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Synthesis, Characterization and Anti-Bacterial Activity of Certain Thermotropic Liquid Crystalline Poly(ester-amides) containing 2,6-bis(3-methoxybenzylidene) Cyclohexanone Moiety in the Main Chain

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ABSTRACT

A series of five thermotropic liquid crystalline poly(ester-amides) were synthesized by polycondensation of varying dicarboxylic acids with a common diamine namely 1,4-diaminobenzene and a common diol namely 2,6-(bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone (CHBV). The viscosity measurements and solubility data were used for the qualitative characterization of these synthesized poly(ester-amides). For investigating the microstructural features of these synthesized poly(ester-amides), the spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR were performed. The thermal phase transition behavior of these poly(ester-amides) were studied by Differential Scanning Calorimetry (DSC). The morphology of these poly(ester-amides) were established with SEM analysis. X-ray diffraction (XRD) patterns were taken to assess the degree of crystallinity in these poly(ester-amides). These poly(ester-amides) displayed potential antimicrobial activity against certain bacterial strains.

KEYWORDS

Bisbenzylidenecyclohexanone, Poly(ester-amides), Polycondensation, Bactericidal

1. INTRODUCTION:

The bisbenzylidene cyclohexanone has both mesogenic and photo-active properties. This can exist in three isomeric structures *EE*, *EZ* and *ZZ*. Among these three isomers, *EE* has a linear rod shaped structure. Synthesis of bisbenzylidene cycloalkanone molecule was first reported by Samdahl [1,2]. This photo-active molecule have attracted the attention of macromolecular chemists. They were found to be a potential mesogen and their incorporation in the polymeric backbone has imparted thermotrophic liquid crystalline property to the polymeric materials. Thermotropic liquid crystalline polymers have wide range of applications from ultrahigh strength fibres to nonlinear optical devices. Polyesters synthesized have been reported to possess intriguing anisotropic, mechanical, electrical and optical properties suitable for technological applications [3]. Poly(ester-amides) attracted scientific interest, since they may be designed to couple the excellent mechanical properties of polyamides and the biodegradability of polyesters [4]. Poly(ester-amides) have found a wide range of applications, such as disposable bags, agricultural films, drug carriers and matrix resins for biomedical materials [5]. Malathi *et al.*[6] reported on the synthesis and antibacterial activity of certain random copolyesters containing arylidene-ketones in the main chain. The random blend

copolymer nanofibers containing arylidene diol moiety have antibacterial activity and photocrosslinking efficacy [7]. Kannapan and Reuben Jonathan [8] reported on the study on the synthesis and bactericidal efficacy of certain poly(ester-amides) containing 2,5-bis(benzylidene)cyclopentanone moiety in the main chain.

However, there is no literature information on the synthesis and bactericidal efficacy of poly(ester-amides) containing bis(arylidene)cyclohexanone moiety in the polymer backbone. The purpose of the current work is to synthesize five poly(ester-amides) containing bis(arylidene)cyclohexanone moiety in the polymer backbone and to evaluate its bactericidal efficacy. Poly(ester-amides) are a category of polymeric materials which contains both ester and amide linkages [9,10] and are synthesized by the copolymerization of a diacid with that of a diamine and a diol in the mole ratio of 2:1:1.

2. EXPERIMENTAL SECTION

2.1. Chemicals

Aldrich samples of diphenyl chlorophosphate, terephthalic acid, isophthalic acid, phthalic acid, adipic acid, azelaic acid, and 1,4-diaminobenzene were used as received. Vanillin (CDH), lithium chloride (SD Fine) and cyclohexanone (Fluka) were used as received. Merck sample of pyridine was used as polymerization medium was refluxed over potassium hydroxide pellets, distilled (b.p. 115°C) and stored over potassium hydroxide pellets. Merck, LR sample of methanol was used as solvent. SD-Fine AR sample of N,N-dimethyl acetamide (DMAc) was used as solvent for finding out the inherent viscosity of the poly(ester-amide). Aldrich spectral grade DMSO-d6 was used as received for recording NMR spectra.

