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RESEARCH ARTICLE

Synthesis, Characterization and Antimicrobial Screening of Coumarin Copolymers

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Abstract: 7-Acryloyloxy-4-methylcoumarin (ACU) monomer was polymerized with *N*-cyclohexyl-acrylamide (NCA) in different feed ratio using AIBN (Azobisisobutyronitrile) as initiator in DMF. 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 using linear methods like Fineman-Ross and Kelen-Tudos. The value showed that ACU is more reactive than NCA. The copolymers contained a higher proportion of ACU units. Mean sequence lengths of copolymers were estimated from r_1 and r_2 values. ACU unit increases in a linear fashion in the polymer chain as the concentration of ACU increases in the monomer feed. The copolymers were tested for their antimicrobial properties against selected microorganisms.

Keywords: 7-Acryloyloxy-4-methylcoumarin, *N*-Cyclohexylacrylamide, Reactivity ratio, Mean sequence length, Antimicrobial activity

Introduction

Coumarins are plant flavonoids widely distributed in nature. Coumarins (2*H*-1-bengopyran-2-ones) are important oxygen containing fused heterocycles used in drugs and dyes[1]. Coumarins be bound their class name to 'coumarou' the vernacular name of the Tonka bean (*Dipteryxodorata willd, Fabaceae)*, from which coumarin itself was isolated in 1820[2]. They are the family of lactones containing benzopyrone skeletal framework that have enjoyed isolation from plant as well as total synthesis in the laboratory. The extract containing coumarin related heterocycles which were employed as herbal remedies in early days, have now been extensively studied for their biological activities[3]. The incorporation group as a fused component into parent coumarin alters the property of parent coumarin and converts it into a more useful product[4] natural coumarins are known to have antidiabetic activity[5], anabolic antioxidant and hepato protective activities[6]. Substituted coumarins derivatives have been reported to have variety of biological activities. The potent antibiotics like novobiocin, coumaromycin and chartesium are coumarin derivatives.

One method of achieving antimicrobial polymers is the preparation of polymerizable monomers containing biocide moieties and then polymerizing subsequently or copolymerizing with another monomer[7-10]. Coumarin polymers have not received considerable attention in the literature. However, the reported coumarin polymers possess variety of functions and appear to be interesting. Although there are a huge number of reports on monomeric coumarin derivatives, there are only a few reports on coumarin polymers[11,12]. Bhrahmbhatt *et al*. prepared poly (3-phenoxycoumarin ethylene) and determined their toxicity effect on various fungal and bacterial strains[13]. These polymers showed good biological activity. Lee and co-workers[14] 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[15]. Lindsay and co-workers[16] 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, our interest was to synthesize acrylic copolymers with 4- methyl coumarin side groups. This work was taken up with a view to synthesizing biocidal polymers, derived from acrylic monomers as these polymers have many commercial applications.

The synthesis and development of antimicrobial polymers is one of the leading frontiers of research in polymer science. With this view, in our earlier work[17] *N*-cyclohexyl-acrylamide was copolymerized with 8-quinolinyl acrylate. Copolymers with different feed ratio were prepared and characterized by ^{1}H-NMR spectroscopy. The reactivity ratios of monomers determined by Fineman-Ross (r_1= 0.84 and r_2=2.86), Kelen-Tudos (r_1=0.84 and r_2=2.82). The $r_1.r_2$=2.42 value indicates the formation of random copolymers. The thermal stability decreases with increasing mole % of 8QA. It shows antimicrobial activity. The activity of copolymers against Fungi (A.N and A.F) increases with increasing mole % of NCA.

In this paper, we report the synthesis of *N*-cyclohexylacrylamide (NCA) and 7-acryloyloxy-4-methylcoumarin (ACU) copolymers in different feed ratio by free radical polymerization. The prepared copolymers were characterized by ^{1}H NMR spectroscopy. Copolymer composition was obtained from ^{1}H NMR data monomer reactivity ratios were determined by Fineman-Ross[18] and Kelen-Tudos[19] methods. The prepared copolymers were characterized by calculating its reactivity ratio; mean sequence length, antibacterial and antifungal studies.

Experimental

The monomer *N*-cyclohexylacrylamide was prepared by the reaction of cyclohexanol with acrylonitrile. *N*-cyclohexylacrylamide was recrystallized in warm dry benzene. The white crystals have mp.115 ^{o}C and the yield was 87%[20]. The monomer was confirmed by ^{1}H NMR spectroscopy (Figure 1).

