Phthalimide Subsidiary Containing Novel Acrylate Copolymers to Use as Antimicrobial Agent

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Abstract:

New cyclic amide acrylate based monomer 2-(N-phthalimido) ethyl acrylate (NPEA) was synthesized by reacting N-(2-hydroxyl ethyl) phthalimide with acryloyl chloride. The free radical solution polymerization of (NPEA) with methyl methylacrylate was carried out in dimethyl formamide (DMF) solution at 70 ± 2°C using 2-2'-azobisisobutytronitrile (AIBN) as an initiator with different monomer feed ratios. The monomer (NPEA), poly(NPEA) and copolymer of poly(NPEA-co-MMA) were characterized by FT-IR and 1H-NMR. The reactivity ratios of monomeric units were evaluated using UV spectroscopy. The reactivity ratios were calculated by Finemann-Ross (F-R) and Kelen-Tudos (K-T) methods. Average molecular weights and polydispersity index were determined by gel permeation chromatography (GPC). Thermogravimetry (TG) was employed to investigate thermal stability of the copolymers. The prepared homo and copolymers were tested for their antimicrobial activity against various bacteria, fungi and yeast.

Keywords: Phthalimide, Reactivity ratio, Thermal study, Antimicrobial screening.

INTRODUCTION

Copolymerization is one of the important techniques being used to impact methodical changes in the properties of the commercially vital polymers. Normally acrylates or methacrylates monomers are copolymerized by free radical solution polymerization. In this technique, initiator used to initiate the polymerization. The study shows (Patel et al, 2007; Senthilkumar et al 2001; Parambil et al,2012) that different types of initiators are available in market, also synthesized in lab and are used as free radical initiators. The studies shows (Lien et al,1968; Lee et al, 2003; Singh et al, 2015, Jeganathan and Prakya, 2014) that acrylate and methacrylate polymers with reactive functional groups are now being synthesized, tested and used not only for their macromolecular properties, but also for the properties of functional group in specific end applications. Attachment of anticancer agents to Phthalimide polymers has been demonstrated to improve their therapeutic profiles (Xin et al, 2009).

Phthalimido based polymers used as semiconductors in solar cells (Konstantinova and Grabchev, 1997). Phthalimido-group-containing polymers have excellent heat resistance and transparency properties. The study (Oh et al, 1994) of Copolymers containing the phthalimide derivatives have been used as optical brightening agents. Copolymers of phthalimidomethyl methacrylate and sodium methacrylate have been used as ion exchanging polymer stabilizer (Shekh et al, 2016). The uniqueness of MMA as a plastic component accounts for its industrial use in this capacity and this far exceeds the combined use of all the other methacrylate. The study (Ahmed et al, 2008) shows the acrylic polymer used in coating of ambroxol hydrochloride pallets. It is observed that (Silvestri et al, 2009), Introducing hydrophilic moiety of 2-hydroxyethyl methacrylate in

the poly(MMA) chain, which is used for control drug delivery. The (Pekel et al, 2001) prepared copolymer of N-vinyl imdazole and ethyl methacrylate with different feed ratio and evaluated for reactivity ratios by using F-R (Fineman and Ross, 1992) and K-T (Kelen and Tudos, 1995) methods.

Present work focuses on synthesis and characterization of 2-(N-Phthalimido) ethylacrylate (NPEA) and its copolymers with methyl methacrylate (MMA). Reactivity ratio values of the monomers were determined. Thermal properties were determined using TGA. The prepared copolymers were tested for their antimicrobial activity against various bacteria, fungi and yeast.

MATERIALS AND METHODS

Acryloyl chloride, AIBN, methyl methacrylate, phthalic anhydride and monoethanol amine were analytical grade reagents and used without further purification.

