RESEARCH ARTICLE

Synthesis, Characterisation and Cytotoxic Activity of Random Copolyester using 1,4- Dithiane- 2,5-diol

B. Kalpana[1],*, R. Nanthini[2]

[1]PG & Research Department of Chemistry, Pachaiyappa's College, Chennai 600030, India
[2]PG & Research Department of Chemistry, Pachaiyappa's College, Chennai 600030, India

***Corresponding author: E-mail: kalpanajanarthanan2005@gmail.com**

DOI: 10.5185/amp.2020.010393

A novel random copolyester Poly(1,4-dithiane-2,5-diol Succinate – Co – 1,2-ethane diol Succinate) PDES containing sulphur atom was synthesised by direct melt polycondensation method using 1,4-dithiane-2,5-diol as a special monomer, succinic acid, and 1,3 ethane diol with Titanium tetra isopropoxide as catalyst. The wide range of application of the special monomer especially as biocontrol material and food additive led us to extend our area of research towards producing copolyester with cytotoxic activity. The various physical properties of the copolyester such as inherent viscosity, glass transition temperature, solubility and crystalline nature was investigated and studied in detail. The ^{1}H-NMR and FT-IR was done to investigate the chemical properties of the PDES copolyester. *In Vitro* cytotoxicity against Normal (Vero cell line) and cancer (A549 Lung cancer cell line) by MTT Assay, Antioxidant activity by Dot-Blot/DPPH scavenging Assay and Antimicrobial by Well diffusion method studies proved that the synthesised copolyester have immense biological properties and could be used in biomedical application.

Introduction

One of the major issues in the world is environmental pollution, hence it is important to produce biodegradable products. As the polymers especially plastic play an important role in environmental degradation it is important to synthesis biodegradable polymers. The synthesis of new biodegradable polyesters has been an area of major priority nowadays as it has wide application in making films, sheets, bottles, tissue engineering systems [**1-3**], drug delivery, dental implant and scaffold for bone TE [**4**]. The Poly(propylene fumerate) was a biocompatible and biodegradeable polyester that finds wide application in drug delivery, bone tissue engineering and also used as bone cement [**5**]. After going through literature survey it has been found that synthesis of polymer with sulphur atom in main chain will have wide biological application, search for special monomer with sulphur atom was carried carefully so that the monomer have biodegradability. The Polyesters containing sulphur atoms in the main chain may find wide application as rubber modifiers and non volatile plasticizers. As the requirement for current study was satisfied by the monomer 1,4-dithiane–2,5-diol, it was selected as special monomer. A number of aliphatic polyesters have been reported with good biocompatible nature [**6-9**], hence it was decided to prepare copolyester using the monomer 1,4-dithiane–2,5-diol. A plastic polarized lens including a polarized film, and a base layer formed over at least one surface of the polarized film is sythesised. The base layer is comprised of a (thio) urethane resin obtained by using polyol containing a sulfur atom such as 1,4-dithiane-2,5-diol [**10**]. Also the polymer prepared by using special monomer 1,4-dithiane-2,5-diol was used as antireflective coating compositions and in image processing and photolithographic techniques [**11**]. The special monomer was used in sulfa-Michael/aldol-type reactions [**12**] and Gewald reactions [**13**] to synthesis thiophene and thiazole derivatives that has wide application in medicinal chemistry [**14, 15**]. The special monomer 1,4 dithiane 2,5 diol was also used in synthesis of aromatic urethane acrylates having a high refractive index [**16**]. An aromatic carbonate polymer containing an effective amount of a stabilizing compound 1, 4 dithiane 2,5 diol inhibits yellowing upon when exposed to sterilizing radiation [**17**] mainly the medical components. The special monomer 1, 4-dithiane-2,5-diol was also an food additive and biocontrol agent which exhibit high antimicrobial activity against various plant pathogens and have high inductive resistance [**18**]. As the monomer 1, 4-dithiane-2, 5-diol is environment friendly and exhibit antimicrobial activity; it led us to synthesis copolyester Poly (1,4 dithiane 2,5 diol succinate – Co – 1,2 ethane diol succinate) PDES and study its cytotoxic activity.

