Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(3):1452-1461

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, characterization and cytotoxicity of certain itaconic acid based biodegradable aliphatic copolyesters

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ABSTRACT

Polymer, poly[butylene succinate-co-butylene itaconate] (PISB) and poly[hexylene succinate-co-hexylene itaconate] (PISH) were synthesized by melt polycondensation using succinic acid, itaconic acid and butanediol/hexanediol in presence of Titanium tetraisopropoxide. Prepared biodegradable polyester based on Itaconic acid was characterized by IR, ^{1}H and ^{13}C-NMR. The inherent viscosity of the polyesters was elucidated by viscometer. Thermal stability of polyesters was studied by using DSC analysis. Anti cancer activity of the synthesized copolymer was tested against human breast cancer (MCF-7) cell lines. Both the polyesters show good IC_{50} value at lower concentration but among them PISB has higher toxicity on MCF-7 cells.

Keywords Succinic acids, Itaconic acids, Copolyesters, Anti cancer, MCF-7.

INTRODUCTION

To date, several copolyesters have been synthesized and notable work has been conducted in the preparation, characterization and use of polymers [1]. Direct melt polycondensation is a very useful method for preparation of polyesters since it is eco-friendly when compared with other polymeric synthesis technique and hence it comes under green chemistry [2].The aliphatic copolyesters are widely used in the fields like pharmaceutical, biomedical and especially in drug delivery. Some polymers are used mainly to carry out the systematic release of antineoplastic drugs. Many polyanhydrides, the poly (lactide), poly (glycolate) and poly(lactide-co-glycolate) are used as a drug carrier [3-5]. Generally drug delivery devices were produced by mixing the polymers with drugs and treated on the affected tissue after surgery. But it has a drawback, which makes tissue illness after surgery which lead to second drug delivery to recrudescent illness such as glicomer [6] and to overcome this difficulty, tablet coating method was used [7-8]. The advantage in tablet coating technique are non surgery, low risk, less expensive, repeatable and controlled release which is similar to injectable drug carrier[6]. The application of polyester in durg delivery system mainly concentrated on degradation of the polymers. The enzymatic degradation of the polyesters doesn't only depend on the structure of the polyesters but also focused on hydrophilic/hydrophobic balance within the structure and their specific solid state morphology [9]. The polyesters prepared from the monomer having six carbon are readily hydrolyzed than from monomers having lesser or higher than six carbons in a chain of polyester leads to less degradation [10-11]. The biodegradation of the polymer is mainly influenced by amorphous nature and chemical structure [12]. In this study, two polymers have been synthesized from succinic acid, itaconic acid and butanediol/hexanediol by direct melt polycondensation method. All the monomers used are non-toxic, biodegradable and biocompatibe which are already reported in the literature [13-15]. The structure of the synthesized polymer was confirmed from FT-IR, ^{1}H-NMR and ^{13}C-NMR. Decomposition temperature of the polymers was determined by

DSC spectra. The anti cancer activity of the polymers was evaluated in MCF-7 cells which are found to have good IC_{50} value.