Synthesis of N-(2-hydroxyethyl) Phthalimide (NHEP) and 2-(N-Phthalimido) ethyl acrylate (NPEA)

Synthesis of N-(2-hydroxyethyl) Phthalimide (NHEP) and 2-(N-Phthalimido) ethyl acrylate (NPEA) by reported Method (Senthilkumar 2001). The formation of the monomer was confirmed by 1H-NMR spectra (Fig 1) and FT-IR (Fig 2).
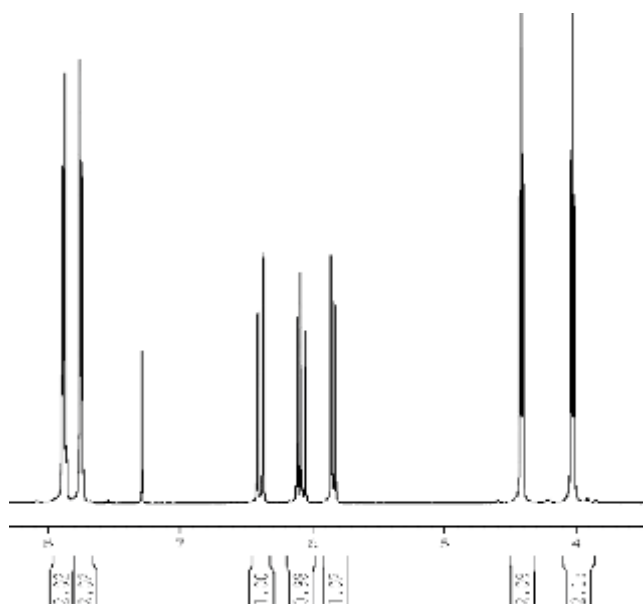

Fig 1. ^{1}H-NMR spectrum of monomer NPEA.

IR (cm^{-1}): 3041 (C-H stretching in olefinic & aromatic ring), 2947 (-C-H stretching vibration due to alkyl group), 1773 ($_{C=O}$ due to ester group), 1725 ($_{C=O}$ due to phthalimide group), 1637 ($_{C=C}$), 1192 (asymmetric $_{C-O-C}$), 1152 (symmetric $_{C-O-C}$), Two strong absorptions at 990

and 910 cm-1 are seen in this type of vinyl moiety due to out of plane bending of C-H. The out of plane C-H bending in aromatic is ~730 cm^{-1}.
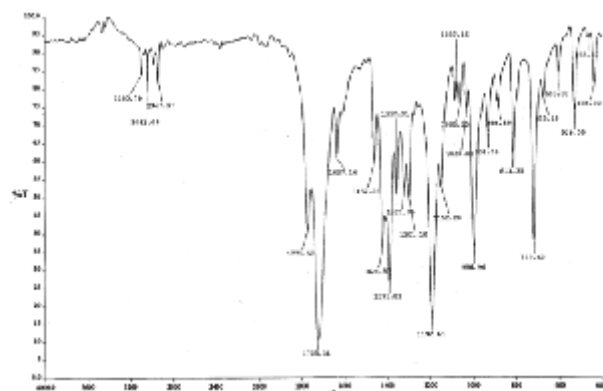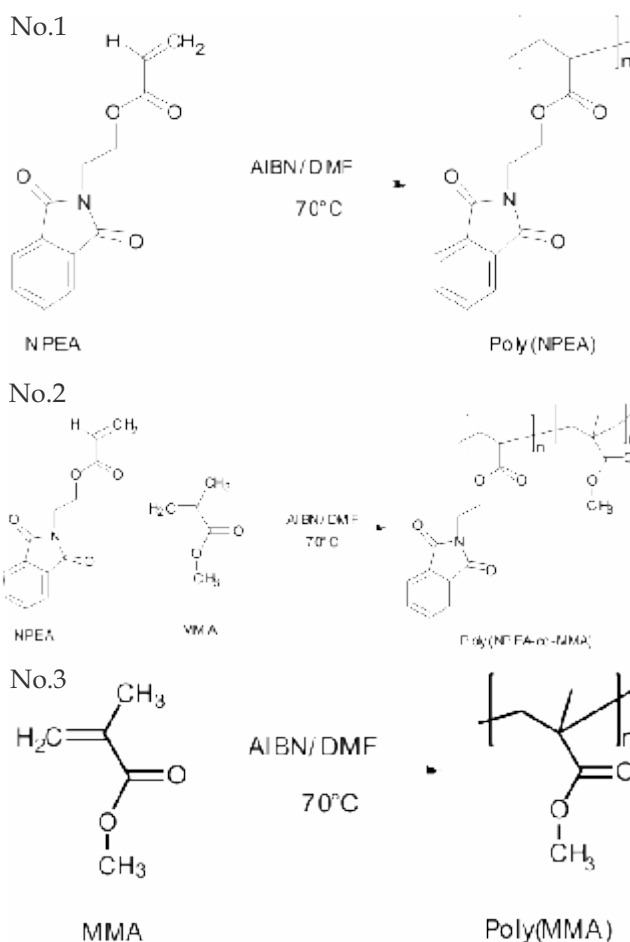