Synthesis of 7-hydroxy-4-methylcoumarin and acryloyl chloride

7-Hydroxy-4-mehtylcoumarin and acryloyl chloride were prepared according to the process reported in the literature[21,22].

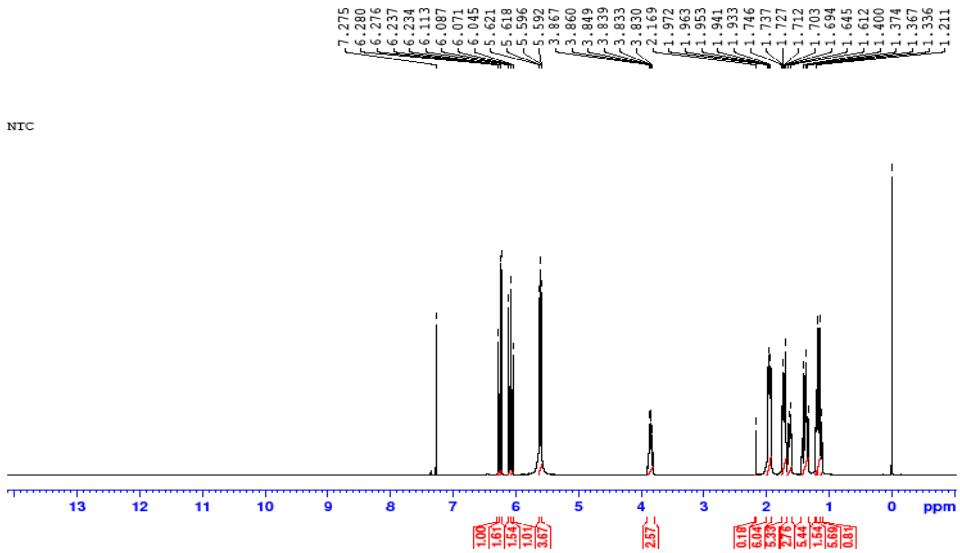


Figure 1. [1]H -NMR spectrum of *N*-cyclohexylacrylamide

Synthesis of 7-hydroxy-4-methylcoumarin

250 mL of concentrated sulphuric acid (H_2SO_4) was added to a one liter three necked flask fitted with a thermometer, mechanical stirrer and a dropping funnel. The flask was immersed in an ice bath. Solution containing (0.23 mol) of resorcinol and (0.26 mol) of ethyl acetoacetate was added drop wise to the flask maintained at temperature less than 10 ºC with constant stirring. The reaction mixture was kept for about 18 hours at room temperature after completion of addition of the reagents. The contents of the flask were poured into crushed ice-water mixture where the product separated out.

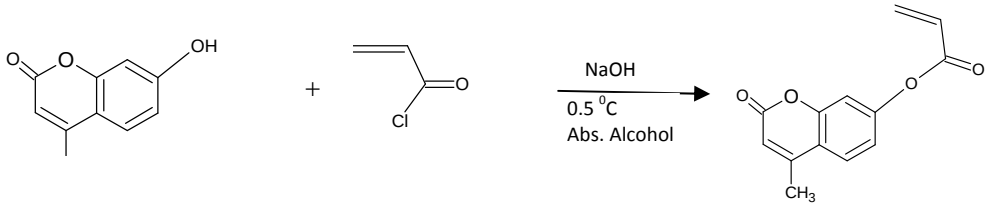
Synthesis of acryloyl chloride

A mixture of acrylic acid (1 mol), Benzoyl chloride (2 mol) and hydroquinone (0.00 25 mol) was distilled at a fairly high rate through an efficient column. The distillate was collected in a receiver containing hydroquinone (0. 0025 mol). The product was obtained at a temperature between 85-100 ºC. The crude product was redistilled through the same column.

Synthesis of 7-acryloyloxy-4-methylcoumarin

To a 1L three-necked flask equipped with stirrer, thermometer and guard tube were added absolute alcohol (550 mL) and NaOH (4 g, 0.1 mol) and the contents were stirred until all NaOH was dissolved. Then, 7-hydroxy-4-methyl coumarin (17.62 g, 0.1 mol) was added to the above solution. The reaction mixture was heated to 60 ºC for 30 min. with stirring, then cooled to room temperature and then to 0-5 ºC. Freshly prepared acryloyl chloride (9.2 mL, 0.11 mol) was added drop wise within 60 min to the cooled reaction mixture. The temperature was maintained around 0-5 °C during the addition. After complete addition, reaction mixture was stirred for 90 min and it was poured into crushed ice water mixture where a white colored product was separated (Figure 2). It was filtered, dried and recrystallized from methanol. mp: 122 ºC. Yield: 89%.