Experimental section

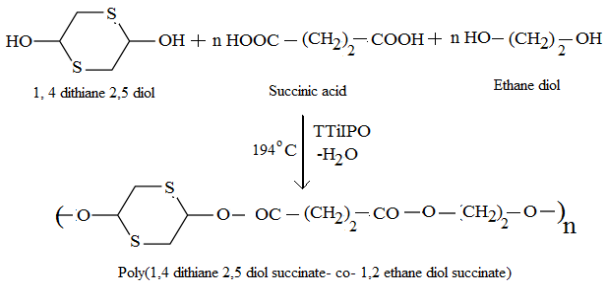
Materials and methods

1,4-Dithiane-2,5-diol, Succinic Acid and 1,2 Ethane diol were purchased from Sigma Aldrich and the catalyst Titanium tetra isopropoxide was purchased from Lancaster. The solvents and all other chemicals used were (AR grade) of Sigma Aldrich. Using $CDCl_3$ solvent ^{1}H - NMR was recorded on a Bruker 400MHz spectrometer. DSC Q200 V23.10 build Differential scanning calorimeter

is used to record DSC thermogram. FT-IR Spectra of the copolyester were recorded on Perkin Elmer 883 spectrophotometer. A Bruker B8 wide angle XRD with Cu/30Kv/15mA was used to find the crystalline nature of copolymer. MTT Assay (Mosmann 1983) method was used to determine the *In vitro* cytotoxicity in Normal cell line and A549 (lung cancer) cell line and *In Vitro* Antioxidant by Dot-Blot/DPPH scavenging Assay (Blois. M.S 1958) and antibacterial activity by Well diffusion method (Perez., C., M. Pauli and P. Bazerque. 1990) were also determined.

Synthesis of copolyester

In a three necked round bottom flask 1,4 dithiane 2,5 diol (0.01mole), 1,2 ethane diol (0.01mole) and succinic acid (0.02mole) were taken in the ratio of 1:1:2 respectively. A magnetic stirrer is added to RB to stir the mixture. The nitrogen gas is passed through left inlet to maintain inert atmosphere and in the middle inlet a guard tube filled with $CaCl_2$ is connected to absorp the water molecules formed during polymerization and the middle inlet is closed with the stopper. The entire set up is kept in the oil bath and heated upto 184°C at which all the constituents melts and then 0.8ml of the catalyst Titanium tetra isopropoxide is added slowly through the middle inlet and the same temperature is maintained for about 1 hour. The temperature is then increased to about 194°C and maintained for 2 hrs. The crude copolyester is dissolved using chloroform and then poured into the 10 fold ice cold methanol to reprecipiate the pure copolyester Poly(1,4 dithiane 2,5 diol succinate - co- 1,2 ethane diol succinate) as per **Scheme 1**.



Scheme 1

Results and discussion

Solubility and viscosity studies

The solubility of the copolyester PDES was determined by dissolving the copolyester PDES in various solvents like DMF, DMSO, THF, $CHCI_3$ etc. The copolyester was found to be soluble in all these solvents.The intrinsic viscosity of the copolyester PDES was determined by using Ubbelonde Viscometer at room temperature. The flow time of the solvent and the 1% solution of the copolyester dissolved in chloroform were determined and the intrinsic viscosity was found to be 0.752dL/g.

IR spectra

FT-IR spectrum of the Poly(1,4 dithiane 2,5 diol succinate – co 1,2 ethane diol succinate) is shown in **Fig. 1**. The absorption bands in the spectrum for corresponding groups are given in **Table 1**. The IR data reveals that a new ester bond was formed during polycondensation of monomers and the presence of dithiane diol moiety was confirmed from the IR spectrum of the 1,4 dithiane 2,5 diol.

