**ABSTRACT**

This paper demonstrates the synthesis and characterization of a new functional acrylic monomer, 7-acryloyloxy 4-methyl coumarin (AOMC) and its copolymers with methyl acrylate (MA). The polymerization was conducted by free radical polymerization technique using AIBN as an initiator at 70±1ºC. The resulting polymers were characterized by FTIR and $^1$H NMR techniques, solubility study and their inherent viscosity measurements. Gel permeation chromatography (GPC) was used to find out the molecular weights of homopolymers and copolymers. The number average molecular weight of copolymers was found to increase with increase in mole fraction of AOMC. The polymers showed moderate thermal stability which were determined by thermogravimetry (TG) and differential thermal anlaysis (DTA). Copolymers' compositions were determined by $^1$H NMR spectra. Further, the linearization method of Finemann-Ross ($r_{AOMC} = 0.7315$; $r_{MA} = 0.8439$), Kelen-Tudos ($r_{AOMC} = 0.7734$; $r_{MA} = 0.8650$), and extended Kelen-Tudos ($r_{AOMC} = 0.7663$; $r_{MA} = 0.8554$) were employed to calculate the monomer reactivity ratios. These values suggest that MA is more reactive than AOMC. Antimicrobial activities of different copolymers synthesized were also studied against different bacteria, fungi, and yeasts and it was observed that their antimicrobial activities differ from each other depending on the AOMC content in copolymers.

**INTRODUCTION**

The polymeric antimicrobial agents have the advantages that they are non-volatile, chemically stable, and do not permeate through skin. Thus, they can reduce losses associated with volatilization, photolytic decomposition, and transportation. In the field of biomedical polymers, infections associated with biomaterials pose a significant challenge to the more widespread application of the medical implants [1,2]. One method of achieving antimicrobial polymers is the preparation of polymerizable monomers containing biocide moieties and then polymerizing subsequently or copolymerizing with...
another monomer [3-6]. 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 [7,8].

Brahmbhatt et al. prepared poly (3-phenoxy-coumarin ethylene)s and determined their toxicity effect on various fungal and bacterial strains [9]. These polymers showed good biological activity. Lee and co-workers [10] 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 photoalignment 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 photo-cross-linking [11]. Lindsay and co-workers [12] synthesized the copolimerization 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.

In this paper, we report the synthesis, characterization, thermal studies, and effect of AOMC/MA copolymers on different micro-organisms. The formation of polymer has been established with the help of IR spectral data. Gel permeation chromatography was employed to determine the molecular weights of the synthesized polymers. The thermal stability of the polymers has been investigated using Broido method. Proton NMR spectroscopy has been employed to study the copolymers' compositions and monomer reactivity ratios.

**EXPERIMENTAL**

**Materials**

Methyl acrylate (MA) (Chiti Chem, Baroda) was freed of inhibitor by washing with 5% NaOH and then with water several times. After being dried over anhydrous Na₂SO₄, it was distilled under reduced pressure. 2,2'-Azobis (isobutyronitrile) (AIBN) (Aldrich) was recrystallized twice from methanol. 7-Hydroxy-4-methyl coumarin and acryloyl chloride were synthesized using procedure previously described in the literature [13,14]. The solvents used were purified by standard reference [15].

**Synthesis of 7-Acryloyloxy-4-methylcoumarin (AOMC) Monomer**

To a 1-litre 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°C for 30 min with stirring, then cooled to room temperature and then to 0-5°C. Freshly prepared acryloyl chloride (9.2 mL, 0.11 mol) was added dropwise 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 coloured product was separated. It was filtered, dried, and recrystallized from methanol. mp: 122ºC. Yield: 89 %, purity (HPLC) > 99 %. Scheme I shows the reaction leading to the formation of AOMC monomer.

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).

1H NMR (δ ppm) (60MHz): 6.26 (1H, -CH=), 2.43 (3H, CH₃), 6.36 (2H, non-equivalent methylene protons), 7.06-7.72 (3H, aromatic protons).

Homopolymerization
One gram of monomer AOMC and 100 mg of AIBN, free radical initiator were dissolved in 10 mL of DMF in a polymerization tube and oxygen free nitrogen was purged through solution for 20 min. then the solution was heated at 70±1ºC for 5 h with stirring. After 5 h the polymer was precipitated in excess of methanol. The polymer was purified by repeated precipitation in methanol from its DMF solution.

Copolymerization
Homopolymers and copolymers of AOMC with MA having different compositions were synthesized by free radical polymerization in DMF solvent using AIBN as a free radical initiator. Predetermined quantities of AOMC, MA, and AIBN (1 w/w(%) based on total monomers) (Table 1) were taken in a polymerization tube and the reaction was carried out under nitrogen atmosphere at 70±1ºC with stirring. After desired time (<10% conversion), the polymer was precipitated by pouring the contents into an excess of methanol. The precipitated polymer was filtered and washed with methanol and were further purified by repeated precipitation by methanol from the DMF solution and finally dried.

