliferation.

Synthesis of random copolyester: The synthesis of aromatic polyesters of novel 4,4′-biphenol and aromatic diacid chlorides such as terephthalic acid chloride and isophthalic acid chloride adopting a unique solution polycondensation method [19]. In brief, into a 250 mL three-necked round bottom flask charged with 150 mL of 1,2-dichlorobenzene as solvent, equipped with a magnetic stirrer, a condenser, a N_2 gas inlet and a $CaCl_2$ guard tube were placed 2.436 mL of 2,5-hexanediol (0.02 mol), 1.862 g of 4,4′-biphenol (0.01 mol) and 6.09 g of isophthaloylchloride (0.03 mol). The polymerization process happened at 150 ºC for 45 h until the product become more viscous. The product mixture was transferred slowly into about 300 mL of petroleum spirit, cooled and left undisturbed for a prolonged period, often overnight to obtain copolyester. The unrefined polymer was further isolated by filtration, purified by washing with deionized water followed by dissolving into sufficient amount of acetone and dried over P_2O_5 under atmospheric temperature and a reduced pressure (**Scheme-I**). The obtained aromatic polyester containing 4,4′-biphenol moiety was labelled as PBHI with a yield of around 75%.

RESULTS AND DISCUSSION

Viscosity and solubility: The chemical properties and physical traits in a polymer is influenced strongly by functional groups present in the polymeric chain, composition and arrangement of the repeating structural units. The solubility of the prepared polyester was examined carefully in common organic solvents such as DMSO, DMF, THF, propanone, trichloromethane, methanol and *o*-chlorophenol. The copolyester found to be comparatively more insoluble in THF and methanol was relatively more soluble in the other solvents under similar conditions.

Viscosity measurement can be correlated to the molecular mass and degree of polymerization in macromolecules. The inherent viscosity value of the copolyester PBHI prepared in the current investigation was found to be 0.360 dL/g at 30 ºC on the basis of comparison between the flow times of polyester sample in solution and *o*-chlorophenol solvent.



Scheme-I: Synthetic route of copolyester PBHI derived from Derived from isophthaloyl chloride and 4,4′-biphenol

UV-visible studies: The UV-visible spectrum (Fig. 1) of copolyester PBHI was investigated in the 200-800 nm range and the absorbance showed a maximum in the wavelength range 290-310 nm, which is in accordance with the literature [18,20] and confirms the presence of a conjugated aromatic moieties in the recurring units in the polyester.

