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Photocrosslinking Property of Certain Synthesized Bis(arylidene)cycloalkanone based Random Copolyesters with Computational Support and their Anticancer Study

B. Kiruba*, S. Chidambaravinayagam

PG & Research Department of Chemistry, Rajeswari Vedachalam Government Arts College, Chengalpattu, Affiliated to University of Madras, Chennai - 603 001, India

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Abstract

The synthesis of random copolyesters involves polycondensation of arylidene diol with curcumin and glutaryl chloride in 1:1:2 ratio. For this monomers, 2,6-bis-(4-hydroxy benzylidene) cyclohexanone, 2,6-bis-(4-hydroxy-3-methoxybenzylidene)cyclohexanone, 2,5-bis-(4-hydroxybenzylidene)cyclopentanone and 2,5-bis-(4-hydroxy-3-methoxy benzylidene)cyclopentanone were synthesized using acid catalyzed Claisen-Schmidt reaction. Qualitative solubility tests reveal that prepared copolyesters dissolve well in polar solvents. The successful formation of copolyesters was confirmed by Fourier transform-infrared spectroscopy and proton nuclear magnetic resonance spectroscopic techniques. Glass transition temperature of prepared polymers was calculated using differential scanning calorimetric analysis. Further, the prepared copolyesters were utilized as in vitro anticancer agents against breast cancer MCF7 cells. The experimental results of phototcrosslinking property of the copolyesters was compared with that of computational method by DFT calculations which shows coincidence of both.

Keywords: Arylidene-ketones; Curcumin, Copolyesters; Glass transition temperature; Photocrosslinking; Computational study; Anticancer activity.

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1. Introduction

Polymers are very attractive materials that can be tailored for specific needs and functionalities. Particularly, aromatic-aliphatic polymers possess wide range of applications in various fields. Aromatic polyesters are biodegradable [1] and they are of greater importance because of the property of withstanding the extreme conditions. Since it has potential mesogenic [2] and photoactive unit, arylidene-ketones have reckoned macromolecular chemists. Their inclusion in the polymeric backbone has passed on thermotropic liquid crystalline property [3,4]. Arylidene cycloalkanones were included in medical therapy and chemotherapy [5]. Bisbenzylidene cycloalkanones are versatile photo-active molecules and have already established their importance in medicinal applications [6], materials science and biomedical applications [7]. They also display

* *Corresponding author*: akpolyesters@gmail.com

photocrosslinking property [8-10], electrical properties [11], crystallinity [12], photo and halochromic responsiveness [13]. Copolyesters containing arylidene-ketones in the main chain were reported to exhibit antibacterial activity [14], antioxidant [15] and anti-inflammatory [16] activities.

Curcumin is a kitchen spice and a dietary phytochemical. It is a hydrophobic polyphenol identified as 1,7-bis(4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione or diferuloylmethane found in the dried rhizomes of plant turmeric, Curcuma longa and also highly pleiotropic [17] molecule with numerous targets and mechanisms of action. Its analogues are found to be cytotoxic [18], antimalarial [19], anticancer [20], antioxidant [21], anti-inflammatory [22] and anti-tumour [23] activities.

Copolyesters synthesized in this work have repetitive ester bonds obtained by the copolymerization of curcumin as a common diol with diacid chloride namely glutaryl chloride. Four polymers were synthesized by varying diols in the mole ratio of 2:1:1 by solution polycondensation method. Characterization of the synthesized copolyesters was carried out using various physical techniques and their photo-crosslinking property was determined and it was supported by computational studies.

Photo crosslinking studies were carried out so far for diacid chlorides used in the synthesis of copolyesters with even number of carbon atoms and in the current work glutaryl chloride is used which has odd number of carbon atoms i.e., three which was found to be successful by both experimental and computational studies.

2. Experimental

2.1. *Materials and methods*

Sigma Aldrich samples of curcumin and glutaryl chloride were used for the copolymerization process. Vanillin (SRL, India), 4-hydroxybenzaldehyde, cyclohexanone (Finar Reagents, India) and cyclopentanone (Spectrochem, India) were used as received. To prepare the four arylidene-diols, concentrated H_2SO_4 was used as catalyst. Ethanol (Merck, India) was used for the precipitation of copolyesters besides the preparation of the aryldene diols. Dimethyl acetamide (DMAc), dimethyl formamide (DMF), dimethyl sulphoxide (DMSO), ethyl acetate (EtOAc), tetrahydrofuran (THF), $CHCl_3$, acetone, benzene and n-hexane were purchased from SD fine AR chemicals, India. DMSO-d6 and $CDCl_3$ (Sigma Aldrich, USA) were used as internal standards to record NMR.

The synthesized copolyesters were characterized by solubility studies in various solvents qualitatively and viscosity measurements at a concentration of 0.1 g dL^{-1} by the usage of Ubbelohde viscometer at 30 ºC. Fourier transform infrared (FT-IR) instrument (Shimadzu, Japan, IR Affinity 1) was used for recording FT-IR spectra of copolyesters. ^{1}H-NMR (BRUKER AV III 500 MHz, Japan,JEOL ECA) spectra was taken in DMSO-d6 solvent. Differential scanning calorimeter (DSC) thermograms were recorded by Polyma 214, Netzsch, Germany. Anticancer activity for the synthesized polymers was carried out by MTT assay. To study the photo-crosslinking behavior of copolyesters, JASCO V650

UV-Visible spectrophotometer (UV-Vis) was used in which the UV light was irradiated from a mercury source (125W - 365 nm) at a distance of 9 cm in HVAR 123, Heber Annular Photochemical reactor model on copolyester samples in DMF solution whose concentration was about 0.02 g dL^{-1} at different intervals of time.

