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Synthesis, Characterization and Biological Applications of Random Aliphatic Copolythioesters Using 3,3'-Thiodipropionic Acid

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The aliphatic random copolyester polycyclohexylthiodipropionate-*co*-cyclohexyldecanedioate (PCTCD) containing sulphur atom in the main chain was synthesized by mixing 3,3'-thiodipropionic acid, decanedioic acid and 1,4-cyclohexanediol monomers with titanium tetra isopropoxide catalyst using direct melt polycondensation method. The different physical properties of copolyester such as inherent viscosity, solubility, differential scanning colorimetry and X-ray diffraction technique was investigated. The chemical structure of the copolyester was investigated by FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy. The synthesized compounds were tested for human pathogenic bacteria using well diffusion method, *in vitro* cytotoxicity against normal (Vero cell line) and cancer (A_{549} lung cancer cell line) by MTT assay. Also *in vitro* antioxidant property of copolymer was studied.

Keywords: Polycyclohexylthiodipropionate-co-cyclohexyldecanedioate, 3,3'-Thiodipropionic acid, Decanedioic acid, 1,4-Cyclohexanediol.

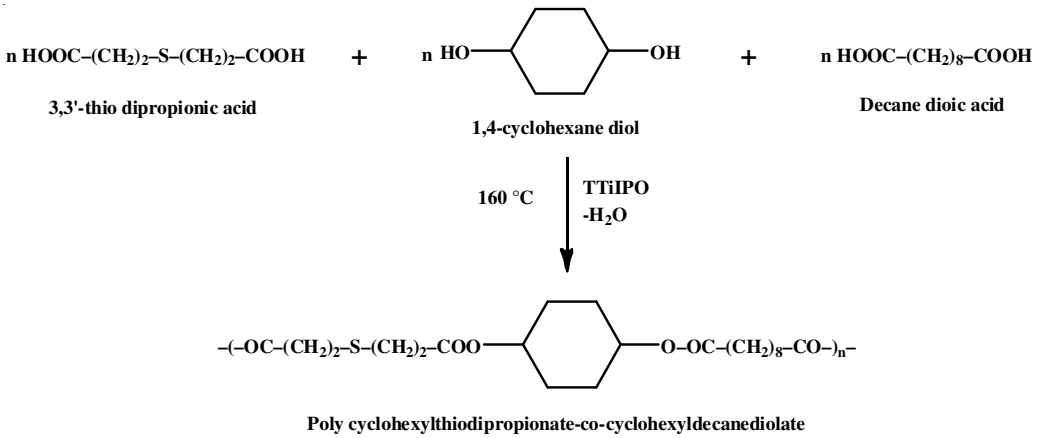
INTRODUCTION

In early studies, low molecular weight aliphatic polyesters are used as plasticizers, stabilizers and very good antioxidants like PBA, PVC *etc*., The only other sulfur-containing biopolymers known are proteins, some complex polysaccharides and recently described PHAs allow various applications in medicine, pharmacy, agriculture, packaging and food industry, as active agents or as coatings or carriers [1]. Hydroxyl terminated aliphatic and aromatic thiopolyesters are used as polyol components in the synthesis of high elasticity polyurethane elastomers [2]. Poly(3HB-co-3MP) is the first biopolymer which are designated as polythioesters contains sulfur in the polymer backbone [3]. Linear copolymeric polyesters *i.e.*, poly(3,3'-thiodipropionic acid-co-1,6-hexanediol) and poly (3,3'-thiodipropionic acid-co-1,12-dodecanediol) by esterification of an equimolar mixture of 3,3'-thiodipropionic acid and 1,6-hexanediol or 1,12-dodecanediol catalyzed by immobilized lipase B from *Candida antarctica* (Novozym 435) were extracted from the reaction mixtures using tetra-hydrofuran and precipitated from tetrahydrofuran-iso-hexane [4]. In the present work, we have synthesized a low molecular weight linear copolythioesters by using special monomer 3,3'-thiodipropionic acid, which has many applications in food, medicine, paint, plastic and pharmaceutical industries.