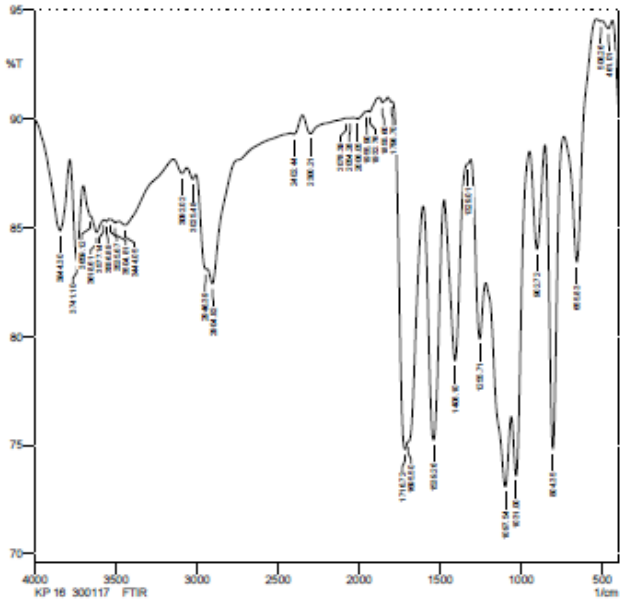


Fig. 1. FT-IR Spectrum of the copolyester PDES.

Table 1. IR Spectral data of the synthesised copolyester in cm^{-1}.

Absorption band (cm^{-1})	Moiety Responsible
1716.72	C=O stretching (Ester group)
1255.71	C-O-C stretching (Ester group)
2904.92	Aliphatic C-H Stretching
1408.1	Aliphatic C-C stretching
804.35	C-H bending (1,4 disubsituted)
1097.54	C-O stretching of dithiane
655.83	C-S Stretching of dithiane

^{1}H-NMR spectra

^{1}H-NMR spectrum of copolyester PDES is shown in **Fig. 2**. The chemical shift value of the characteristic peaks are assigned according to the protons present in the copolyester and given in **Table 2**. The chemical shift values of the special monomer are in accordance with ^{1}H-NMR spectrum of 1,4-dithiane-2,5-diol. The copolyester PDES synthesised by polycondensation method have random distribution of monomeric units as the monomers have equal reactivities [**19**] and the spectral data are in confirmative with the random distribution of monomeric units in copolyester PDES.

Table 2. ^{1}H-NMR data of PDES copolyester.

Chemical shift (ppm)	Proton Type
1.21, 1.53-1.61	$-CH_2-$ Protons
2.55-2.61	$-CH_2-CO-$ Protons
3.42 – 3.95	$-CH_2-O$ Protons
2.89- 3.18	$-CH_2-S$ Protons
3.37 – 3.39	$-CH_2-O-CO$ Protons
4.25	methylene proton attached to –OH of 1,4 dithiane 2,5 diol

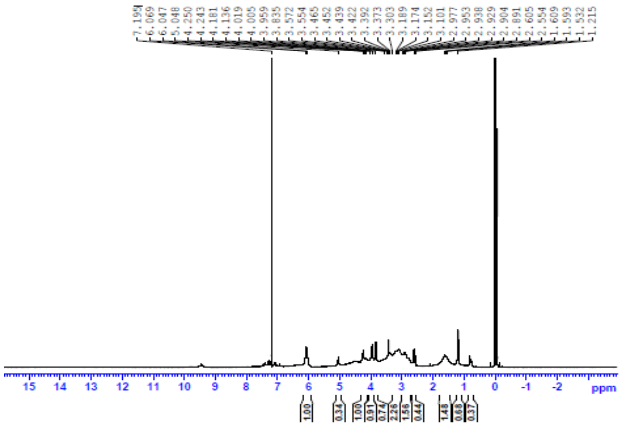


Fig. 2. H-NMR spectrum of PDES.

