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Synthesis, Characterization and *in vitro* Antimicrobial, Antioxidant and Anticancer Activity of Random Copolyester Using 1,4-Dithiane-2,5-diol

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A random copolyester was prepared by direct melt polycondensation method using 1,4-dithiane-2,5-diol, 1,4-cyclohexane diol and Sebacic acid in the mole ratio 1:1:2 using titanium tetra isopropoxide as catalyst. The synthesized copolyester poly(1,4-dithiane-2,5-diol sebacate–co–1,4-cyclohexane diol sebacate) (PDCSe) was characterized by FT-IR, ^{1}H NMR and ^{13}C NMR to determine the chemical structure, while wide angle XRD, differential scanning spectroscopy and Inherent viscosity were determined to study its physical properties. Further the antioxidant, antimicrobial and anticancer activities of the synthesized copolyester were determined to study its biological property.

Keywords: 1,4-Dithiane-2,5-diol, Anticancer, Copolyester, Polycondensation.

INTRODUCTION

Aliphatic polyesters plays an important role in solving white pollution as it can easily be biodegraded and constitute nowadays an important group of polymers that finds wide application in biomedical field [1-6]. As the aliphatic polyester are non-toxic and can enter the metabolic cycles of bio organism, the poly(lactic acid) and poly(β-hydroxyalkanoates) have been commercially synthesized and used in field of medicine from long period [7]. A careful selection of monomers is required to synthesize copolyester with desired properties such as biodegradability, biocompatibility and biomedical application. As a result of intensive research, 1,4-dithiane-2,5-diol have been selected as special monomer to synthesize random copolyester. Several reports indicate that sulphur containing polymers exhibit both high refractive indices and high Abbe's number as sulphur atom have large atomic refraction and hence used in making optical polymers and for use in photoresist formulation for 193 nm immersion lithograph [8]. An optical polymer using 1,4-dithiane derivatives have been reported by Okubo *et al.* [9]. A resin obtained by curing polymerizable composition comprising sulpur containing cyclic compound like dithiane derivatives have high refractive index, transparancy, low brittleness and high impact resistance, hence used in making optical material. 1,4-Dithiane-2,5-diol also used as resin modifier for controlling optical physical properties [10]. Polyester resin obtained by polymerization of carboxy containing compound and hydroxyl compounds and polymerizable composition containing 1,4-dithiane-2,5-diol as monomer have been used in the preparation of antireflective coating composition [11,12]. An ester obtained from dihydric alcohol such as 1,4-dithiane-2,5-diol and poly basic aliphatic carboxylic acid is used as plasticizer in cyanoacrylate adhesive [13]. It is reported that 1,4-dithiane-2,5-diol is the main source to synthesize sulphur containing heterocyclic compounds like thiophene and 1,3-thiazole families that have broad application in medicinal chemistry [14,15]. The 2-aminothiophene derivatives have interesting properties like antiviral and antitumour activites [16-18], GluR6 Antagonism [19], inhibition of p53-Mdm2 interaction [20]. The thiophene derivatives obtained from 1,4-dithiane-2,5-diol have excellent antiproliferative activity and also inhibits the tubulin polymersation for the treatment of cancer [21]. Available report confirms that 1,4-dithiane-4,5-diol derivative have been used as drug to eject zinc ions from Human Papillomavirus Type 16 E6 Oncoprotein that is responsible for cervical cancer [22] and also attacks retroviral zinc fingers

and inhibits the HIV Type 1 replication [23]. The 1,4-dithiane derivatives were highly active against Junin Virus and also exhibited Virucidal activity [24]. Sebacic acid used as monomer in current study has been reacted with multifunctional alcohols giving rise to materials with good properties for cell growth and control over physico-chemical and degradation properties through the use of different polyols [25]. Hence new copolyester poly(1,4-dithiane-2,5-diol sebacate–co–1,4-cyclohexane diol sebacate) (PDCSe) with sulphur in main chain possessing desired biomedical property is synthesized by direct melt polycondensation method.