Fig 2. FT-IR spectrum of monomer NPEA.

Synthesis of Homopolymer and Copolymer

Reported procedure (Patel, 2007) was employed to prepare homo and copolymer of NPEMA with CMPMA. Scheme 1 shows the reactions leading to the formation of homopolymers as well as copolymers of NPEMA with CMPMA.

Scheme 1. (1) poly(NPEA), (2-6) poly(NPEA-co-MMA) and (7) poly(MMA).

CHARACTERIZATION TECHNIQUES

Infra-red spectra of the monomer, homo and copolymer in KBr pellet were recorded on Nicolet 400D FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded in CDCl$_3$ on a Bruker Avance 400 (MHz) spectrometer using solvent peak as internal standard at 400 MHz. The number average and weight average molecular weight of the polymers were determined by GPC technique using polystyrene as standard and DMF as eluent at 1.0 ml/min flow rate. TGA curves of the homo and copolymers were recorded on TA instrument (USA) 2690 thermogravimetric analyzer in nitrogen atmostphere at a heating rate of 10°C/min.

Determination of Copolymer Composition

Shimadzu-160A-recording UV visible spectrophotometer was employed to obtain UV spectra to find the composition of NPEA in the copolymers. The wavelength λ_{max}, at which the absorbance is maximum, was determined by scanning the dilute solution of NPEA in the wavelength range of 200-350 nm using chloroform as solvent. The characteristic wavelength (λ_{max}) of NPEA in chloroform was found to be 294 nm.

Antimicrobial Activity

The homopolymer and its copolymers were tested against different bacterial strains (*Bacillus subtilis, Escherichia coli and staphylococcus citreus*), fungal strains (*Aspergillus niger, Sportichum pulveruletum and Trichoderma lignorum*), yeast strains (*Candida utilis, Saccharomyces cerevisial and Pichia stipitis*) which were grown in Nutrient broth (N-broth) and sabouraud's dextrose broth and YEPD (yeast extract potato dextrose) medium, respectively, with or without indicated polymers. The antimicrobial properties were screened using the procedure reported in reference (Patel, 2007).

RESULTS AND DISCUSSION

Table 1. Reaction parameters for homo and copolymers of NPEA with MMA.

| Sample No. | Monomer feed ratio | | | | | | Composition of NPEMA in Copolymer |
| | NPEA | | | MMA | | | |
	mole	Gms.	Wt. %	Mole	Gms.	Wt. %	
1	1	245	100	-	-	-	100
2	0.2	49	20	0.8	80	80	39
3	0.4	98	40	0.6	60	60	54
4	0.5	122.5	50	0.5	50	50	66
5	0.6	147	60	0.4	40	40	80
6	0.8	196	80	0.2	20	20	92
7	-	-	-	1	100	-	-

Synthesis of Homopolymer and Copolymer

Different homo and copolymers were obtained by the free radical solution polymerization technique with the mole fractions of NPEA ranging from 1.0 to 0.2 in the feed were studied. The reaction time was selected in trials to give conversions less than 10% in order to satisfy the differential copolymerization equation. The data on the composition of the feed and copolymers are given in Table 1.