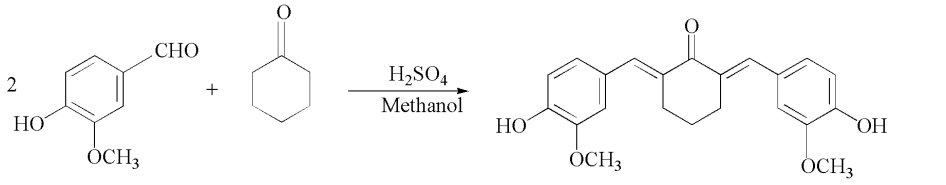
2.2. PREPARATION OF MONOMER

2.2.1. Synthesis of Arylidene-keto Diol

The arylidene-keto diol namely 2,6-(bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone (CHBV) were synthesized by the method reported in literature [11,12].

Preparation of 2,6-(bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone (CHBV)

A solution containing vanillin (4-hydroxy-3-methoxybenzaldehyde) (0.2 mol) in 100 mL of dry methanol was taken in a 250 mL conical flask. Cyclohexanone (0.1 mol) was added drop wise and the mixture was shaken well. Fuming sulphuric acid (5 mL) was added drop wise with external cooling in an ice bath. An exothermic reaction took place and the reaction mixture turned bright yellow, dark green and finally pink. The precipitated diol was filtered and washed with aqueous methanol. It was filtered and recrystallised from aqueous methanol. Yield: 95% (m.p.: 180 °C).



2,6-bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone

2.2.2. Synthesis of Poly(ester-amides)

Depending upon the type of dicarboxylic acid monomer (aromatic or aliphatic), two methods were employed to synthesize the poly(ester-amides).

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Method 1: Synthesis of poly(ester-amides) derived from aromatic dicarboxylic acid monomer

The procedure for the synthesis of a typical poly(ester amide) was reported by Arulmoli and coworkers [13] is represented here.

Three poly(ester-amides) were prepared by the direct polycondensation of a diamine, a diol with one diacid in the respective mole ratio 1:1:2 in pyridine solution using diphenyl chlorophosphate (DPCP) as the condensation agent. The yield of the polymer is high, and this method avoids the tedious preparation of acid chloride.

Diphenyl chlorophosphate (12 mmol) in pyridine (10 mL) was added to a solution containing LiCl (10 mmol) and aromatic dicarboxylic acid (5 mmol) in pyridine (10 mL) were taken in a 100 mL round-bottomed flask. The reaction mixture was continuously stirred at room temperature for a span of 30 minutes. Then the temperature was raised to 115°C and stirring was carried out at this temperature for about 10 minutes. The solution containing the diamine 1,4-diaminobenzene (2.5 mmol) and the diol (2.5 mmol) was added over a period of 10 minutes with constant stirring. The reaction mixture was maintained at this temperature for a time frame of 3 hours. Then reaction mixture was cooled to room temperature and poured into 300 mL of methanol when the poly(ester-amide) was precipitated. It was filtered, washed with methanol and dried in vacuum.

Method 2: Synthesis of poly(ester-amides) derived from aliphatic diacid chloride monomer

The procedure for the synthesis of a typical aliphatic diacid-based polymer is given here. Two aliphatic diacid-based poly(ester-amides) were prepared by the polycondensation of a diamine, a diol with one diacid in the respective mole ratio of 1:1:2 in DMF [14].

The common diamine 1,4-diaminobenzene (2.5 mmol) and the diol (2.5 mmol) were dissolved in DMF (30 ml) were taken in a 100 mL round-bottomed flask. Diacid chloride (5 mmol) was added dropwise with stirring to the solution at 100°C. The reaction mixture was heated at 115°C for 8 hours with stirring at nitrogen atmosphere. The contents were cooled and poured into methanol. The mixture was kept in a refrigerator overnight and then filtered. The poly(ester-amide) was dissolved in minimum amount of acetone, filtered and the clear solution was poured into water. The precipitated poly(ester-amide) was dried in vacuum over phosphorus pentoxide.

