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Research Article

Evaluation of antimicrobial property of few co-polyesters

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ABSTRACT

Aliphatic random co-polyesters Poly (Propane1,3-diol Fumaric acid 1,4-cyclohexanedimethanol) (PPFCDM), Poly (Propane1, 3-diol Fumaric acid Pimelic acid (PPFPi), Poly (Butane1,4-diol Fumaricacid 1, 4-cyclohexanedimethanol (PBFCDM), Poly (1,4-Cyclohexanedimethanol Pimelicacid 1,4-Cyclohexanedicarboxylic acid) (PCDMPiCDC) Poly (1,4-Cyclohexanedimethanol Fumaricacid 1,4-Cyclohexanedicarboxylicacid (PCDMFCDC) were synthesized from propane1, 3-diol and 1, 4-cyclohexane dimethanol with various carboxylic acids in the presence of catalyst using microwave technique. The antimicrobial efficacy was evaluated using well diffusion method against gram positive bacteria (*Bacillus cereus*), gram negative bacteria (*Escherichia coli*) and yeast (*Candida albicans*). Streptomycin and Ketoconazole were used as a positive control against the studied bacteria and yeast respectively. These results show that random copolyesters possess mild activities against microorganisms selected.

Keywords: Polymers; Antibacterial; *Bacillus cereus; Escherichia coli; Candida albicans*

INTRODUCTION

Polyesters offer versatile physical and chemical properties for a wide variety of applications in day to day life. Changing the molecular structure of polyesters permits tailoring the thermo mechanical properties and biodegradation behavior (Sriromreun et al, 2013; Müller et al, 2010; Han et al, 2009). The physical properties of the copolyesters and degradability are significantly influenced by the incorporation of different diols and diacids in the main chain (Makadia et al, 2011, and Chen et al, 2008) and also depends on the crystallinity, the chain length and hydrophilicity / hydrophobicity balance (Shimao, 2001 and Müller et al, 2010). Polyesters are macromolecular substances characterized by the presence of carboxylate ester groups in the repeating units of their main chain and their biodegradability is due to the susceptibility of the ester bond to hydrolysis. In general, preparation of linear aliphatic polyesters consists in the thermal poly condensation of diacids with diols, or in the self-condensation of hydroxy acids followed by the elimination of water. The nature, molecular weight, molecular weight distribution of polymers, degree of cross linking, stereo configuration, etc. have a significant role on biological activities (Muñoz-Bonilla et al, 2012 and Lee et al, 2003)

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Recently, cationic agents of the quaternary ammonium type proved to be potential antiseptics and disinfectants (Frier, 1971) for a variety of clinical purposes (e.g., preoperative disinfections and disinfection of non critical surfaces). Few microwave assisted polymers were synthesized and these polymers were subjected antimicrobial efficacy on *Bacillus cereus, Escherichia coli* and *Candida albicans* and the results are presented in this paper.

MATERIALS AND METHODS

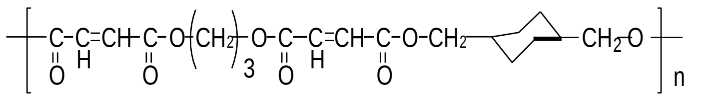
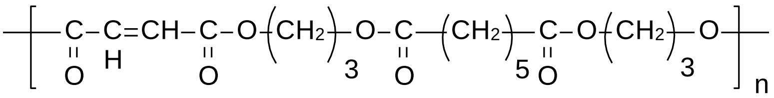
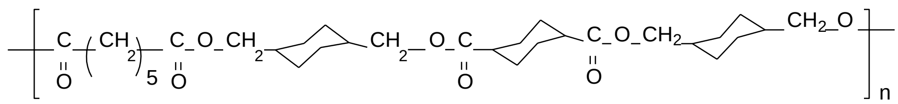
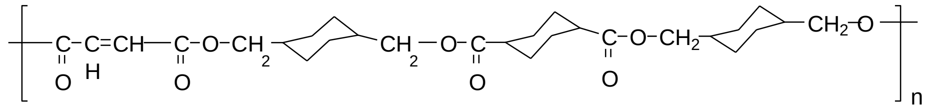
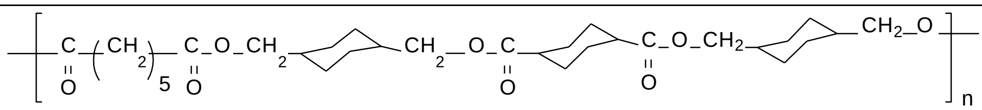
Materials