Density functional theory (DFT) calculations have been used for the optimization of the crosslinked polymer at BP86 [24,25] level and TZP [26-30] basis set using the Amsterdam Density Functional (ADF2019.105) [31] software. Frequency analysis was done for the confirmation of the optimized structures as there was no imaginary frequencies that correspond to their lowest energy conformation. Frontier Molecular Orbital (FMO) analysis was performed to study the chemical reactivity and kinetic stability of the molecule. The UV-absorption spectra of the cross-linked polymer in DMF were obtained using Time dependent density functional theory (TDDFT).

MTT assay [32] was used for determining the anti-cancer activity of samples on MCF7 cells. Cell lines were acquired from NCCS Pune. In 96-well plates of 0.2 mL of medium/well, cells (1×10^5/well) were plated and incubated in a 5 % CO_2 incubator for 72 h. Subsequently, different concentrations of polymers in 0.1 % DMSO were added and incubated for 24 h in a 5 % CO_2 incubator. Images were taken using an Inverted microscope 40X. After the sample solution was removed, MTT reagent (20 µL) was added to all wells. Viable cells were determined by measuring the absorbance at 540 nm. Lethal concentration, i.e., 50 % inhibition of cell viability (IC50 value) was calculated from the graph by applying the formula:

% cell viability = A540 of treated cells / A540 of control cells × 100 %

2.2. *Synthesis of arylidene-keto diols*

The monomers 2,6-bis(4-hydroxybenzylidene)cyclohexanone (BHCH), 2,6-bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone (BVCH), 2,5-bis(4-hydroxybenzylidene) cyclopentanone (BHCP) and 2,5-bis(4-hydroxy-3-methoxybenzylidene)cyclopentanone (BVCP) were synthesized using the procedure reported by Arumugasamy [33].

2.3. *Preparation of 2,6-bis(4-hydroxybenzylidene)cyclohexanone*

4-hydroxybenzaldehyde (0.05 mol) was dissolved in 100 mL of ethanol followed by the addition of cyclohexanone (0.0254 mol). To this ethanolic solution, 1mL of Conc. H_2SO_4 was added dropwise with constant shaking and the mixture was maintained at normal temperature for 12 h and the precipitate thus obtained as a crude product (BHCH) was filtered, washed few times with water and then crystallized using chloroform. Yield: 90 %; m.p. > 250 °C; FT-IR (KBr pellet, cm^{-1}) 3383, (b, O-H), 1651(s, C = O); ^{1}H-NMR (400 MHz, in DMSO-d6, δ) 3.11 (s, 4H), 6.88– 7.65 (m, 10H) and 9.61 (s, 2H).

2.4. *Preparation of 2,6-bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone*

By adopting the same procedure as described above, cyclohexanone (0.02 mol) and vanillin (0.04 mol) instead of 4-hydroxy benzaldehyde was used to synthesize BVCH. Yield: 88 %, m.p. 182 °C; FT-IR (KBr pellet, cm^{-1}) 3383 (b, OH), 1652 (s, C = O); ^{1}H-NMR (400 MHz, in DMSO-d6, δ) 2.93 (s, 4H), 3.91 (s, 6H), 6.92–7.79 (m, 10H) and 9.61 (s, 2H).

2.5. *Preparation of 2,5-bis(4-hydroxybenzylidene)cyclopentanone*

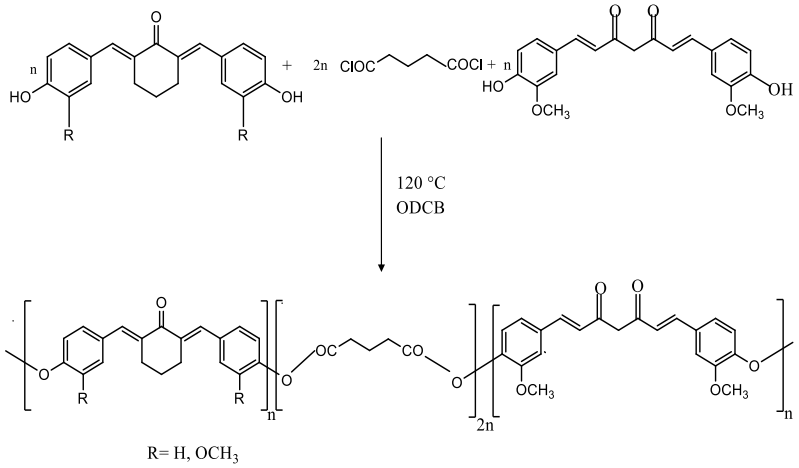
BHCP was synthesized using the same procedure as that of BHCH**.** Cyclopentanone (0.0254 mol) instead of cyclohexanone and 6 g of 4-hydroxybenzaldehyde (0.05 mol) was used. Yield: 93 %, m.p. > 250 °C; FT-IR (KBr pellet, cm^{-1}) 3300 (b, O-H), 1667 (s, C = O); ^{1}H-NMR (400 MHz, in DMSO-d6, δ) 3.11 (s, 4H), 6.85–7.67 (m, 10H) and 9.61 (s, 2H).

2.6. *Preparation of 2,5-bis(4-hydroxy-3-methoxybenzylidene)cyclopentanone*

To prepare BVCP, cyclopentanone (0.033 mol) and vanillin (0.066 mol) were used and the procedure is same as mentioned for the above monomers. Yield: 88 % m.p. 212 °C; FT-IR (KBr pellet, cm^{-1}) 3448 (b, OH), 1667 (s, C = O); ^{1}H-NMR (400 MHz, in $CDCl_3$, δ) 3.10 (s, 4H), 3.94 (s, 6H), 6.63–7.94 (m, 10H) and 9.61 (s, 2H).

2.7. *Synthesis of copolyesters*

Random copolyester was prepared by solution polycondensation method shown in Scheme 1.



