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N-tert-butylacrylamide based Copolymers –I :Synthesis and Characterization of Poly(N-tert-butylacrylamide - co -7-acryloyloxy-4-methyl coumarin)

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Abstract: Copolymerization of N-tert-butylacrylamide (NTB) and 7-acryloyloxy-4-methyl coumarin (AMC) was carried out in DMF medium at 60°C using AIBN as initiator. The copolymers were characterized by ^{1}H-NMR spectroscopy and the copolymer compositions were determined by ^{1}H-NMR analysis. The reactivity ratios of monomers were determined by Fineman -Ross and Keleln-Tudos methods. The reactivity ratios were found to be r_1= 1.15 and r_2 = 0.70 by Fineman –Ross and r_1 = 1.16 and r_2 = 0.72 by Kelen-Tudos . These values suggest that NTB is more reactive than AMC. The $r_1.r_2$ = 0.805 value indicates the formation of random copolymers. Antimicrobial activities of different copolymers synthesized were also studied against different bacteria and fungi.
Keywords: N-tert-butylacrylamide, copolymer composition, reactivity ratios,antimicrobial activity.

Introduction

Many polymers with reactive functional groups are now being synthesized, tested and used not only for the macromolecular properties but also for the properties of functional groups. These functional groups provide an approach to a subsequent modification of the polymers for specific end application [1]. In recent years, some comprehensive work has been published on functional monomers and their polymers [2, 3].

The N-substituted acrylamides are used to prepare thermo sensitive polymers like poly(N-isopropylacrylamide) and copolymers of N-alkyl acrylamide and styrene [4]. Thermosensitive polymers have great potential in applications as drug delivery system [5] human gene vector [6] and biocatalysts [7].

The determination of copolymer composition and reactivity ratios of the monomers is important in evaluating the specific application of the copolymer [8]. The monomer reactivity ratios determined by conventional linearization methods are not always accurate and several non-linear methods have been attempted to determine their value [9–11]. ^{1}H-NMR spectroscopic analysis has been established as a powerful tool for the estimation of copolymer composition [12, 13].

The use of antimicrobial polymers offers promise for enhancing the efficacy of some existing antimicrobial agents and minimizing the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity of the agents, increasing their efficiency and selectivity, and prolonging the lifetime of the antimicrobial agents. Kenawy and coworkers discussed on the requirements of

antimicrobial polymers, factors affecting the antimicrobial activities, methods of synthesizing antimicrobial polymers, major field of application, and future and perspectives in the field of antimicrobial polymers in their review article [14].
Brahmbhatt et al. prepared poly (3-phenoxycoumarin ethylene) s and determined their toxicity
effect on various fungal and bacterial strains [15]. These polymers showed good biological activity. Lee and co-workers [16] prepared coumaryl acrylate and further they synthesized poly (cinnam-4'-yl methyl methacrylate). They investigated optical anisotropy using UV-Vis spectrometer and also studied the thermal stability of polymer films as photo alignment layer. Huyck et al. have synthesized coumarin functionalized poly (alkyl acrylate) and poly(alkyl methacrylate) random copolymers and studied the influence of copolymer composition on photocross-linking [17]. Lindsay and co-workers [18] synthesized the copolymerization of coumarin methacrylate with isobornyl methacrylate. These polymers showed tremendous non-linear optical properties. There are reports on the antifungal activities on monomeric coumarin, although works on coumarin polymers are rare. Since, coumarin and its derivatives have attracted considerable interest because of various physiological and biochemical properties.

Due to various physiological and biochemical properties of coumarin, our interest was to synthesize copolymers containing coumarin pendent groups. The Present work focuses on synthesis and characterization of N-tert-butylacrylamide (NTB) and 7-acryloyloxy-4-methyl coumarin (AMC) . The prepared copolymers were tested for their antimicrobial activity against various bacteria and fungi.

Experimental

Materials

Acrylonitrile was first washed with 5% NaOH solution in water to remove the inhibitor and then with 3% Orthophosphoric acid solution in water to remove basic impurities. Then the Acrylonitile was washed with double distilled water and dried over anhydrous $CaCl_2$. The acrylonitrile was then distilled in an atmosphere of nitrogen and reduced pressure. It was then collected in a clean dry amber colored bottle and kept in the refrigerator at 5^oC. AIBN was recrystallized from chloroform. N,N'- dimethyl formamide (DMF) was dried in magnesium sulphate.