EXPERIMENTAL

1,4-Dithiane-2,5-diol, sebacic acid, 1,4-cyclohexane diol were purchased from Sigma Aldrich. The catalyst titanium tetra isopropoxide were purchased from Lancaster. The solvents and chemicals AR grade were used as such. Using Ubbelohde viscometer the inherant viscosity of copolyester was determined. FT-IR spectra of synthesized copolyester was recorded using Perkin Elmer 883 Spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra of the copolyester using $CDCl_3$ as a solvent was recorded on a Bruker 400 MHz and Bruker 100 MHz spectrometer respectively. DSC thermogram was recorded to determine the thermal behaviour using DSC Q200 V23.10 Build 79 differential scanning calorimeter. Crystalline nature of the synthesized copolyester were determined by wide angle XRD using Bruker B8 wide angle XRD with Cu/30kv/15mA. By MTT assay method the *in vitro* cytotoxicity against normal (vero cell line) and cancer (A_{549} lung cancer cell line) were determined. The antioxidant activity by DPPH scavenging assay and antimicrobial activity by well diffusion method have been determined.

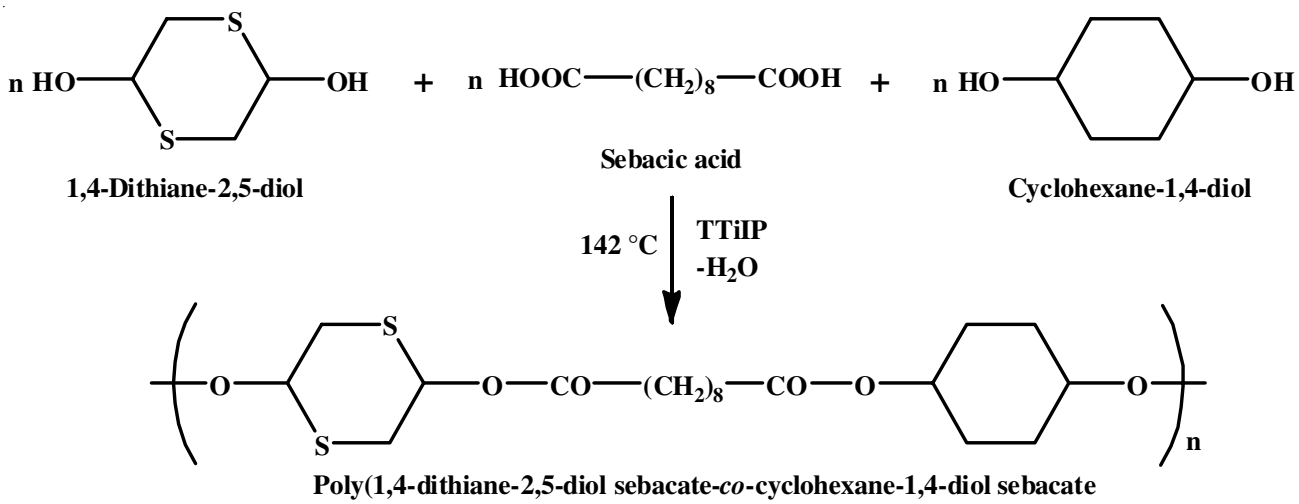
Synthesis of copolyester: In a three necked round bottom flask about 0.01 mol of 1,4-dithiane-2,5-diol, 0.01 mol of 1,4-cyclohexane diol and 0.02 mol of sebacic acid is taken and kept in an oil bath. The left inlet was connected to the nitrogen cylinder and the middle inlet to the guard tube filled with calcium chloride and the right inlet was closed with a stopper. The mixture was heated along with magnetic stirring till the constituents get melted and then about 0.8 mL of titanium tetra isopropoxide was added and the temperature is maintained at 132 °C upto 1 h. Then the temperature was increased to 142 °C and maintained for 2 h. The crude formed in the reaction flask is dissolved in chloroform and then poured in ice cold methanol and stirred. The reprecipitated copolyester PDCSe is filtered and dried. The polymerization reaction occurs as shown in **Scheme-I**.