Scheme 1. Synthesis of the random copolyester.

The monomer BHCH (1.6 mmol) and curcumin (1.6 mmol) were dissolved in 10 mL of ODCB in a 100 mL round-bottomed flask. To the mixture, glutaryl chloride (3.2 mmol) was added and the stirring was continued at 120 °C for 12 h. The obtained copolyester was precipitated in 100 mL of n-hexane, filtered, recrystallized in ethanol and dried under vacuum. Table 1 reveals the information about the monomers used for the preparation of copolyesters with their corresponding codes.

Table 1. Monomers used for polymerization, copolyester code of the copolyesters with their inherent viscosities and glass transition temperatures

S. No.	Diol-I	Diol – II	Diacid Chloride - I	Copolyester Code	η_{inh} (dL/g)	T_g (°C)
1	BHCH	Curcumin	Glutaryl Chloride	PGCA	0.47	115.87
2	BVCH	Curcumin	Glutaryl Chloride	PGCB	1.36	117.72
3	BHCP	Curcumin	Glutaryl Chloride	PGCC	0.24	120.24
4	BVCP	Curcumin	Glutaryl Chloride	PGCD	1.15	134.72

The copolyesters PGCB, PGCC and PGCD were prepared by adopting the same procedure using the diols BVCH, BHCP and BVCP respectively.

3. Results and Discussion

The synthesized copolyesters are completely soluble in solvents such as DMAc, DMF, DMSO and EtOAc which are highly polar, partially soluble in THF, ethanol, $CHCl_3$ and acetone, and insoluble in benzene and hexane. Table 2 represents the solubility results of the copolyesters.

Table 2. Solubility of the copolyesters in common organic solvents.

Copolyester	Hexane	Benzene	$CHCl_3$	THF	Acetone	Ethanol	DMAc	DMF	DMSO	EtOAc
PGCA	– –	– –	+ –	+ –	+ +	+ –	+ +	+ +	+ +	+ +
PGCB	– –	– –	+ –	+–	+ –	– –	+ +	+ +	+ +	+ +
PGCC	– –	– –	+ –	+ –	+ +	+ +	+ +	+ +	+ +	+ +
PGCD	– –	– –	+ –	+ –	+ –	+ –	+ +	+ +	+ +	+ +

+ + = Soluble; – – = Insoluble; + – = Partially soluble/soluble on warming

The inherent viscosity values η_{inh} for all four copolyesters were measured using the flow time measurements with Ubbelohde viscometer. The η_{inh} values were calculated to be in the range of 0.24–1.36 dL g^{-1} that are shown in Table 1.

FT-IR spectra is used extensively for the characterization of polymers that reveals the chemical composition in polymers. Fig. 1 shows representative FT-IR spectra of PGCC, a characteristic absorption peak in the range of 1740-1770 cm^{-1} is attributed to C=O stretching (from ester) in the polymer confirms the formation of copolyester. In addition to that, absorption bands at 1427 cm^{-1} and 1583 cm^{-1} are observed due to C=C bonds from

the aromatic ring. The bending frequency of C-H bonds from aromatic rings is confirmed from bands at 825 cm^{-1}. A peak at 1255 cm^{-1} is responsible for C-O stretching. Rais *et al* [34] , Jasmine *et al.* [35] and Perundevi *et al.* [36] also reported similar observations for copolyesters containing chalcone diol moiety in the copolyester main chain.

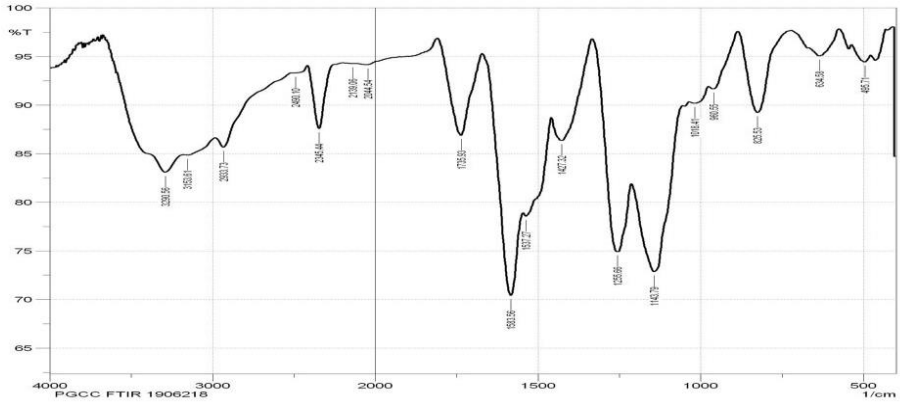


Fig. 1. FT-IR spectrum of the copolyester PGCC.

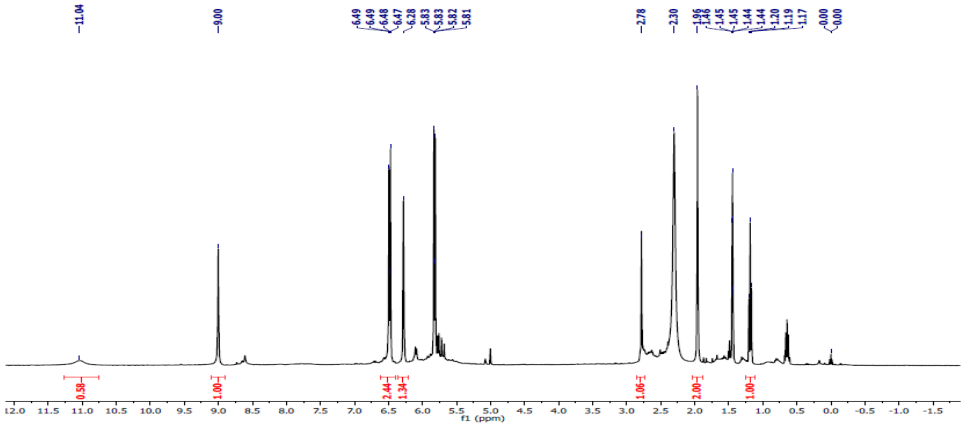


Fig. 2. ^{1}H-NMR of the copolyester PGCC.