Pimelicacid (Merck) was purchased and used fumaric acid (Merck), 1,4-cyclohexanedimethanol, 1,4-cyclohexanedicarboxylicacid (Sigma Aldrich) were used as received, titanium (IV) isopropoxide ($Ti(OC_3H_7)_4$, reagent grade, Alfa Aesar Chem. Co., Ward Hill, MA) Propane1,3-diol, Butane1,4-diol (Sigma Aldrich) were purchased and used. The following solvents were used in the characterization of all the polyesters. Chloroform (Merck, AnalaR) was used as such. Spectral grade $CDCl_3$ and DMSO-d_6 was used for recording NMR spectra of the polyesters.

Synthesis of random copolyesters

The microwave assisted synthesis has been carried out using co-polyesters from propane1, 3-diol and 1, 4-cyclohexane dimethanol with various carboxylic acids in the presence of Titanium isopropoxide as the catalyst. The influence of reaction time, reaction temperature, catalyst concentration and monomer ratio of the polymer yield and the molecular weight has been studied for bulk and solution poly condensation. A reac-

Table 1: Structure of random copolyesters

Polymers	Structure
Polymer 2 (PPFCDM)	 Poly (Propane1, 3-diol Fumaric acid 1, 4-cyclohexanedimethanol)
Polymer 3 (PPFPi)	 Poly (Propane 1, 3-diol Fumaric acid Pimelic acid)
Polymer 5 (PCDMPiCDC)	 Poly (1,4-Cyclohexanedimethanol Pimelic acid 1,4-Cyclohexanedicarboxylicacid)
Polymer 8 (PCDMFCDC)	 Poly (1,4-Cyclohexanedimethanol Fumaric acid 1,4-Cyclohexanedicarboxylicacid)
Polymer 9 (PBFCDM)	 Poly (Butane1,4-diol Fumaricacid 1, 4-cyclohexanedimethanol)

tion temperature of 290°C is fixed two dicarboxylic acid/diol ratio of 1:1:2 and a MW irradiation time of 20 min on 300-500 watts. All the random copolyesters were characterized by IR spectral measurements, ^{1}H – NMR, ^{13}C NMR, viscosity measurements and solubility studies. Thermal studies were also made on these polyesters and confirmed.

Standard Culture and Maintenance:

The antimicrobial potency of the polymer, 2, 3, 5, 8 and 9 were screened against different microbes. The standard cultures were procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The microbes include gram-positive bacteria, Bacillus cereus (MTCC 430), gram-negative bacteria, *Escherichia coli* (MTCC 443) and a yeast *Candida albicans* (MTCC 183). The stock cultures were maintained in slants of Nutrient agar (Peptic digest of animal tissue, 5.0gms; Sodium chloride, 5.0gms; Beef extract, 1.5gms; Yeast extract 1.5gms; Agar, 17gms / Litre of distilled water) and Sabouraud's Dextrose Agar (Dextrose, 40gms; Mycological, peptone, 10gms; Agar, 17gms / Litre of distilled water) (SDA) and stored at 4˚C in a refrigerator for further use. Active cultures for the screening were prepared by transferring loopful of cells from stock cultures to test tubes containing Nutrient Broth and Sabouraud's Dextrose Broth and were incubated at 37˚C for 24 hours. The media compositions mentioned above were prepared without amending agar and were used as Broth.