RESULTS AND DISCUSSION

Solubility and viscosity studies: The synthesized copolyester was dissolved in various solvents like DMF, DMSO, THF, Chloroform and acetone to find their solubility. The copolyester was soluble in chloroform, DMF and THF while partially soluble in other solvents. The inherent viscosity of the polymer PDCSe was determined by noting the flow time of the polymer at the concentration of 1 mg/mL and the pure solvent using Ubbelohde viscometer. The inherent viscosity of polymer was found to be 0.82 dL/g.

FT-IR spectral studies: The IR spectra of the synthesized copolyester showed a strong absorption band at around 1710.12 cm^{-1}, which corresponds carbonyl stretching vibration of ester group. The absorption bands at 2976.07 cm^{-1} and 1152 cm^{-1} was assigned to aliphatic C-H stretching of the diacids/diols and C-O-stretching of ester group. The absorption bands at 710 and 1450 cm^{-1} was attributed to C-S stretching and aliphatic C-C stretching, respectively. The formation of the ester bond during polycondensation was confirmed from the report.

^{1}H NMR and ^{13}C NMR spectral studies: The ^{1}H NMR spectrum exhibited characteristic peak at 1.26 ppm and 1.616 ppm corresponding to the methylene protons of sebacic acid and $–CH_2$-CO protons in copolyester. The peak at 2.2-2.5 ppm and 2.6-2.8 ppm is due to the $–CH_2$-S and –CH-S protons of 1,4-dithiane-2,5-diol, while the peak 5.1-5.2 ppm is attributed to –CH-O protons. The peak around 0.9 ppm is due to the methylene protons of 1,4-cyclohexane diol. The ^{13}C NMR spectrum of the copolyester exhibited a characteristic peaks at 76.7 ppm and 31.9 ppm corresponding to methylene carbon attached to oxygen of $–O-CH_2$ and methylene carbon attached to ester of $–O-CH_2$ respectively. The peak at 14.12 ppm and 29.58 ppm is attributed to the methylene carbon in sebacic acid and 1,4-cyclohexane diol respectively. The peaks at 22.6 and 77.3 ppm is attributed to the $–CH_2$-S and –CH-S carbon present in 1,4-



Poly(1,4-dithiane-2,5-diol sebacate-*co*-cyclohexane-1,4-diol sebacate

Scheme-I

dithiane-2,5-diol. Based on the spectral data it is confirmed that the monomeric units are distributed in the random copolyester.

DSC studies: The thermal properties of the copolyester is determined by the differential scanning calorimetry. The DSC thermogram shows glass transistion temperature T_g as 94.08 °C and its melting point as 258.11 °C (Fig. 1).

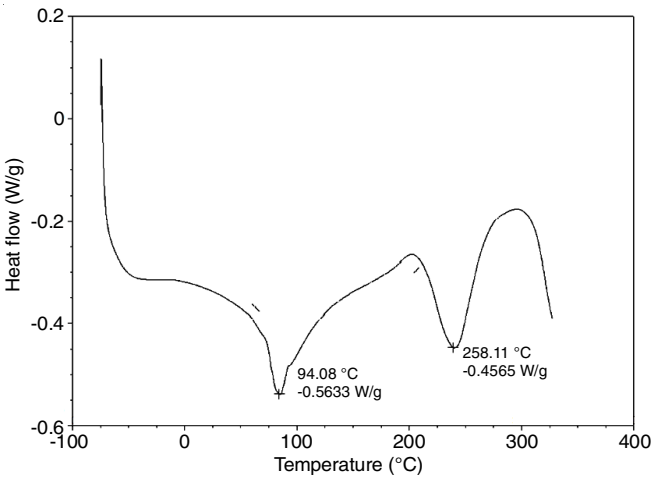


Fig. 1. DSC thermogram of PDCSe

Wide angle X-ray diffraction studies: The degree of crystallinity of copolyester is determined by wide angle X-ray diffraction analysis. It is observed from the X ray diffractogram (Fig. 2) that PDCSe is amorphous in nature.