Fig. 2 illustrates a representative ^{1}H NMR spectra of polymer PGCC. The peaks in the range of 7.3–7.5 ppm are responsible for aromatic protons. The peaks of C=C protons which are attached to carbonyl groups are observed in the range of 6.8–6.9 ppm. The presence of $–OCH_3$ in the arylidene moiety is confirmed from peaks in the range of 3.3–3.6 ppm. The methylene protons are observed in the range of 2.5–2.7 ppm. NMR result confirms the successful formation of the polymer PGCC and shows consistent results with literature [37-39].

The differential scanning calorimetry (DSC) thermograms of the prepared copolyesters are presented in Fig. 3. The glass transition temperatures, (T_g) for polymers were determined and shown in Table 1. The overall T_g values of prepared polymers are above 110 ºC. The T_g of polymers are in the order of PGCB (117.72 ºC) > PGCA (115.87 ºC) and PGCD (134.72 ºC) > PGCC (120.24 ºC). The polymers prepared using cyclopentanone (PGCC and PGCD) showed higher T_g values than that of polymers from cyclohexanone (PGCA and PGCB). This is accredited to the interlocking effect of the $–OCH_3$ substituents in the aromatic ring. These results are consistent with the reported results [40-42]. The lower T_g values for PGCC and PGCB are attributed to the flexibility offered by cyclohexanone rings than that of cyclopentanone rings [43]. Further, DSC results suggest that PGCC, PGCD, PGCA and PGCB are stable up to 325 ºC without any decomposition.

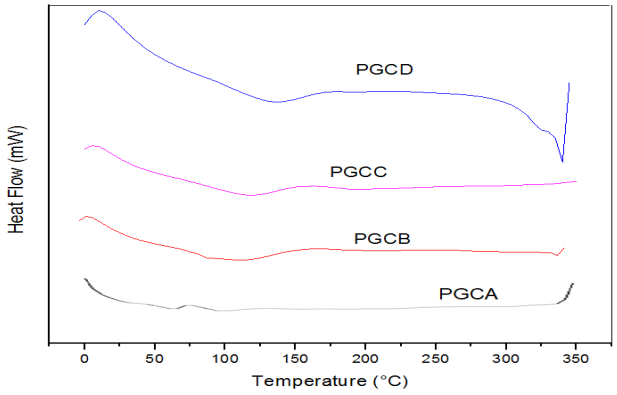


Fig. 3. DSC Thermogram for all the four copolyesters.

3.1. *Photocrosslinking*

The effect of irradiation on the polymers was monitored by UV visible spectrophotometric method. The copolyesters possessing arylidene moiety absorb between 350 nm and 420 nm which is due to $\pi \rightarrow \pi^*$ transition in double bond of C = C that are conjugated with keto group. The copolyesters are crosslinked by $2\pi + 2\pi$ cycloaddition of C=C bond on UV irradiation. The exposure of the polymers dissolved in DMF (10 mg/10 mL) to visible light resulted in the lowering of absorbance in their UV-vis spectra. This is due to the photochemically allowed $2\pi+2\pi$ addition of olefinic bonds in the bisbenzylidene systems and curcumin incorporated in the polymers. This was monitored in a continuous exposure and the resulting is overlaid and presented in Fig. 4a-b.

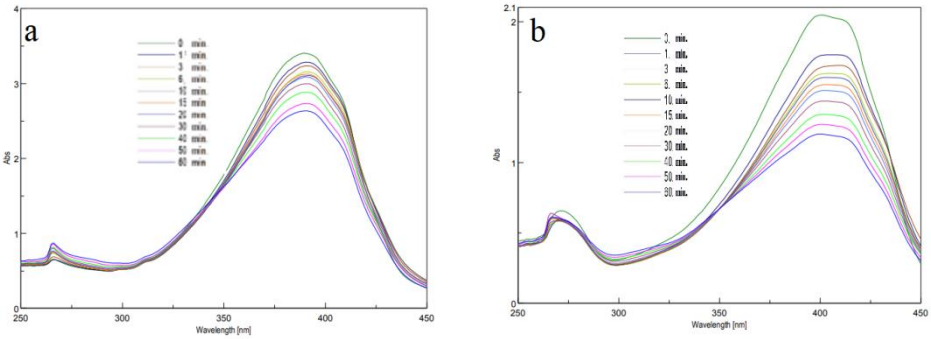


Fig. 4. UV-visible spectra of copolyesters (a) PGCC (b) PGCD at different intervals of exposure to light.

The plot of $[A_0\text{-}A_t]\ /A_0$ versus the time of irradiation (where A_0 and A_t are absorbance at 0 min and at time 't' of irradiation) is presented in Fig. 5. This gives an insight into the rate of photolysis on the copolyesters.

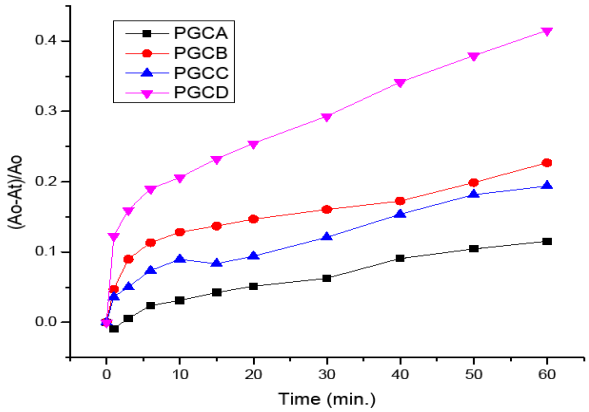


Fig. 5. Rates of photocrosslinking of copolyesters.

