

ANTIMICROBIAL ACTIVITY OF COPOLYMERS OF 2,4-DICHLOROPHENYL ACRYLATE WITH STYRENE: SYNTHESIS, CHARACTERIZATION AND REACTIVITY RATIOS

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Abstract Some copolymers of 2,4-dichlorophenyl acrylate (2,4-DCPA) with styrene (St) of different feed compositions were prepared by free radical polymerization technique using 2,2'-azobisisobutyronitrile (AIBN) as an initiator, and the copolymers were characterized by IR spectroscopy. The copolymer composition obtained by UV-spectra led to determination of reactivity ratio by employing Fineman-Ross (F-R) and Kelen-Tudos (K-T) methods. Average molecular weight, as well as intrinsic viscosity, was obtained by vapor pressure osmometry (VPO) and Ubbelohde viscometer. Thermo gravimetric analysis (TGA) and differential thermal analysis (DTA) of copolymers were carried out under nitrogen atmosphere. Antimicrobial effects of the homo and copolymers were also investigated against various microorganisms such as bacteria, fungi and yeasts.

Keywords: Copolymers; Reactivity ratios; Vapor pressure osmometry; Thermal analysis; Antimicrobial screening.

INTRODUCTION

There has been considerable interest during the past ten years to synthesize a multifunctional macromolecular material for their potential usage in different polymer fields. Many polymers with reactive functional groups are now being synthesized, tested and used not only for their macromolecular properties, but also for the properties of the functional groups^[1]. Phenyl acrylate polymers are relatively newly developed materials compared to commercial polymers such as vinylics, acyl amides, alkyl amides, *etc.* Phenyl acrylate, phenolic esters of acrylic/methacrylic acid are considered as reactive monomers primarily because of presence of the aromatic ring^[2]. Functionalized polymers have wide applications for various biological and technological purposes^[3].

Due to their varied applications, chlorine containing phenyl methacrylate and its polymers have received considerable attention in recent years^[4, 5]. These chemicals find application as biocide also. Many chlorine containing polymers possessing antimicrobial properties have been reported^[6–9]. Soykan and coworkers^[10] synthesized acrylic copolymers and tested antimicrobial activity of prepared copolymers against different strains of bacteria and fungi. They reported that all the copolymers showed moderate activity. Patel and co-workers synthesized acrylic copolymers derived from 2,4-dichlorophenyl methacrylate as well as 4-chloro-3-methylphenyl methacrylate and tested their antimicrobial properties against various bacteria, fungi and yeasts^[11, 12]. The polymers having antimicrobial properties are suitable in variety of applications such as film, packaging materials, food stuffs, sanitary applications and many others^[13, 14].

Knowledge of the copolymer composition is an important step in the evaluation of its utility. Copolymer composition and its distribution are dependent on the reactivity ratios. The most common mathematical model of copolymerization is based on finding the relationship between composition of copolymers and composition of monomer feed in which the monomer reactivity ratios are the parameters to be determined^[15, 16]. Vijayanand^[17] and coworkers prepared copolymers with various contents of 4-nitro-3-methylphenyl methacrylate and GMA

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in methyl ethyl ketone solvent using benzoyl peroxide as free radical initiator at 70°C. Characterization of the resulting polymers was done by FT-IR, ^1H -NMR and ^{13}C -NMR spectroscopic techniques. Reactivity ratios were also calculated by Fireman-Ross (F-R) & Kelen-Tudos (K-T) methods. Thermal analysis was also carried out for these polymers.

The present work deals with the synthesis and characterization of the monomer 2,4-dichlorophenyl acrylate (2,4-DCPA) as well as homo and copolymers of 2,4-DCPA with St using different feed ratios. The copolymer composition was determined by UV spectroscopy, and molecular weight was determined by VPO. Thermal analyses are also included over here. The homo and copolymer have been used to screen their antimicrobial activity against microorganism such as bacteria (*Bacillus subtilis*, *Escherichia coli* and *Staphylococcus citreus*), fungi (*Aspergillus niger*, *Sporotichum pulveruleum* and *Trichocerma lignorum*) and yeasts (*Candida utilis*, *Saccharomyces cerevisiac* and *Pichia stipitis*).