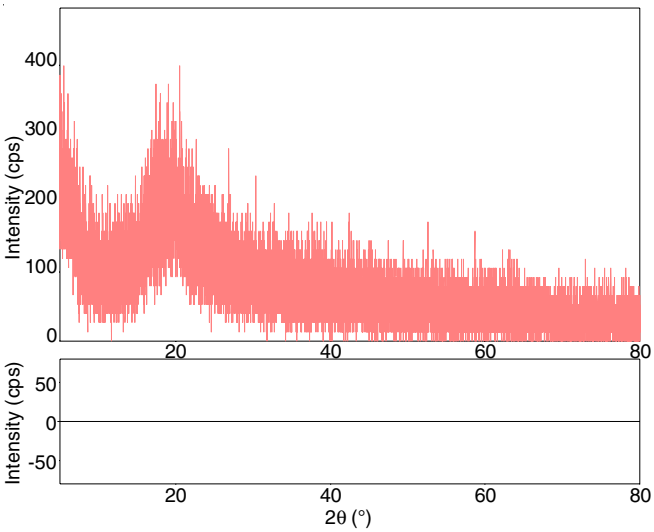


Fig. 2. X-ray diffractogram of PDCSe

Antioxidant activity: The antioxidant activity of the synthesized copolyester PDCSe is determined by DPPH scavenging assay on TLC by dot blot method and by spectrophotometer. The synthesized copolyester PDCSe showed radical scavenging activity on TLC by turning purple colour of DPPH radical into yellow which confirms that PDCSe having antioxidant activity. The DPPH scavenging assay by spectrophotometer for synthesized copolyester PDCSe showed various ranges of percentage of inhibitions. The copolyester at the concentrations (15.62-1000 µg/mL) were tested and maximum percentage of inhibition activity was determined. The maximum percentages of inhibition activity of copolyester was 78.49 % (Table-1) 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. The IC_{50} (µg/mL) values was 425.53.

TABLE-1
in vitro DPPH ACTIVITIES OF PDCSe

Concentration (µg/mL)	Inhibition of PDCSe (%)
1000	78.49 ± 5.49
500	58.75 ± 4.11
250	42.28 ± 2.96
125	26.11 ± 1.83
62.5	22.11 ± 1.55
31.25	20.77± 1.45
15.62	9.20 ± 0.64
IC_{50} (µg/mL)	425.53

Antimicrobial activity: The *in vitro* antimicrobial activity of the synthesized copolyester PDCSe is determined by well diffusion method using Mueller Hinton agar (MHA) medium. Synthesized copolyester have exhibited antimicrobial activities with inhibition zones ranging from 12 to 18 mm, as shown in Table-2. Three concentrations (250, 500 and 1000 µg) of copolyester were tested against the pathogenic microbes and they showed various range of zone of clearance.

Anticancer activity: The *in vitro* anticancer activity of the synthesized copolyester PDCSe is determined by MTT assay method. The % cell viability of copolyester at different concentrations on the vero cell line and A549 cell line were determined. The effect of polymer at various concentration on the vero cell line and A549 cell line is expressed as % cell viability. It is explicit from Table-3 that the copolyester showed more anticancer activity on A549 (lung cancer) cell line at low concentration (125 µg/mL).

