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ANTIMICROBIAL ASSESSMENT OF COPOLYMER DERIVED FROM 2-AMINOTHIOPHENOL, ETHYLENEDIAMINE AND FORMALDEHYDE

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Abstract

Copolymer (2-ATEAF) was prepared by condensing the 2-aminothiophenol, ethylenediamine, and formaldehyde in the presence of hydrochloric acid. To analyse the elemental composition of the copolymer, the CHNS analysis was conducted, and the number-average molecular weight (Mn) and polydispersity index were precisely determined through gel permeation chromatography (GPC) analysis, utilizing either dimethylformamide (DMF) or tetrahydrofuran (THF) as the mobile phase and employing polystyrene standards for accurate calibration. The use of the Huggins and Kraemer equations, intrinsic viscosity, and viscometric constants (k and a) were determined in dimethyl sulfoxide (DMSO). The structure of the copolymer was explained by a range of spectroscopy techniques including UV–Visible spectroscopy, FTIR spectroscopy, and ^{1}H NMR spectroscopy were employed for characterization. Besides the SEM and the powder x-ray diffraction (PXRD), it was also established that the material was amorphous using scanning electron microscopy (SEM). Antimicrobial assay was done by using *aspergillus niger, staphylococcus aureus and bacillus subtilis* through agar well diffusion. Gram-positive species of bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-negative species of bacteria (*Escherichia coli*) were also tested. Ethylenediamine moiety was evaluated as a factor of antimicrobial and antifungal effect.
Keywords: Copolymer, Antimicrobial, Fungal strain, Synthesis, polycondensation, Gram-negative, Gram-positive.

Introduction

The challenge of microbial contamination is an important issue in numerous sectors, such as food preservation, pharmaceutical compound preparation, medical instruments, packaging systems, textiles, medical facilities, and protective coatings. In order to deal with these challenges, research efforts have been channeled to the creation of antimicrobial polymers at large scale [1]. These polymeric biocides are the macromolecular systems which are normally used to prevent microbial growth. Biocidal effect is usually characterized by the first stage charge-based attraction of the positively ionized polymer to the negatively ionized cell membrane of the microbes and secondary perturbation of the membrane and subsequent cell lysis[2-4].

Increased concern has been on the development of environmentally friendly polymeric materials with inbuilt antifungal and antibacterial properties. The microbial colonization of polymeric materials often results in the loss of mechanical integrity, surface damage, color sometimes distortion of the substance, staining, and other undesired changes [5-7]. Over the last few years, synthetic polymers are being designed and developed to work as active biocidal agents. There are also such polymeric biocides that generally include cationic functional groups including quaternary ammonium, phosphonium, tertiary sulfonium, and guanidinium moieties. Given the anionic character of most microbial surfaces, these cationic polymers readily adsorb onto cell envelopes, compromising membrane integrity and inducing microbial death.

Various terpolymer systems have been prepared by polycondensation of o- and m-hydroxyacetophenone, m-aminoacetophenone, p-chlorobenzoic acid, 4-hydroxyphenacyl bromide, or other substituted aromatics with formaldehyde, and these materials have displayed pronounced bactericidal and fungicidal performance. Contemporary investigations have also highlighted the utility of resinous materials derived from hydroxy-substituted aromatic ketones, phenacyl bromides, and chalcones owing to their broad-spectrum biological activity[8-9]. Literature surveys further reveal that the antimicrobial potential of p-hydroxybenzaldehyde oxime-based terpolymers, acetophenone-derived terpolymers, lanthanide(III)-containing phenolic resins, and poly[(2-hydroxy-4-methoxybenzophenone)ethylene] resins has been systematically explored [10-13]. Numerous condensation polymers based on hydroxy- and amino-aromatic monomers have garnered attention for their ion-exchange capabilities[14-17]. In addition, the fabrication, spectroscopic evaluation heat-induced degradation behaviour of terpolymers, and incorporating 8-hydroxyquinoline or substituted amines with formaldehyde was documented[18-19]. Terpolymer resins obtained from salicylic acid, p-hydroxybenzoic acid, biogenic amines, and formaldehyde have been reported by Masram and co-workers[20-21], whereas Jadhao et al. described the preparation and evaluation of 2-hydroxy-1,4-naphthoquinone-based terpolymers, including their anticancer properties[22-23].

In the present study, the antimicrobial efficacy of the 2-ATEAF copolymer was examined, revealing moderate inhibitory activity against selected bacterial and fungal strains.