EXPERIMENTAL

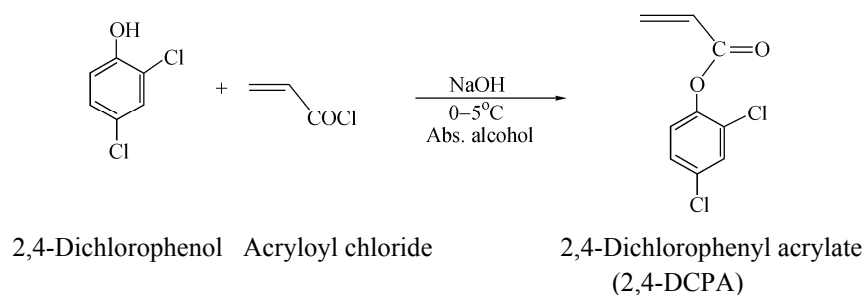
Materials

2,4-Dichlorophenol (S.D. Fine chemicals), acrylic acid (Merck), benzoyl chloride (S.D. Fine chemicals) and AIBN (Aldrich) were used as received. Styrene was freed from inhibitor by washing with 5% NaOH solution followed by distilled water, and after drying over anhydrous sodium sulfate it was distilled under vacuum. Solvents purified by fractional distillation were used in reaction.

Synthesis of 2,4-Dichlorophenyl Acrylate (2,4-DCPA)

Acryloyl chloride was prepared following the standard procedure given by Stempel^[18]. Further the esterification was performed between acryloyl chloride and 2,4-dichloro phenol (2,4-D) using following procedure:

Absolute alcohol (400 mL) and NaOH (0.2 mol) were added to a three necked flask, equipped with stirrer, condenser and thermometer. The contents were stirred until all the NaOH dissolved and 2,4-D (0.2 mol) was added to this. The reaction mixture was heated to 60°C for 30 min with stirring, and then cooled to room temperature and then 0–5°C by ice. Freshly prepared acryloyl chloride (0.21 mol) was added to the cooled reaction mixture and stirred for 90 min and then poured into crushed ice-water mixture where a light brown colored liquid settled down. It was extracted with ether. The ether layer was separated out and evaporated. The liquid monomer obtained after evaporation of ether was dried over anhydrous calcium chloride in vacuum desiccator. The product yield was 82% (Reaction Scheme 1).



Scheme 1 Synthesis of monomer 2,4-dichlorophenyl acrylate (2,4-DCPA)

The formation of monomer was confirmed by IR (Fig. 1a) and ^1H -NMR spectroscopy (Fig. 1b).

IR of 2,4-DCPA (cm^{-1}): 3015 ($-\text{CH}$ stretching vibration of the aromatic ring), 2978 ($\nu_{-\text{CH}_3}$), 1756 ($\nu_{\text{C=O}}$), 1640 ($\nu_{\text{C=C}}$), 1230 (asymmetric $\nu_{\text{C-O-C}}$), 1150 (symmetric $\nu_{\text{C-O-C}}$), 890 ($-\text{CH}$ bending mode of vinyl group), 730 (rocking mode of vinyl group), 667 ($\nu_{\text{C-Cl}}$). The two absorption bands at 1593 cm^{-1} and 1480 cm^{-1} may be assigned as the characteristic absorption of *o*-substituted phenyl rings.

^1H -NMR of 2,4-DCPA (60 MHz, δ): 5.977 (1H, $-\text{CH=}$), 6.357 (1H) and 6.533 (1H) (non-equivalent methylene protons), 7.158–7.432 (3H, aromatic protons).