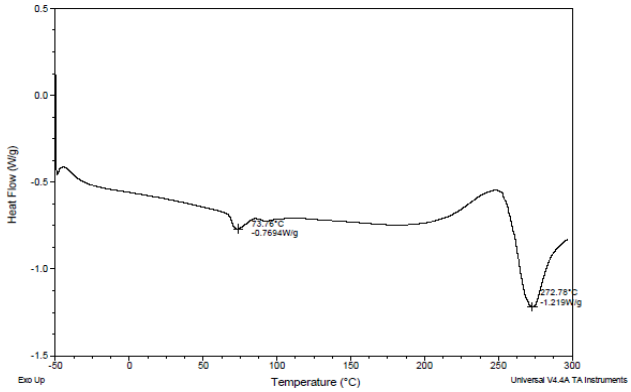


Fig. 3. DSC Thermogram of PDES.

DSC thermogram and Xray diffractogram

The DSC thermogram of PDES copolyester shown in **Fig. 3**. exhibits that the glass transition temperature (T_g) as -50ºC and the melting point temperatue (T_m) as 73.76ºC and the decomposition temperature (T_d) as 272.78ºC. The low glass transition temperature of the copolyester makes it vital for drug delivary applications [**20**]. The X ray difractogram of the PDES copolyester as shown in **Fig. 4**. exhibits amorphorus halo at 18ºC which indicates that the copolyester is amorphous in nature and showed some degree of crystallinity due to introduction of methylene group (diol/diacid) in polymer chain [**21**]. The crystalline nature of the polymer may be increased with the increase in the length of flexibe segments [**22**].

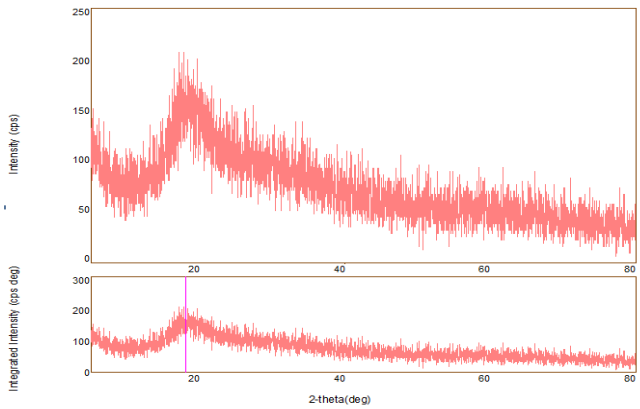


Fig. 4. X ray diffractogram of PDES.

Table 3. Antimicrobial activity of copolyester PDES against Human Pathogens.

Human Pathogen	Concentration (µg/ml)	Zone of inhibition (% of Inhibition)
Escherichia coli	1000	18±1.26 (20.00±1.4)
	500	14±0.98 (15.56±1.08)
	250	12±0.84 (13.33±0.9)
Klebsiella pneumoniae	1000	14±0.98 (15.56±1.0)
	500	12±0.84 (13.33±0.9)
	250	11±0.77 (12.22±0.8)
Bacillus subtilis	1000	13±0.91 (14.44±1.0)
	500	12±0.84 (13.33±0.9)
	250	10±0.7 (11.11±0.7)
Staphylococcus aureus	1000	13±0.91 (14.44±1.01)
	500	12±0.84 (13.33±0.93)
	250	10±0.77 (11.11±0.77)

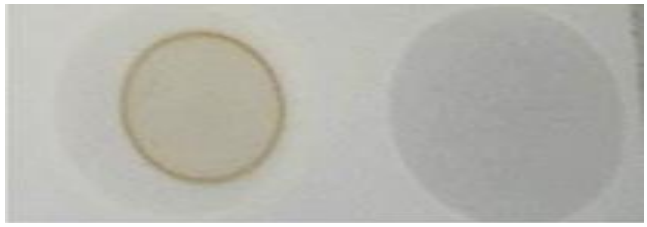


Fig. 5. Antibacterial activity of PDES by Dot blot assay.