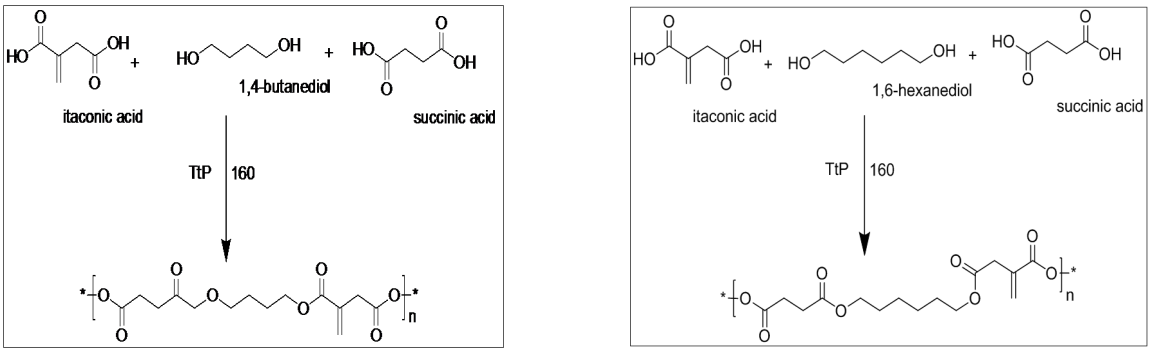
EXPERIMENTAL SECTION

2.1. Materials

Itaconic acid (IA), succinic acid (SA), 1,4-butanediol (BD) and 1,6-hexanediol (HD) were purchased from Sigma Aldrich. Titanium tetraisopropoxide (TtiPO), used as a catalyst was purchased from Lancaster. All other chemicals and solvents (AR Grade) were used as such.

2.2. Synthesis of copolyesters

The aliphatic copolyesters were synthesized by a two-step melt polycondensation and transesterification method as follows. A mixture of SA (0.01 mol), IA (0.01 mol) and BD/HD (0.02 mol) with 0.1 mmol of TtiPO as catalyst taken in reaction flask was slowly heated to 160°C and stood for 2 h under dry nitrogen atmosphere, after 2 h the temperature was further increased to 190°C and kept under *vacuum* for 1 h to remove the traces amount of water. The copolyester obtained were purified by dissolution in $CHCl_3$ and reprecipitated in 10 fold of ice cold methanol, then dried in a *vacuum* at 40°C.



PISB **PISH**

Scheme 1. Synthetic route of copolyester

2.3. Instruments

FT-IR spectra of copolyesters were recorded using Perkin Elmer IR spectrometer in the range of 4000-400 cm^{-1} using KBr pellets. ^{1}H NMR and ^{13}C-NMR spectra were recorded on a Bruker 300 MHz instrument. Viscosity of polyesters was performed in $CHCl_3$ at room temperature using Ubbelohde Viscometer. DSC thermograms were recorded on a DSC Q200 V24.10 Build 122 differential scanning calorimeter. About 2-4 mg of the polymer sample was heated in an aluminium pan with pierced lid under air atmosphere at a scanning rate of 10°C/ min between the temperatures 0°C and 500°C.

2.4. Anti-cancer evaluation

The biocompatibility (cytotoxicity) of polymers on MCF-7 was determined by MTT assay (cell viability). The cells were incubated in the presence of different concentration of the sample in 0.1 % DMSO for 48 h at 37°C, then the sample solution is removed and washed with pH 7.4 phosphate buffered saline after 4 h the sample was incubated in 0.04 M HCl/isopropanol. Viable cells were determined by absorbance at 570 nm using UV- spectrometer. IC_{50} was determined graphically from the effects of polymers on MCF-7 which was expressed as the % cell viability using the formula,

$$\% \text{ cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of controlled cells}} \times 100$$

RESULTS AND DISCUSSION

3.1. Viscosity measurement

Inherent viscosity of the polymers was reported using chloroform at RT at the concentration of 1mg/ml, in presence of Ubbellohde viscometer by studying the values of flow time of pure solvent and polymer. The inherent viscosity of the polymer PISB and PISH is 0.68dL/g and 0.72dL/g respectively.

3.2. IR spectral studies

FT-IR of synthesized copolyesters are taken and presented in **Fig 1 & 2**. The synthesized copolyesters showed characteristic absorption band for ester carbonyl stretching, C-O-C stretching and methylene groups as shown in the **Table 1.** Formation of ester bond during the polycondensation can be confirmed from the specral data.

3.3. NMR spectral studies

^{1}H NMR spectra are shown in **Fig 3 & 4.** The assignments of the characteristic peaks are given in **Table 2**. ^{13}C NMR Spectra are shown in **Fig 5 & 6** and the chemical shift value of characteristic peaks along with their assignment are given in **Table 3**. All these characteristic peaks in the spectra of polymers show a random distribution of the monomers in it.

3.4. Thermal studies

The DSC thermograms of the polymers PISB and PISH are presented in **Fig 7 & 8.** These thermograms show glass transition temperature (Tg) at -26ºC and -28ºC, melting temperature (Tm) at 60ºC and 56ºC and decomposition temperature (Td) at 406ºC and 378ºC for the polyesters PISB and PIHS respectively. The decomposition temperatures observed from the TGA of polymers are in good agreement with the TGA values.