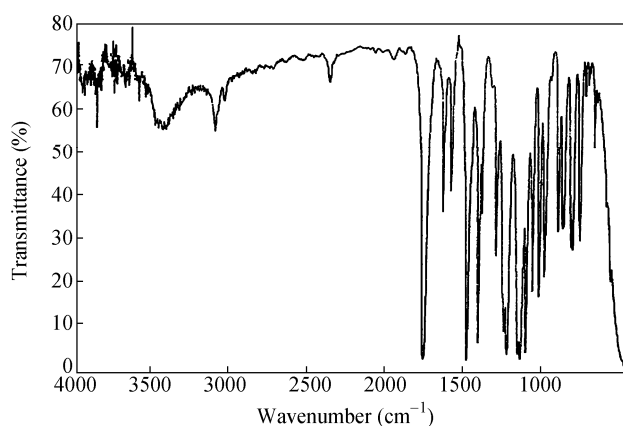


Fig. 1a FT-IR spectrum of monomer 2,4-DCPA

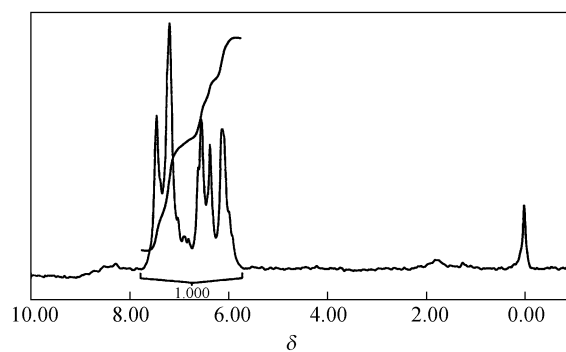
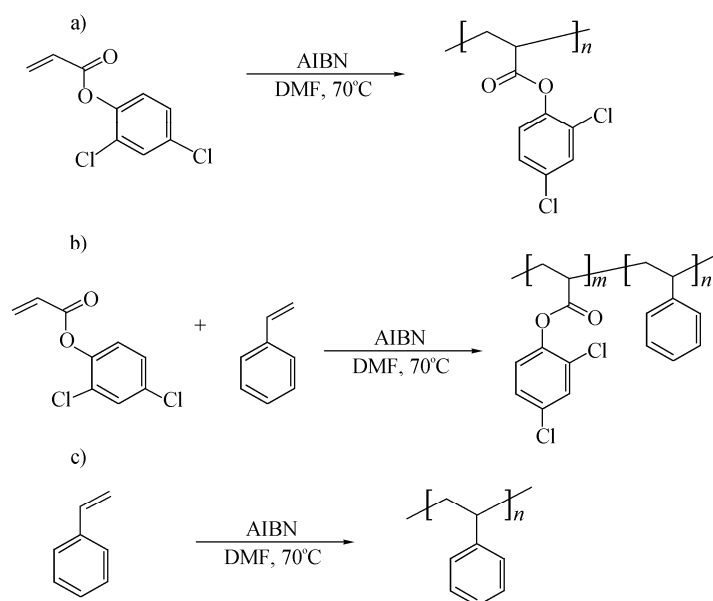


Fig. 1b ^1H -NMR spectrum of monomer 2,4-DCPA

Homo and Copolymerization

Homo and copolymerization were carried out in *N,N*-dimethyl formamide (DMF) using AIBN as an initiator. The feed composition of monomer and comonomer is given in Table 1. Predetermined quantities of 2,4-DCPA,



Scheme 2 Synthesis of (a) poly(2,4-DCPA), (b) poly(2,4-DCPA-co-St) and (c) poly(St)

St, DMF (10 mL) and AIBN (1 wt% based on total monomers 1 and 2) were taken in a polymerization tube (equipped with mechanical stirrer and reflux condenser). The reaction mixture was heated at 70°C for 5 h with constant stirring. After that, it was cooled to room temperature and slowly poured into a large excess of methanol used as a nonsolvent with constant stirring. The polymer precipitated out was purified by repeated precipitation by methanol from solution in DMF and finally dried. Reaction Scheme 2 shows the reaction leading to the formation of homopolymer as well as copolymers of 2,4-DCPA with St.