In all the polymers, two absorption bands due to bisbenzylidene and curcumin chromophores merged together are visible. It is also witnessed that both the absorptions bands decreased in intensity during irradiation. That means both of them undergo crosslinking. All polymers except PGCA showed faster photolysis up to 6 min. Later on, the rate decreases. This phenomenon is attributed to the greater flexibility of the uncrosslinked polymers in the initial stages, and once the crosslinking sets in, the flexibility and the rate of crosslinking are decreased consequently. In the case of PGCA, in the initial stages, the absorbance has increased, which means that there is an increase in the amount of chromophore itself. The chromophores can have EE, EZ and ZZ isomers.

By absorbing light ZZ and EZ isomers convert to EE isomers which are active in cycloaddition. The increase in the EE isomers results in high absorbance in the beginning [44].

The polymers containing cyclohexanone moiety have shown higher rate of photolysis than their cyclopentanone counterparts (PGCA > PGCC; PGCB > PGCD). Similar observation was reported by Sidharthan *et al.* [10]. It is also an evident that the methoxy groups in the bisbezylidene part enhances the rate of photolysis (PGCB > PGCA; PGCD > PGCC). The absorption of light by the polymers continues even after 60 min. This may be due to the mismatching offered by copolymerization [10].

3.2. *Computational work*

The BP86 optimized geometry of the cross linking polymers is deposited in Fig. 6. The Frontier molecular orbitals (LUMO & HOMO) of the crosslinked polymers have been given in Fig. 7. The FMO diagram of the cross-linked polymer reveals that HOMOs of all the polymers are stabilized by the dimethoxy cyclohexanone moiety and benzene moiety. The LUMOs of PGCA, PGCB, PGCC and PGCD are stabilized by benzylidene-cyclohexanone, methoxy-benzylidene-cyclohexanone, benzylidene-cyclopentanone and methoxy-benzylidene-cyclopentanone respectively.

To confirm the proposed structure of the cross-linked polymer, time dependent density functional theory (TDDFT) calculations were performed. In TDDFT calculations, the singlet vertical excitation energy (λ_{max} nm), electronic transition energies (ΔE eV), and oscillator (f_0) values were calculated with their transitions (Table 3). The crosslinked polymer PGCA gives a peak at 380.93 nm with oscillator strength of 0.4365. This peak is mainly due to HOMO→LUMO which contributes about 97 %. In PGCB, the λ_{max} is observed at 425.36 nm having an electron transition energy of 2.9148 eV. This transition is due to HOMO-4→LUMO which contributes about 51 %. The crosslinked polymer PGCC shows a peak at 392.28 nm with oscillator strength of 1.1464. It is arising mainly due to two transitions HOMO-3→LUMO+1 and HOMO-3→LUMO which contribute 31 and 25 % respectively. In PGCD, the peak is observed at 429.49 nm with electron transition energy of 2.8091 eV. This arises due to HOMO-2→LUMO+1 which gives 36 % contributions. All these values lie in the range of experimental results and thus support the formation of the cross-linking polymers.

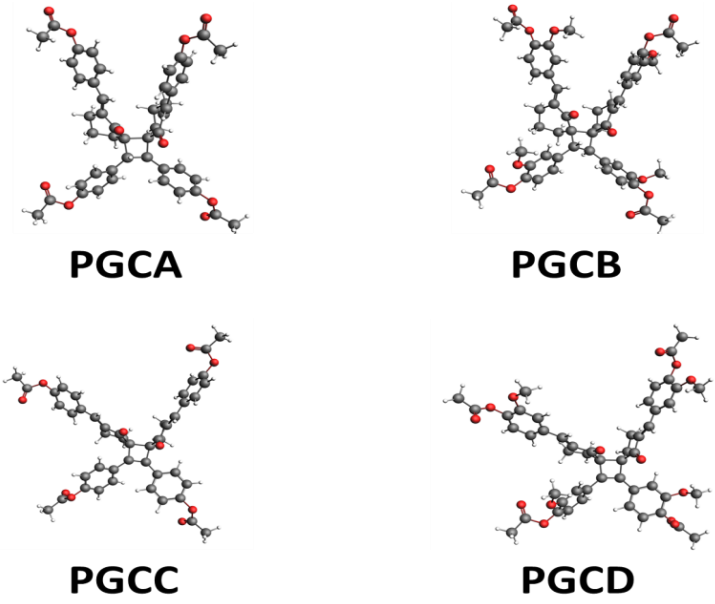


Fig. 6. Optimized geometries of the crosslinked polymers.