3.5. Anti-cancer evaluation of synthesized polymers

For polymers PISB and PISH, IC_{50} is determined graphically and are shown in **Fig 10 & 12** and the affected MCF-7 cell line at different concentration is shown in **Fig 9 & 11**. The effect of polymers against MCF-7 cell line is determined and shown in **Table 4 & 5.**

Table 1 IR Spectral data of copolyesters PISB & PISH

S.No.	Absorption frequency cm^{-1}		Assignment
	PISB	PISH	
1	1745	1747	C=O stretching of ester group
2	1128	1131	C-O stretching of ester group
3	2999	2998	aliphatic C-H stretching
4	2384	2378	aliphatic C-CH stretching of IA

Table 2 ^{1}H NMR spectral data of copolyesters PISB&PISH

S.No.	Characteristic peak ppm		Assignment
	PISB	PISH	
1	1.6-4	1.2-3.8	CH_2
2	5.8	6.3	HCC(O)O of IA
3	6.2	7.4	HCC(O)O of SA

Table 3 ^{13}C-NMR spectral data of copolyesters PISB & PISH

S.No.	Characteristic peak (ppm)		Assignment
	PISB	PISH	
1	171.9	171	O-C=O
2	63.5	71	CH_2 (O)
3	28.5	27-30	CH_2
4	133.8	131	C=C of IA

Fig. 1 FT-IR spectrum of copolyester PIFB

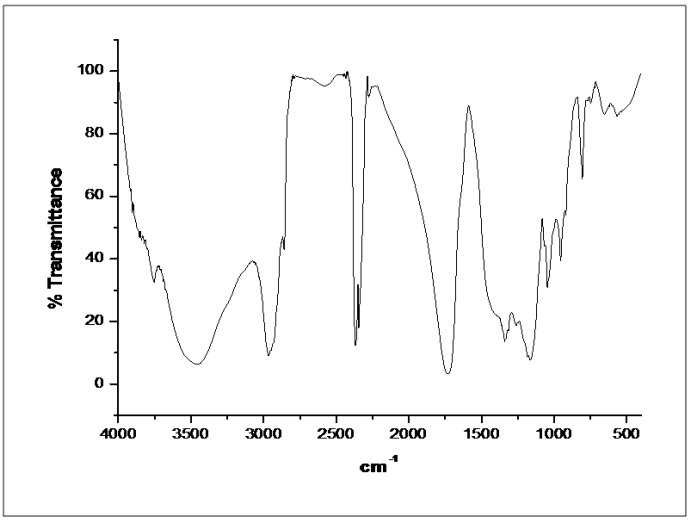


Fig. 2 FT-IR spectrum of copolyester PIFB

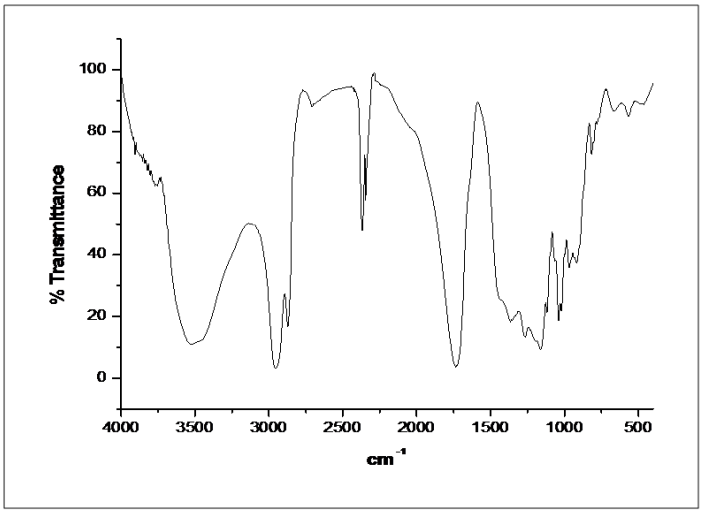


Table 4 Anticancer effect of copolyesters PISB on MCF7 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.09	16.66
2	500	1:1	0.15	27.77
3	250	1:2	0.21	38.88
4	125	1:4	0.26	48.14
5	62.5	1:8	0..34	62.96
6	31.2	1:16	0.42	77.77
7	15.6	1:32	0.46	85.18
8	7.8	1:64	0.50	92.59
9	Cell control	-	0.54	100