Measurements

Infrared spectra of solid samples in the KBr pellets were recorded with a NICOLET-400_D FT-IR spectrophotometer. A Shimadzu-160A recording UV-Visible spectrophotometer was used to determine copolymer composition and reactivity ratio. Molecular weights of the polymers were determined by VPO (Knaur, Germany). TGA and DTA were performed with TA Instruments (U.S.A.)-2900 thermal analyzer at a heating rate of 10 K min⁻¹ in nitrogen atmosphere.

Determination of Copolymer Composition by UV-Spectroscopy

Determination of $\lambda_{\max}$ for 2,4-DCPA

The maximum absorption wavelength ($\lambda_{\max}$) of the dilute solution of 2,4-DCPA was determined by scanning the UV spectrum in the wavelength range of 200–350 nm using chloroform as solvent and standard reference. The characteristic $\lambda_{\max}$ of 2,4-DCPA in chloroform was found at 272 nm.

Construction of calibration curve of 2,4-DCPA

Ten dilute solutions of 2,4-DCPA with known and exact concentration were prepared in chloroform. The absorption of each solution at $\lambda_{\max}$ of 2,4-DCPA was recorded using chloroform as a reference standard. The values of absorption were plotted against corresponding concentration values.

Determination of the 2,4-DCPA content in the copolymers

A dilute solution of each copolymer, containing unknown amount of 2,4-DCPA, was prepared by dissolving exact amount of the polymer into the chloroform. The weight of the polymer sample taken should be such that the concentration of the resulting solution is remaining within the concentration range of the calibration curve. The absorption of each solution was recorded, at $\lambda_{\max}$ of 2,4-DCPA, using chloroform as a reference standard. By using these absorption values, the corresponding concentration values for 2,4-DCPA composition in the copolymers were obtained from the calibration curve. The values of 2,4-DCPA content in the copolymer were converted into the appropriate units.

Antimicrobial Activity

The synthesized homo- and co-polymers were tested against different microorganism that is commonly employed for biodegradability tests. Bacterial strain (*Bacillus subtilis*, *Escherichia coli* and *Staphylococcus citreus*), fungal strain (*Aspergillus niger*, *Sporotichum pulveruleum* and *Trichocerna lignorum*) and yeast strain (*Candida utilis*, *Saccharomyces cerevisiae* and *Pichia stipitis*) were grown in Nutrient broth (N-broth) and Sabourands dextrose broth medium.

Screening of acrylic copolymers for antibacterial activity

A 5 vol% inoculum of bacterial culture was used to inoculate 100 mL solution of N-broth (control, *i.e.*, it does not contain polymer) and test media (100 mL solution of N-broth + 50 mg polymer sample) and incubated on rotary shaker (200 r/min) at room temperature. A 0.5 mL liquid was withdrawn at specific time intervals (24-48 hr) from test-media and after suitable dilution with 3,5-dinitrosalicylic acid (DNS) reagent^[19] and distilled water, absorbance was measured at 660 nm.

The activity was calculated as absorbance per milliliter (*i.e.* growth). This method is based on the principle that as growth proceeds cell number increases, which leads to increase in absorbance of medium.

Screening of acrylic copolymers for antifungal activity

Since fungal culture shows filamentous growth, an optical method cannot be used to monitor the growth. Gravimetric analysis was carried out to determine the dry cell mass. A 10 vol% inoculum was added to the

sterile control medium (without polymer) and test medium (100 mL control + 50 mg polymer sample). Flasks were incubated at room temperature on a rotary shaker (200 r/min) for 48 h. Contents of the flasks were filtered using cheese cloth and cell pellets were dried to constant weight.