Measurements
Fourier transform infrared (FTIR) spectra were measured using Nicolet Impact-400D on carefully dried samples embedded in KBr pellets. NMR spectra were recorded on Hitachi-R-1500 in CDCl₃ solution. The inherent viscosities were measured with an Ubbelohde viscometer thermostated at 30ºC. Molecular weights were determined by gel permeation chromatography (GPC) equipped with Jasco-PU 1580 pump, two PL gel column packed with styrene divinylbenzene bead and R.I. detector (RI-71 Shodex) was employed. Dimethylformamide (DMF) at 1.0 mL/min flow rate was used as a mobile phase throughout the analysis. All the measurements were carried out at 30ºC. Thermogravimetric (TG traces) was carried out with DuPont-951 thermal analyzer at a heating rate of 10ºC/min in static air atmosphere. Differential thermal analysis (DTA) was done with DuPont-9900 differential thermal analyzer at heating rate of 10ºC/min in nitrogen atmosphere.

Table 1. Composition data for free radical copolymerization of AOMC with MA in DMF at 70±1ºC.

<table>
<thead>
<tr>
<th>Polymer code</th>
<th>M₁</th>
<th>M₂</th>
<th>Conversion (%)</th>
<th>Intensities of protons</th>
<th>C</th>
<th>m₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>9.20</td>
<td>7.21</td>
<td>32.33</td>
<td>0.223</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>0.6</td>
<td>8.15</td>
<td>6.42</td>
<td>34.33</td>
<td>0.187</td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>0.7</td>
<td>8.75</td>
<td>5.43</td>
<td>36.93</td>
<td>0.147</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>0.8</td>
<td>8.60</td>
<td>4.21</td>
<td>40.87</td>
<td>0.103</td>
</tr>
<tr>
<td>6</td>
<td>0.1</td>
<td>0.9</td>
<td>7.22</td>
<td>2.61</td>
<td>49.24</td>
<td>0.053</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(a) Mole fraction of AOMC in feed. (b) Mole fraction of MA in feed. (c) Mole fraction of AOMC in copolymer.
commonly employed in biodegradability examinations. Bacterial strains (*Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus citreus*), fungal strains (*Aspergillus niger*, *Sporotrichum pulveruletum*, and *Trichocerma lignorum*) and yeast strains (*Candida utilis*, *Saccharomyces cerevisiae*, and *Pichia stipitis*) were taken for antimicrobial activity study. The bacterial strains were grown in nutrient broth (N-broth) and fungal strains were grown in Sabourand’s dextrose broth. Yeast extract peptone dextrose (YEPD) was added to N-broth to grow yeast strains, with or without the above mentioned polymers. The content of the flasks was incubated in a shaker at room temperature. At specific time intervals (20-48 h), the absorbance was measured at 660 nm for bacteria and yeast cultures. Inhibition percentage (I) is obtained from the following equation:

\[
I = \frac{100(X - Y)}{X}
\]

where, X and Y are the absorbance of bacterial suspension in control set and the absorbance of bacterial suspension in test set, respectively. The fungal cultures were harvested after 48 h and the dry cell mass was determined gravimetrically. The inhibition percentage (I) is obtained from eqn (1) where, X and Y are the weight of dry fungal cell mass in control set and the weight of dry fungal cell mass in test set, respectively. The details of experimental procedure have been reported elsewhere [16,17].

RESULTS AND DISCUSSION

Homopolymerization and copolymerization of AOMC with MA with various mole fractions ranging between 0.5 and 0.1 in the feed were carried out by free radical solution polymerization in DMF using AIBN as an initiator. The reaction time was selected to give conversion less than 10% in order to obtain polymer samples having homogeneous composition as far as possible. The structures of homopolymer and copolymers are shown in Scheme II.

**Scheme II.** Synthesis of (a) poly(AOMC), (b) poly(AOMC-co-MA) and (c) poly(MA).
copolymer of monomeric units are shown in Scheme II. The data on the composition of the feeds and the copolymers are presented in Table 1. In the present study, newly prepared monomer AOMC was copolymerized with comonomer MA. Initial feed ratios were varied as shown in Table 1 to obtain polymers of different compositions.

Characterization of Copolymers
Copolymers obtained are solid white powders and were found to be soluble in dimethylformamide, dimethylsulphoxide, tetrahydrofuran, toluene, and chloroform whereas being insoluble in water, methanol, ethanol, hexane, and acetone. IR Spectra of polymers showed characteristic absorption bands of the repeat units present in the polymer chain.