Materials and Methods

All chemicals used in the investigation were of analytical reagent (AR) grade and were used without further purification, requiring no additional purification. 2-Aminothiophenol and ethylenediamine was purchased from Fisher India, whereas formaldehyde (as a 37–41% w/v aqueous solution) was supplied by S.D. Fine Chemicals Ltd. and Sigma-Aldrich.

Synthesis of 2-ATEAF copolymer

An equimolar quantity of 2-aminothiophenol and ethylenediamine was reacted with twice the molar proportion of formaldehyde through an acid-catalyzed polycondensation route to obtain the 2-ATEAF copolymer. Hydrochloric acid served to promote the condensation process. The reaction system was maintained at an elevated temperature of approximately 126 °C under continuous agitation for five hours to ensure effective chain formation. The mixture was then slowly cooled under stirring following thermal treatment and then left to stand under ambient conditions to enable precipitation of the polymer[24-27].

Filtration allowed the isolation of the resulting solid material with an appearance of a light-yellow material, which was then completely washed with heated water to get rid of the catalyst and unreacted species. Additional purification was done by dissolving the crude polymer in an alkaline dilute environment and carrying out a reprecipitation by carefully introducing acidity using a solution of aqueous hydrochloric acid. The polymer was recovered and repeatedly washed using distilled water till the neutrality was achieved and dried under controlled heating. This step yielded the 2-ATEAF purified copolymer of about eighty percent.

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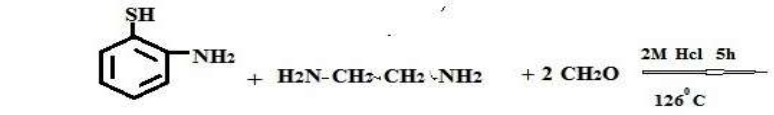
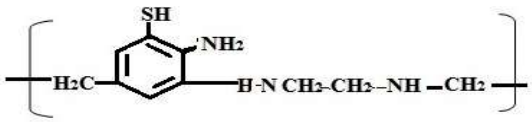


Figure 1.



2-ATEF-I COPOLYMER

Synthesis of 2-ATEAF Copolymer

2-ATEAF copolymer characterisation

Characterisation The 2-ATEAF copolymer was characterised using solid-state ^{1}H nuclear magnetic resonance spectrophotometry combined with solid-state NMR spectrophotometry. 1H solid-state NMR Spectrophotometry The solid-state NMR spectrophotometry was performed combined with Proton solid-state magnetic resonance spectrophotometry. The solid-state proton NMR spectrophotometry was conducted at ^{1}H with solid-state NMR spectra.

The 2-ATEAF copolymer was analysed using an infrared instrument of Shimadzu FTIR-1800S. The samples were made in the form of potassium bromide discs and spectral measurements were made across the wavenumber band of 4000-400 cm^{-1}. To conduct the proton nuclear magnetic resonance, Bruker Avance-II of 400 MHz was used as a solvent with deuterated dimethyl sulfoxide and tetramethyl silane as a chemical shift calibration. All the characterization was carried out at the state-of-the-art Sophisticated Analytical Instrumentation Facility housed within Cochin University of Science and Technology, Kochi, Kerala, India. Analysis of 2-ATEAF copolymer with antimicrobial properties.

Antimicrobial Efficacy

An antimicrobial investigation in 2-ATEAF copolymer was conducted against bacteria of both Gram classes like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis* using well diffusion method on solid media. Fresh clean bacterial isolates were used in all experiments.

Growth medium: This medium was developed by mixing peptone, sodium chloride, yeast extract, and agar in distilled water at the right proportions after which sterilization in pressurized steam conditions was performed. To get the test material which is polymeric, it was solubilized in dimethyl sulfoxide to get a stock solution out of which one got various working concentrations. An antibiotic with the fluorine group allotted standard was taken as reference drug and the solvent in its pure form

was associated to verify the lack of any inherent antimicrobial activity[28-31].
The developed bacterial cultures were used to obtain bacterial inoculate through the transfer of preserved cultures into liquid nutrient medium and incubation using controlled temperature to obtain active growth. An aliquot of the standardized cell suspension which had been standardize in relation to cell density was inoculated uniformly onto the surface of solidified agar plates. Agar uniform cavities were to be prepared with the help of sterile borer; volumes of the polymer solutions and control agents were to be carefully added.
After a brief pre-incubation at ambient temperatures to ensure that the test substances had migrated radially; the plates were then incubated under optimal conditions in order to enable the proliferation of bacteria. The ability of the copolymer to inhibit microbial growth was determined by measuring the diameter (in millimetres) of the transparent zones created around each well, which represented its antibacterial activity. entities that were examined.