Screening of acrylic copolymers on yeast

A 5 vol% inoculum of yeast culture was used to both sterile control medium and test medium (100 mL solution of N-broth + 50 mg polymer sample), and the same procedure as mentioned in antibacterial activity was followed.

RESULTS AND DISCUSSION

The copolymerization of 2,4-DCPA with St in DMF was studied in a wide composition interval with a mole fraction of 2,4-DCPA ranging from 0.2 to 0.8 in the feed. The reaction time was selected to give conversion less than 10 wt% in order to satisfy the differential copolymerization equation. The copolymers were found to be soluble in acetone, chloroform, DMF, dimethyl sulfoxide, toluene and methyl ethyl ketone.

Characterization of Copolymers

The IR spectra (Fig. 2) confirmed the structure of copolymers. Bands at 2993 cm^{-1} and 2947 cm^{-1} may be attributed to the symmetric and asymmetric $\nu_{\text{C-H}}$ stretching vibration of methylene groups where as the strong absorption at 1471 cm^{-1} may be due to $\nu_{\text{C-H}}$ bending vibration of $-\text{CH}_2-$ groups. Two sharp and distinct bands at 1770 cm^{-1} and 1215 cm^{-1} may have contribution from $\nu_{\text{C=O}}$ and $\nu_{\text{C-O}}$ stretching vibration of ester groups. The bands at 763 cm^{-1} and 702 cm^{-1} may have contribution from $\nu_{\text{C-H}}$ out of plane bending vibration of mono-substituted aromatic rings^[20]. The $\nu_{\text{C-Cl}}$ stretching frequency is assign to the band at 670 cm^{-1} ^[21]. The disappearance of the band at 1640 cm^{-1} indicates the formation of polymers.

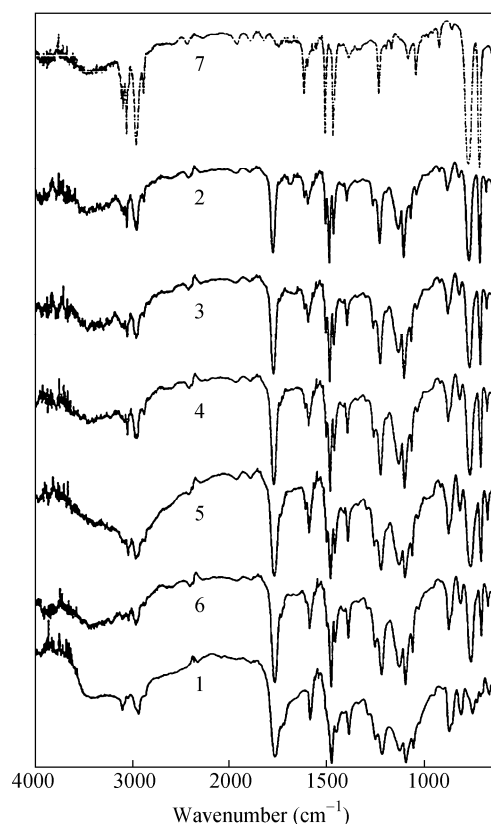


Fig. 2 FT-IR spectra of (1) poly(2,4-DCPA), (2–6) poly(2,4-DCPA-co-St) and (7) poly(St)

Copolymer Composition and Reactivity Ratios

The average composition of each copolymer sample was determined from the corresponding UV-spectrum (Fig. 3). The assignment of the absorption in the UV-spectrum allows for the accurate determination of the content of each kind of monomeric unit incorporated into the copolymers chains. The reactivity ratios of 2,4-DCPA and St were determined by the F-R^[22] and K-T^[23] methods. These values are shown in Table 1. The reactivity ratio obtained by these two methods agrees well. The reaction time was selected in such a way that conversion was less than 10% in weight.