IR (KBr, cm\(^{-1}\)): 2966 and 2932 (asymmetric and symmetric C-H stretching of methylene group), 1736 (broad, three C=O groups in monomers AOMC and MA), 1260 and 1148 (asymmetric and symmetric vibrations of C-O-C). The main evidence of polymer formation is the disappearance of C=C band at 1640 and the appearance of absorption band at 890 and 720 (C-H bending and C-H rocking modes of vinyl group). A representative IR spectrum of polymers is given in Figure 1.

\(^{1}\)H NMR (\(\delta\) ppm) (60 MHz): 6.96-7.48 (aromatic protons), the formation of polymer is evident from the disappearance of signals at 6.26 (1H, -CH=), 2.43 (3H, -CH\(_3\)), 6.36 (2H, non-equivalent methylene protons) and appearance of broad signals at 3.35 (1H, -CH) and 2.97-3.10 (2H, -CH\(_2\)).

Molecular Weight and Viscosity Measurement
The number and weight average molecular weights of poly (AOMC), poly (MA) and poly (AOMC-co-MA) obtained are presented in Table 2. The polydispersity index and intrinsic viscosity lie in the range 1.4 - 1.5 and 0.14 - 0.21 dL.g\(^{-1}\), respectively.

![Figure 1. IR Spectra of poly(AOMC), poly(AOMC-co-MA) and poly(MA).](image)

Thermal Degradation of Copolymers
The thermal behaviour of copolymers was characterized using TG and DTA traces. The measured results are shown in Tables 3 and 4. The TGA curves (Figure 2) clearly indicates that all polymers undergo single

<table>
<thead>
<tr>
<th>Polymer code</th>
<th>M(_n)</th>
<th>M(_w)</th>
<th>M(_z)</th>
<th>Polydispersity (M(_w)/M(_n))</th>
<th>Intrinsic viscosity [(\eta)] (dL.g(^{-1}))</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>31230</td>
<td>47070</td>
<td>63470</td>
<td>1.507</td>
<td>0.212</td>
</tr>
<tr>
<td>2</td>
<td>27200</td>
<td>41170</td>
<td>56040</td>
<td>1.514</td>
<td>0.167</td>
</tr>
<tr>
<td>4</td>
<td>24520</td>
<td>35210</td>
<td>47150</td>
<td>1.436</td>
<td>0.154</td>
</tr>
<tr>
<td>6</td>
<td>21230</td>
<td>30030</td>
<td>40190</td>
<td>1.414</td>
<td>0.136</td>
</tr>
<tr>
<td>7</td>
<td>20720</td>
<td>29050</td>
<td>36240</td>
<td>1.402</td>
<td>0.124</td>
</tr>
</tbody>
</table>
In all polymers the weight loss was found to occur in a single step, starting at ~280°C and ending at ~425°C. The maximum weight loss occurred at around 370°C. Activation energy ($E_a$) and integral procedural decomposition temperature were determined by Broido’s method [18] and Doyle’s method [19] which were around 48 kJ/mol and 380°C, respectively.

From DTA traces the decomposition of polymers occurs at around 298 to 396°C. The activation energy for thermal degradation and reaction order were determined by Reich’s method [20]. Activation energy of polymers ranged between 28 and 60 kJmol⁻¹; whereas the reaction order for all the polymers was being one.

**Copolymer Composition Determination by $^1$H NMR**

The $^1$H NMR technique is well established as a sim-
ple, rapid, and accurate method for the determination of copolymer composition. The assignment of the resonance peaks in $^1$H NMR spectrum allows for the accurate evaluation of the content of each kind of monomeric unit incorporated into the copolymer chain. Thus, the mole fraction of AOMC in the copolymer chains was calculated from integrated intensities of aromatic protons of AOMC and aliphatic protons of AOMC and MA units.

The following expression applies to copolymers. Let $m_1$ be the mole fraction of AOMC and $1-m_1$ that of MA. There are three aromatic protons in AOMC and seven aliphatic protons in AOMC and another six aliphatic protons in MA.

The following expression applies to copolymers. Let $m_1$ be the mole fraction of AOMC and $1-m_1$ that of MA. There are three aromatic protons in AOMC and seven aliphatic protons in AOMC and another six aliphatic protons in MA.

$$C = \frac{\text{Intensity of aromatic protons} (I_{Ar})}{\text{Intensity of aliphatic protons} (I_{Al})}$$ (2)

$$C = \frac{3m_1}{7m_1 + 6(1-m_1)}$$ (3)

$$m_1 = \frac{6C}{3-C}$$ (4)

From eqn (3) the mole fractions of AOMC in copolymer were determined by measuring the intensities of aromatic proton signals and aliphatic proton signals from the spectra of all copolymers samples. Table 1 gives the value of C and corresponding mole fraction of AOMC on the copolymers. All copolymer samples exhibited higher concentration of MA units.