The various poly(ester-amides) prepared by these methods and the monomers are given in table 1 along with the polymer code, percentage of yield and inherent viscosities.

Table 1. Monomers used and the polymer code of poly(ester-amides) with percentage of yield and inherent viscosities (η_{inh})

S. No.	Common diamine: 1,4-diaminobenzene		Polymer Code	Yield (%)	η_{inh} (dL/g)
	Diol	Diacid / Diacid chloride			
1	CHBV	Terephthalic acid	PVBT	74	1.20
2	CHBV	Isophthalic acid	PVBI	71	1.10
3	CHBV	Phthalic acid	PVBP	70	1.05
4	CHBV	Adipoyl dichoride	PVBA	57	0.46
5	CHBV	Azelaoyl dichloride	PVBAz	59	0.58

CHBV: 2,6-(bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone.

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2.3. Characterization Methods

All the poly(ester-amides) were characterized by solubility studies, viscosity measurements, and spectral data. Thermal investigations were made on these poly(ester-amides) by differential scanning calorimetry. X-ray diffraction (XRD) patterns were taken to assess the degree of crystallinity in these poly(ester-amides). The morphology of these poly(ester-amides) were established with SEM analysis. Antibacterial activity and antifungal activity were carried out by disc diffusion method on these poly(ester-amides).

2.3.1. Solubility

The solubility of these poly(ester-amides) were tested in various solvents qualitatively. About 50 mg of the polymer was taken in a small stoppered test tube containing 1 ml of the solvent. The mixture was kept for 24 hours with occasional shaking.

2.3.2. Viscosity

The inherent viscosity (η_{inh}) of the five poly(ester-amides) was determined in N,N-dimethylacetamide (DMAc) solution at 30°C using Ubbelohde viscometer in which the pure solvent had a flow rate of 470 seconds. In each case, 25 mg of dry poly(ester-amides) sample was dissolved in 25 ml of DMAc, kept aside for 12 hours with occasional shaking. The η_{inh} was calculated from the flow time measurement at 30°C.

2.3.3. Fourier Transform Infrared (FT-IR) Spectroscopy

The FT-IR spectra were recorded using Shimadzu FT-IR instrument in the form of potassium bromide pellet method.

2.3.4. ^{1}H and ^{13}C NMR Spectra

The ^{1}H and ^{13}C NMR spectra were recorded with Bruker AVANCE III 500 MHz instrument in DMSO-d6 solvent with TMS as internal reference.

2.3.5. Differential Scanning Calorimetry (DSC)

In thermal analysis of these poly(ester-amides), DSC thermograms were recorded in NETZSCH DSC 200F3 differential scanning calorimeter using 5mg samples under nitrogen atmosphere at a heating rate of 10°C/min.

2.3.6. X-Ray Diffraction (XRD)

The X-ray diffraction measurements were taken to assess the degree of crystallinity of these powdered poly(ester-amides) using GE-Inspection Technology Diffractometer System XRD 3003TT model made in Germany with a source of copper target operating voltage 40Kv 300mA^{o} current rate.

2.3.7. Scanning Electron Microscopy (SEM)

The ***morphology*** of these poly(ester-amides) were investigated using Hitachi S-3000 Hz scanning electron microscopy (*SEM*).

2.3.8. Antibacterial Activity (Agar Disc Diffusion Method)

Preparation of inoculums

Stock cultures were maintained at 4°c on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24hrs at 37^{o}c. The assay was performed by disc diffusion method.