Preparation of N-tert-butylacrylamide(NTB)

The Monomer N-tert-butylacrylamide was prepared by the reaction of t-butyl alcohol with acrylonitrile[19]. N-tert-butylacrylamide was recrystallized in warm dry benzene. The white crystals have a mp. 94^oC and the yield was 87 %. The monomer was confirmed by both ^{1}H-NMR and ^{13}C-NMR spectroscopy.

^{1}H-NMR Spectroscopy $CDCl_3$, δ(PPM)

The ^{1}H-NMR spectra of monomers and copolymers were recorded on the GSX-400 spectrometer (JEOL, Tokyo, Japan) operating at 400 MHz respectively in $CDCl_3$. The following peaks appear in NTB spectrum; at 1.42 ppm for tert-butyl protons, at 5.59-6.28 ppm for vinyl protons and at 7.27 ppm for N-H proton

^{13}C-NMR($CDCl_3$), δ(ppm) :

δ 164.80(CH_2 = C(H)-<u>CO</u>-NH..);
δ 132.93(CH_2 =<u>C</u>(H)-CO-NH…);
δ 122.82(<u>CH_2</u>=C(H)-CO-NH..) ;
δ 51.37(-<u>C</u>(CH_3)$_3$;
δ 28.77 (-C (<u>C</u>H$_3$)$_3$

Preparation of 7-acryloyloxy-4-methyl coumarin (AMC)

Acryloyl chloride was prepared by reacting acrylic acid with benzoyl chloride. The 7-acryloyloxy-4-methyl coumarin (AMC) comonomer was prepared by esterfiation of 7-hydroxy-4-methyl coumarin and acrloyl chloride. Absolute ethanol (400mL) and NaOH (0.2 mol) were added to a three-necked flask that was equipped with stirrer, condenser and thermometer. The flask was placed in a water bath and the contents were stirred until all the NaOH was dissolved. Next, 7-hydroxy-4-methyl coumarin (0.2 mol) was added to the reaction mixture, which was then heated to room temp. and then cooled to 0-5^oC by ice. Freshly prepared acryloyl chloride (0.2 mol) was added in a drop wise manner to the cooled reaction mixture and stirred for 90 min. The mixture was then poured in to a crushed-ice-water mixture where a white colored product was separated out. The product was filtered, washed thoroughly with cold water and recrystallized from methanol.

IR (KBr, cm^{-1}): 3073(-CH stretching vibration of the aromatic ring), 2986 (-CH_3), 1737 (broad, C=O of acrylate and of coumarin moiety), 1630 (C=C), 1240 (asymmetric C-O-C), 1142 (symmetric C-O-C), 890 (-CH bending mode of vinyl group), 730 (rocking mode of vinyl group).

^{1}H-NMR (δ ppm): 6.26 (1H, -CH=), 2.43 (3H, CH_3), 6.36 (2H, non-equivalent methylene protons), 7.06-7.72 (3H, aromatic protons).

Copolymerization

A total feed of 5g of monomers N-tert-butylacrylamide, 7-acryloyloxy-4-methyl coumarin and 50 mg of AIBN initiator were dissolved in 25ml of

DMF placed in a standard reaction tube to obtain a homogenous solution. The mixture was flushed with oxygen free dry nitrogen gas. The inlet and outlet of the reaction tube were closed by means of rubber tubing's and pinch cock. The reaction vessel is then immersed in a thermostatic water bath maintained at 60°C. The copolymerization reaction was allowed to proceed for an appropriate duration that would give a conversion below 10%. After the reaction vessel was removed from the thermostat and cooled under the tap. The solution poured in ice cold water to precipitate the copolymer and the copolymer washed with methanol to remove unreacted monomers. It was then dried in vacuum oven for 24 hours.