Characterization of Polymers

The IR spectra of the homo and copolymers are shown in Fig 4. The –CH stretching vibration of the aromatic ring is seen at 3100 cm^{-1}, the peak at 1773 cm^{-1} is due to ester group, the C=O stretching of phthalimide is assigned to 1725 cm^{-1}, 675 and 996 cm^{-1} for –CH out of plane bending, 1763 cm^{-1} for c=c. The absorption at 1192 cm^{-1} is traced to asymmetric c-o-c and 719 cm^{-1} is there for rocking mode of vinyl group. The main evidence of the polymer formation is the disappearance of some characteristic peaks of the double bond in the spectrum, thus the absorption bands at 996 cm^{-1} and 675 cm^{-1} assigned respectively to the C-H out of plane bending and C-H rocking mode of vinyl group and the stretching vibration of C=C at 1637 cm^{-1} disappeared in the IR specta of poly (NPEA) and its copolymers. IR spectra of poly (NPEA-co-MMA) shows that as NPEA content increases in the copolymers the intensity of the band at 720 cm^{-1} due to C-H out of plane bending in phenyl moiety of NPEA and 1393 cm^{-1} due to C-H bending of methylene protons also increases.

Fig 4. FT-IR spectra of (1) poly(NPEA), (2-6) poly(NPEA-co-MMA) and (7) poly(MMA).
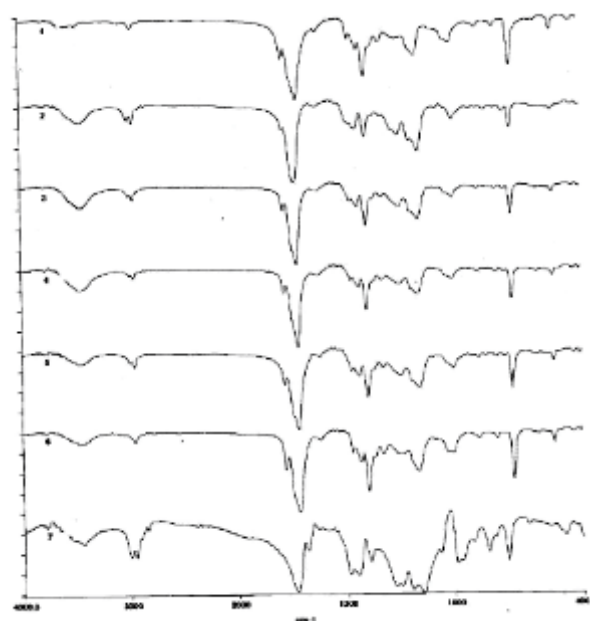
Reactivity Ratios

The monomer reactivity ratios, r_1 and r_2, were calculated using F-R(fig 5) and K-T(fig 6) methods are shown in Table 2. F-R method gives the values of r_1 (1.06) which is greater than that of r_2 (0.61) for poly (NPEA-c0o-MMA). NPEA is found to have higher reactivity than MMA and the values of r_1 is greater than 1. The product of r_1r_2 is less than one, which indicates that the system follows a random distribution of monomeric unit. Copolymer formed will be richer in NPEA monomeric unit.
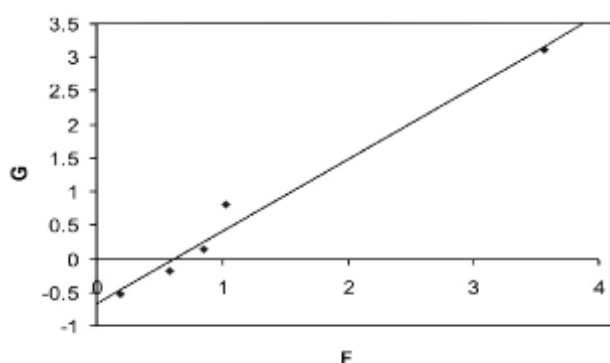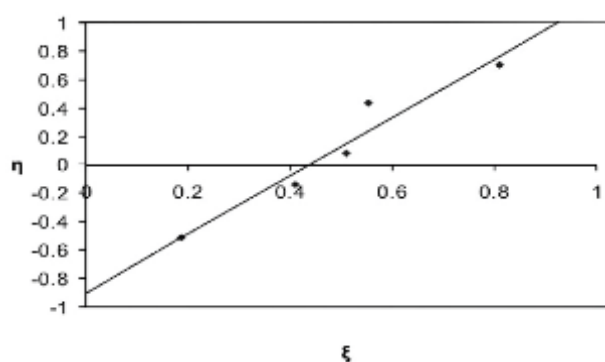
Fig 5. F-R plot for poly(NPEA-co-MMA).