Determination of antibacterial activity

Antibacterial activity of sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium was weighed as 3.8gms and dissolved in 100ml

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of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. Discs were prepared with 20 µl sample of respective concentrations (5, 10, 15, 20, 25 and 30 µg) and positive control 10 µl (10 µg) norflaxin and placed on MHA plates. These plates were incubated for 24 hrs at 37ºc .Then the microbial growth was determined by measuring the diameter of zone of inhibition.

3. RESULTS AND DISCUSSION

3.1. Solubility

The poly(ester-amides) reported here are found to be soluble in highly polar solvents such as dimethyl sulphoxide, DMAc and trifluoroaceticacid, partially soluble in moderately polar solvents and thoroughly insoluble in non-polar solvents such as benzene and hexane. This might be recognized due to the inter-molecular interactions of polar solvents with ether linkages of the polymer backbone. Similar explanation was offered by Sathish and coworkers [15] in a series of Copolyesters. Also, the poly(ester-amides) with methoxy substituent in the phenyl ring of the arylidenecyclohexanone moiety was found to be highly soluble because of their capacity to disrupt the macromolecular polymer chain molecules. The results of the solubility of the poly(ester-amides) are represented in table 2.

Table 2. Solubility of poly(ester-amides) in common organic solvents

S.No.	Polymers	Hexane	Benzene	$CHCl_3$	EtOAc	THF	Acetone	DMF	CH_3CN	DMAc	TFA	DMSO
1	PVBT	--	--	--	--	+-	+-	++	++	++	++	++
2	PVBI	--	--	--	--	+-	+-	++	++	++	++	++
3	PVBP	--	--	--	--	+-	+-	++	++	++	++	++
4	PVBA	--	--	+-	+-	++	++	++	++	++	++	++
5	PVBAz	--	--	+-	+-	++	++	++	++	++	++	++

++ =Freely Soluble; -- = Insoluble; +- = Partially soluble

3.2. Viscosity

The η_{inh} values of all the five poly(ester-amides) were found to be in the range of 0.58–1.20 dL/g and are represented in table 1. It may be pointed out that the poly(ester-amides) synthesized from aromatic dicarboxylic acids have higher η_{inh} values than those prepared from aliphatic diacid chloride monomers. The data shows higher viscosity values and hence they are reasonably of high molecular weight.

3.3. Spectral Studies

The ester and amide functional groups present in the poly(ester-amide) chain were identified by FT-IR spectra. The IR spectra of all the five poly(ester-amides) showed characteristic absorption at $\bar{\upsilon}$ = 1640–1730 cm^{-1} due to ester and amide C=O stretching frequency and an absorption at $\bar{\upsilon}$ = 3250–3380 cm^{-1} due to the amide N-H stretching frequency. Similar observations were made by Khairou et al [16] in a new series of poly(ester-amides) containing diarylidenecyclohexanone in the main chain. They are represented in figures 1(a) and 1(b) for PVBT and PVBAz. The structural units present in the poly(ester-amide) chain were identified by ^{1}H and ^{13}C NMR spectra. The secondary amide proton appeared as a singlet in the range of 9.5–10.15 ppm. Related observation was made by

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Kannapan and Reuben Jonathan [8]. The aromatic protons are observed in the range of 6.5–8.54 ppm. The methoxy protons in the vanillin moiety are indicated by a signal at 3.8 ppm. They are represented in figures 2(a) and 2(b) for PVBT and PVBAz.

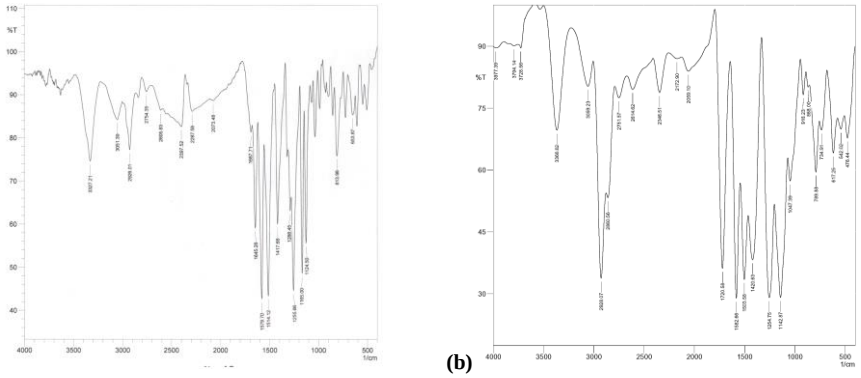

Fig 1(a,b): FT-IR spectrum of random poly(ester-amides) PVBT (a) and PVBAz (b).