Copolymer	**C content % Actual (Pred)**	**H content % Actual (Pred)**	**N content % Actual (Pred)**	**S Content % Actual (Pred)**	**Repeating Unit Composition**	**Formula Weight (FW)**
2-ATEAF	57.15 (57.38)	7.32 (7.22)	19.81 (19.97)	15.49 (15.31)	$C_{10}H_{15}N_3.H_2O$	209.31

RESULTS AND DISCUSSION

Solubility Studies

The purified 2-ATEAF copolymer was obtained as a yellowish-white amorphous solid. Its solubility was examined qualitatively the solubility was tested in organic solvents of varying polarity at room temperature. Good solubility of the copolymer was observed in DMSO and THF, whereas remaining insoluble in methanol, ethanol, isopropanol, ethyl acetate, chloroform, chlorobenzene, dimethylformamide (DMF), dimethylacetamide (DMAc), toluene, benzene, n-hexane, n-heptane, cyclohexane, carbon tetrachloride, and diethyl ether.

Elemental Analysis of 2-ATEAF copolymer

Elemental microanalysis was conducted on the synthesized copolymer to determine its carbon, hydrogen, nitrogen, and sulphur content. The empirical weight of a single repeating unit was calculated based on the empirical formula provided in Table 1.

Table. 1 Elemental analysis of 2-ATEAF copolymer

Spectral studies of 2-ATEAF copolymer

The optical absorption spectrum of the 2-ATEAF copolymer in DMSO (neat solvent) was recorded over the 190 to 800 nm wavelength range. Two distinct absorption bands were observed, one prominent band between 280–300 nm and another moderate band in the range of 300–330 nm. The intense absorption feature occurring between 280 and 300 nm is due to an allowed $\pi \rightarrow \pi^*$ transition of the conjugated aromatic ring, meanwhile, the band seen at 300–330 nm results from a

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forbidden n → π* transition of the thiol (–SH) group. The observed bathochromic shift from the standard values (257 nm and 320 nm) can be attributed to the influence of conjugation and the presence of thiol and amide groups, which act as auxochromes in the copolymer[32-37].

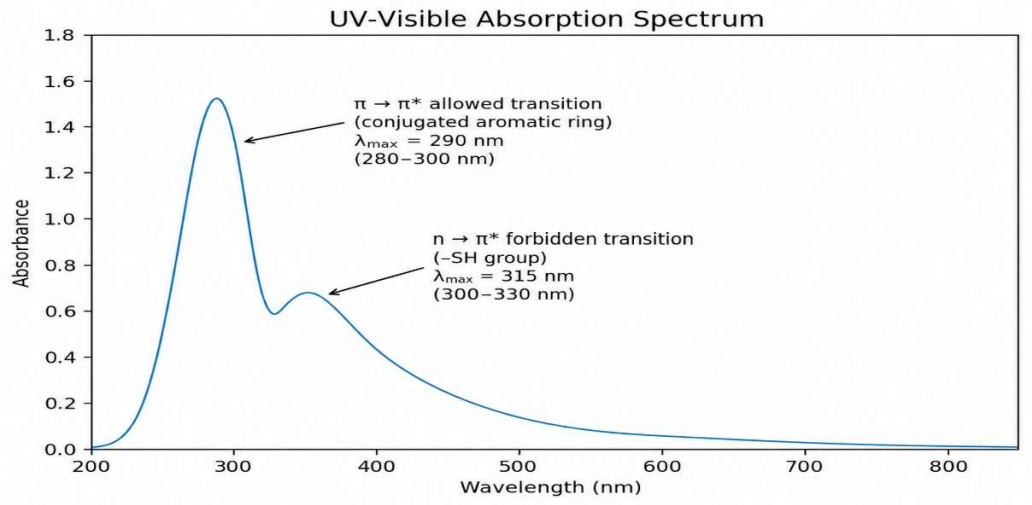

Figure 2. UV–visible spectrum of the 2-ATEAF copolymer

Figure 3 presents the IR spectra of the 2ATEAF copolymer, offering valuable information about the functional groups and linkages within the polymer structure. A strong and wide absorption band centred at 3426.73 cm^{-1} is assigned to >NH stretch mode, characteristic of amide or imide groups. The weak band at 2922.96 $^{cm-1}$ indicate the –SH moiety, as found in phenolic thiols. The absorption band centred at 1670.02 cm^{-1} is assigned to the stretching of >C=O, originating from ketones and biuret functional groups. A medium-intensity band at 1487 cm^{-1} reveals aromatic >C=C< groups. Bands appearing at 1340.29 cm^{-1} and 1060.84 cm^{-1} are associated with $–CH_2–$ bridges within the polymer chain. Additionally, bands at 1120.64 cm^{-1} and 781.09cm^{-1} confirm tetra-substitution at the 1,2,3,4,5 positions on the benzene ring[38-40].