**Reactivity Ratios**

From the monomer feed ratios and the copolymer composition, the reactivity ratios of AOMC and MA were determined by the application of conventional linearization methods due to Finemann-Ross [21], Kelen-Tudos [22] and extended Kelen-Tudos [23]. The significance of the parameters of F-R and K-T for the copolymers is presented in Table 5 and that of extended K-T is shown in Table 6. The reactivity ratios of AOMC and MA are denoted as $r_1$ and $r_2$, respectively and the values obtained from various methods are presented in Table 7. Since the $r_1$ and $r_2$ values are less than one, this system gives rise to azetotropic polymerization at a particular composition of the monomers which is calculated using the following equation [24]:

$$N_1 = \frac{(1-r_2)}{(2-r_1-r_2)} = 0.372$$ (5)
Table 7. Copolymerization parameters for the free radical copolymerization of AOMC with MA.

<table>
<thead>
<tr>
<th>Method</th>
<th>( r_1^a )</th>
<th>( r_2^a )</th>
<th>( r_1 r_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fineman-Ross</td>
<td>0.7315</td>
<td>0.8439</td>
<td>0.6173</td>
</tr>
<tr>
<td>Kelen-Tudos</td>
<td>0.7734</td>
<td>0.8650</td>
<td>0.6690</td>
</tr>
<tr>
<td>Extended Kelen-Tudos</td>
<td>0.7633</td>
<td>0.8554</td>
<td>0.6530</td>
</tr>
</tbody>
</table>

(a) \( r_1 \) and \( r_2 \) are the reactivity ratios of AOMC with MA, respectively.

when, the mole fraction of AOMC in the feed is 0.372, the copolymer formed will have the same composition as that of feed. When the mole fraction of the feed is less than 0.372 with respect to AOMC, the copolymer is richer in this monomeric unit. When the mole fraction of AOMC in the feed is above 0.372, the copolymer is relatively richer in MA monomeric unit.

**Antimicrobial Screening**

The antimicrobial activity of homopolymers and copolymers of AOMC and MA was investigated. The results observed are presented in Figures 3, 4, and 5. All the copolymers system showed almost similar antimicrobial properties against bacteria, fungi, and the yeasts. Poly (AOMC) allowed about 17-19% growth of bacteria; whereas its copolymers favoured 28-75% growth. Poly (AOMC) allowed 14-16% growth of fungi; whereas its copolymers favoured 27-82% growth. Yeast, however, in the presence of poly (AOMC) registered around 12-14% growth; whereas 27-79% growth for yeast was observed in the copolymers. It was observed that copolymers of AOMC and MA show strong inhibitory effects against tested microorganisms than the homopolymer of MA. Interestingly, the homo(MA) shows antimicrobial property to very lesser extent. It appears that polymers having no coumarin group might also demonstrate antimicrobial activity, although the presence of coumarin group enhances this activity by many folds.

The benzene ring of the coumarin moiety will have electron rich as well as electron deficient centres. As the number of coumarin moiety increases these centres also increase. The microorganism depending on its nature will attack the electron rich or electron deficient centres. Thus, it is concluded that as the percentage of AOMC in the copolymer increases, the effectiveness of the copolymers to inhibit the growth of microorganisms increases and as expected the homo (AOMC) shows maximum growth inhibition.

**Figure 3.** Effect of homopolymers and copolymers on percentage growth of bacteria.

**Figure 4.** Effect of homopolymers and copolymers on percentage growth of fungi.

**Figure 5.** Effect of homopolymers and copolymers on percentage growth of yeast.
CONCLUSION

Copolymers of AOMC with MA having various compositions were synthesized in solution by free radical polymerization. The structures of the copolymers were confirmed by FTIR and $^1$H NMR spectroscopic techniques. The copolymers were found to be soluble in dimethylformamide, dimethylsulphoxide, tetrahydrofuran, toluene, and chloroform; whereas they are insoluble in water, methanol, ethanol, hexane, and acetone. GPC results show that as the content of AOMC in the copolymers increases the molecular weight also increases. Thermal analysis indicated that the copolymers showed moderate thermal stability. Variation in monomer composition in the copolymer results in changes in thermal stability, rate of decomposition, and activation energy. Copolymer compositions were calculated following their $^1$H NMR analysis. The reactivity ratios were determined by F-R, K-T, and extended K-T methods. The values of $r_1$ and $r_2$ are less than one thus the system gives rise to azeotropic polymerization at a 0.372 mole fraction of AOMC in feed. The AOMC content is important to impart antimicrobial property in these polymers. Amongst the polymers investigated, the homopolymer of AOMC is the most effective antimicrobial agent which tends to support this view.

REFERENCES