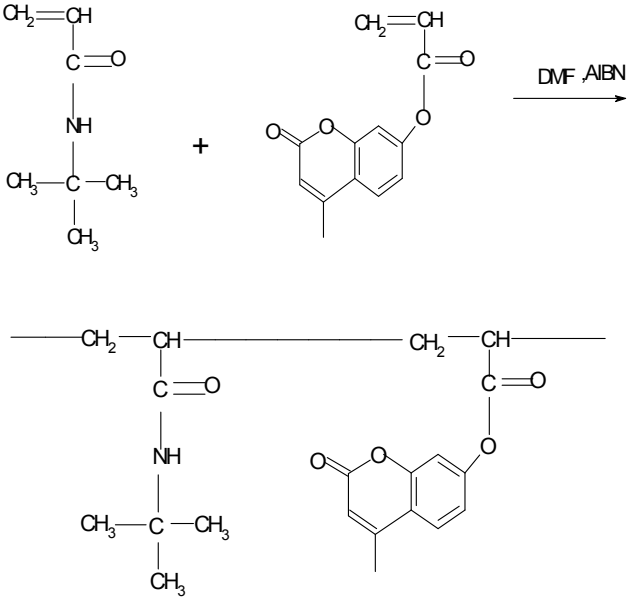
Antimicrobial Activity

The synthesized compounds in the present investigation have been tested for antimicrobial activity by well diffusion method. The organisms selected for the antifungal activity was carried out by using *Aspergillus flavus , Candida albicans and Cryptococcus*.The organisms selected for the antibacterial activity was carried out by using *Escherichia coli* , *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The plates are prepared as per the standard methods [20].

Results and Discussion

Copolymerization

A series of copolymers of N-tert-butylacrylamide(NTB) and 7-acryloyloxy-4-methyl coumarin (AMC) were prepared by free radical polymerization in DMF at 60°C using AIBN as initiator. The schematic representation of the copolymer is given below:



Characterization

^{1}HNMR Spectroscopy

The ^{1}H-NMR spectrum of copolymer, Poly(N-tert-butylacrylamide - co -7-acryloyloxy-4-methyl coumarin (0.5 : 0.5) is shown in Figure 1 . The following peaks appear in the copolymer spectrum: at 1.1 -1.4 ppm for tert-butyl group , at 2.2-2.4 ppm for coumarin CH_3 , at 3.1 ppm for backbone –CH_2 & water peak, at 6.9-7.7 ppm due to coumarin aromatic protons and at 8.1 ppm for N-H proton.

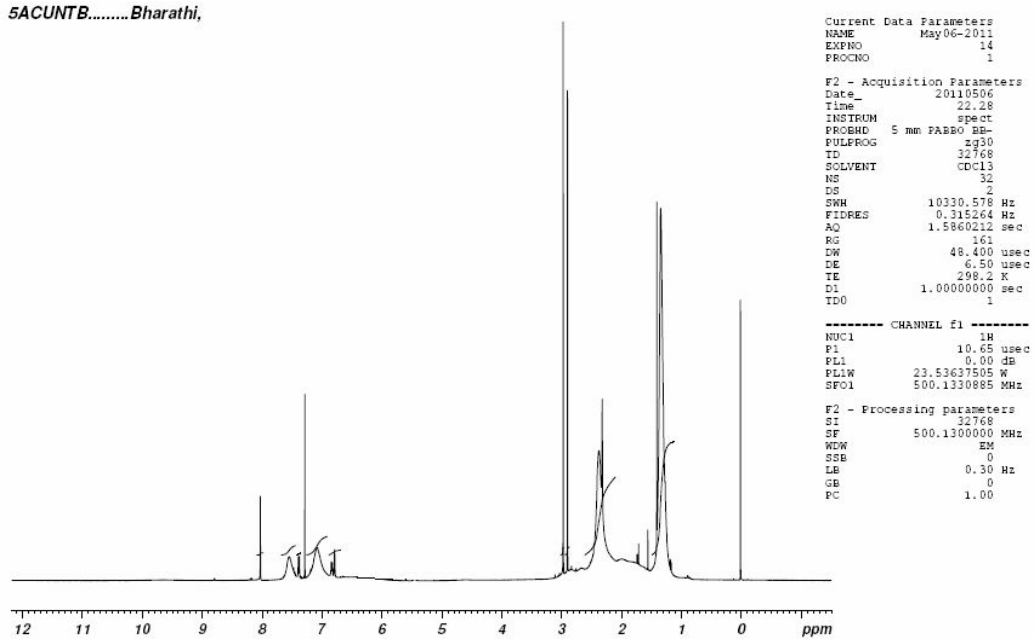


Figure-1: Poly(N-tert-butylacrylamide - co -7-acryloyloxy-4-methyl coumarin)(0.5 : 0.5)

Determination of copolymer composition

The copolymer composition was determined by ^{1}H-NMR spectral analysis of the copolymer. The Assignment of the resonance peaks in the ^{1}H-NMR spectrum allows the accurate evaluation of the content of each kind of monomer incorporated in to the copolymer chain.