Fig. 3 ^{1}H-NMR spectra of copolyester PISB

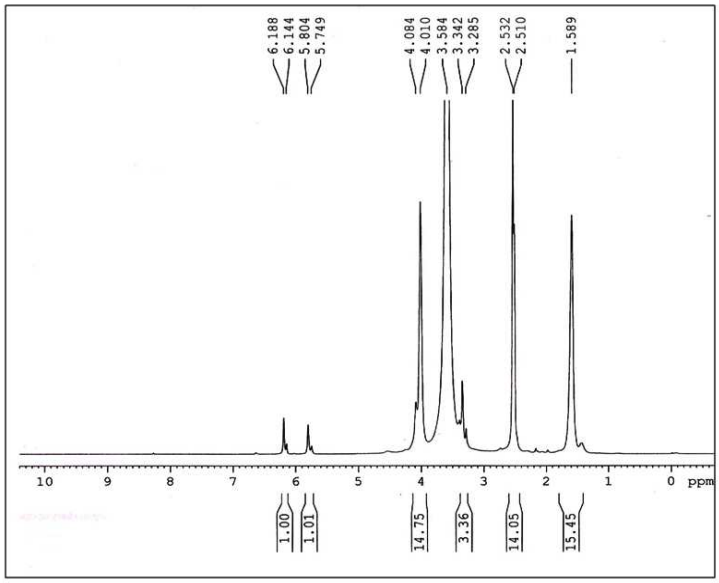


Fig. 4 ^{1}H-NMR spectra of copolyester PISH

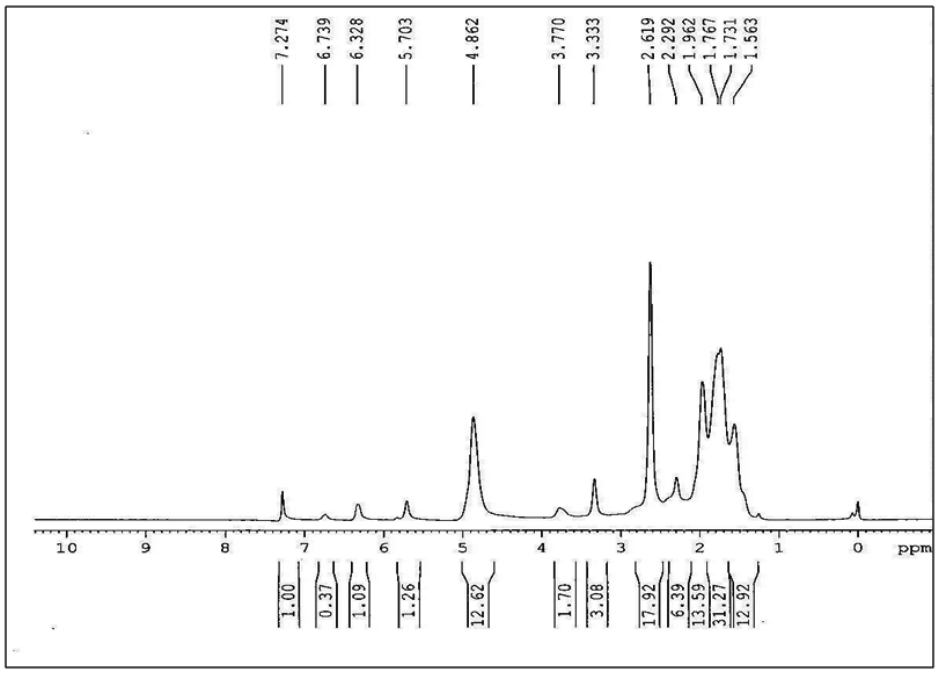


Fig. 5 ^{13}C-NMR spectra of copolyester PISB

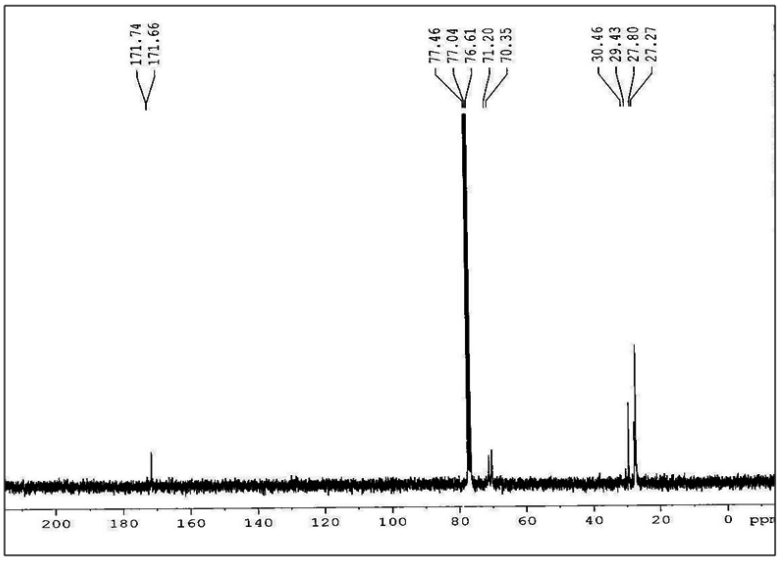


Fig. 6 ^{13}C-NMR spectra of copolyester PISH

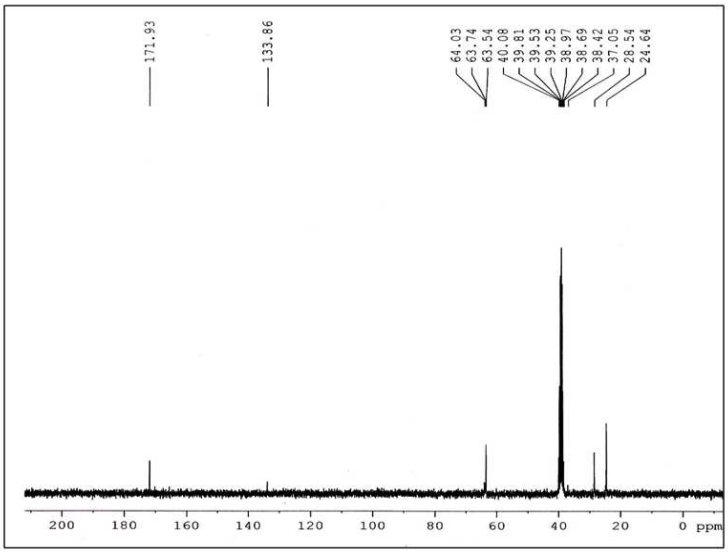


Table 5 Anticancer effect of copolyesters PISH on MCF7 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.10	17.85
2	500	1:1	0.13	23.21
3	250	1:2	0.19	33.92
4	125	1:4	0.23	41.07
5	62.5	1:8	0.28	50.00
6	31.2	1:16	0.35	62.50
7	15.6	1:32	0.41	73.21
8	7.8	1:64	0.47	83.92
9	Cell control	-	0.56	100