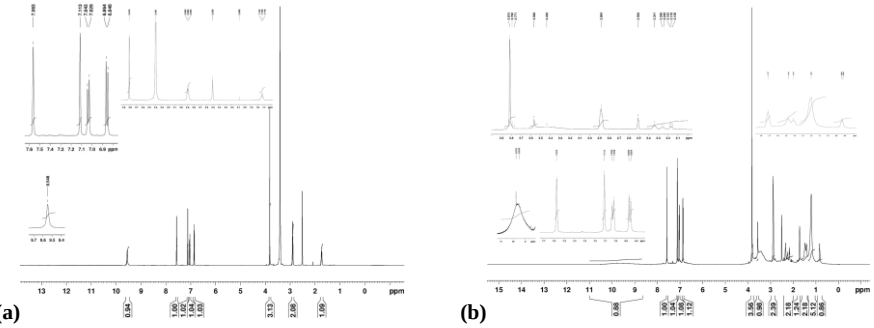

Fig 2(a,b): ^{1}H NMR spectrum of random poly(ester-amides) PVBT (a) and PVBAz (b).

The signal in the range of δ = 188–200 ppm in the ^{13}C NMR spectra of the poly(esteramides) is due to the carbonyl carbon of the α,β-unsaturated ketone. The signals in the range of δ = 171–185 ppm and δ = 160 –175 ppm are due to the carbonyl carbon of the amide and ester groups [16] which indicates the formation of poly(ester-amide). They are represented in figures 3(a), and (b) for PVBT and PVBA.

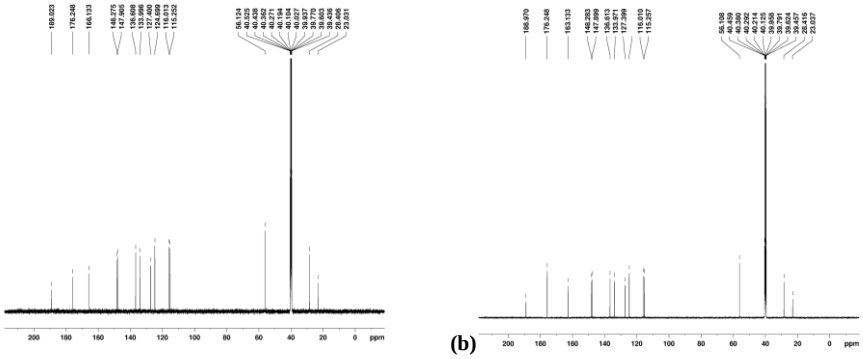

Fig 3(a,b): ^{1}C NMR spectrum of random poly(ester-amides) PVBT (a) and PVBA (b).

3.4. Thermal Analysis and Phase Behaviour

The thermal and phase behaviours of the five poly(ester-amides) were investigated by Differential Scanning Calorimetry (DSC). The glass transition temperature (T_g), crystal to crystal transition

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($T_{K\rightarrow K}$), melting temperature (T_m), isotropization temperature (T_i) and liquid crystalline range are tabulated in table 3.