Fig 6. K-T plot for poly(NPEA-co-MMA).

Molecular Weights

The GPC data of poly (NPEA) gave nM, wM and poly dispersity index as 29400, 39600, and 1.3 respectively (Table 3). The intrinsic viscosity [] was found to be 0.21 dl.g^{-1}. The GPC data for poly(NPEA-co-MMA) provided the values of nM, wM and poly dispersity index which range from 17200 to 22000, 25000 to 39000 and 1.4 to 1.9 are shown in Table 3 respectively. The intrinsic viscosity was in the range 0.13 to o.16 dl.g^{-1} and for poly (MMA) values of intrinsic viscosity is 0.14 dl.g^{-1}.

Thermal analysis

TGA characteristics for poly (NPEA), poly (MMA), and poly (NPEA-co-MMA) are shown in Fig 7 and Table 4. It is observed from the data that poly (NPEA)

Table 3. GPC data for homo & copolymers.

Sample No.	Mn	Mw	Polydispersity index
1	29400	39600	1.3
2	20156	39000	1.9
3	21126	38100	1.8
4	22000	36500	1.6
5	21956	33900	1.5
6	17200	25000	1.4
7	32549	60527	1.8

shows one step decomposition in the range 269 to 498°C. Copolymers of monomers NPEA and MMA obtained using different feed ratios, show thermal decomposition temperature in the range 200 to 402°C. The values of T_{max} and T_{50} for poly (NPEA) obtained from the thermogram are 371°C and 370°C respectively and T_{max} and T_{50} for its copolymers with MMA from 339 to 366°C and 342 to 368°C respectively. Broido's method (Broido, 1969) was employed to obtain activation energies which varied from 43-49 kJ.mole^{-1} for poly (NPEA-co-MMA). The activation energy of homopolymers of NPEA and MMA are 51 and 38 kJ.mole^{-1} respectively. The values of integral procedural decomposition temperature (IPDT) calculated by Doyle's method [Doyle, 1961] for poly (NPEA) is 547°C and for its copolymers with MMA are in the range of 434 to 475°C.

**Fig 7. TGA graph of homo and copolymer of NPEA/MMA.**
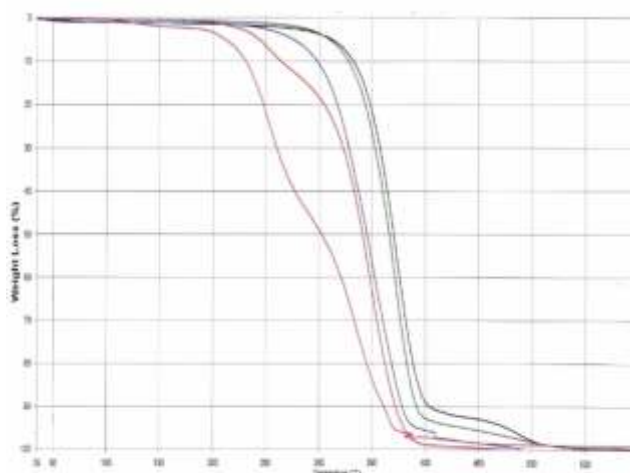
Antimicrobial Activity

The results obtained for antimicrobial activity of the poly (NPEA), poly (NPEA-co-MMA) and poly (MMA) are presented in Fig 8, 9 and 10 respectively. Poly (NPEA) allows 46-51% growth of bacteria, where as its copolymers favors 67-86 % growth. Fungi in

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Table 4. TGA data for homo and copolymers of NPEA with MMA

Sample No.	% weight loss at different temperature (°C)			Decomposition temperature range (°C)	T_{max}^{a} (°C)	T_{50}^{b} (°C)	$IPDT^{c}$ (°C)	Activation energy^{d} (KJ.mole^{-1})
	300	400	500					
1	4	89	97	269-498	371	370	547	51
2	18	99	100	210-388	339	342	475	43
3	14	90	98	219-389	344	345	452	45
4	11	96	100	236-389	350	346	448	46
5	7	85	96	244-401	357	354	441	48
6	4	90	98	255-402	366	368	434	49
7	49	89	99	200-383	325	304	414	38

presence of poly (NPEA) register around 57-60 % growth, while 63-85 % growth for fungi is observed in the copolymers. Yeast in presence of poly (NPEA)

