Synthesis,characterization and antibacterial studies of certain copolysters containing noncoplanarbiphenyl moiety

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Abstract:Seven novel random copolyesters have been synthesized by direct polycondensation of 2,2 '-biphenyldicarboxylic acid with certain diols and specific monomers namely 4,4'-oxybis(benzoic acid), terephthalic acid and 2,6-naphthalene dicarboxylic acid. The structural features of the copolyesters were investigated by FT-IR,^{1}H NMR and^{13}C NMR spectroscopy. All the copolyesters of this series have been screened for their antibacterial activity studies. The result revealed that most of the copolyestersshowed broad spectrum activities that are equipotent to the standard antibiotic ampicillin.

Keywords:2,2'-biphenyl dicarboxylic acid, diphenic acid,diphenyldichlorophosphate, 4,4'oxybis(benzoicacid)

I INTRODUCTION

Microbial infection is a threat constantly faced in numerous appliances including hospital and dental surgery equipments,child care articles, sportswear,household sanitation and water purification systems [1] -[3]. Bacteria have remarkable ability to develop resistance to the antibacterial agents with which they are treated. Antimicrobial agents of low molecular weight used for the sterilization of water and soil, as antibacterial drugs and as food preservatives have the limitation of having residual toxicity even when small amounts of the antimicrobial agent are applied [4]. Moreover, when used for clothing, such low molecular weight antimicrobial agents generally leach out from the fabrics towards the environment and to the skin of the wearers with harmful effects. The use of antimicrobial polymers over promise for enhancing the effcacy of some existing antimicrobial agents and minimizing the environmental problems accompanying the use of conventional antimicrobial agents, by reducing the residual toxicity of the agents, increasing their efficiency and selectivity and prolonging the lifetime of the antimicrobial agents. In addition, polymeric antimicrobial agents have the advantage of being nonvolatile, chemically stable, and do not permeate through the skin [5]. NuritBeyth*et al*., reported the antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against Streptococcus mutans [6]. Tom Anthierens*et al*., synthesized alkyne-containing poly(butylene adipate) functionalized with quaternary phosphonium groups as potential antimicrobial packaging material [7]. Kannapan*et al*., synthesized a series of four poly (ester-amides) by direct polycondensation of 4,4´-oxybis(benzoicacid) with arylidenediol and a diamine and studied the bactericidal efficacy [8]. Malathy*et al*., synthesized six nonlinear random copolyesters by direct polycondensation ofmesogenic 4,4'-oxybis(benzoic acid) with certain aliphatic diols and arylidenediols and investigated the bactericidal activity [9].

Prompted by the biological activities of 4,4'-oxybis(phenylene) moiety we envisioned our approach towards the synthesis of random copolyesters containing 2,2'-biphenyl dicarboxylic acid as a monomer with certain diols and specific aromatic dicarboxylic acids such as terephthalic acid, 4,4'-oxybis(benzoic acid)and 2,6-naphthalene dicarboxylicacid.

II MATERIALS AND METHODS

Terephthalic acid (TA) (98%),4,4'–oxybis (benzoic acid) (OBBA) (99%), 2,6 -naphthalenedicarboxylic acid (NDA) (99%), diphenic acid (DA) (97%),diphenylchlorophosphate (DPCP) (99%), 1,4-cyclohexanediol (99%) (CHD), 1,8-dihydroxyanthraquinone (AQ), hydroquinone(≥99%) (HQ), bisphenol A (≥99%) (BP), hydroquinone bis (2-hydroxyethyl) ether (98%) (HE) purchased from Sigma Aldrich were used as received. Lithium chloride anhydrous (Merck, India) was dried at 130^{o}C under vacuum for 4 h and at 180^{o}C for 10 h. Pyridine was distilled before use.