Table 3. Differential Scanning Calorimetry (DSC) data of poly(ester-amides)

S.No.	Polymers	Differential Scanning Calorimetry (DSC)				
		T_g (°C)	$T_{K\rightarrow K}$ (°C)	T_m (°C)	T_i (°C)	ΔT
1	PVBT	51.16	183.16	359.16	407.16	48
2	PVBI	46.21	178.21	330.21	446.21	116
3	PVBP	40.21	177.21	347.21	504.21	157
4	PVBA	39.21	167.21	293.21	443.21	150
5	PVBAz	30.87	180.87	323.87	412.87	89

The DSC thermograms of all poly(ester-amides) are shown in figure 4. The analysis of DSC data shows that the poly(ester-amides) synthesized from aliphatic diacid chloride have lower glass transition temperature (T_g) values than those prepared from aromatic dicarboxylic acids monomers. In these polymers, an endothermic peak appears before melting transition, which seems t o be related to crystal to crystal transition resulted from different crystalline polymorphs [17].The poly(ester-amide) derived from adipic acid monomer maintain broader liquid crystalline range (ΔT) than the poly(ester-amide) derived from azelaic acid monomer. This may be due to the coplanar geometry that would favour effective molecular packing. Also, while comparing the liquid crystalline range (ΔT) of PVBT, PVBI, and PVBP, the poly(ester-amide) derived from phthalic acid monomer maintain broader liquid crystalline range (ΔT). This may be due to the rigidity of o-phenylene moiety when compared to m-phenylene or p-phenylene moiety in the polymer chain. moiety present in the polymer chain. It is observed that these poly(ester-amides) have higher T_g values which could be due to interlocking effect of the methoxy substituent present in the arylidene keto moiety. There are reports on such interlocking effects on the thermal properties of polymers by Lenz and coworkers [18]. They suggested that the interlocking effect depends on the size of the substituent. This was further supported by reported work of Kannappan and coworkers [12] by ultrasonic method.

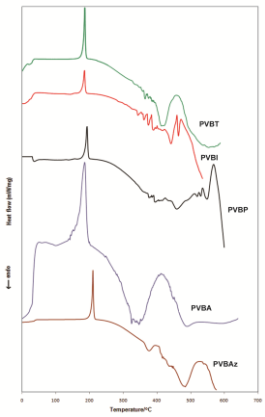

Fig 4: DSC thermograms of random poly(ester-amides).

3.5. X-Ray Diffraction Studies. The X-Ray diffraction pattern of poly(ester-amides) PVBT and PVBA are shown in figures 5(a) and 5(b) which indicates the semicrystallinity of these polymers

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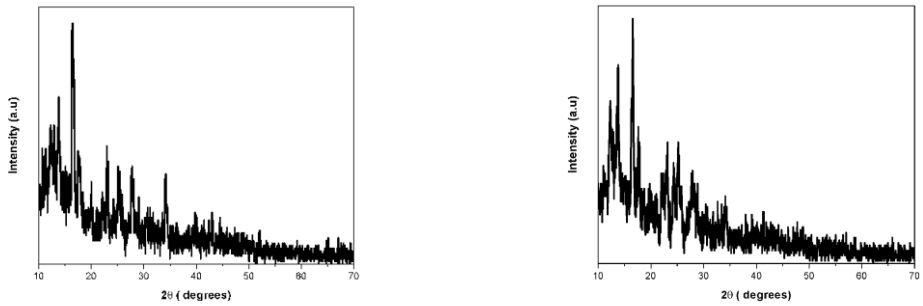
with an amorphous background. This may be due to the presence of C=O and C=C groups. These poly(ester-amides) showed few reflection peaks in the region 2θ = 10–50° [16].