Biological activity

The antimicrobial activity of the PDES was probed against four human pathogens and the results were given in the **Table 3**. The copolyester showed different zones of inhibition from 10-18mm for different concentration against human pathogens. The higher value for zone of inhibition indicates that the PDES has good antimicrobial activity [**23**]. The dot blot assay showed color change from yellow to purple as shown in **Fig. 5**, indicate that the copolyester have hydrogen donating tendency to reduce DPPH to DPPH-H form and thus its antioxidant property. The synthesised copolyester showed remarkable antioxidant property by *In Vitro* DPPH Scavenging assay method as it has lower IC_{50} value (58.35) and higher % of inhibition [**24**], and its activity in various concentration of copolyester is given in **Table 4**. The cytotoxic effect of copolyester at different concentration on A549 (lung cancer cell line) by MTT Assay was shown in **Fig. 6**. The effect of the copolyester on A549 (lung cancer cell line) and Vero (normal cell line) are expressed as the % cell viability as shown in **Table 5 & 6**. The IC_{50} value of the polymer for vero cell (510.10) and A549 cancer cell (57.67) indicates that the copolyester have more toxic effect on cancer cells than the normal cells. Further the lower value of IC_{50} exhibits that the copolyester have more anticancer activity against lung cancer cell line [**25**].

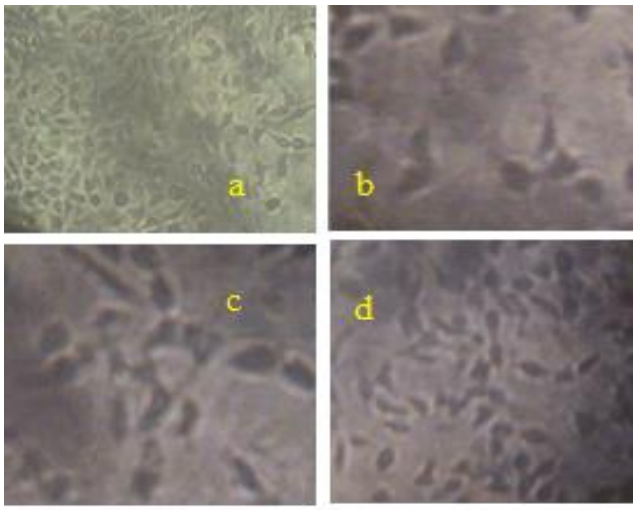
Table 4. *In Vitro* DPPH Activity of PDES.

Conc. of Copolyester (µg/mL)	% inhibition
1000	80.11 ± 5.60
500	70.62 ± 4.94
250	65.28 ± 4.56
125	55.78 ± 3.90
62.5	53.56 ± 3.74
31.25	32.93 ± 2.30
15.62	24.03 ± 1.68
IC_{50} (µg/mL)	58.35

Table 5. Anticancer activity of copolyester PDES on A549 Cell line.

Concentration (µg/ml)	Absorbance (O.D)	Cell viability (%)
1000	0.236	14.03
500	0.484	28.79
250	0.595	35.39
125	0.715	42.53
62.5	0.911	54.19
31.2	1.097	65.25
15.6	1.228	73.05
7.8	1.387	82.51
Cell control	1.681	100
IC_{50} (µg/mL)	-	57.67

Fig. 6. Anticancer Effect of PDES on A549 cell line.



a. Normal A549 cell line
b. Toxicity-1000 µg/ml
c. Toxicity-62.5 µg/ml
d. Toxicity-7.8 µg/ml

Table 6. Anticancer activity of copolyester PDES on Vero Cell line.

Concentration (µg/ml)	Absorbance (O.D)	Cell viability (%)
1000	0.607	40.82
500	0.714	49.01
250	0.836	56.22
125	0.939	63.14
62.5	1.028	69.14
31.2	1.156	77.74
15.6	1.228	82.58
7.8	1.347	90.58
Cell control	1.487	100
IC_{50} (µg/mL)		510.10

Conclusion

The structure of the repeating units in the copolyester PDES was confirmed based on the FT-IR and NMR Spectral data. The amorphorus nature of the copolyester was confirmed by XRD data.The copolyester PDES exhibited good antimicrobial and antioxidant property. The cytotoxic effect of PDES on A549 cell line is remarkable and futher studies can be carried to know its drug delivary applications.