Fig. 7 DSC thermogram of polymer PISB

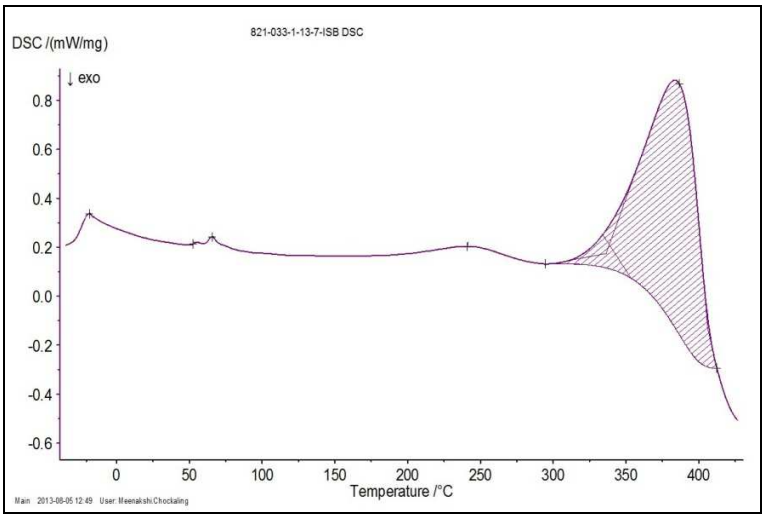


Fig. 8 DSC thermogram of polymer PISH

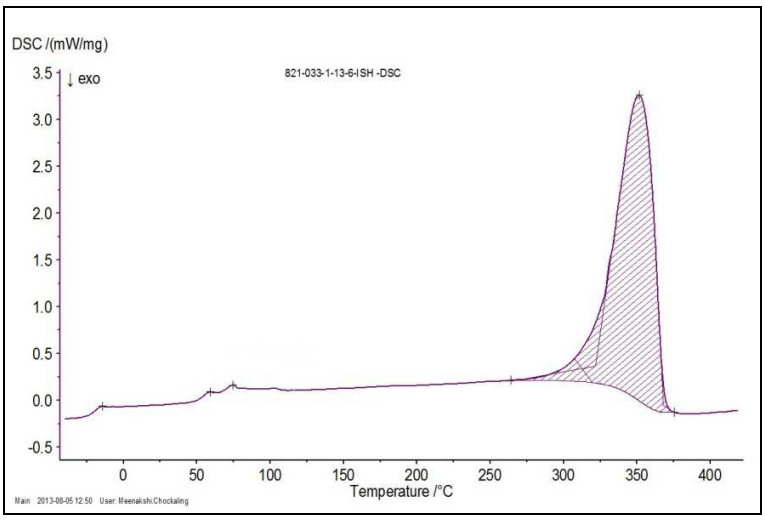


Fig. 9 Cytotoxic anticancer evaluation of polymer PISB on MCF-7 cell line at different concentrations (a) Normal MCF-7 cell line (b) 1000µg/ml (c) 125µg/ml (d) 62.5µg/ml(IC50) and (e) 31.2µg/ml