TABLE-2
ANTIMICROBIAL ACTIVITY OF PDCSe BY WELL DIFFUSION METHOD

Human pathogen	Concentration	Zone of inhibition (percentage of inhibition)	
		PDCSe	Kannamycin (30 µg)
Klebsiella pneumoniae	1000	18 ± 1.26 (20 ± 1.40)	30.67 ± 1.52 (34.07 ± 1.38)
	500	11 ± 0.7 (12.22 ± 0.85)	
	250	–	
Bacillus subtilis	1000	14 ± 0.84 (15.55 ± 1.08)	27.00 ± 1.00 (30.00 ± 0.90)
	500	12 ± 0.84 (13.33 ± 0.93)	
	250	–	
Staphylococcus aureus	1000	13 ± 0.91 (14.44 ± 1.01)	26.00 ± 1.00 (29.25 ± 0.52)
	500	–	
	250	–	

TABLE-3
ANTICANCER EFFECT OF PDCSe ON VERO AND A549 CELL LINE

Concentration (μg/mL)	Vero cell line			A549 cell line		
	Dilutions	Absorbance (O.D)	Cell viability (%)	Dilutions	Absorbance (O.D)	Cell viability (%)
1000	Neat	0.206	44.11	Neat	0.216	26.43
500	1:1	0.236	50.53	1:1	0.282	34.51
250	1:2	0.269	57.60	1:2	0.349	42.71
125	1:4	0.303	64.88	1:4	0.413	50.55
62.5	1:8	0.330	70.66	1:8	0.479	58.62
31.2	1:16	0.362	77.51	1:16	0.535	65.48
15.6	1:32	0.395	84.58	1:32	0.594	72.70
7.8	1:64	0.427	91.43	1:64	0.649	79.43
Cell control	–	0.467	100	–	0.817	100

Conclusion

The copolyester PDCSe was synthesized by direct melt polycondensation method and its chemical structure was confirmed by FT-IR and NMR spectroscopy. The inherent viscosity of the PDCSe shows that it has high degree of polymerization. The X-ray diffraction studies indicated that copolyester PDCSe is amorphorus in nature. As the dithiane derivatives have wide properties related to biomedical field, the synthesized copolyester PDCSe using 1,4-dithiane-2,5-diol also have exhibited antioxidant, antimicrobial and anticancer activity, hence it can further investigated to make it suitable for biomedical application.