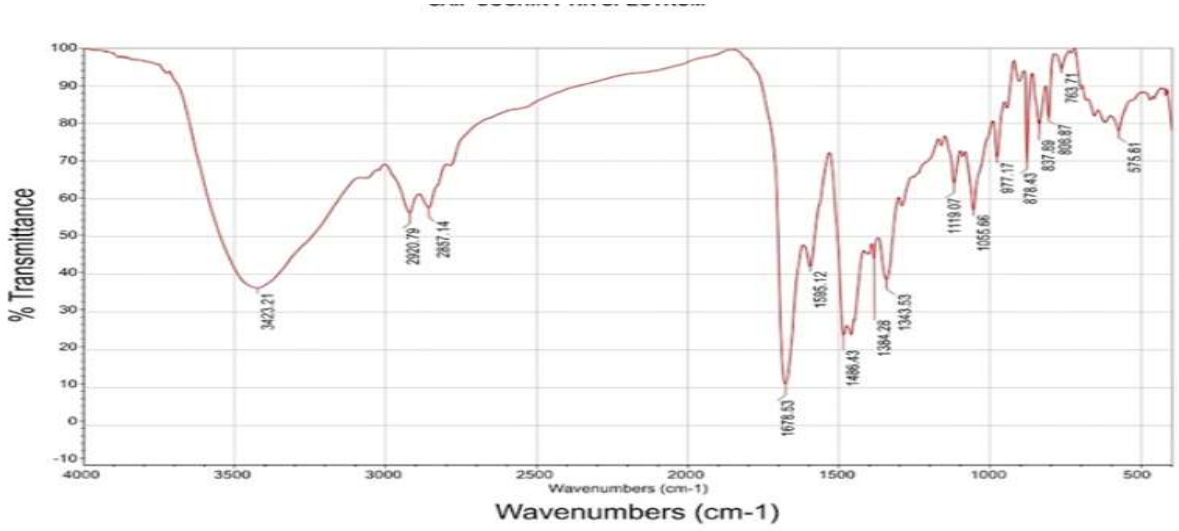

Figure 3. IR spectra of 2-ATEAF copolymer

Sample	Test Bacteria Zone of Inhibition (mm)				
	E.Coli	*S.aureus*	*Pseudomonas*	*Bacillus*	*Aspergillus Niger*
2-ATEAF	10	12	17	10	17
Control (DMSO)	11	Non-positive	Non-positive	Non-positive	Non-positive

Antimicrobial Activity

The copolymeric composition 2-ATEAF had a wide activity in the inhibition bacteria of both Gram classes strains, such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis. Comparative antimicrobial analysis of the ability of this copolymer to control the growth of E. coli, S. aureus and P. aeruginosa indicated that the growth inhibitory properties of this copolymer were more effective than the traditional antibiotic ciprofloxacin as indicated by bigger clear zones around the test sample. This improved performance highlights the possibilities of the material as an effective antibacterial agent to medically significant microorganisms. *Escherichia coli* is a well-known pathogen that causes urinary tract infections, together with gastrointestinal and systemic diseases. S. aureus causes a variety of severe clinical diseases, including respiratory and bone infections, involvement in bloodstream, and the syndromes caused by toxins. Furthermore, *Pseudomonas* aeruginosa is a common opportunistic bacterium responsible for nosocomial infections, serious wound complications, ocular infections, and chronic lung diseases especially in immunocompromised patients, and in patients with cystic fibrosis.

Bacillus subtilis which is a non-pathogenic model organism was also inhibited potently by the copolymer. The 2-ATEAF copolymer demonstrated moderate antifungal activity against *Aspergillus niger*, which is a ubiquitous saprophytic mould that is typically involved in otomycosis, pulmonary aspergillosis and biodeterioration processes[41-42].

Though in the present study, the assessment was done only on the metal-free polymeric ligand, the structure of 2-ATEAF, composing of several donor sites (both nitrogen and sulfur) indicates the possibility of an increased biocidal activity when the ligand is coordinated with the metal ions. These improvements, as it has commonly been reported, occurring with increased lipophilic character due to delocalization of 5 is seen in the chelate rings, some positive charge sharing between the metal cation and the donor atoms as well as favourable changes in solubility, conductivity, dipole moment, and cell membrane permeability. All of those help in more efficient penetration of the microbial cell envelope and disruption of its interference with essential cell work[44-46].