IR (KBr, cm^{-1}): 3073(-CH stretching vibration of the aromatic ring), 2986 (CH3), 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, CH3), 6.36 (2H, non-equivalent methylene protons), 7.06-7.72 (3H, aromatic protons).



7- Hydroxy-4-methylcoumarin Acryloyl chloride 7- Acryloyloxy-4-methylcoumarin

Figure 2. Synthesis of 7-acryloyloxy-4-methylcoumarin

Copolymerization

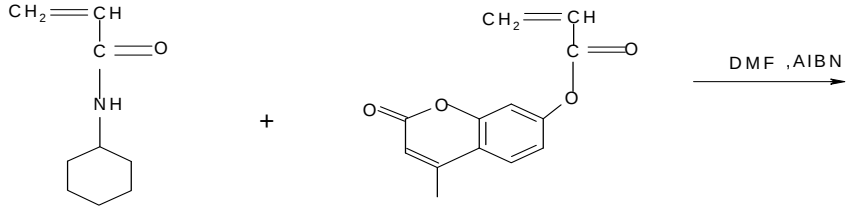
A total feed of 5 g of monomers *N*-cyclohexylacrylamide, 7-acryloyloxy-4-methyl coumarin and 50 mg of AIBN initiator were dissolved in 25 mL of DMF placed in a standard reaction tube to obtain a homogenous solution. The mixture was flushed with oxygen free dry nitrogen gas. The reaction vessel is then immersed in a thermostatic water bath maintained at 60 ^{o}C. The copolymerization reaction was allowed to proceed for an appropriate duration that would give a conversion below 10%. 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 assay

Antimicrobial analysis was followed using standard agar well diffusion method to study the antimicrobial activity of compounds[24-26] . Each bacterial and fungal isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 10^5 colony forming unit (CFU) per mL. They were flood-inoculated onto the surface of BHI agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 30 µL (5 µg compound in 500 µL DMSO) of the sample solution were poured into the wells. The plates were incubated for 18 h at 37 °C for bacteria and at room temperature for fungi. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition in mm against the test microorganisms. DMSO was used as solvent control. Ciprofloxacin was used as reference antibacterial agent. Ketoconazole was used as reference antifungal agent. The tests were carried out in triplicates.

Results and Discussion

The schematic representation of the copolymer is given below (Figure 3):



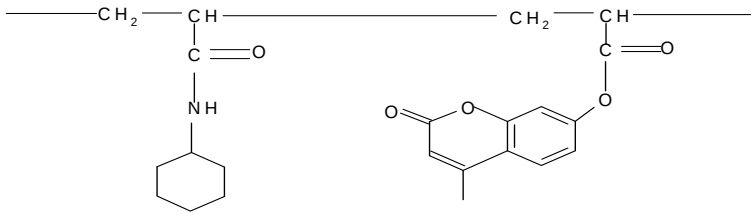


Figure 3. Copolymerization of NCA and ACU

Characterization of copolymer

The ^{1}H NMR spectrum of copolymer, poly (NCA-co-ACU) (0.8: 0.2) is shown in Figure 4 and the following peaks appear in the copolymer spectrum; at 1.14 -2.96 ppm for cyclohexyl CH_2 group, at 3.68 ppm for backbone CH_2, at 7.07-8.01 ppm due to ACU aromatic protons.