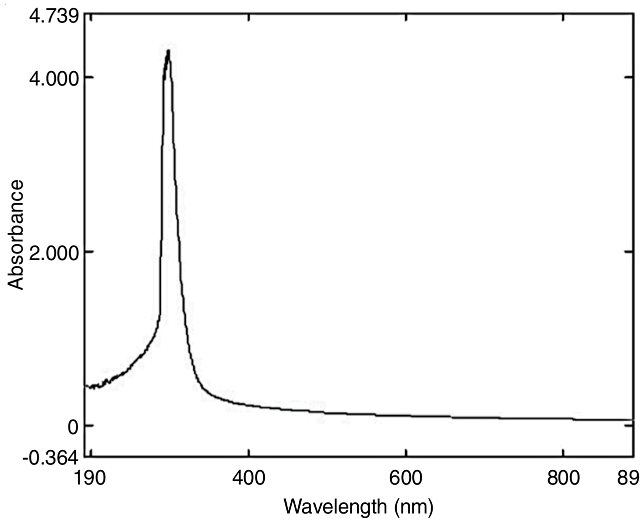


Fig. 1. UV-Visible spectrum of PBHI

FTIR studies: The vibrational frequencies of poly(4′-oxy-[1,1′-biphenyl]-4-yl(5-oxyhexan-2-yl)isophthalate (PBHI) displays a very intense band at 1745 to 1718 cm^{-1}, which is the characteristic absorption of C=O stretch in polyesters and two peaks at 2935 & 2976 cm^{-1} correlated with the C-H stretching modes, respectively (Fig. 2). The peak around 1205 cm^{-1} can be ascribed to the stretching vibration of the ester C–O group. These observations were confirmed from the reports of Zaidi *et al.* [21]. The absorption band at 3074 cm^{-1} was assigned to the aromatic nucleus in the copolyester while the characteristic absorption bands near 1205 cm^{-1} and at 1490 cm^{-1} were assigned to Ar–O–Ar stretching and aromatic ring (–Ar– stretching) in accordance with findings by Guan *et al.* [22]. The out of plane bending of the ring C-H bonds in the ring is appeared below 858 cm^{-1}, while C=C ring stretch and O-H stretching are indicated by bands at 1602 cm^{-1}, 1490 cm^{-1} and a broad band found around 3464 cm^{-1}, which is in accordance with outcomes by Sun *et al.* [23]. The FTIR results further confirmed the successful synthesis of copolyester macromolecule incorporated with the aliphatic and aromatic monomers used.

^{1}H NMR studies: Fig. 3 displays a resonance at δ = 1.3 to 2 ppm, which is attributed to methylene protons and the protons connected to oxygen are observed at δ = 5.3 to 5.5 ppm. The multiplet observed at δ = 6.6 to 9.5 ppm is indicative of aryl protons present in copolyester sample, which is accordance with the earlier research work [24,25].

Thermal studies: The thermogram of the prepared polyester PBHI indicates that the macromolecule undergoes two stage decomposition (Fig. 4), which may be due to the aromatic units present in the polyester [26]. The TGA plot is analogous to the previous findings involving polyesters comprising of isophthalate units [27]. In the first stage of decomposition completed before 400 ºC involves 66.45% weight loss and ensuing decomposition results in a weight loss of about 24.71% with 8.847% residue around 790 ºC.

Differential scanning calorimetry (DSC): The thermogram of the copolyester sample (Fig. 5) revealed that the T_g value less than 50 ºC, which is comparable to the observations on biphenyl polyesters [28]. The T_m value of the copolyester at 334.4 ºC matched with the reported values [29].