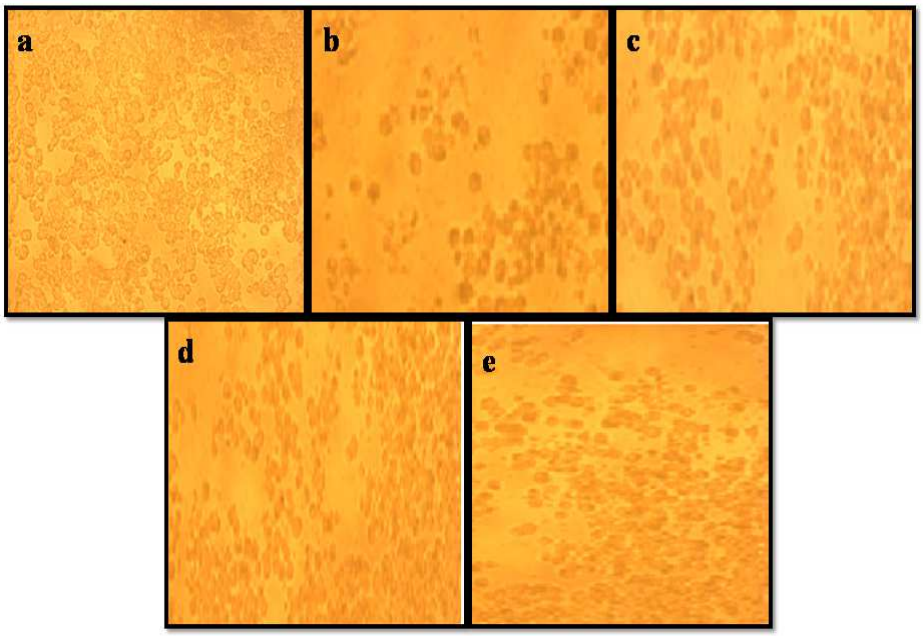


Fig. 10 Graphical representation of polymer PISB on MCF-7 cancer cell line

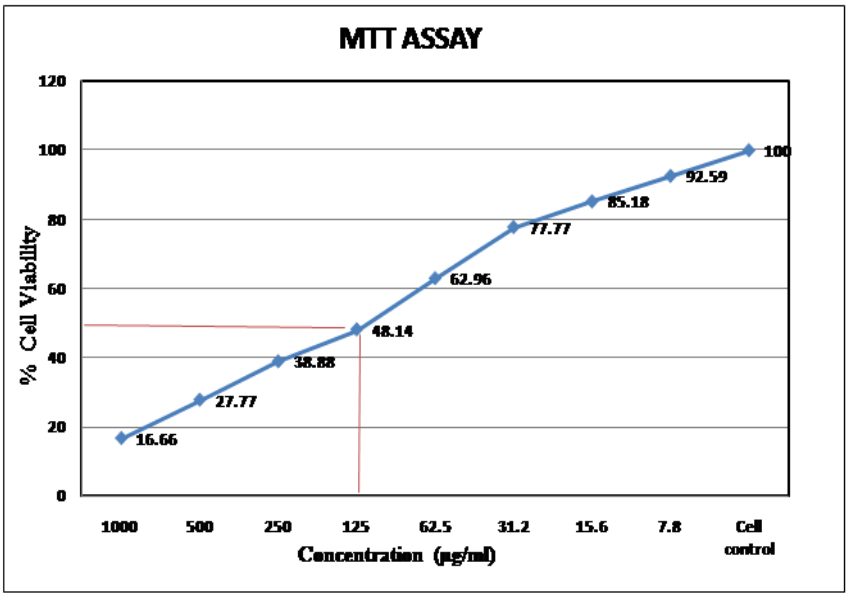


Fig. 11 Cytotoxic anticancer evaluation of polymer PISH on