Table 2. Antimicrobial Response of 2-ATEAF copolymer.

ISSN: 2096-3246
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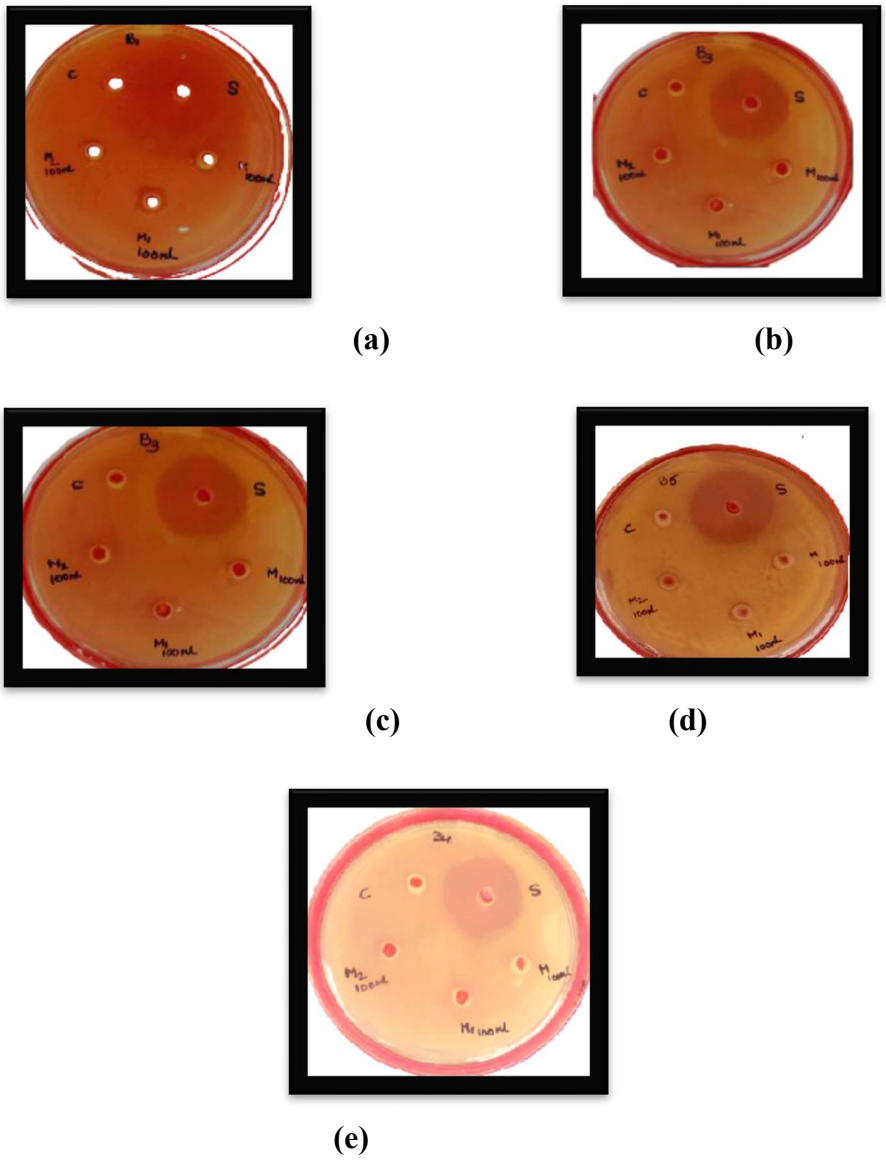

Figure 4. **(a)** ***Escherichia coli*** **(b)** ***Staphylococcus Aureus*** **(c)** ***Pseudomonas aeruginosa*** **(d)** ***Bacillus subtilis*** **(e)** ***Aspergillus niger***

Conclusion

The copolymers have a creamy white appearance. They are partially soluble in Dimethylformamide (DMF), completely soluble in Dimethyl Sulfoxide (DMSO), and do not dissolve in conventional organic solvents or concentrated acids. The structure of the copolymers was elucidated by considering the characteristics and reactive sites of the monomers, together with the results from elemental analysis, electronic IR, and UV spectroscopy. These polymers exhibit superior antimicrobial activity compared to standard drugs and may have diverse applications in the field of material science.

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