EXPERIMENTAL

3,3'-Thiodipropionic acid, decanedioic acid and 1,4-cyclohexanediol was purchased from Sigma Aldrich. The catalyst titanium tetra isopropoxide was purchased from Lancaster. All other chemicals and solvents (AR Grade) were purchased from Sigma Aldrich, Mumbai. FT-IR spectra were recorded on Perkin Elmer 883 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra for copolyester was recorded on a Bruker 400 MHz and Bruker 100 MHz spectrometer respectively using $CDCl_3$ as a solvent. DSC thermogram was recorded on DSC Q200 V23.10 Build 79 differential scanning calorimeter. Bruker B8 wide angle XRD with Cu/30 kv/15 mA was used for assessing crystalline or amorphous nature of copolymer. Biological application of PDTDD carried out such as *in vitro* antioxidant activity by dot blot assay [5], *in vitro* antioxidant activity with spectrophotometer at 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/mL *in vitro* antibacterial activity well diffusion method [6] with 250, 500 and 1000 µg/mL against the human pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus* and *in vitro* cytotoxicity of vero (normal) cell line and lung cancer (A_{549}) line with various concentrations of PCTCD like 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/mL [7].

Synthesis of copolyester: A mixture of 3,3'-thiodipropionic acid (0.01 mol), decanedioic acid (0.01 mol) and 1,4-



Scheme-I: Synthesis of copolyester

cyclohexanediol (0.02 mol) was taken in a three-neck round bottom flask. One of the left inlet of round bottom flask connected to nitrogen cylinder, right inlet with stopper and middle inlet with $CaCl_2$ guard tube. The set up is kept in oil bath and heated at its melting point. After the complete melt of mixture 0.8 mL of titanium tetraisopropoxide catalyst is added and kept for 1 h. Later the temperature is increased by 25 °C and maintained for 2 h. At 0.5 h interval the recrystallization is checked with the reaction sample. The obtained crude copolymer sample is dissolved in chloroform/THF and poured into ice-cold methanol, a pure copolymer PCTCD is reprecipitated as given in **Scheme-I**.

RESULTS AND DISCUSSION

Characterization of physical parameters: Solubilities of the synthesized copolymer were determined in various solvents. It has been found that the copolymer is soluble in chloroform, dimethyl formamide, THF and DMSO. Inherent viscosity of the copolymer PCTCD is 0.796 and was determined by flow times of solvent and one percent solution of the copolymer dissolved in chloroform and taken in Ubbelohde viscometer. The DSC thermogram of the colpolymer PCTCD (Fig. 1) shows glass transition temperature (T_g) at-50 °C and melting temperature (T_m) at 72.43 °C which signifies the copolymer has low molecular weight and may be used as plasticizers, stabilizers and very good antioxidants like PBA, PVC *etc*., Wide XRD of the synthesized copolymer in Fig. 2 gives the value of 2θ (°) = 21-23° which confirms that the polymer is amorphous in nature.

Structural elucidation: The FT-IR spectrum (Fig. 3) of the synthesized copolyester PCTCD showed characteristic absorption band for ester carbonyl stretching at 1730 cm^{-1}. Also the polymer was observed peaks at 640, 1250, 2910 and 1460 cm^{-1} due to C-S stretching, C-O-C asymmetric stretching, aliphatic C-H stretching of methylene group and aliphatic C-C stretching respectively. The C-C stretching vibrations of the cyclohexyl ester give rise to absorption band at 1090 cm^{-1}. A new ester bond that was formed during polycondensation can be revealed from the report.