MCF-7 cell line at different concentrations (a) Normal MCF-7 cell line (b) 1000µg/ml (c) 125µg/ml (d) 62.5µg/ml(IC50) and (e) 31.2µg/ml

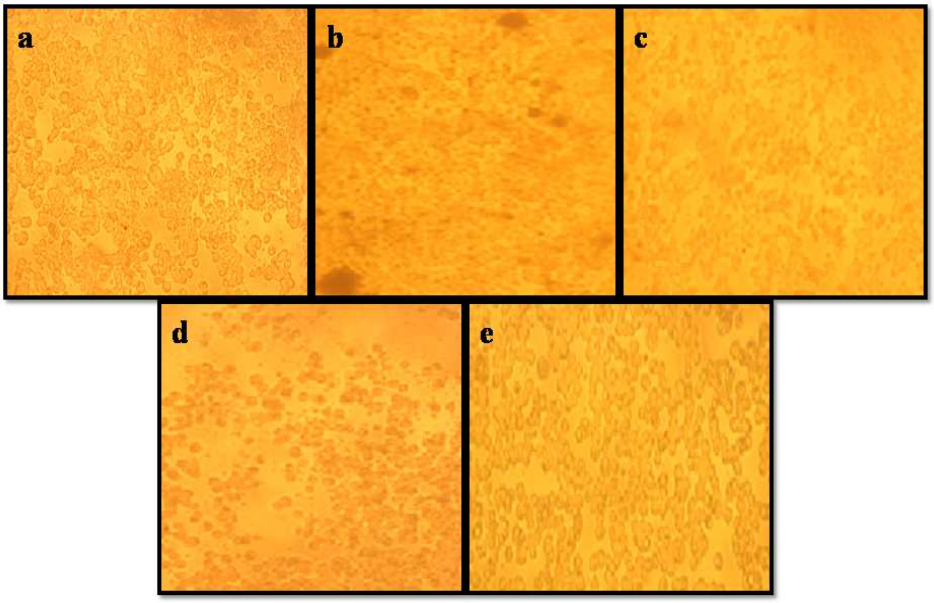
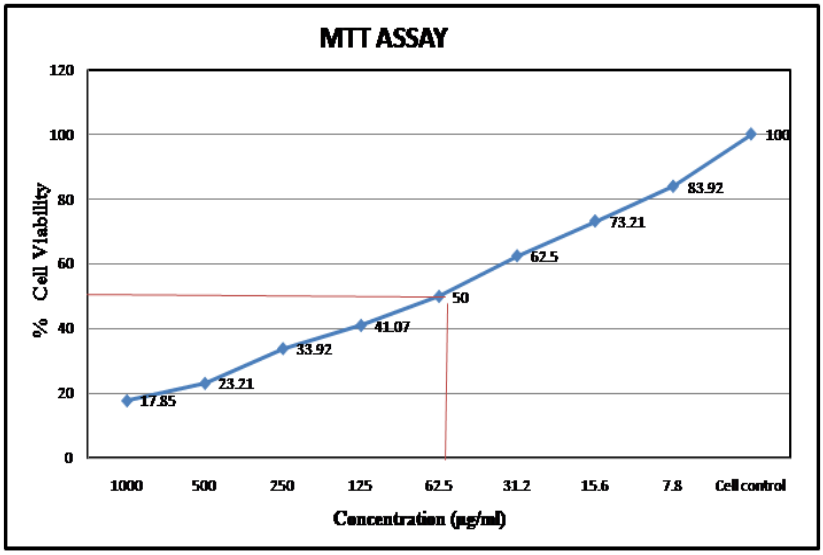