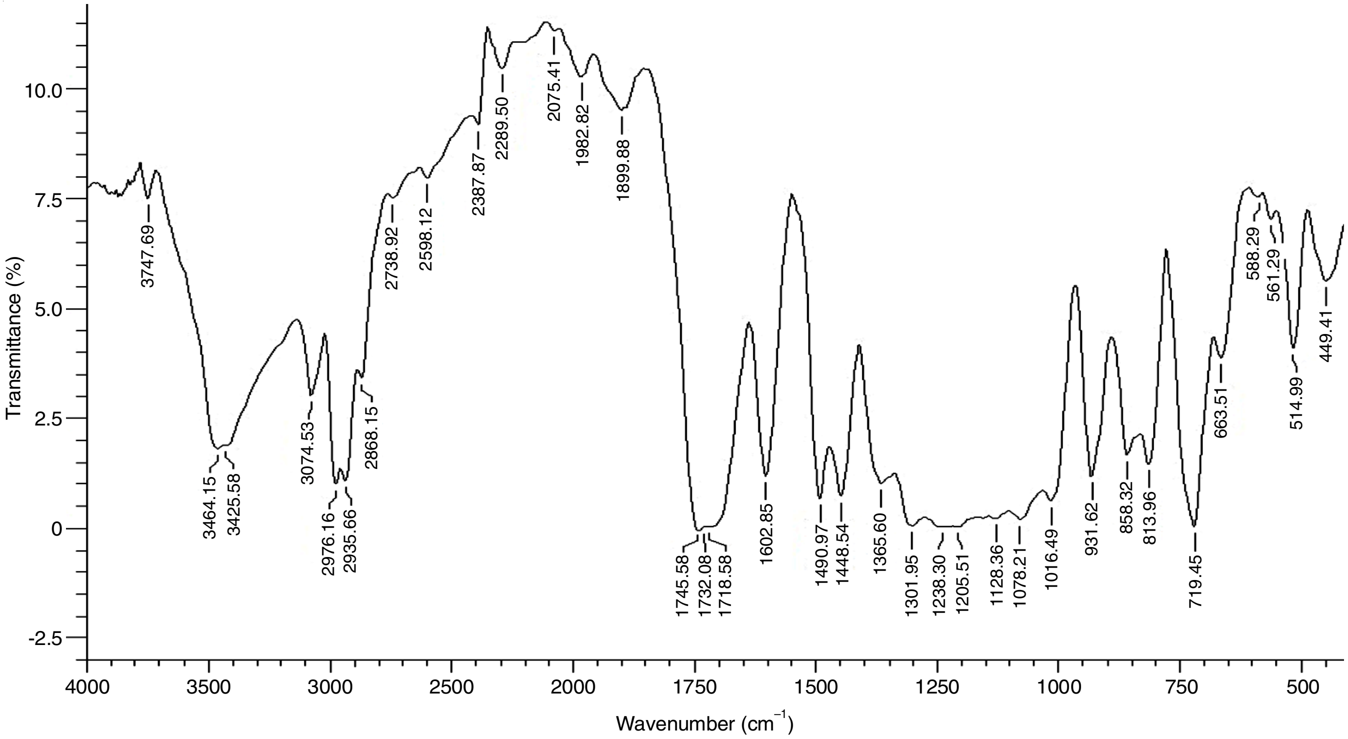


Fig. 2. FTIR spectrum of copolyester PBHI

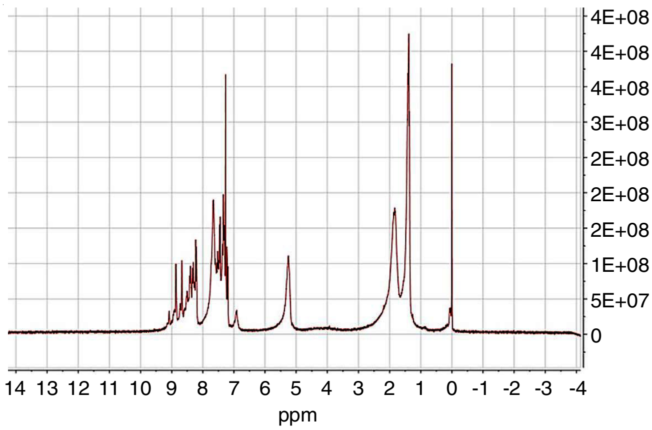


Fig. 3. ^{1}H spectrum of copolyester PBHI

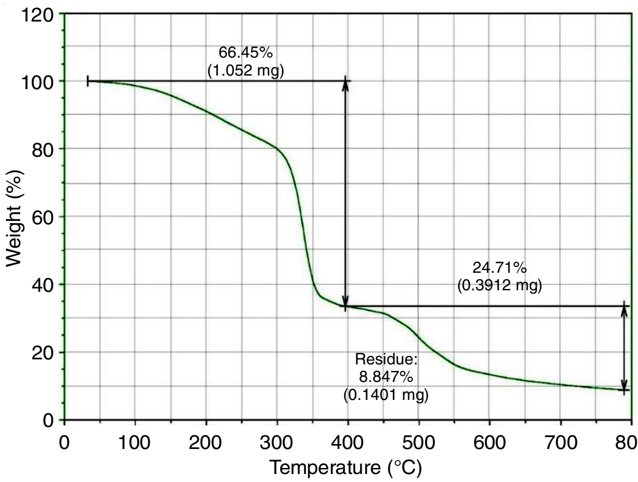


Fig. 4. TG thermogram of copolyester PBHI

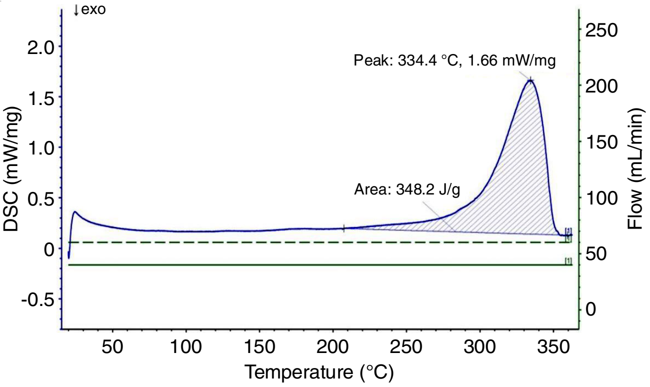


Fig. 5. DSC thermogram of copolyester PBHI

Wide angle X-ray scattering (WAXS): The crystalline nature of prepared copolyester (PBHI) can be analyzed by wide-angle X-ray scattering method. PBHI diffractogram revealed the two peaks at $2\theta = 19.27°$ and $23.82°$ (Fig. 6) with interplanar spacing ranging from 4.60518 to 3.73617 Å suggesting the amorphous nature combined with moderate degree of crystallinity when compared with earlier findings [30]. The XRD patterns are similar with a reported literature [31], which has revealed that a crystalline polyester is gesnerally fabricated by chain growth in the *para*-position compared to a chain growth involving *meta*-position as observed in polyisophthalates, which triggers disorder in the backbone of the macromolecule leading to an amorphous polymer showing a wide peak around 23º.

Scanning electron microscopy (SEM): Morphology and microstructure of the copolyester surface was investigated *via* scanning electron microscopic study. From the SEM micrographs of PBHI (Fig. 7), it is apparent that sizeable number of

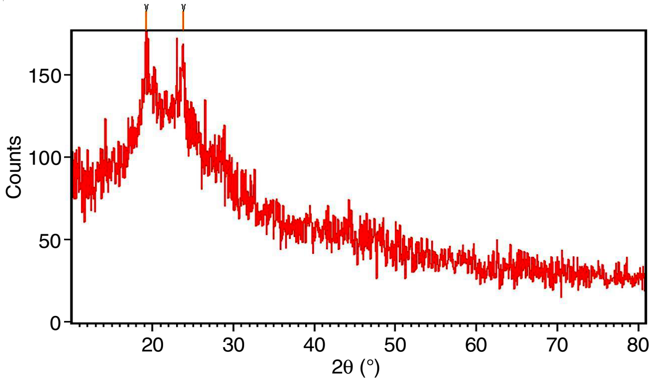


Fig. 6. X-ray diffraction of PBHI

distinct flaws are observed throughout the surface inclusive of granularity with irregular distribution of particles, which can be distinguished from each other. The surface also reveals the features including voids and cracks.