Fig 8. Effect of poly(NPEA-co-MMA) homo and copolymers on growth (%) of Bacteria.
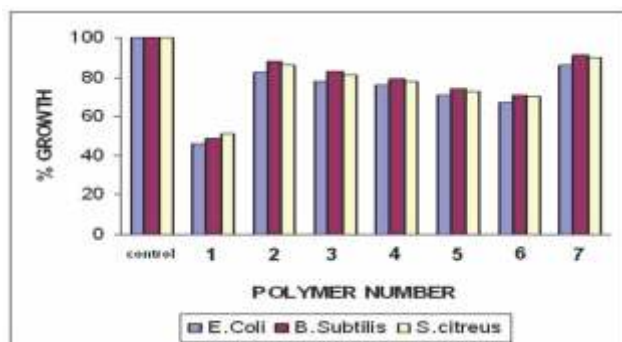
Fig 9. Effect of poly(NPEA-co-MMA) homo and copolymers on growth (%) of Fungi.
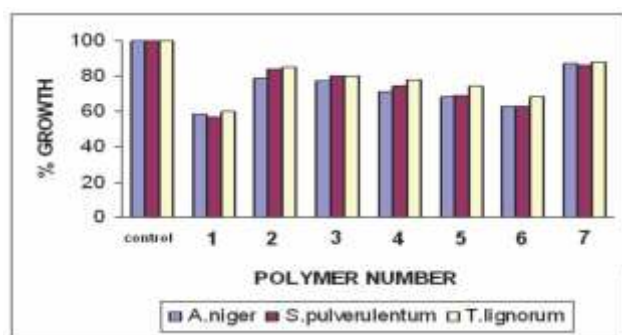
Fig 10. Effect of poly(NPEA-co-MMA) homo and copolymers on growth (%) of Yeast.
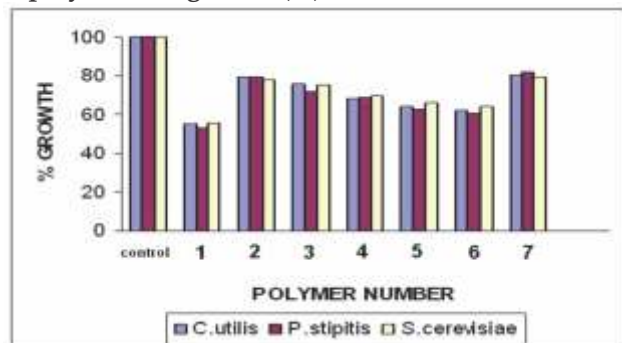
shows 53-56 % growth, while 61-80 % growth for the same is seen in the copolymers. As NPEA content in the copolymers increases the antimicrobial activity decreases. The homopolymer of NPEA inhibits more than their copolymers with MMA. Under the toxicity free environmental condition, all the microorganisms exhibited almost identical growth pattern.

CONCLUSIONS

New acrylate based homo and copolymers were synthesized by free radical solution polymerization technique. The monomers and copolymers were characterized by spectroscopic methods. TGA showed that poly (NPEA), poly (NPEA-co-MMA) and poly (MMA) undergoes single step decomposition. The value of IPDT represents an overall thermal stability of the polymers. The activation energy compares well with the decomposition temperature range. The reactivity ratio of NPEA (r1) is greater than that of MMA (r2) and the product of r1r2 is less than one. This shows that the monomers are distributed in the copolymer chain in random fashion. The results of antimicrobial activity showed that the copolymers containing NPEA have less growth inhibition than homopolymer of NPEA against all microorganisms and as NPEA content in the copolymers increases the growth inhibition of microorganism's decreases, that means homopolymer of NPEA is more preferable to use as antimicrobial agent. GPC results indicate that molecular weight decreases and polydispersity index changes randomly as the NPEA content increases in copolymer.

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