The N-tert-butyl peak area is used to determine the copolymer composition. Resonance signals at 1.1 -1.4 ppm corresponds to tert-butyl proton, and their integrated intensity of this peak was compared to the total intensities of all the peaks in the copolymer spectrum, which is a measure of their relative areas.

The copolymer compositions can be obtained using –

$$X_{(NTB)} = \frac{10\,A(\text{t-butyl})}{9A_{total} - 3A(\text{t-butyl})} \quad (1)$$

Where X= mole fraction and A=peak area. Table. 1 gives the values of the corresponding mole fraction in the copolymers.

Reactivity ratios

From the monomer feed ratios and the resultant copolymer compositions, the reactivity ratios of monomer 1 (NTB) and monomer 2 (AMC) were evaluated by the methods of Finemann –Ross (F-R) Kelen-Tudos (K-T).The significant parameters of F-R and K-T and equation are presented in **Table- 1 and Table -2** respectively .The reactivity ratios for NTB (r_1) and AMC (r_2) from the F-R plot (not shown) and K-T plot(Figure 2) are r_1= 1.15 and r_2 = 0.70 and r_1 = 1.16 and r_2 = 0.72 respectively. The values of r_1 is greater than 1 and r_2 is less than 1.r_1 shows that NTB favors homo-propagation as opposed to cross propagation and r_2 shows that AMC favors cross propagation over homo-propagation. The r_1 and r_2 together show that NTB is generally more reactive than AMC, hence the copolymer contains a higher proportion of NTB units. The $r_1.r_2$ = 0.805 value indicates the formation of random copolymers. Although the product of r_1 and r_2 is 0.805 which is less than unity, this can only be truly said indicate a random distribution of the monomeric units where the values of r_1 and r_2 are close to unity themselves . The more the diverse from unity, the less random the distribution will be [12].

Table 1: Fineman–Ross parameters of Poly(N-tert-butylacrylamide - co -7-acryloyloxy-4-methyl coumarin

Mole fraction of NTB in feed (M_1)	Mole fraction of AMC in feed (M_2)	Mole fraction of NTB in copolymer(m_1)	Mole fraction of AMC in copolymer(m_2)	$F=M_1/M_2$	$f=m_1/m_2$	$(f-1)/F$	f/F^2
0.3	0.7	0.3577	0.6423	0.4286	0.5569	-1.0338	3.0316
0.4	0.6	0.4411	0.5589	0.6667	0.7892	-0.3162	1.7755
0.5	0.5	0.6150	0.3850	1.0000	1.5974	0.5974	1.5974
0.6	0.4	0.6473	0.3527	1.5000	1.8353	0.5569	0.8157
0.7	0.3	0.7373	0.2627	2.3333	2.8066	0.7743	0.5155

Table 2 : Kelen- Tudos parameters for the copolymers of Poly(N-tert-butylacrylamide - co -7-acryloyloxy-4-methyl coumarin)

$G = F(f-1)/f$	$H=F^2/f$	$\eta = G/\alpha + H$	$\varepsilon = H/\alpha + H$
-0.3410	0.3299	-0.3018	0.2920
-0.3186	0.5632	-0.2337	0.4132
0.3740	0.6260	0.2623	0.4390
0.6823	1.2260	0.3368	0.6050
1.5019	1.9398	0.5482	0.7080

$$\alpha = \sqrt{H_{max} \times H_{min}} = \sqrt{1.9398 \text{ X } 0.3299} = 0.8000$$