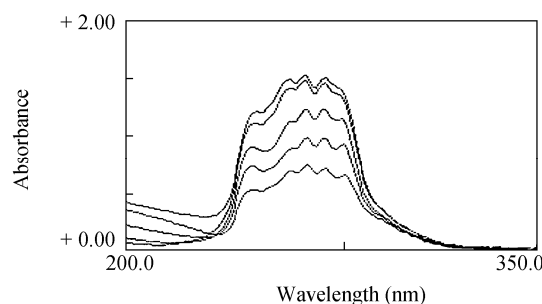


Fig. 3 UV spectra of poly(2,4-DCPA-co-St)

Table 1. Composition data, F-R and K-T parameters for copolymers of 2,4-DCPA with St

Sample code no.	2,4-DCPA [M ₁] (mol)	St [M ₂] (mol)	Conversion (%)	Composition of P(2,4-DCPA) in copolymer [m ₁]	<i>X</i>	<i>Y</i>
1	1.0	—	—	—	—	—
2	0.2	0.8	8.20	0.219	0.25	0.280
3	0.4	0.6	9.13	0.386	0.67	0.629
4	0.5	0.5	7.16	0.468	1.0	0.880
5	0.6	0.4	8.62	0.596	1.5	1.475
6	0.8	0.2	8.89	0.761	4.0	3.184
7	—	1.0	—	—	—	—

Sample code no.	<i>F</i>	<i>G</i>	ξ	η	Reactivity ratio			
					F-R		K-T	
					<i>r</i> ₁	<i>r</i> ₂	<i>r</i> ₁	<i>r</i> ₂
1	—	—	—	—				
2	0.223	−0.64	0.16	−0.47				
3	0.714	−0.39	0.38	−0.21				
4	1.136	−0.13	0.50	−0.06	0.72	0.82	0.71	0.83
5	1.525	0.48	0.57	0.18				
6	5.025	2.74	0.81	0.44				
7	—	—	—	—				

Where, $m_2 = 1 - m_1$; $X = M_1/M_2$; $Y = m_1/m_2$; $F = X^2/Y$; $G = X(Y - 1/Y)$; $\xi = F/\alpha + F$; $\eta = G/\alpha + F$ and $\alpha = [F_M \cdot F_m]^{1/2}$

A plot of *G* versus *F* in F-R method was a straight line as shown in Fig. 4(a). The intercept at *Y* axis gives the value of $-r_2$ and the slope gives value of r_1 . Similarly in K-T method, plot of η against ξ gives a straight line as shown in Fig. 4(b), and extrapolation to $\xi = 0$ and $\xi = 1$ gives $-r_2/\alpha$ and r_1 , respectively. The value of reactivity ratios for 2,4-DCPA (r_1) and St (r_2) from F-R plot is 0.72 and 0.82, respectively. In poly(2,4-DCPA-co-St), the value of r_2 is greater than r_1 . St is found to be slightly more reactive than 2,4-DCPA. The values of r_1 and r_2 are less than 1. The system gives rise to azeotropic polymerization at particular composition of monomer using the following equation^[24].

$$N_1 = \frac{(1 - r_2)}{(2 - r_1 - r_2)}$$

From above equation the value of N_1 is 0.385 (by F-R method). When the mole fraction of the monomer 2,4-DCPA is 0.385 in the feed, the copolymer formed will have the same composition as that of the feed. When the mole fraction of the 2,4-DCPA in the feed is below 0.385, the copolymer is richer in 2,4-DCPA monomeric units. When the mole fraction of 2,4-DCPA in the feed is above 0.385, the copolymer formed will be richer in St monomeric units.