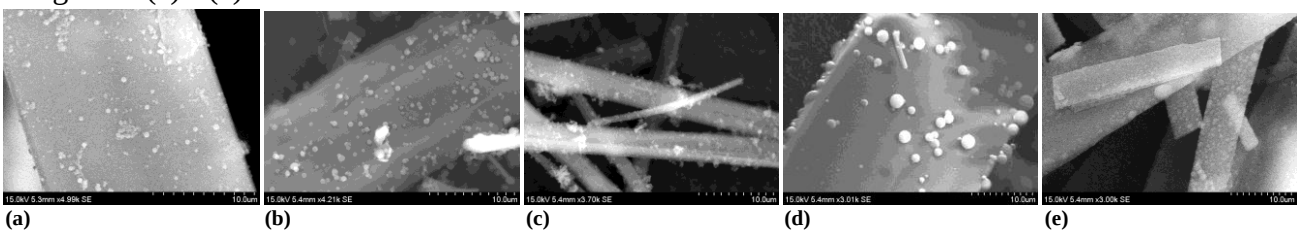


(a) **(b)**

Fig 5(a,b): X-ray diffraction pattern of random poly(ester-amides) PVBT (a) and PVBA (b).

3.6. Morphological Study by SEM

The SEM images illustrates the morphology of all the five poly(ester-amides). They are represented in figures 6(a)-6(e).



(a) **(b)** **(c)** **(d)** **(e)**

Fig 6(a-e): Scanning electron micrograph of random poly(ester-amides) PVBT (a), PVBI (b), PVBP (c), PVBA (d), and PVBAz (e).

3.7. Bactericidal Study

The antibacterial activity of all the five poly(ester-amides) were assayed against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris, Proteus mirabilis,* and *Klebsiella pneumonia* by disc diffusion method. The zone of inhibition for each concentration against all the test bacteria is depicted in table 4. The zone of inhibition for the standard positive control norflaxin disc 10 µg/µl is shown in table 4. They are represented in figures 7(a)-7(e). Analysis of the data in table 4 suggests that the poly(ester-amide) derived from aliphatic diacid choride exhibited substantial antibacterial activity towards all the test bacteria *E. coli, S. typhi, S. aureus, P. vulgaris, P. mirabilis,* and *K. pneumonia* in all the concentrations. S. Kothai and coworkers [19] reported on the antibacterial activity of certain copolyesters derived from aliphatic diacid chloride. Similar observation was reported by V. Kannapan and D. Reuben Jonathan [8] in a series of copolyesteramides derived from arylidene diols.

Comparing the poly(ester-amides) derived from aromatic dicarboxylic acids, the poly(ester-amide) derived from phthalic acid exhibited greater antibacterial activity.

Table 4. Inhibition effects of the five poly(ester-amides) on the growth of *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris, Proteus mirabilis,* and *Klebsiella pneumonia*

Plate No.	Polymer code / Microorganisms	Zone of inhibition (mm)						
		5 µg/µl	10 µg/µl	15 µg/µl	20 µg/µl	25 µg/µl	30 µg/µl	Norflaxin
	PVBT							

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1	*E. coli*	0	0	0	0	0	6.7	6.1
2	*S. typhi*	0	0	0	0	0	6.3	6.2
3	*S. aureus*	0	0	0	0	0	6.1	6.1
4	*P. vulgaris*	0	0	0	0	0	6.1	6.1
5	*P. mirabilis*	0	0	0	0	6.1	6.3	6.1
6	*K. pneumonia*	0	0	0	0	0	6.1	6.1
	PVBI							
1	*E. coli*	0	0	0	0	6.5	7	7
2	*S. typhi*	0	0	0	0	0	6.1	7
3	*S. aureus*	0	0	0	0	0	6.1	7.2
4	*P. vulgaris*	0	0	6.1	6.3	6.5	6.7	6.2
5	*P. mirabilis*	0	0	0	0	0	6.3	6.1
6	*K. pneumonia*	0	0	0	0	6.4	6.7	6.1
	PVBP							
1	*E. coli*	6.2	6.7	6.8	6.9	7.1	7.3	18
2	*S. typhi*	0	0	6.3	6.5	6.9	7.5	14
3	*S. aureus*	0	0	0	0	0	6.4	15
4	*P. vulgaris*	6.4	6.9	7.1	7.4	7.6	8.1	15
5	*P. mirabilis*	6.2	6.5	6.8	8.1	8.7	9.6	14
6	*K. pneumonia*	0	6.4	6.7	6.9	7.2	7.6	17
	PVBA							
1	*E. coli*	6.3	6.5	6.9	7.3	7.6	7.9	21
2	*S. typhi*	6.4	6.6	6.8	7.0	7.4	7.6	14
3	*S. aureus*	6.2	6.4	6.9	7.1	7.5	8.2	20
4	*P. vulgaris*	7.2	7.6	8.1	8.9	9.7	10.5	13
5	*P. mirabilis*	7.3	7.5	7.9	8.2	9.1	9.8	13
6	*K. pneumonia*	6.1	6.3	6.4	6.8	6.9	7.2	15
	PVBAz							
1	*E. coli*	6.2	6.4	6.6	6.8	7.3	7.7	16
2	*S. typhi*	7.3	7.6	7.9	8.4	9.2	9.7	24
3	*S. aureus*	7.8	8.1	8.9	9.8	10.3	10.8	26
4	*P. vulgaris*	6.8	7.6	7.9	8.4	9.3	9.7	24
5	*P. mirabilis*	7.4	7.8	8.1	8.5	9.1	9.6	14
6	*K. pneumonia*	6.3	6.4	6.5	6.7	6.8	6.9	13