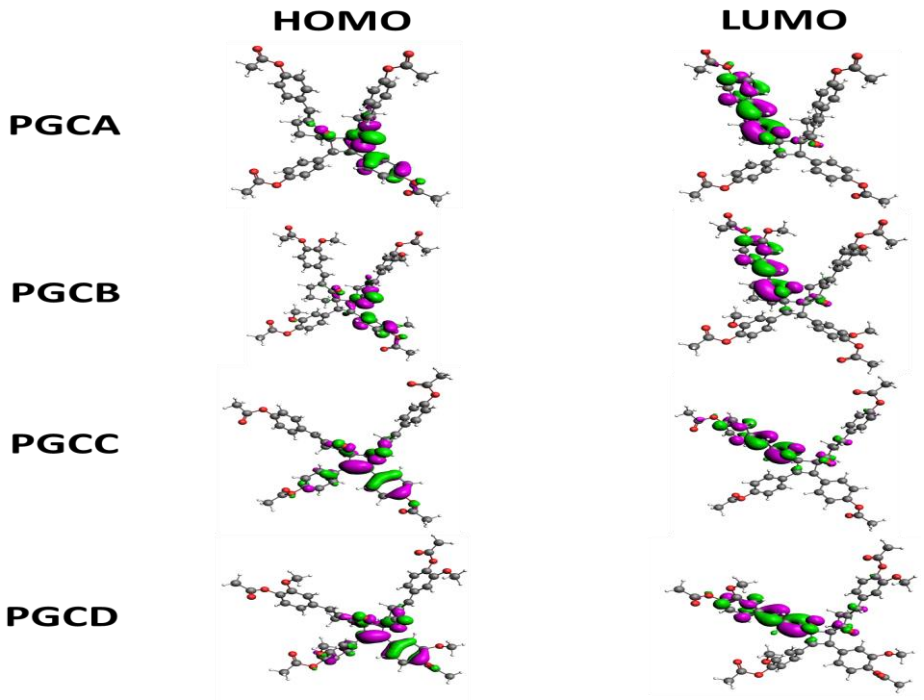


Fig. 7. Frontier molecular orbitals of the crosslinked polymers.

Table 3. Absorption maxima, energy transitions and oscillator strength values of the prepared cross-linked polymer in DMF solvent using TDDFT method at the BP86/TZP Level.

Polymers	λ_{max}(nm)	ΔE (eV)	f_0	Assignment
PGCA	380.93	2.5199	0.4365	HOMO→LUMO (97 %) HOMO→LUMO+1 (2 %)
PGCB	425.36	2.9148	0.1765	HOMO-4→LUMO (51 %) HOMO-2→LUMO (16 %)
PGCC	392.28	3.1606	1.1464	HOMO-3→LUMO+1 (31 %) HOMO-3→LUMO (25 %)
PGCD	429.49	2.8091	0.1403	HOMO-2→LUMO+1 (36 %) HOMO-2→LUMO (22 %)

3.3. *Anticancer activity*

The anticancer activity of the copolyesters PGCB and PGCC were investigated by MTT assay method. The effect of the prepared copolyesters on the MCF7 cell line was expressed as percent cell viability. IC_{50} values for both the copolyesters PGCB and PGCC are shown in Tables 4 and 5 respectively. Fig. 8 shows the images of anticancer activities of PGCB on breast cancer cell lines with 1000, 500, 250, 125, 62.5 and 31.2 µg/mL concentrations respectively. Fig. 9 shows the images of anticancer activities of PGCC on breast cancer cell lines with 1000, 500, 250, 125, 62.5 and 31.2 µg/mL concentrations, respectively.

Table 4. Cytotoxic effect of the polymer PGCB on MCF7 cell lines.

S.No.	Concentration of PGCB (µg/mL)	Absorbance at 540 nm	% cell Viability
1	1000	0.02	1.7
2	500	0.05	4.4
3	250	0.08	7.0
4	125	0.19	16.8
5	62.5	0.37	32.7
6	31.2	0.63	55.7
7	DMSO	1.12	99.1
8	Control Cells	1.13	100

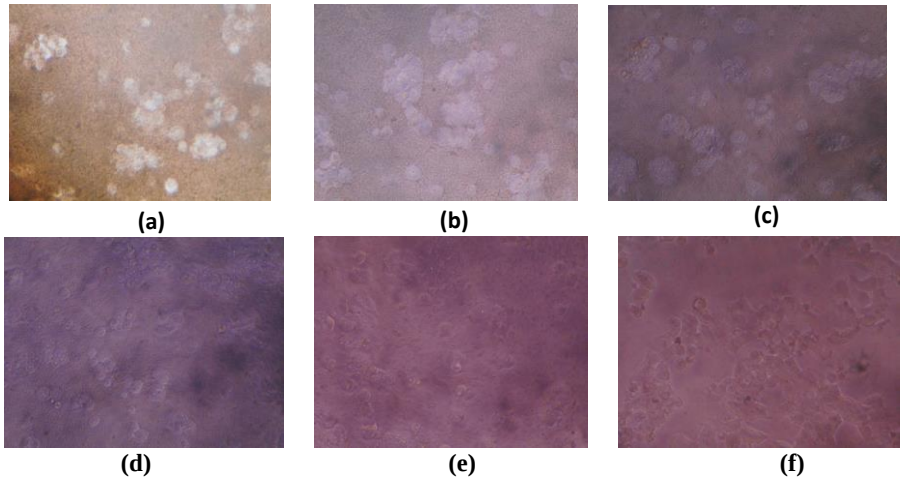


Fig. 8. Images using inverted microscope of MCF-7 cell line with PGCB polymer at different concentrations (µg/mL): (a) 1000, (b) 500, (c) 250, (d) 125, (e) 62.5 and (f) 31.2.