Antimicrobial activity: The well-diffusion method employing Mueller Hinton Agar (MHA) as the growth medium was conducted to evaluate the *in vitro* antimicrobial activity of the copolyester PBHI against *Salmonella typhi* (Gram-negative) and *Bacillus cereus* (Gram-positive) at the concentrations ranging from 1 mg/mL to 3 mg/mL [32]. The copolyester PBHI displayed zone of inhibition for only the studied Gram-negative bacteria ranging from 10 to 13 mm (Table-1). The inhibition zone was shown to grow with the concentration of the copolyester, which is consistent with previous observations on other copolyesters [33].

TABLE-1
ANTIMICROBIAL ACTIVITY OF PBHI

Concentration (15 μL)	Zone of inhibition (mm)	
	Bacillus cereus	*Salmonella typhi*
1 mg/mL	–	9
2 mg/mL	–	12
3 mg/mL	–	13
Chloroform	–	5
Streptomycin	27	18

Anticancer activity: The MTT reduction assay and the proliferation of HeLa cells utilizing various polymeric materials have been extensively examined [34,35]. The MTT assay was used to assess the anticancer activity of the copolyester PBHI. It was tested against the HeLa cell line, a cervical carcinoma cell line, following the standard protocol [36]. Fig. 8 represents the percentage cell viability at various concentrations in the copolyester. Three different concentrations namely 100, 90 and 50 μg/mL revealed 90% to 75% cell viability against HeLa cell line when compared to 60, 70 and 80 μg/mL concentrations, which showed relatively superior percentage cell viability. The chart discloses that 90 and 100 μg/mL may act as potential candidates against HeLa cell line.