^{1}H NMR spectrum of the copolyester is shown in Fig. 4. The peaks at δ = 1.533-1.698 ppm and δ = 1.717-1.894 ppm was due to methylene protons of copolyester and cyclohexane ring respectively. In addition the peak at δ = 2.186-2.822 ppm

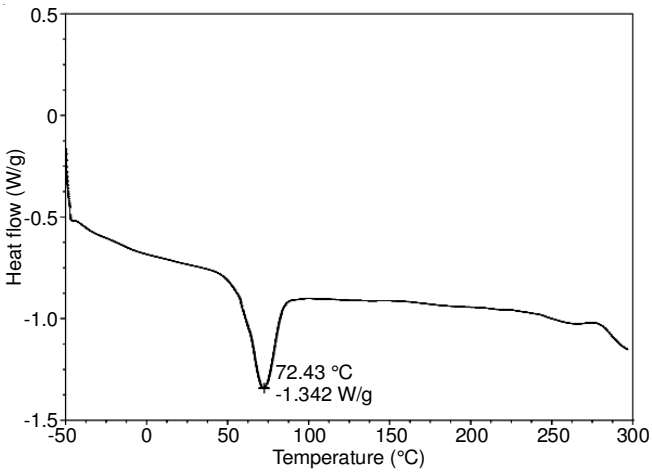


Fig. 1. DSC thermogram of the copolymer

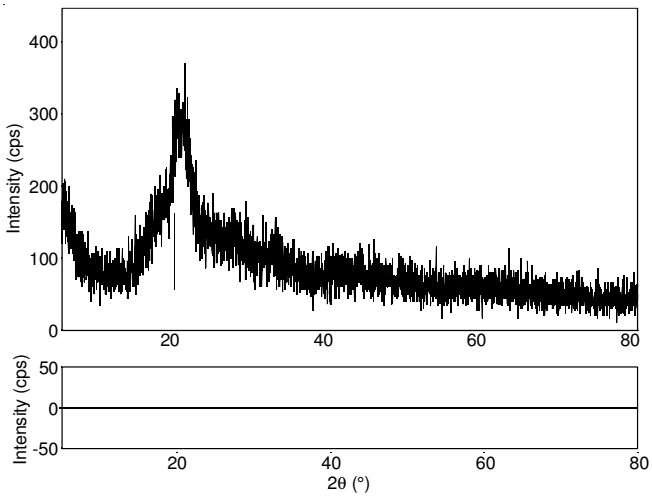


Fig. 2. Wide XRD of the copolymer

was attributed to-CH_2-CO-protons, the signal δ = 2.709-2.771 was due to C-CH_2-S protons wile δ = 4.746-4.779 ppm is due to C-CH_2-O protons.

^{13}C NMR spectrum of the copolyester (Fig. 5) shows the signals at δ = 24.49-25.44 ppm was attributed to methylene protons of copolyester and at δ = 27.33-27.94 ppm due to methylene protons of cyclohexane ring respectively. In addition the peaks at δ = 28.03-35.03 ppm and δ = 69.42-71.38 was attributed to-CH_2-S-and-CH_2-O-respectively, while δ = 171.17 and 173.22 ppm is due to ester groups.