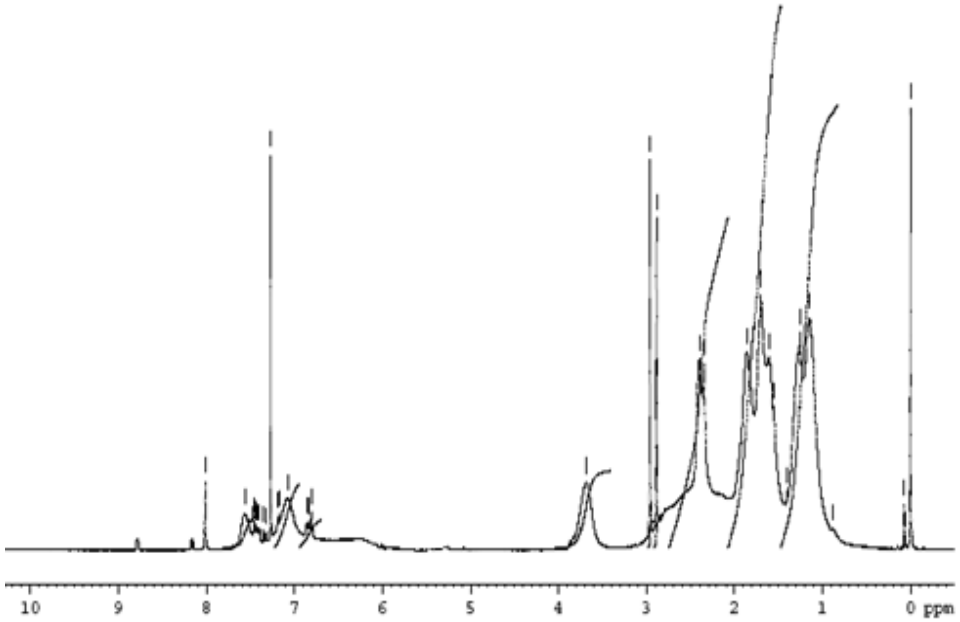


Figure 4. ^{1}H NMR spectrum of poly (NCA-co-ACU) (0.8: 0.2)

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 into the copolymer chain. The area of coumarin moiety was used to determine the copolymer composition. Resonance signal at 7.07-8.01 ppm corresponds to aromatic proton, and their integrated intensity of this peak is 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;

$$X_{ACU} = \frac{15\ A\ (aryl)}{3A_{total} + 5A(aryl)} \quad (1)$$

Where X= mole fraction and A= peak area.

Reactivity ratios

From the monomer feed ratios and the resultant copolymer compositions, the reactivity ratios of monomer 1 (NCA) and monomer 2 (ACU) were evaluated by the methods of Fineman-Ross (FR) and Kelen- Tudos (KT). The significant parameters of F-R and K-T and equation are presented in Table 1. The reactivity ratios for NCA (r_1) and ACU (r_2) from the F-R plot (Figure 5) and K-T (Figure 6) plot are given in Table 2. The value of r_1 is less than 1 and r_2 is greater than 1. r_1 shows that NCA favors cross-propagation as opposed to homopropagation and r_2 shows that ACU favors homopropagation over cross-propagation. The r_1. r_2 = 1.4 value indicates the formation of random copolymers.

Table 1. Finemann-ross and Kelen-tudos parameters for the copolymers

Mole fraction of NCA in feed , M_1	Mole fraction of ACU in feed , M_2	Mole fraction of ACU in copolymer , m_2	$F=M_1/M_2$	$f=m_1/m_2$	$G = F(f-1)/f$	$H=F^2/f$	$\eta=G/(\alpha+H)$	$\xi=H/(\alpha+H)$
0.2	0.8	0.8757	0.25	0.1419	-1.51	0.4404	-0.7284	0.2124
0.3	0.7	0.7913	0.427	0.2637	-1.1922	0.6914	-0.5131	0.2976
0.4	0.6	0.6874	0.667	0.4547	-0.7999	0.9784	-0.3066	0.3748
0.5	0.5	0.5468	1	0.8288	-0.2065	1.2065	-0.0727	0.4251
0.6	0.4	0.4362	1.5	1.2925	0.3394	1.7408	0.1006	0.5161
0.7	0.3	0.3899	2.333	1.5647	0.8419	3.4785	0.1647	0.6807
0.8	0.2	0.2743	4	2.6456	2.4881	6.0477	0.3239	0.7875

$$\alpha = (H_{min} X H_{max})^{1/2} = 1.6391$$

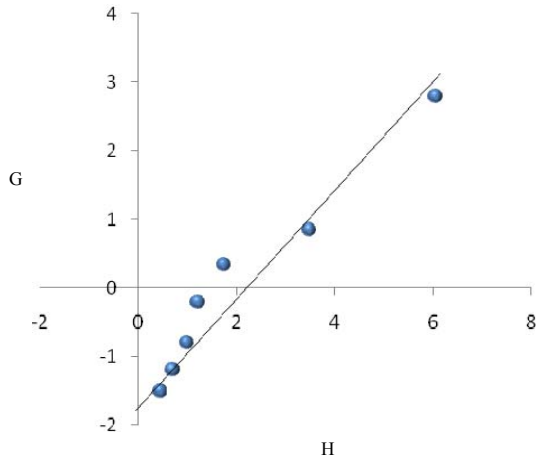


Figure 5. Fineman-Ross plot of Poly (NCA-co-ACU)