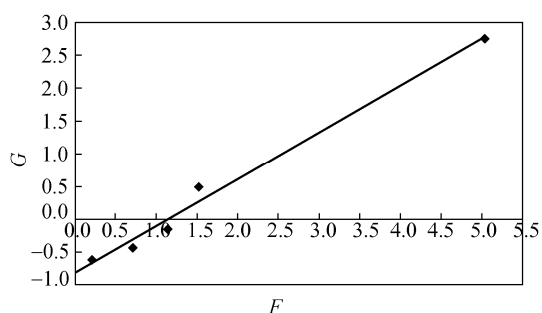


Fig. 4a F-R plot for poly(2,4-DCPA-co-St)

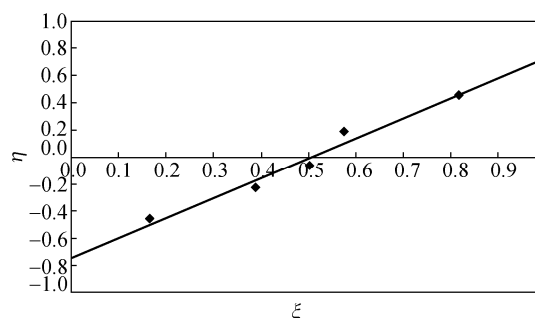


Fig. 4b K-T plot for poly(2,4-DCPA-co-St)

Molecular Weights and Viscosity Measurements

The VPO data for poly(2,4-DCPA-co-St) shows that M_n lies in the range of 4600–7900. From these data it is concluded that as 2,4-DCPA content increases the molecular weight increases or decreases randomly. The intrinsic viscosity lies in the range of 0.009–0.021 dL·g⁻¹. Viscosity follows the same trends as that of molecular weight for poly(2,4-DCPA-co-St) *i.e.* as 2,4-DCPA content in copolymers increases, viscosity increases or decreases randomly (Table 2).

Table 2. VPO and viscosity data for poly(2,4-DCPA), poly(St) and poly(2,4-DCPA-co-St)

Sample code no.	M_n	Intrinsic viscosity $[\eta]$ (dL·g ⁻¹)
1	7900	0.021
2	5100	0.010
3	4600	0.009
4	6000	0.014
5	5500	0.012
6	6900	0.017
7	5000	0.010

Thermal Analysis

The results of TGA analysis of homo- and copolymers are presented in Table 3. The data clearly indicate that all polymers undergo double step decomposition. The first decomposition occurs between 191°C and 340°C where as the second step between 303°C and 455°C. Activation energy (E_A) and integral procedural decomposition temperature (IPDT) were determined by Broido's method^[25] and Doyle's method^[26], respectively, and the data are represented in the table.

The DTA data of homo- and copolymers are presented in Table 4. The activation energy for thermal degradation and reaction order were obtained by Reich's method^[27]. The activation energy for thermal degradation of polymers ranges from 38–87 kJ·mol⁻¹, and all the copolymers undergoes a first order reaction.

Table 3. TGA data for homo- and copolymers of 2,4-DCPA with St

Sample code no.	% Weight loss at various temperature (°C)					Decomposition temperature range (°C)
	200	300	400	500	600	
1	1	6	79	87	92	191–340 341–424
2	3	6	86	95	98	234–324 325–415
4	5	10	83	93	97	255–320 321–445
6	5	14	76	90	95	215–324 325–428
7	0	2	32	99	100	303–455

Sample code no.	$T_{\max}^a$ (°C)	T_{50}^b (°C)	IPDT ^c (°C)	Activation energy ^d (E_A) (kJ·mol ⁻¹)
1	389	383	403	75
2	389	385	409	72
4	387	382	408	74
6	394	389	414	76
7	414	411	418	79

^aTemperature for maximum rate of decomposition; ^bTemperature for 50% weight loss;^cIntegral procedural decomposition temperature; ^dBy Broido's method**Table 4.** DTA data for homo- and copolymers of 2,4-DCPA with St

Sample code no.	T_1^a (°C)	T_2^b (°C)	T_b^c (°C)	Activation energy ^d (E_A) (kJ·mol ⁻¹)	Reaction order
1	385	440	412	76.44	1
2	390	467	418	76.11	0.5
	531	600	582	38.24	1
4	391	470	422	79.33	1
	538	600	580	44.34	1
6	396	600	572	83.72	1
7	417	511	462	87.04	1