Keywords

Copolyester, 1,4-dithiane-2,5-diol, anticancer, antioxidant.

References

1. Kulkarni, R.K.; Pani, K.C.; Neuman, C.; Leonard, F.; *Arch. Surg.*, **1966**, *93*, 839.
2. Schneider, E. E.; Polistina, R. A.; U.S. Patent 3463158, **1969**.
3. Schneider, A. K.; U.S. Patent 3636956, **1972**.
4. Wasserman, D.; Versfelt, C. C.; U.S. Patent 3839297, **1974**.
5. Kasper, F. K.; Tanahashi, K.; Fisher, J. P.; Mikos, A. G.; *Nat. Protoc.*, **2009**, 4, 518.
6. Nikolic, M. S.; Poleti, D.; Djonlagic, J.; *Eur. Polym. J.*, **2003**, *39*, 2183.
7. Tserki, V.; Matzinos, P.; Pavlidou, E.; Panayiotou, C.; *Polym. Degrad. Stab.*, **2006**, *91*, 377.
8. Liu, C.; Zeng, J. B.; Li, S. L.; He, Y. S.; Wang, Y. Z.; *Polym. J.*, **2012**, *53*, 481.
9. Du, J.; Zheng, Y.; Chang, J.; Xu, L.; *Eur. Polym. J.*, **2007**, *43*, 1969.
10. Miura, T.; Akutsu, N.; U.S. Patent 0058089 A1, **2017**.
11. Huirong Yao.; Shuji Ding-Lee.; U.S. Patent 0020557 A1, **2007**.
12. Baricordi, N.; Benetti, S.; Bertolasi, V.; De Risi, C.; Pollini, G. P.; *Tetrahedron*, **2012**, 68, 208.
13. Carl, J. M.; Lukas, E.; Gary, C. W.; Ian, R. B.; *Beilstein J. Org. Chem.*, **2015**, *11*, 875.
14. Zamberlan, F.; Fantinati, A.; Trapella, C.; *Beilstein J. Org. Chem.*, **2018**, *25*, 1707.
15. Khalil, A. M.; Gouda, M. A.; Berghot, M. A.; *Eur. J. Med. Chem.*, **2009**, *44*, 4434.
16. Stockel, N.; Bruder, F. K.; Weikard, J.; U.S. Patent 7981987 B2, **2011**.
17. Nelson, L. H.; Avakian, R. W.; Factor, A.; U.S. Patent 4880850, **1989**.
18. Young Soon Kim.; Jin-Cheol Kim.; Hyo Hyun Seo.; Gyung Ja Choi.; Ae Ran Park.; W.O. Patent 120955 A1, **2008**.
19. Ahn, B. D.; Kim, S. H.; Kim, Y. H.; Yang, J. S.; *J. Appl. Polym. Sci.*, **2001**, *82*, 2808.
20. Yamini, B.; Nanthini, R.; *Rasayan J. Chem.*, **2018**, 11, 413.
21. Narendran, K.; Nanthini, R.; *Int. Sch. Res. Notices.*, **2014**.
22. Chen, B. K.; Tsay, S. Y.; Chen, J. Y.; *Polym. J.*, **2005**, *46*, 8624.
23. Hossein, J.; Jelas Haron, M. D.; Mohd Halim Shah Ismail.; Roshanak Rafiee, M.; Leili Afsah, H.; Yadollah, A.; Majid, R.; Nazanin, V.; *Dig. J. Nanomater. Bios.*, **2013**, *8*, 1263.
24. Sinaga, S. M.; Sudarmi, S.; Iksen, I.; Kevin, K.; Sari, M. P.; *Rasayan J. Chem.*, **2018**, *11*, 1604.
25. Ariharan, V. N.; Prasad, P. N.; Rasayan. *J. Chem.*, **2014**, *7*, 260.