Table 2. Copolymerization parameter for the NCA (r_1) and ACU (r_2)

Methods	r_1	r_2	$r_1.r_2$
Fineman-Ross (FR)	0.80	1.75	1.40
Kelen-Tudos (KT)	0.85	1.75	1.49

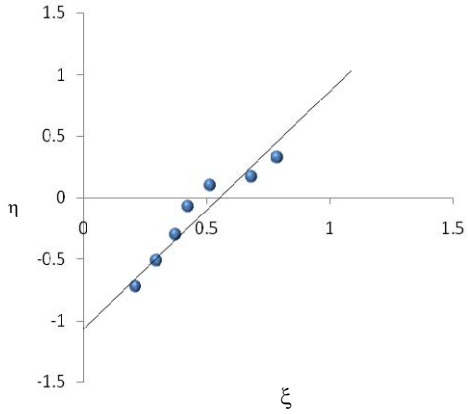


Figure 6. Kelen-Tudos plot of poly (NCA-co-ACU) mean sequence length

The mean sequence length[23] can be determined using the pertinent equations;

$$l_1 = r_1 \frac{M_1}{M_2} + 1 \tag{2}$$

$$l_2 = r_2 \frac{M_2}{M_1} + 1 \tag{3}$$

Where r_1 and r_2 are the reactivity ratios and M_1 and M_2 represent the concentration of NCA and ACU respectively, in the monomer feed. The mean sequence lengths of copolymers are given in Table 3. It is significant to note from Table 3 that the ACU units increases in a linear fashion in the polymer chain as the concentration of ACU increases in the feed.

Table 3. Mean sequence lengths in (NCA-co-ACU)

Mole fraction of ACU in feed M_2	l_1	l_2	l_1: l_2	Distribution
0.8	1.2	8	1:8	N CCCCCCCCN
0.7	1.3	5	1:5	NCCCCCN
0.6	1.5	3.6	2:4	NNCCCCNN
0.5	1.8	1.75	2:2	NNCCNN
0.4	2.2	2.1	2:2	NNCCNN
0.3	2.8	1.75	3:2	NNNCCNNN
0.2	4.2	1.43	4:1	NNNNCNNNN

N=NCA; C=ACU

Antimicrobial activities

The results of antibacterial activities (Figure 7 & 8) of copolymers were shown in Table 4 and Table 5. The activity of copolymers against bacteria and fungi increases with increasing mole% of NCA. All the three copolymers impart almost similar activity against all the three bacteria. The polymers showed higher activity than the reference against in all the three bacteria. From the Table 5, it is observed that the activity of copolymers against the fungi increases with increasing amount of NCA feed. When the content of NCA at 0.7 mole fraction, the polymers showed strong inhibitory effect against *Aspergillus niger* and *Candida tropicalis*.

Table 4. Antibacterial study of copolymers (values in mm)

S.No	Compounds	*Escherichia coli*	*Salmonella typhi*	*Bacillus cereus*
1.	ACU-NTC (0. 3:07)	19	18	19
2.	ACU-NTC (0. 5:05)	18	16	17
3.	ACU-NTC (0. 7:03)	11	11	11
4.	Ciprofloxacin	10	23	32

Table 5. Anti fungal study of copolymers (values in mm)

S.No	Compounds	*Aspergillus niger*	*Candida albicans*	*Candida tropicalis*
1.	ACU-NTC (0. 3:07)	28	18	26
2.	ACU-NTC (0. 5:05)	25	14	23
3.	ACU-NTC (0. 7:03)	06	13	07
4.	Ketoconazole	26	24	30

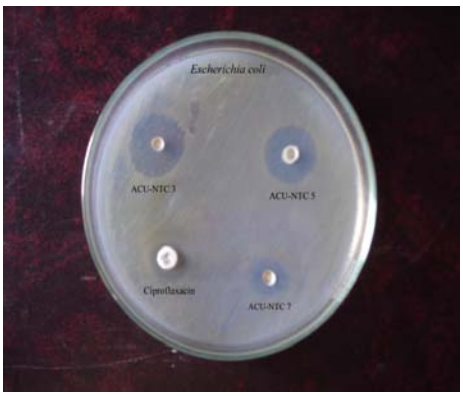


Figure 7. Antibacterial activity of NCA-ACU copolymers against *E.coli*

Figure 8. Anti fungal activity of NCA-ACU copolymers against *C.albicans*

Conclusion

Copolymers of *N*-cyclohexylacrylamide and 7-Acryloyloxy-4-methylcoumarin in various feed ratio prepared were characterized by ^{1}H NMR method. The reactivity ratios of the monomers calculated by Finemann-Ross and Kelen-Tudos methods indicate that the copolymers are random copolymers. Calculation of mean sequence lengths showed that the ACU units increases in a linear fashion in the polymer chain as the concentration of ACU increases in the feed. The organisms antifungal activity was carried out by using *Aspergillus flavus, Candida albicans and Candida tropicalis*. The antibacterial activity was carried out by using *Escherichia coli*, *Salmonella typhi and Bacillus cereus.* It was observed from the results the both antibacterial and antifungal activity increases with increase in the concentration of NCA moiety.