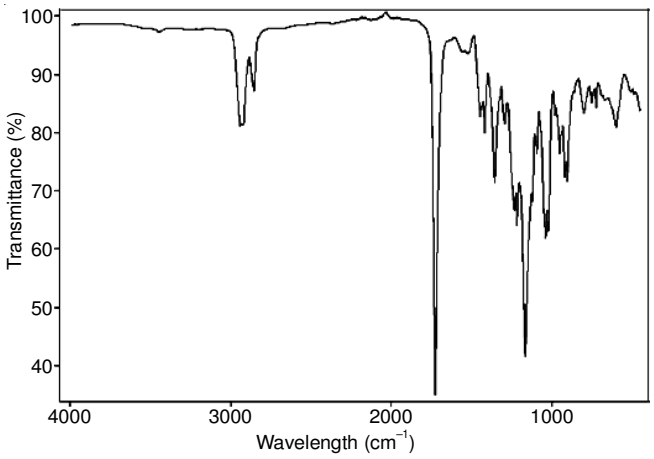


Fig. 3. FT-IR spectrum of the copolyester

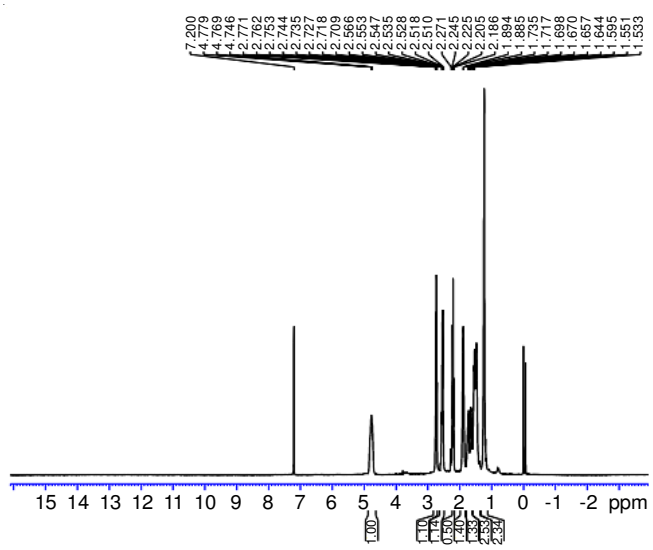


Fig. 4. ^{1}H NMR spectrum of the copolyester

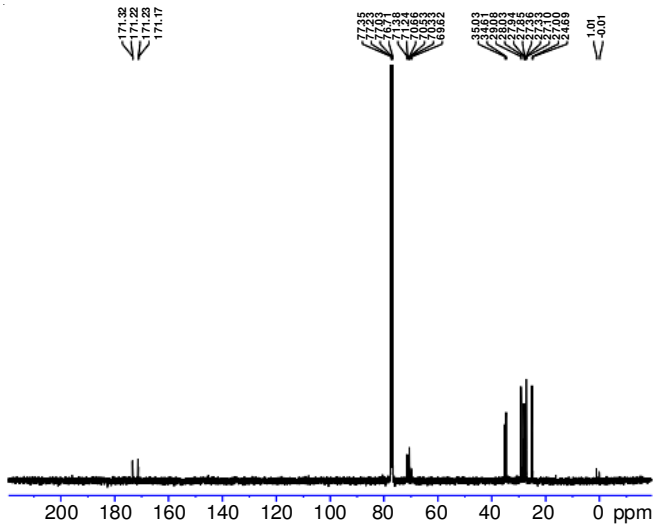


Fig. 5. ^{13}C NMR spectrum of the copolyester

Biological studies: Synthesized compounds were tested for cytotoxicity on Vero (normal) cell line and lung cancer (A549) line with various concentrations of PCTCD like 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/mL. The compound showed different ranges of viability, cell shrinkage in dose dependent manner (Tables 1-3), observed under stereomicroscope. At the maximum concentration of PCTCD (1000 µg/mL), the cell viability were 51.37 % for Vero cell line and 17.01 % for lung cancer A549 cell line. The copolymer PCTCD showed more toxic on lung cancer cell than normal Vero cell line. Fifty percent death was calculated at the concentration of 973.33 µg/mL Vero cell and 55.77 µg/mL lung cancer cell. *In vitro* antioxidant property of PCTCD copolymer showed radical scavenging activity on thin layer chromatography purple colour of DPPH radical turned into yellow which confirms the polymer has antioxidant activity by Dot-Blot assay (Tables 4 and 5). The maximum percentages of inhibition activity of compound PCTCD is 63.64 % at the concentration of 1000 µg/mL, whereas standard showed maximum percentage of Inhibition activity 75.85 % (quercetin) at the concentration of 10 µg/mL. IC_{50} (µg/mL) value for PCTCD is 425.33, while for standard quercetin it was (6.59 µg/mL). *In vitro* antimicrobial activities exhibited 10 to 15 mm and inhibition percentage 11.22 to 16.67 % against the pathogens (Table-6) by well diffusion method.