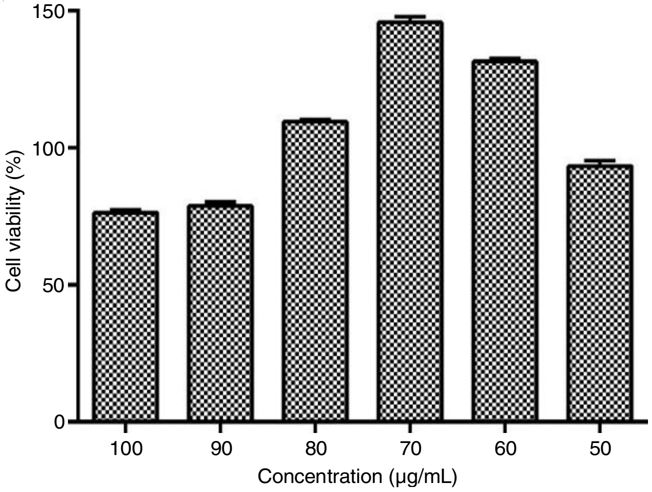


Fig. 8. Anticancer activity of copolyester PBHI

Activation energy for thermal degradation: The comparison of the activation energy values obtained *via* Murray-White and Coats-Redfern models can be valuable in identification of probable kinetic thermal degradation mechanism in the polymer as indicated by several reports [37,38]. Table-2 shows the thermogravimetric analysis of polyester based on two standard kinetic methods used to determine the thermal decomposition

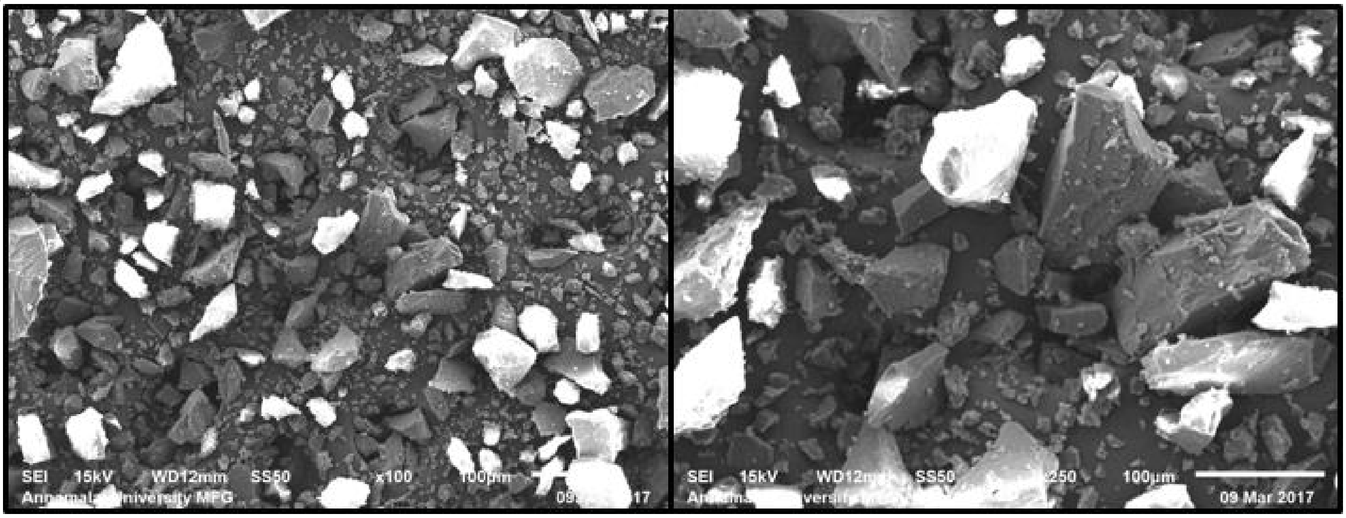


Fig. 7. SEM micrographs of prepared copolyester PBHI

TABLE-2
COMPUTATION OF ACTIVATION ENERGY FROM TGA THERMOGRAM OF PBHI

Degradation (%)	T (K)	$T^{-1} \times 10^{-3}$ K^{-1}	1–C	2 ln T	T^2	ln(1–C)	log [ln(1–C)]	ln [ln(1–C)]	ln [ln(1–C)]-2 ln T	log [ln(1–C)]/T^2
10	483	2.0703	90	12.3600	233289	4.49	0.652	1.501	-10.9678	2.79×10^6
20	573	1.7452	80	12.7017	328329	4.38	0.641	1.477	-11.0421	1.95×10^6
30	598	1.6722	70	12.7871	357604	4.24	0.627	1.444	-11.0987	1.75×10^6
40	611	1.6366	60	12.8301	373321	4.09	0.611	1.408	-11.1667	1.63×10^6
50	618	1.6181	50	12.8529	381924	3.91	0.592	1.363	-11.2448	1.55×10^6
60	623	1.6051	40	12.8690	388129	3.68	0.565	1.302	-11.3283	1.45×10^6
70	733	1.3642	30	13.1942	537289	3.40	0.531	1.223	-11.4282	9.88×10^7
80	793	1.2610	20	13.3516	628849	2.99	0.475	1.095	-11.5751	7.55×10^7
90	973	1.0277	10	13.7607	946729	2.30	0.361	0.832	-12.0752	3.81×10^7