2.1*Polymerization*

All the polymers were prepared by direct polycondensation [10] of two diacids and one diol in the respective molar ratio 1:1:2 as shown in the Table 1. To a four-necked 250mL round bottomed flask fitted with a condenser, thermometer, mechanical stirrer and an oil bath, 2.5 mmol each of DA and another dicarboxylic acid, 10mL pyridine and 13mmol DPCP were added. After stirring for 20 min, 10 mmol of LiCl in 10mL pyridine was added and stirring was continued at room temperature for 30 min. The reaction mixture was slowly heated and maintained at 120^{o}C for 20 min.To this mixture 5 mmol of diol HQ in 5mL pyridine was added drop wiseat 120^{o}C for 20 min and the whole solution was further stirred undersame condition for 3 h. The solution was cooled to room temperature and thepoured into 500mL water/methanol (1:1 v/v).The product was filtered, washed with hot methanol and dried in vacuum oven at 50^{o}C.

All the seven copolyesters were prepared in the same manner with the same feed ratio.

2.2*Antibacterial studies*

The agar diffusion method was followed for antibacterial susceptibility testing. Muller Hinton Agar (MHA) medium is poured into the petri plate. After the medium was solidified, the inoculums were spread on the MHA plates with a sterile swab moistened with the bacterial suspension. Stock solutions of the synthesized copolyesters were diluted in dimethyl sulfoxide.The stock solutions were prepared at a concentration of 1mg/mL. The sample containing Disc and 20µl of the Standard antibiotic disc was placed on MHA plates. The plates were incubated at 37ºC for 24 hrs. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition. The bacterial activity of the random copolyesters was assayed against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi*. The zone of inhibition was recorded in millimeters (mm) at the completion of the incubation and the meandiameter was recorded for each compound at three different concentrations of 250µg mL^{-1}, 500µg mL^{-1}, and 1000µg mL^{-1}. The diameters of the zone of inhibition produced by the copolyesters were compared with the standard antibiotic ampicillin.

2.3*Characterization*

The solubility of the copolyesters was determined using 0.005 g of the copolyester in 1mL of the solvent. The inherentviscosities of the copolyesters were determined with an Ubbelohde Viscometer at a concentration of 0.1 g dL^{-1} in N, N-dimethylformamide.

Infrared spectra from 4000–600 cm^{-1} of solid samples of the synthesized polymers were obtained by the KBr pellet method using a Shimadzu, IR Affinity 1, Japan spectrophotometer. High resolution ^{1}H NMR spectra were recorded on a BrukerAvaceIII 500 MHz NMR for ^{1}H and 125 MHz for ^{13}C nucleus in DMSO-d_6 solvent with TMS as an internal reference.

III RESULTS AND DISCUSSION

In the present work we report the synthesis of seven new random copolyesters and their antibacterial activity. The random copolyesters described in this work were synthesized by a direct polycondensation method using DPCP as condensing agent. This method of polymerization required lesser time duration under mild conditions. The moieties present in these polyesters along with their yield and inherent viscosities at 30°C in DMF solution are furnished in Table I. The inherent viscosities were found to be in the range of 1.04–1.30 dl/g. The copolyesters exhibit excellent solubility in a variety of polar organic solvents such as THF, DMF,DMSO, NMP and pyridine. The increase in the solubility of all the polyestersmay be attributed to the presence of2,2'-biphenylene moiety in the polymer backbone.

Table I. Physical properties of random copolyesters and their IR data

Polymer code	Monomers*	Inherent Viscosity g dL^{-1}	Yield%	IR spectral values(cm^{-1})			
				Aromatic C-H stretching	C=O stretching	Aromatic C=C stretching	C-O-C stretching
DOHE	DA+ HE+OBBA	1.28	68	3061.6	1716.72	1599.06	1225.82
DOAQ	DA+ AQ+OBBA	1.17	99	3072.6	1741.72	1589.34	1219.01
DOHQ	DA+ HQ+OBBA	1.11	96	3066.82	1734.01	1595.13	1246.02
DTHQ	DA+ HQ+TA	1.07	98	3066.82	1741.72	1591.27	1253.73
DTAQ	DA+ AQ+TA	1.30	99	3076.46	1743.65	1585.49	1273.02
DNHQ	DA+ HQ+NDA	1.17	87	3066.82	1735.93	1597.06	1247.94
DNAQ	DA+ AQ+NDA	1.04	97	3070.68	1739.79	1589.34	1230.58