TABLE-1
CYTOTOXICITY EFFECT OF SYNTHESIZED COMPOUND PCTCD ON VERO (NORMAL) CELL LINE

Concentration of compounds (µg/mL)	Cell viability (%)
1000	51.37
500	58.64
250	63.41
125	69.06
62.5	74.71
31.2	80.49
15.6	88.83
7.8	92.60
Cell control	100
IC_{50} value (µg/mL)	973.330

TABLE-2
CYTOTOXICITY OF SYNTHESIZED COMPOUNDS PCTCD AGAINST LUNG CANCER (A_{549}) CELL LINE

Concentration of compounds (µg/mL)	Cell viability (%)
1000	17.01
500	30.22
250	38.78
125	48.30
62.5	56.03
31.2	67.63
15.6	75.13
7.8	86.31
Cell control	100
IC_{50} value (µg/mL)	55.77

TABLE-3
ANTICANCER EFFECT OF PCTCD ON VERO CELL LINE

Concentration (µg/mL)	Dilutions	Absorbance (O.D)	Cell viability (%)
1000	Neat	0.764	51.37
500	1:1	0.872	58.64
250	1:2	0.943	63.41
125	1:4	1.027	69.06
62.5	1:8	1.111	74.71
31.2	1:16	1.197	80.49
15.6	1:32	1.321	88.83
7.8	1:64	1.377	92.60
Cell control	–	1.487	100

TABLE-6
ANTIMICROBIAL ACTIVITY OF COMPOUND PCTCD AGAINST HUMAN PATHOGENS BY WELL DIFFUSION METHOD

Human pathogens	Concentration (µg)	Zone of inhibition (%)	
		PCTCD	Kannamycin (30 µg)
Escherichia coli	1000	15 ± 1.05 (16.67 ± 1.17)	26.33 ± 1.52 (29.25 ± 1.38)
	500	13 ± 0.91 (14.44 ± 1.0)	
	250	10 ± 0.7 (11.11 ± 0.7)	
Klebsiella pneumoniae	1000	13 ± 0.91 (14.44 ± 1.0)	30.67 ± 1.52 (34.07 ± 1.38)
	500	12 ± 0.84 (13.33 ± 0.9)	
	250	10 ± 0.7 (11.11 ± 0.7)	
Bacillus subtilis	1000	12 ± 0.84 (13.33 ± 0.93	27.00 ± 1.00 (30.00 ± 0.90)
	500	10 ± 0.77 (11.11 ± 0.7)	
	250	–	
Staphylococcus aureus	1000	14 ± 0.98 (15.55 ± 1.08)	26.00 ± 1.00 (29.25 ± 0.52)
	500	11 ± 0.77 (12.22 ± 0.85)	
	250	–	

All values are mean values of triplicates (mean ± standard deviation)

TABLE-4
In vitro DPPH ACTIVITIES OF COMPOUNDS PCTCD

Concentration of compounds (µg/mL)	PCTCD inhibition (%)
1000	63.64 ± 4.45
500	58.75 ± 4.11
250	44.21 ± 3.09
125	40.94 ± 2.86
62.5	33.97 ± 2.37
31.25	20.77 ± 1.45
15.62	14.24 ± 0.99
IC_{50} (µg/mL)	425.53

All values are mean values of triplicates (mean ± standard deviation).

TABLE-5
In vitro DPPH ANTIOXIDANT ACTIVITIES
OF STANDARD (QUERCETIN)

Concentration of compounds (µg/mL)	Inhibition (%)
1.0	7.58 ± 0.53
2.0	15.17 ± 1.06
3.0	22.75 ± 1.59
4.0	30.34 ± 2.12
5.0	37.92 ± 2.65
6.0	45.51 ± 3.18
7.0	53.09 ± 3.71
8.0	60.68 ± 4.24
10.0	75.85 ± 5.30
IC_{50} (µg/mL)	6.59

All values are mean values of triplicates (mean ± standard deviation).

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