Table 5. Cytotoxic effect of the polymer PGCC on MCF7 cell lines

S.No.	Concentration of PGCC (µg/mL)	Absorbance at 540 nm	% cell Viability
1	1000	0.03	2.6
2	500	0.05	4.4
3	250	0.09	7.9
4	125	0.17	15.0
5	62.5	0.34	30.0
6	31.2	0.59	52.2
7	DMSO	1.12	99.1
8	Control Cells	1.13	100

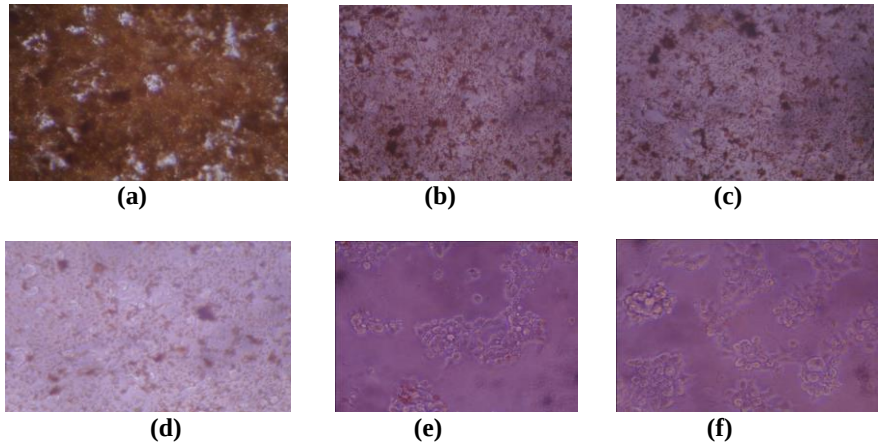


Fig. 9. Inverted microscope images of MCF-7 cell line with PGCC polymer at different concentrations (µg/mL): (a) 1000, (b) 500, (c) 250, (d) 125, (e) 62.5 and (f) 31.2.

PGCB and PGCC have 50 % cell viability at concentrations of 37.4 µg/mL and 34.3 µg/mL. From the observed values, it is inferred that PGCB and PGCC may be potential candidates for pharmaceutical application with a concentration less than 40 µg/mL. The copolyesters reported here have a substantial anticancer effect, which can be suggested useful for anticancer treatment. Gowsika *et al.* [45], Narendran *et al.* [46] and Sivaramakrishnan *et al.* [47] also reported similar observations for certain random copolyesters.

4. Conclusion

Copolyesters were synthesized by solution polycondensation method. The prepared polymers PGCA, PGCB, PGCC and PGCD are highly soluble in polar organic solvents. Under UV irradiation, the photocrosslinking of bisbenzylidene and curcumin occurred by the 2π+2π cycloaddition. The DSC analysis shows that the prepared polymers have good thermal stability characteristics. The successful formation of copolyesters is further confirmed by the DFT calculations. Anticancer studies by MTT assay reveals that the polymers may emerge as a potential anticancer agent against breast cancer cells.

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References

1. A. Larranaga and E. Lizundia, Eur. Polym. J. **121**, ID 109296 (2019). https://doi.org/10.1016/j.eurpolymj.2019.109296
2. K. I. Aly, M. A. Abdel-Rahman, and A. H. Tolba, Liq. Cryst. **45**, 187 (2017). https://doi.org/10.1080/02678292.2017.1309699
3. P. Wei, W. Zhang, Y. Zhang, Y. Wang, Z. Guo, H. Yu, Y. Xia, Y. Wang, and Y. Wang, Liq. Cryst. **46**, 1780, (2019). https://doi.org/10.1080/02678292.2019.1600751
4. K. I. Aly, M. A. Abdel-Rahman, and M. M. Q utai, J. Polym. Res. **25**, 185 (2018). https://doi.org/10.1007/s10965-018-1579-2
5. C. Rajam and D. R. Singh, WWJMRD **3**, 252 (2017).
6. P. N. Ramanan and M. N. A. Rao, Indian J. Pharm. Sci. **51**, 207 (1989).
7. D. Xiang, Q. Meng, H. Liu, M. Lan, and G. Wei, Talanta **146**, 851 (2016). https://doi.org/10.1016/j.talanta.2015.05.066
8. B. Krishnasamy, B. P. Shanmugaraj, S. C. Murugavel, L. Dai, and D. F. S. Petri, Int. J. Polym. Anal. Ch. **25**, 1 (2020). https://doi.org/10.1080/1023666X.2020.1780849
9. A. Muthusamy, K. Balaji, S. C. Murugavel, C. Yuan, and L. Dai, Polym. Sci. Ser. B **62**, 245 (2020). https://doi.org/10.1134/S1560090420030112
10. J. Sidharthan and T. P. Amaladhas, J. Polym. Res. **24**, 1 (2017). https://doi.org/10.1007/s10965-017-1206-7
11. M. S. Abdelaty, A. H. M. Hussien, M. S. El-gaby, Gad-Elkareem, M. Abas, S. M. Saddeek, Y. B. Kuckling, and D. K. I. Ali, Int. Res. J. Pure Appl. Chem. **16**, 1 (2018). https://doi.org/10.9734/IRJPAC/2018/40643