Antibacterial susceptibility test

The well diffusion method was used to screen the antibacterial activity of the polymers 1-5. Antibacterial studies were carried out using Mueller Hilton Agar (Beef Extract, 2gms; Acid Hydrolysate of Casein, 17.5gms; Starch, 1.5 gms; Agar, 17gms / Litre of distilled water) (MHA) and antifungal studies using SDA obtained from Hi-Media. The MHA plates were prepared by pouring about 25 ml of molten agar into sterile petri plates. The plates were allowed to solidify for 30 minutes and 0.1 ml of the inoculum of both gram positive (*Bacillus cereus*) and gram negative (*Escherichia coli*) bacterial suspension was swabbed uniformly and allowed to dry for 15 minutes. Four different wells of 5mm diameter size were cut into the solidified media for the dispersal of polymers. The well was cut using a stainless steel, sterile cork borer of 5 mm diameter. Different concentrations of the polymers dissolved in 3 % of Dimethyl Sulfoxide (DMSO) (50 µl, 75 µl and 100 µl) were loaded to the respective wells each. Streptomycin was used as a positive control for bacteria and Ketoconazole of 25µl was used as a control for evaluation of anticandidal effect. The plates were incubated at 37˚C for 24-36 hours and the diameters of the zone of inhibition (clearance of growth) were recorded in millimeters. All assays were carried out in duplicate.

RESULTS AND DISCUSSION

Synthesis and characterization of random copolyesters

The aliphatic random copolyesters were synthesized by microwave assisted polycondansation of 1,4-butanediol with either two different aliphatic diacids or a diacids and diol. The structure of the repeating units present in the polymer chains determined by FT-IR, ^{1}H - NMR and ^{13}C - NMR spectra spectral studies. The thermal characterization of all the polyesters would be

Table 2: Zone of inhibition recorded for different Polymers against bacteria and Candida

Microbe		50µl	75µl	100µl	Control
		Average Zone of inhibition recorded (In mm)			
Bacillus cereus	Polymer 2	7	8	10	17
	Polymer 3	7	8	10	
	Polymer 5	7	9	10	
	Polymer 8	7	9	11	
	Polymer 9	11	11	14	
Escherichia coli	Polymer 2	7	7	10	19
	Polymer 3	8	10	12	
	Polymer 5	9	11	12	
	Polymer 8	-	7	7	
	Polymer 9	11	12	13	
Candida albicans	Polymer 2	7	7	10	22
	Polymer 3	7	7	9.5	
	Polymer 5	7	7	9	
	Polymer 8	-	-	11	
	Polymer 9	-	7	9	

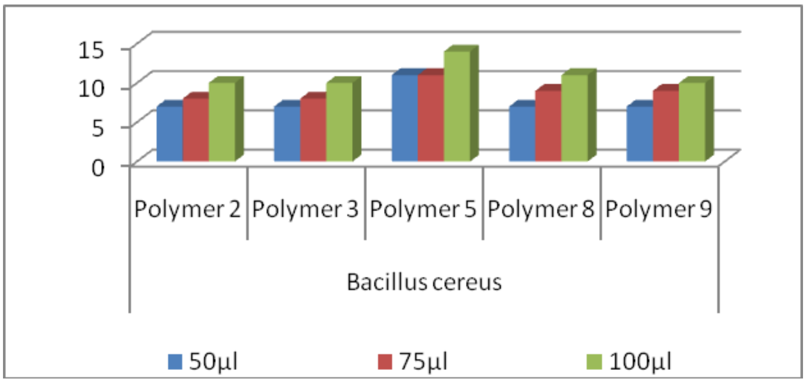


Figure 1: Zone of inhibition recorded for different Polymers against *Bacillus cereus*

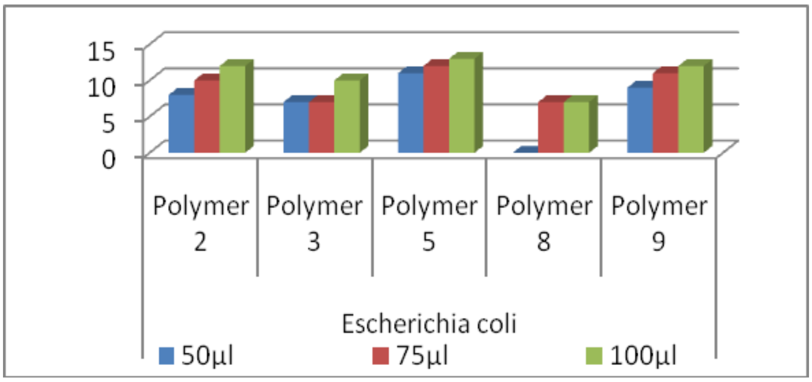


Figure 2: Zone of inhibition recorded for different Polymers against *Escherichia coli*

carried out by DSC studies and were reported. The structures of the random copolyesters used in the present study are given in Table 1.