^aStarting Temperature of DTA trace; ^bEnding Temperature of DTA trace;^cPeak maxima Temperature of DTA trace; ^dActivation Energy by Reich's method

Antimicrobial Activity

It has already reported^[28] that 2,4-dichlorophenol is a very effective antimicrobial agent. The results of present investigation shows that poly(2,4-DCPA) has maximum growth inhibition against all microorganisms. All the copolymer systems showed almost similar antimicrobial properties against bacteria, fungi and yeasts. Poly(2,4-DCPA) allowed almost 48%–51% growth of bacteria, 43%–48% growth of fungi and 42%–44% growth of yeasts. When 2,4-DCPA is replaced by St (*i.e.* in copolymer of 2,4-DCPA and St) the extent of antimicrobial property is reduced. It has been generally observed that as the amount of St increases, the antimicrobial properties decrease. As expected, the growth of the microorganism in poly(St) is quite substantial (Figs. 5, 6 and 7). The inhibition properties of polymers are as follows:

$$2,4\text{-DP} > 1 > 6 > 5 > 4 > 3 > 2 > 7$$

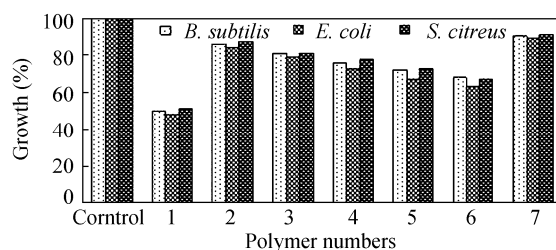


Fig. 5 Effect of homo- and copolymers of 2,4-DCPA with St on growth (%) of bacteria

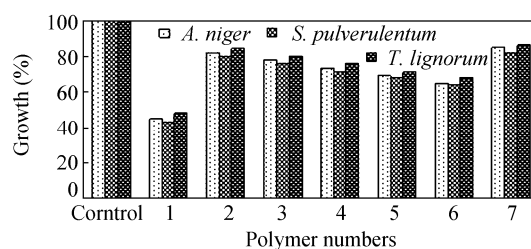


Fig. 6 Effect of homo- and copolymers of 2,4-DCPA with St on growth (%) of fungi

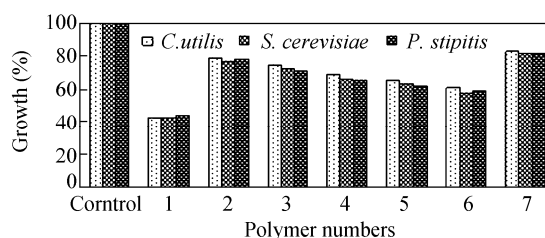


Fig. 7 Effect of homo- and copolymers of 2,4-DCPA with St on growth (%) of yeast

CONCLUSIONS

New acrylate based homo and copolymers were synthesized by free radical solution polymerization technique. The monomers and copolymers were characterized by spectroscopic methods. The reactivity ratio of 2,4-DCPA (r_1) is less than that of St (r_2), and the product of reactivity ratio was less than one. This shows that the monomers were distributed in the copolymer chain in a random fashion. The number average molecular weight (M_n) was in the range of 4600–7900. Thermal analyses showed that all copolymers of 2,4-DCPA undergo two step decomposition whereas poly(St) decompose in a single step. The antimicrobial activity of the homo- and copolymers of 2,4-DCPA with St was studied. The poly(2,4-DCPA) was found to be excellent inhibiting the growth of microorganisms. This may be traced due to high chlorine content of the homopolymer. As the percentage of St in the copolymers increases, the effectiveness of the copolymer in inhibiting the growth of microorganism's decreases. Although the chlorine content of the polymers appears to be the most important component to impart antimicrobial properties, other factors also need to be investigated.

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