12. K. E. Kalappa, N. Ramalakshmi, J. Drug Deliv. Ther. **9(4-s)**, 210 (2019). https://doi.org/10.22270/jddt.v9i4-s.3273
13. M. Homocianu, D. Serbezeanu, A. M. Macsim, and T. Vlad-Bubulac, J. Mol. Liq. **316**, ID 113888 (2020). https://doi.org/10.1016/j.molliq.2020.113888
14. K. E. Kalappa, S. Arul Antony, and N. Ramalakshmi, Int. J. Eng. Technol. Sci. Res. **4**, 1 (2017). https://doi.org/10.30799/jacs.187.18040202
15. B. O. Al-Najjar, Jordan J. Biol. Sci. **11**, 307 (2018).
16. B. Wijianto, R. Ritmaleni, H. Purnomo, and A. Nurrochmad, J. Appl. Pharm. Sci. **10**, 99 (2020). https://doi.org/10.7324/JAPS.2020.10613
17. A. Allegra, V. Innao, S. Russo, D. Gerace, A. Alonci, and C. Musolino, Cancer Invest. **35**, 1 (2017). https://doi.org/10.1080/07357907.2016.1247166
18. N. A. Razak, M. N. Akhtar, N. Abu, W. Y. Ho, S. W. Tan, S. Zareen, and S. K. Yeap, RSC Adv. **7**, ID 36185 (2017). https://doi.org/10.1039/c7ra06580a
19. A. Andromeda, S.Ekawardhani, and A. Berbudi, Syst. Rev. Pharm. **11**, 18 (2020). https://doi.org/10.31838/srp.2020.7.05
20. A. Giordano and G. Tommonaro, Nutrients **11**, 2376 (2019). https://doi.org/10.3390/nu11102376
21. M. K. Oglah, Y. F. Mustafa, M. K. Bashir, M. H. Jasim, and Y. F. Mustafa, Syst. Rev. Pharm. **11**, 472 (2020). https://doi.org/10.5530/srp.2020.3.60
22. M. Catanzaro, E. Corsini, M. Rosini, M. Racchi, and C. Lanni, Molecules **23**, 2778 (2018). https://doi.org/10.3390/molecules23112778
23. S. I. A. Mohamed, I. Jantan, and M. A. Haque, Int. Immunopharmacol. **50**, 291 (2017). https://doi.org/10.1016/j.intimp.2017.07.010
24. E. V. Lenthe and E. J. Baerends, J. Comput. Chem. **24,** 1142 (2003). https://doi.org/10.1002/jcc.10255
25. C. Wang, Q. Y. Wu, X. H. Kong, C. Z. Wang, J. H. Lan, C. M. Nie, and W. Q. Shi, Inorg. Chem. **58**, 10047 (2019). https://doi.org/10.1021/acs.inorgchem.9b01200
26. M. Roger, L. Belkhiri, P. Thuery, T. Arliguie, M. Fourmigue, A. Boucekkine, and M. Ephritikhine, Organometallics **24**, 4940 (2005). https://doi.org/10.1021/om050329z
27. A. Zaiter, B. Amine, Y. Bouzidi, L. Belkhiri, A. Boucekkine, and M. Ephritikhine, Inorg. Chem. **53**, 4687 (2014). https://doi.org/10.1021/ic500361b
28. B. Teyar, S. Boucenina, L. Belkhiri, B. Le Guennic, A. Boucekkine, and M. Mazzanti, Inorg. Chem. **58**, 10097 (2019). https://doi.org/10.1021/acs.inorgchem.9b01237
29. F. Kias, F. Talbi, A. Elkechai, and A. Boucekkine, Comput. Theor. Chem. **1118**, 133 (2017). https://doi.org/10.1016/j.comptc.2017.09.002
30. S. O. Odoh, Ph.D Thesis, University of Manitoba, Winnipeg, Canada (2013).
31. G. T. T. Velde, F. M. Bickelhaupt, E. J. Baerends, C. F. Guerra, S. J. van Gisbergen, J. G. Snijders, and T. Ziegler, J. Comput. Chem. **22**, 931 (2001). https://doi.org/10.1002/jcc.1056
32. T. Mosmann, J. Immunol. Meth. **65**, 55 (1983). https://doi.org/10.1016/0022-1759(83)90303-4
33. E. Arumugasamy, B. Baskar, and V. Kannappan, J. Polym. Mater. **17**, 421 (2000). https://doi.org/10.1046/j.1525-1470.2000.01812-4.x
34. M. I. Rais, S. J. A. Ali, and D. R. Jonathan, Asian J. Chem. **30**, 1675 (2018). https://doi.org/10.14233/ajchem.2018.21324
35. S. J. Francis, D. R. Jonathan, and D. R. Singh, J. Chem. Pharm. Res. **6**, 1155 (2014).
36. T. S. Perundevi, D. R. Jonathan, and S. Kothai, Chem. Sci. Trans. **6**, 199 (2017). https://doi.org/10.7598/cst2017.1357
37. E. Jaisankar, M. E. Pavithra, S. Krishna, M. Thirumarimurugan, and R. S. Azarudeen, Int. J. Biol. Macromol. **145**, 42 (2020). https://doi.org/10.1016/j.ijbiomac.2019.12.038
38. S. Sathya, D. R. Jonathan, S. J. Francis, D. Roopsingh, and G. Usha, J. Opt. **46**, 116 (2017).
39. N. Kandaswamy, Macromol. Res. **27**, 593 (2019). https://doi.org/10.1007/s13233-019-7082-8
40. K. Deepa and J. S. Viswanathan, Trends Biomater. Artif. Organs **32**, 105 (2018).
41. S. J. Kim, H. W. Kwak, S. Kwon, H. Jang, and S. I. Park, Polymers **12**, 2389 (2020). https://doi.org/10.3390/polym12102389

42. M. Konstantopoulou, Z. Terzopoulou, M. Nerantzaki, J. Tsagkalias, D. S. Achilias, D. N. Bikiaris, S. Exarhopoulos, D. G. Papageorgiou, and G. Z. Papageorgiou, Eur. Polym. J. **89**, 349 (2017). https://doi.org/10.1016/j.eurpolymj.2017.02.037
43. S. Vasanthi, J. A. Moli, and D. R. Singh, Asian J. Res. Chem. **4**, 1343 (2011).
44. G. Deepa, R. Balamurugan, and P. Kannan, J. Mol. Struct. **963**, 219 (2010). https://doi.org/10.1016/j.molstruc.2009.10.038
45. U. Veerabagu, G. Jaikumar, and L. A. Fushen, J. Polym. Environ. **27**, 2756 (2019). https://doi.org/10.1007/s10924-019-01562-4
46. N. Kandaswamy and N. Raveendiran, Res. Chem. Intermed. **41**, 7189 (2015). https://doi.org/10.1007/s11164-014-1806-3
47. T. Sivaramakrishnan, G. Annamalai, and G. Elango, Asian J. Res. Chem. **13**, 103 (2020). https://doi.org/10.5958/0974-4150.2020.00021.8