activation energy of copolymer. The plots of activation energy based on Murray-White and Coats-Redfern for PBHI are revealed in Figs. 9 and 10. The activation energy values derived from these two methods were found to be 11.14 and 10.65 Kcal mol^{-1}, respectively, and show a good degree of agreement with each other. These findings are also comparable to the research conducted by Chrissafis *et al*. [39], which utilized the different kinetic approaches.

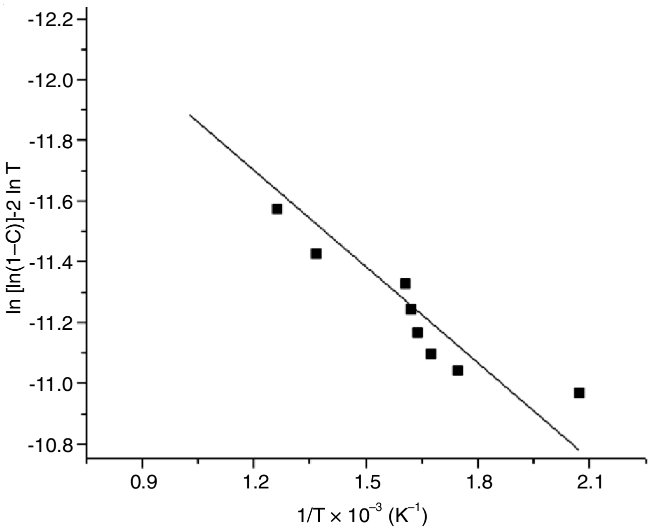


Fig. 9. Activation energy graph based on Murray and White method for copolyester PBHI

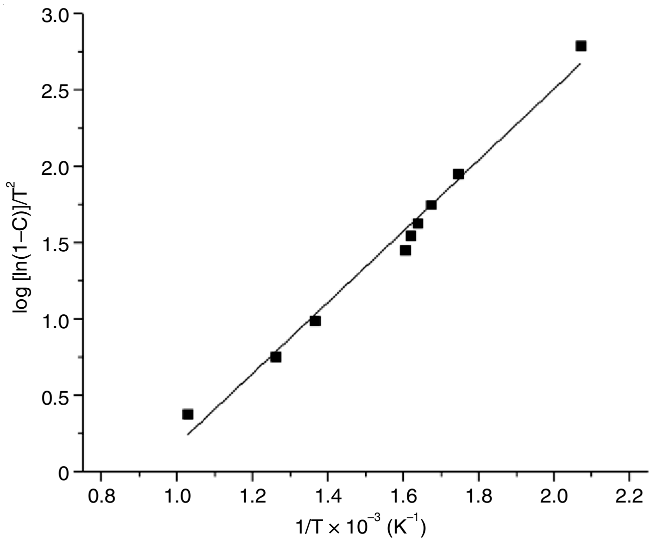


Fig. 10. Activation energy graph based on Coats and Redfern method for copolyester PBHI

Conclusion

The synthesis of a copolyester derived from isophthaloyl chloride and 4,4′-biphenol by polycondensation method using 1,2-dichlorobenzene as solvent. The viscosity data revealed that the polyester was of moderate molecular mass. The spectral data obtained were consistent with the suggested arrangement of the repeating units in the copolyester. The TGA and DSC results revealed the significant thermal stability of polyester, which was further confirmed by the comparatively elevated activation energy values obtained from conventional kinetic models for thermal degradation. The semi-crystalline nature of the copolyster was disclosed by its WAXD patterns. The SEM images of copolyester exposed the expected dissimilar surface morphology. The antimicrobial and anticancer properties exhibited by the copolyester indicate that it may serve as a promising candidate for important biological applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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