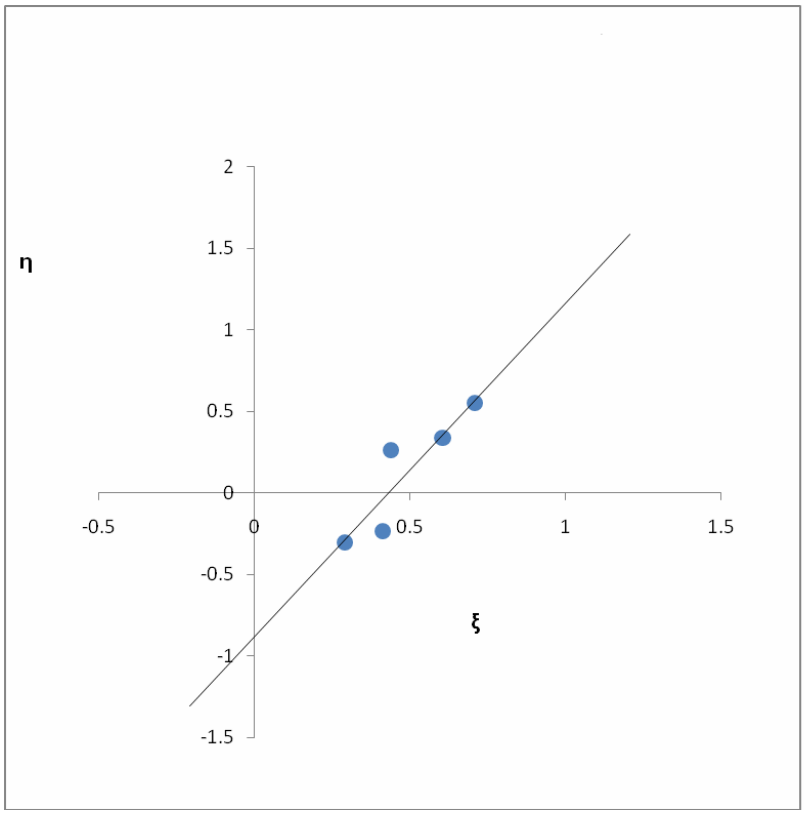


Figure 2: K-T plot of Poly(N-tert-butylacrylamide - co- 7-acryloyloxy-4-methyl coumarin)

Mean sequence length

The mean sequence length was determined using the pertinent equation:

$$l_1 = r_1 \frac{M_1}{M_2} + 1 \quad \text{----------} (2)$$

$$l_2 = r_2 \frac{M_2}{M_1} + 1 \quad \text{----------} (3)$$

Where r_1 and r_2 are the reactivity ratios and $[M_1]$ and $[M_2]$ represents the concentration of NTB and AMC respectively, in the monomer feed. The mean sequence lengths of copolymer are given in Table 3.It is significant to note that from the Table -3, the AMC units decreases in a linear fashion in the polymer chain as the concentration of AMC decreases in the monomer feed. In other words the NTB units increases in a linear fashion in the polymer chain as the concentration of NTB increases in the monomer feed. This suggests that the copolymers are becoming more ordered and less random in nature.

Table 3: Mean sequence lengths in copolymers of Poly(N-tert-butylacrylamide - co – 7-acryloyloxy-4-methyl coumarin)[a]

AMC in feed M_2 (mol %)	l_1	l_2	l_1: l_2	Distribution
0.7	1.49	2.63	1:3	NAAAN
0.6	1.33	2.05	1:2	NAAN
0.5	2.15	1.70	2:2	NNAANN
0.4	2.73	1.47	3:1	NNNANNN
0.3	3.68	1.30	4:1	NNNNANNNN

[a] **$r_1 = 1.15$ and $r_2 = 0.70$**

Antimicrobial Activity

The synthesized compounds in the present investigation have been tested for antimicrobial activity by well diffusion method. The organisms selected for the antibacterial activity was carried out by using *Escherichia coli* , *Pseudomonas aeruginosa* and *Klebsiella pneumonia* as mentioned in Table 4. From the Table 4 it noticed that the activity of polymers against bacteria increases with increasing mole % of NTB , only in the case of *Escherichia coli* . But for other bacteria had almost same activity. The organisms selected for the antifungal activity was carried out by using *Aspergillus flavus , Candida albicans and Cryptococcus* as shown in Table 5. *Cryptococcus* only has increasing activity in the order of increasing NTB contents and the other fungi have same activity.

Table 4 : Antibacterial Activity of copolymers

Bacteria zone of inhibition in mm				
S.No.	Organisms	3AMC-NTB	5 AMC-NTB	7AMC-NTB
1.	Escherichia coli	10	8	7
2.	Pseudomonas aeruginosa	8	6	7
3.	Klebsiella pneumonia	7	-	8
DMSO Solvent		No zone of inhibition		

Table 5 : Antifungal Activity of copolymers

Fungi zone of inhibition in mm				
S.NO.	Organisms	3AMC-NTB	5 AMC-NTB	7 AMC NTB
1.	Aspergillus flavus	-	6	13
2.	Candida albicans	17	7	-
3.	Cryptococcus	14	10	10
DMSO Solvent		No zone of inhibition		

3AMC-NTB : 30 mol% AMC and 70 mol% NTB
5 AMC-NTB : 50 mol% AMC and 50 mol% NTB
7AMC-NTB : 70 mol% AMC and 30 mol% NTB

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