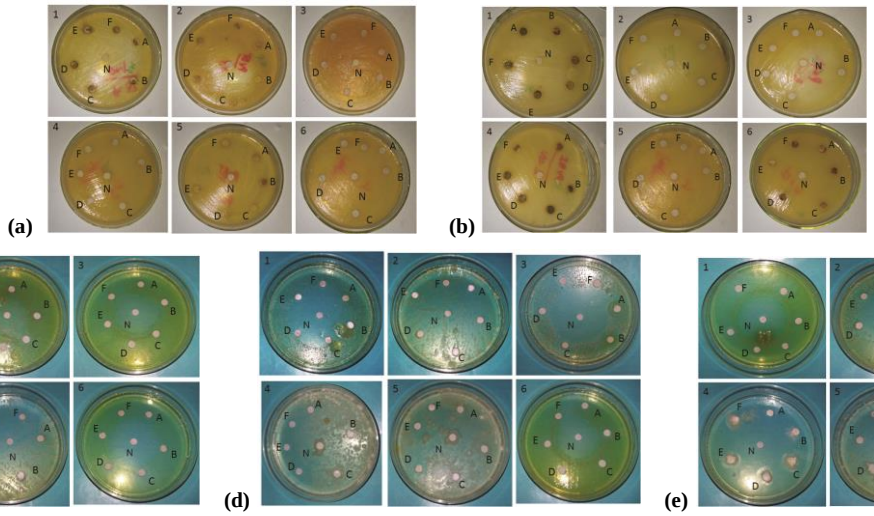

Fig 7(a-e): Inhibition effects of random poly(ester-amides) PVBT (a), PVBI (b), PVBP (c), PVBA (d), and PVBAz (e) on the growth of pathogenic bacteria.

(Fig Label: A = 5 µg/µl, B = 10 µg/µl, C = 15 µg/µl, D = 20 µg/µl, E = 25 µg/µl, F= 30 µg/µl, and N = Standard (positive control) norflaxin).

4. CONCLUSION

The five poly(ester-amides) have been synthesized successfully by direct polycondensation method. These poly(ester-amides) are highly soluble in polar organic solvents. The viscosity measurements reveal that the poly(ester-amides) synthesized are of high molecular weight. The synthesized poly(ester-amides) have been characterized by FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectral studies. The structural assignment of these poly(ester-amides) are supported by these spectral data. The liquid crystalline property of these poly(ester-amides) were recognized from DSC. X-ray diffractograms of these poly(ester-amides) showed the degree of crystallinity. The morphology of all the five poly(ester-amides) are investigated by SEM. These poly(ester-amides) exhibited significant bactericidal activity against pathogenic bacteria.

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