Fig. 12 Graphical representation of polymer PISH on MCF-7 cancer cell line



CONCLUSION

The polyesters PISB and PISH were synthesized by transesterification and melt polycondensation method and was characterized by the inherent viscosity values indicate the formation of polymers, IR spectral data confirmed the ester linkage and NMR spectral data ascertain the structure of the polymer repeat unit. The thermal data reveals that they are thermally stable and biodegradable. The anti-cancer property of the polyesters are expected a long way in the present research field of biomedical polymers.

Acknowledgements
The authors are thankful to the PG & Research Department of Chemistry, Pachaiyappa's College, Chennai for providing laboratory facilities to carry out this work.

REFERENCES

[1] F Migneco; YC Huang; RK Birla; SJ Hollister, *Biomaterials*, **2009**, 30, 6479-6484.
[2] T Kajiyama; T Taguchi; H Kobayashi; K Kataoka; J Tanaka, *Polymer Degradation and Stability*, **2003**, 81, 525-530.
[3] KE Uhrich; A Gupta; TT Thomas; C Laurencin; R Langer, *Macromolecules*, **1995**, 19, 2184-2193.
[4] DS Muggli; AK Burkoth; SA Keyser; HR Lee; KS Anseth, *Macromolecules*, **1998**, 31, 4120-4125.
[5] P Sampath; H Brem, *Cancer control*, **1998**, 2, 130-137.
[6] WX Guo; KX Huang; R Tong; HB Xu, *Journal of Controlled Release*, **2005**, 107, 513-522.
[7] LL Augsburger; RP Shangra; RP Gannini; VP Shah; VK Prasas, D. *Bro Pham. Sci*., **1983**, 72, 876.
[8] British Pharmacopoeia, **1988**, Vol II, A 141.
[9] G Montawdo; P Rizzarelli, *Polymer Degradation and Stability*, **2000**, 70, 305-314.
[10] R Field; F Rodriguez; RK Finn, *Journal applied polymer science*, **1974**, 18, 357.
[11] R Field; F Rodriguez; JM Sharply; AM Kaplan, *International Biodegradation symposium, applied science*, **1976**, 775.
[12] XH Li; SC Tjong; YZ Meng; Q Zhu, *Journal of polymer science part B: polymer physics*, **2003**, 41, 1806-1813.
[13] W Guo; Z Shi; K Liang; Y Liu; X Chen; W Li, *Polymer Degradation and Stability*, **2007**, 92, 407-413.
[14] J Margaret Marie; R Puvanakrishnan; R Nanthini, *Journal of Chemical Pharmaceutical Research*, **2012**, 4, 175-179.
[15] L Sowbagyalakshmi Prabha; R Nanthini; G Krishnaveni, *Journal of Chemical Pharmaceutical Research*, **2012**, 4, 2442-2457.