* DA: 2,2'-biphenyldicarboxylic acid ,TA: terephthalic acid ,OBBA: 4,4'-oxy(bisbenzoic acid), NDA : 2,6-naphthalenedicarboxylicacid , HE : hydroquinone bis(2-hydroxyethyl) ether , HQ: hydroquinone , AQ: 1,8-dihydroxyanthraquinone

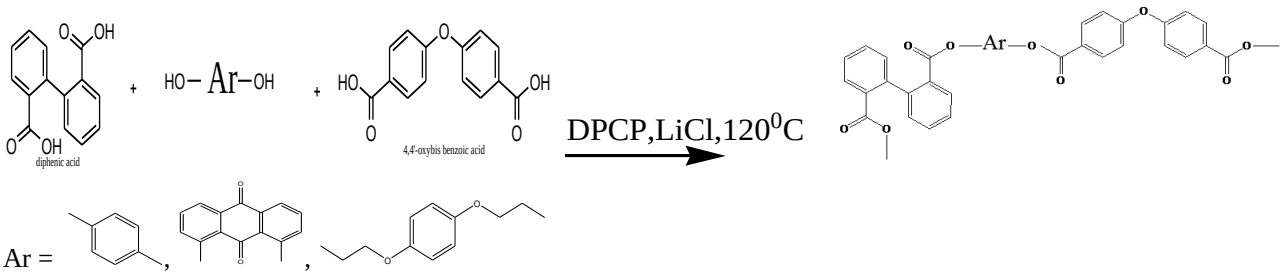


Fig. 1 Scheme of synthesis of random copolyester

3.1*Spectral studies*

The chemical structures of the random copolyesters were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR. The Fourier transform infrared spectra of the synthesized copolyesters indicate, the formation of ester in the products (Table 1). Figure 2 is the characteristic FT-IR spectrum of DOHQ. The peak at 3450.65 cm^{-1} is due to the overtone of the C=O group from ester [11]. The small peak at 3066.82 cm^{-1} is due to =C-H stretching of aromatic protons. The presence of a sharp band at 1728.22 cm^{-1} in DOHQ may be attributed to the C=O stretching of the ester group [12]. The band at 1246.02 cm^{-1} is assigned for the C-O stretching of the aromatic carbon–O linkage [13]. The bands at 1595.13 cm^{-1} and 1500.62 cm^{-1} indicates C=C stretching in aromatic rings.

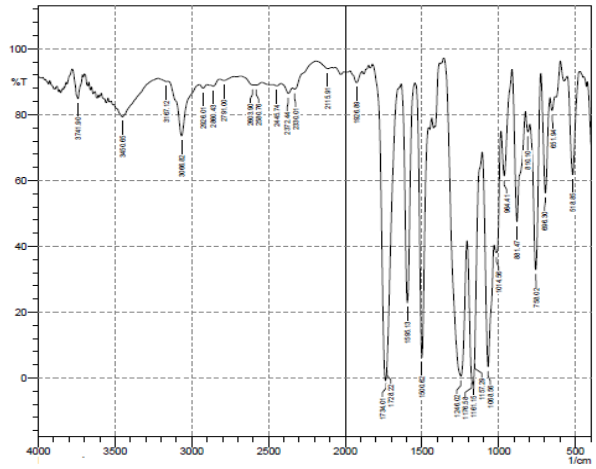


Fig. 2 FT-IR spectrum of copolyester DOHQ

To have an understanding into the chemical structure, the ^{1}H NMR investigation of the samples was performed. From the ^{1}H NMR spectrum of DOHQ shown in Fig. 3 it can be found that the chemical shifts between 6.70-8.64 ppm, correspond to aromatic protons. The various protons associated with the two respective monomer diacidscan be clearly distinguished by ^{1}H NMR. Thus, for the diacid portion of the polymer molecule, the resonances associated with the oxybenzoate moiety occur at δ 7.16 (H_a) and δ 8.04 (H_b)and that of 2,2'-biphenyl dicarboxylate moiety four resonance signals occur at δ 8.07(H_g), δ 7.69 (H_f), δ 7. 46(H_e) and δ 7.30(H_d). For the diol portion of the polymer molecule, the resonances associated with the aromatic protons displayed a chemical shift of δ 7.44 (H_c)[14]. Broad peak at δ 12.71 ppm and δ 9.3 ppm are due to the end –COOH group and phenolic OH respectively.