References

1. Rajasekaran S, Rao Gopal Krishna, Pai Sanjay P N and Ranjan Amit, *Int J Chem Tech Res.,* 2011, **3(2)**, 555-559.
2. Dighe Nachiket S, Patton Shashikant R, Dengale Santosh S, Musmade Deepak S, Shelar Madhuri, Tambe Vishal and Hole Mangesh B, *Der Pharma Chemica***,** 2010, **2(2),** 65-71 .
3. Ajani Olayinka O. and Nwinyl Obinna C, *J Heterocycl Chem*., 2010, **47**, 179-187**.**
4. Brahmbhatt D I, Gajera J M, Pandya V P and Patel M A, *Indian J Chem*., 2007, **46(B),** 869-871 .
5. Sharma Rohini and Arya Vikrant, *J Chem Pharm Res*., 2011, **3(2),** 204-212,
6. Murrey R D H, Medez D and Brown S A, The Natural Coumarins Occurrences, Chemistry and Biochemistry, John Wiley Interscience, Newyork, 1982.
7. Kanazawa A, Ikeda T and Endo T, *J Polym Sci A Polym Chem.*, 1993, **31**, 1467-1472.
8. Patel M M, Kapadia M A, Patel G P and Joshi J D, *Iran Polym J*., 2007, **16**, 113-122.
9. Patel M V, Patel R M, Patel J N and Dolia M B*, Iran Polym J.*, 2005, **14(10)**, 899-908.
10. Bankova M, Petrova T, Manolova N and Rashkov I, *Eur Polym J.,* 1996, **32**, 569-578.
11. Ren B, Zhao D, Liu S, Liu X and Tong Z, *Macromolecules,* 2007, **40**, 4501-4508.
12. Kim C, Trajkovska A, Wallace J U and Chen S H, *Macromolecules,* 2006, **39(11)**, 3817-3823.
13. Brahmbhatt D I, Singh S and Patel K C, *Euro Poly J.,* 1999, **35(2)**, 317-324.
14. Lee J, Kim H and Kim H, *Bull Korean Chem Soc.*, 2001, **22**, 179-182.
15. Huyck R H, Trenor S R, Love B J and Long T E, *J Macromol Sci A Pure Appl Chem.,* 2008, **45(1)**, 9-15.
16. Lindsay G A, Henry R A and Hoover J M, *Near Poly Prepr (Amer Chem Soc Div Poly Chem.),* 1993, **34**, 771-771.
17. Chitra R, Jeyanthi P and Pazhanisamy P, *Int J Chem Tech Res*., 2010, **2(4)**, 1871-1880.
18. Fineman M and Ross S D, *J Poly Sci*., 1950, **5(2**), 259.
19. Kelen T and Tudos F, *J Macromol Sci Chem.,* 1975, A**9,** 1.
20. Pazhanisamy P and Reddy B S R, *eXPRESS Poly Lett.*, 2007, **1(11),** 740-747.
21. Brain S, Furniss AJ, Hannaford P W, Smith G and Tatchell A R, Vogel's textbook of Practical Organic Chemistry; 5th Eds., Pearsion Education Pvt. Ltd. Singpore, 1989, 1193.
22. Stempel G H, Cross R P and Mariella R P, *J Am Chem Soc*., 1950, **72**, 2299-2300.
23. Pazhanisamy P and Reddy B S R, *eXPRESS Poly Lett*., 2007, **1(6),** 391-396.
24. Perez C, Pauli M and Bazerque P, *Acta Biol Med Exp.,* 1990, **15**, 113-115**.**
25. Erdemogˇlu N, Ku Peli E, Yes E and Ilada R, *J Ethnopharmacol.,* 2003, **89**, 123-131.
26. Bagamboula C F, Uyttendaele M and Debevere J, *Food Microbiol.,* 2004, **21(1)**, 33-42.