CONFLICT OF INTEREST

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

REFERENCES

1. T.S. Lu, Y.M. Sun and C.S. Wang, *J. Polym. Sci.*, **33**, 2841 (1995); https://doi.org/10.1002/pola.1995.080331618.
2. D. Byrom, *Trends Biotechnol.*, **5**, 246 (1987); https://doi.org/10.1016/0167-7799(87)90100-4.
3. J.M. Raquez, Y. Nabar, R. Narayan and P. Dubois, *Macromol. Mater. Eng.*, **293**, 310 (2008); https://doi.org/10.1002/mame.200700352.
4. B.D. Ulery, L.S. Nair and C.T. Laurencin, *J. Polym. Sci.*, **49**, 832 (2011); https://doi.org/10.1002/polb.22259.
5. H. Seyednejad, A.H. Ghassemi, C.F. van Nostrum, T. Vermonden and W.E. Hennink, *J. Control. Rel.*, **152**, 168 (2011); https://doi.org/10.1016/j.jconrel.2010.12.016.
6. M. Vert, *Biomacromolecules*, **6**, 538 (2005); https://doi.org/10.1021/bm0494702.
7. A.K. Mohanty, M. Misra and G. Hinrichsen, *Macromol. Mater. Eng.*, **276-277**, 1 (2000); https://doi.org/10.1002/(SICI)1439-2054(20000301)276:1<1::AID-MAME1>3.0.CO;2-W.
8. I. Blakey, W. Conley, G.A. George, D.J.T. Hill, H. Liu, F. Rasoul and A.K. Whittaker, Synthesis of High Refractive Index sulphur containing Polymers for 193 nm Immersion Lithography; A Progress Report, *Proc. SPIE 6153*, Advances in Resist Technology and Processing XXIII, 61530H (2006); https://doi.org/10.1117/12.659757.
9. T. Okubo, S. Kohmoto, M. Yamamoto and T. Nakahira, *J. Mater. Sci.*, **34**, 337 (1999); https://doi.org/10.1023/A:1004417908638.
10. S. Kobayashi and H. Morijiri, Polymerizable Composition Containing Novel Cyclic Sulfur Compound and Resin Obtained by Curing the Polymerizable Composition, US Patent 7132501 B2 (2006).
11. G.B. Wayton, P. Trefonas III, S. Coley and T. Kurihara, Coating Compositions for Use with an Overcoated Photoresist, US Patent 6,852,421 B2 (2005).
12. H. Yao and S.S. Ding-Lee, Organic Bottom Anti Reflective Polymer Compositions, US Patent 7470500B2 (2008).
13. W. Klauck, J. Klein, L. Duhm, W. Maler, Cyanacrylate Adhesive with Ester and Polymer Additives, US Patent 6617385B1 (2001).
14. F. Zamberlan, A. Fantinati and C. Trapella, *Eur. J. Org. Chem.*, 3248 (2018); https://doi.org/10.1002/ejoc.201701785.
15. S. Vivek Kumar, S. Muthusubramanian, J. Carlos-Menendez and S. Perumal, *Beilstein J. Org. Chem.*, **11**, 1707 (2015); https://doi.org/10.3762/bjoc.11.185.
16. C.E. Stephens, T.M. Felder and J.W. Sr, *Bioorg. Med. Chem.*, **9**, 1123 (2001); https://doi.org/10.1016/S0968-0896(00)00333-3.
17. R. Romagnoli, P.G. Baraldi, M.G. Pavani, M.A. Tabrizi, F. Fruttarolo, D. Preti, L. Piccagli, M.K. Jung, E. Hamel, M. Borgatti and R. Gambari, *J. Med. Chem.*, **49**, 3906 (2006); https://doi.org/10.1021/jm060355e.
18. R. Romagnoli, P.G. Baraldi, M.D. Carrion, C.L. Cara, F. Fruttarolo, D. Preti, M.G. Pavani, M.A. Tabrizi, M. Tolomeo, S. Grimaudo, A. Di Cristina, J. Balzarini, J.A. Hadfield, A. Brancale and E. Hamel, *J. Med. Chem.*, **50**, 2273 (2007); https://doi.org/10.1021/jm070050f.
19. D. Briel, A. Rybak, C. Kronbach and K. Unverferth, *Eur. J. Med. Chem.*, **45**, 69 (2010); https://doi.org/10.1016/j.ejmech.2009.09.025.
20. W. Wang, D. Lv, N. Qiu, L. Zhang, C. Hu and Y. Hu, *Bioorg. Med. Chem.*, **21**, 2886 (2013); https://doi.org/10.1016/j.bmc.2013.03.070.
21. R. Romagnoli, P.G. Baraldi, O. Cruz-Lopez, M. Tolomeo, A.D. Cristina, R.M. Pipitone, S. Grimaudo, J. Balzarini, A. Brancale and E. Hamel, *Bioorg. Med. Chem.*, **21**, 2746 (2011); https://doi.org/10.1016/j.bmcl.2010.11.083.
22. W. Beerheide, H.-U. Bernard, Y.-J. Tan, A. Ganesan, W.G. Rice and A.E. Ting, *J. Natl. Cancer Inst.*, **91**, 1211 (1999); https://doi.org/10.1093/jnci/91.14.1211.
23. W.G. Rice, D.C. Baker, C.A. Schaeffer, L. Graham, M. Bu, S. Terpening, D. Clanton, R. Schultz, J.P. Bader, R.W. Buckheit Jr., L. Field, P.K. Singh and J.A. Turpin, *Antimicrob. Agents Chemother.*, **41**, 419 (1997);
24. C.C. Garcia, N.A. Candurra and E.B. Damonte, *Antiviral Chem. Chemother.*, **11**, 231 (2000); https://doi.org/10.1177/095632020001100306.
25. J.A. Stammen, S. Williams, D.N. Ku and R.E. Guldberg, *Biomaterials*, **22**, 799 (2001); https://doi.org/10.1016/S0142-9612(00)00242-8.