^{13}C NMR spectrum of DOHQ along with assignments is presented in Fig. 4 and is in good agreement with the structure. The carbonyl carbons of the ester groups gave rise to two distinct resonances at 164.34 ppm and 168.42 ppm. For the diol portion of the polyester, peaks at δ 119.6 ppm (C-6) and δ 131.09 ppm (C-7) could be assigned to the aromatic carbons. For the diacid portion of the polymer molecule, the resonances associated with the aromatic ring carbons of diphenate moiety occur at 127.85 ppm (C-8), 129.59 ppm (C-9, C-10), 130.29 ppm (C-11), 130.86 ppm (C-12) and the quaternary carbon adjacent to –COO- resonated at 142.91 ppm (C-13). The carbonyl carbon of diphenate segment appear at 168.42 ppm (C-16) [15]. Aromatic carbons of oxybis(benzoate) moiety appear at 120.39 ppm (C-1), 124.91 ppm (C-2), and the quaternary carbon adjacent to –COO- appear at 133.02 ppm (C-3). The copolyester exhibit resonances associated with the quaternary carbon attached to the oxygen at δ 160.68 ppm (C-4) and the carbonyl carbon at δ 164.34 ppm (C-5) [16]. The presence of two different ester carbonyls in the polymer backbone was confirmed by the appearance of more than two different carbonyl absorptions at δ 168.42, 165.72,164.41 and 164.34, which in turn indicates that both the monomeric units are incorporated into the polymeric backbone.

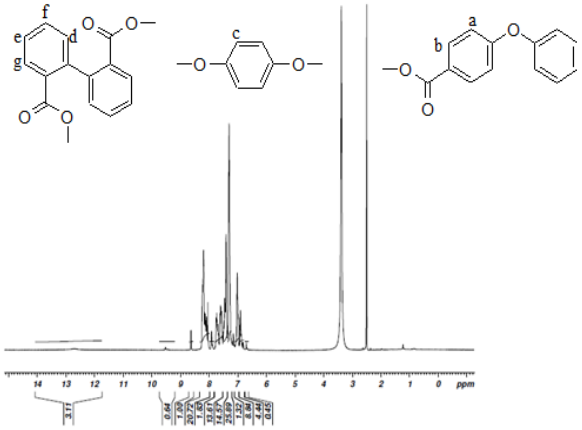


Fig. 3 ^{1}H NMR spectrum of copolyester DOHQ

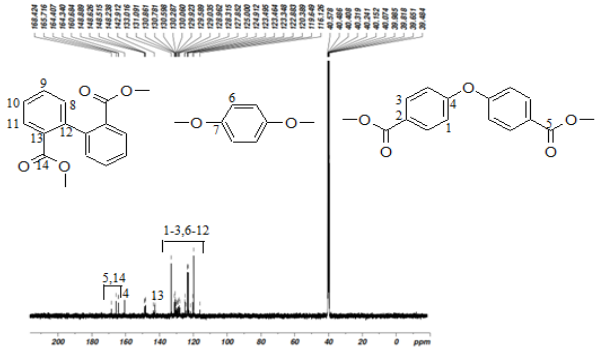
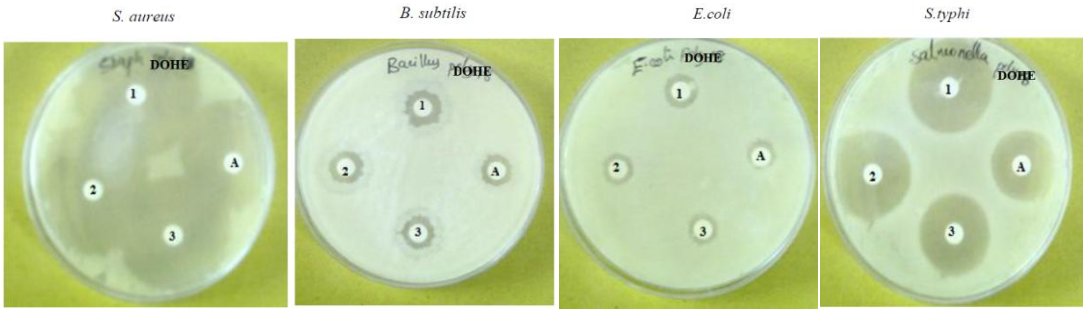