In general, the length of the carbon chain present in the polymer backbone affects the antimicrobial activity of the polymer. Polymer containing longer alkyl chains showed that higher activity is due to availability of more active sites for adsorption with the bacteria cell wall and cytoplasmic membrane. Increasing the number of methylene group and introduction of an aromatic moiety in the polymer backbone has a significant effect on its physical and thermal properties.

Antimicrobial activity

All the polymers studied have shown the zone of inhibition against the gram positive bacteria, *Bacillus cereus.* They have recorded higher zone of inhibition only at the concentration of 100µl. Polymer 5 has recorded more zone of inhibition when compared to all other polymers. When compared with that of control, they have not recorded any significant level of zone of inhibition against the bacteria, *Bacillus cereus*. The average zone of inhibition recorded for different polymers against the bacteria, *Bacillus cereus* is given in Figure 1.

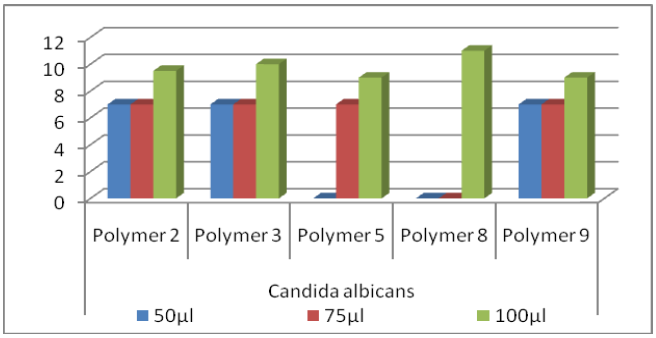


Figure 3: Zone of inhibition recorded for different Polymers against *Candida albicans*

Similar result was observed with the polymers reacting in a same way against, the gram negative bacteria, *Escherichia coli.* However, polymer 4 has not recorded any significant zone of inhibition when compared with other polymers. Polymer 5 has recorded maximum zone of inhibition against the bacteria, *Escherichia coli* when compared with other polymers. Control, Streptomycin has recorded 19 mm as its zone of inhibition when compared with the polymers studied. The average zone of inhibition recorded for different polymers against the bacteria, *Escherichia coli* is given in Figure 2.

The antifungal ability of the polymers studied showed that none of the polymers have recorded any significant level of zone of inhibition against the yeast fungus, *Candida albicans.* They have recorded their zone of inhibition only at the concentration of 100µl. Among the polymers, polymer 1 has recorded higher zone of inhibition when compared to others. The average zone of inhibition recorded for different polymers against the yeast fungus, *Candida albicans* is given in Figure 3. Average Zone of inhibition recorded for different polymers against, the gram positive, gram negative and yeast fungus are represented in Table 2.

Muňoz-Bonilla et al 2012 and Salton et la (1968) proposed a five step mechanism for the antimicrobial action of this agents (I) adsorption and penetration of the agent into the cell wall; (ii) reaction with the cytoplasmic membrane followed by its disorganization; (iii) leaking of intracellular low molecular weight material; (iv) degradation of the proteins and nucleic acids; and (v) wall lysis caused by autolytic enzymes.

CONCLUSION

Present study describes the microwave assisted synthesis and characterization of aliphatic random copolyester and its application towards the antimicrobial activity. The present study has demonstrated the antimicrobial efficacy of 5 different co-polyesters synthesized through assistance of microwave. Among the polymers, polymer Poly (Butane1,4-diol Fumaricacid 1, 4-cyclohexanedimethanol) has shown better bactericidal activity of both gram positive and gram negative bacteria. The polymer Poly (1,4-Cyclohexanedi methanol Fumaricacid 1,4-Cyclohexane dicarboxylic acid) has given better candidicidal activity. However, they are subservient to the standard antibiotics used as a control.

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