Fig. 4 ^{13}C NMR spectrum of copolyester DOHQ

3.2 *Antibacterial studies*

The potentiality of the synthesized polyesters as antibacterials was appraised for their antibacterial studies against different strains of human pathogens of both gram positive bacteria, namely *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria like *Escherichia coli* and *Salmonella typhi* by agar disc diffusion assay(Fig.5). The diameters for the zone of inhibitions at different concentrations against the test bacteria are given in Table 2. The antibiotic (ampicillin, 20 µL/disc) inhibited the growth of *Staphylococcus aureus* by 36mm, *Bacillus subtilis* by 16mm, *E.coli* by 16mm and *Salmonella typhi* by 28 mm. The inhibition zone diameter data analysis indicated that copolyesterscontaining 4,4'-oxybis(phenylene) moiety show very high activity at 1000µg/mL comparable to the standard drug ampicillin. It also has moderate activity at concentrations of 250,500 µg/mL. The antibacterial activity of the random copolyesters derived from 4,4'-oxy bis(benzoic acid) are due the presence of ether linkage [17] in the 4,4'-oxybis(phenylene) moiety. Inspection of the copolyesters containing 4,4'-oxybis(phenylene) moiety further revealed that the copolyester DOHE is more active than the standard antibiotic ampicillin. This may be due to the presence of more ether linkages in 1,4-bis (2-hydroxybenzoyloxy) benzene moiety in addition to 4,4'-oxybis(phenylene) moiety. It is also interesting to note that if 4,4'-oxybis(phenylene) moiety is replaced by the terephthalate or the naphthalate moiety the activity is reduced significantly. This result clearly indicates that the antibacterial activity is mainly due to the presence of the ether linkage in 4,4'-oxy (bisphenylene) moiety. The order of activity of the synthesized copolyesters against *Staphylococcus aureus* is found to be in the order DOHE> DOAQ≈DOHQ> DNHQ> DTAQ≈DNAQ> DTHQ.



1-1000µg/ml , 2- 750µg/ml, 3- 500 µg/ml,A-Antibiotic

Fig. 5 Antibacterial activity of random copolyester DOHE at different concentrations

Table II Inhibition effects of random copolyesters on the growth of pathogenic bacteria

Test material	S. aureus (Zone of inhibition in mm)			B. subtilis (Zone of inhibition in mm)			E. coli (Zone of inhibition in mm)			S. typhi (Zone of inhibition in mm)		
	Concentration µg/mL											
	1000	500	250	1000	500	250	1000	500	250	1000	500	250
DOHE	38	34	27	22	19	14	14	13	13	42	38	35
DOAQ	34	32	30	14	13	11	12	11	11	27	26	22
DOHQ	34	32	28	13	12	9	15	14	12	28	26	24
DTHQ	21	18	15	-	-	-	9	8	7	20	14	14
DTAQ	24	21	18	-	-	-	9	8	7	25	20	14
DNHQ	27	24	21	-	-	-	8	5	5	25	20	14
DNAQ	24	21	18	-	-	-	12	9	8	22	22	17

IV CONCLUSION

Seven novel random copolyesters have been synthesized successfully in appreciable yields. The copolyesters were characterized by FT IR, ^{1}H NMR and ^{13}C NMR spectroscopy. They were evaluated for*in vitro* antibacterial activity against four bacterial strains. From the activity studies, it was concluded that among all the synthesized polyesters DOHE, DOHQ and DOAQ registered high inhibitory activity against all bacterial strains. The results of the *in vitro* antibacterial evaluation emphasized that copolyesters generated from the incorporation of 4,4'-oxybis(phenylene) moiety have high potential as useful antibacterial agents.

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