5.1 INTRODUCTION

Infections by pathogenic microorganisms are of great concern in many fields, particularly in medical devices, drugs, hospital surfaces/furniture, dental restoration and surgery equipment, health care products and hygienic applications, water purification systems, textiles, food packaging and storage, major or domestic appliances, aeronautic, etc. Infectious diseases kill worldwide more people than any other single cause. These diseases are of particular significance in hospitals where great efforts and important overheads are consumed for struggling against infections. These are triggered by germs (bacteria, viruses, fungi and protozoa), which are found everywhere, in air, soil and water. Mainly, the infections are produced by touching, eating, drinking or breathing something that contains a germ. Generally, these infections are combated with antimicrobial agents, which are susceptible to their action. Particularly problematic is the resistant microorganisms that rapid and easily mutate their genes, making difficult their elimination. For instance, *Pseudomonas aeruginosa* bacterium is one of the most common causes of healthcare-associated infections and is increasingly resistant to many antibiotics. *Staphylococcus aureus* is also a bacterium that commonly colonises human skin and mucosa without causing severe problems. However, serious illnesses that range from mild to life threatening can be developed if the bacteria enter the body. These include skin and wound infections, infected eczema, abscesses infections, heart valves infections or endocarditis, pneumonia and blood stream infection or bacteraemia. Some of *S. aureus* are resistant to the antibiotic meticillin, meticillin resistant *S. aureus*, and often require different types of antibiotic to treat them.

On the other hand, fungal infections in humans can range from common, mild superficial infections such as athlete's foot and candidiasis or thrush (both vaginal and oral) to serious life-threatening diseases such as invasive aspergillosis derived from the diversity of *Aspergillus* fungi. Belonging to the kingdom fungi are yeasts, eukaryotic unicellular microorganisms, and molds, pluricellular, and in that respect, humans are exposed to molds every day, usually by touching or breathing them. Because molds naturally exist outdoors and indoors, their excessive growth is also a health concern.

In brief, the use of potent and/or specific antimicrobial systems will help to mitigate, combat and/or eradicate these infections, which means an improvement in
the state of well-being. In this sense, polymers due to their intrinsic properties are extensively and efficiently employed in all of these fields. Therefore, the use of polymeric materials with antimicrobial properties gains an increasing interest from both academic and industrial point of view. Polymers can act as matrix of the materials holding the antimicrobial agents. In this case the characteristics of the polymer such as its hydrophilicity or its molecular weight have a great influence on the final antimicrobial activity concerning aspects from the rate of biocide release to even conferring synergistic activities.

In addition, the development of polymers with antimicrobial activity themselves is also an important area of research focused on solve the problem of contamination by microorganisms. This alternative avoids the inconvenience of the diffusion of the low molecular weight biocides through the polymeric matrix, which often causes toxicity to the human body. Besides, antimicrobial polymers usually present longer-term activity. During the last decade there have been notable reviews in the field of polymeric materials with antimicrobial activity, which have considered definite families, aspects and/or applications [1-22].

The use of antimicrobial polymers offers promise for enhancing the efficiency 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. Also, polymeric antimicrobial agents have the advantage that they are nonvolatile and chemically stable and do not permeate through skin. Therefore, they can reduce losses associated with volatilization, photolytic decomposition and transportation. In the area of health care and hygienic applications, biocidal polymers may be incorporated into fibers or possibly extruded into fibers themselves and used for contact disinfectants in many biomedical applications such as sterile bandages and clothing. For example, antimicrobial surgical gowns and antifungal polymeric coatings on surfaces such as shower walls and many kinds of tubing minimize the problems of biofouling and release of pathogenic microorganisms into streams of flowing fluids [23].

In the field of biomedical polymers, infections associated with biomaterials represent a significant challenge to the more widespread application of medical implants [24-28]. Infection is the most common cause of biomaterial implant failure.
in modern medicine. Antimicrobial polymers play an important role in reducing the incidences of such failures. Significant advances in the past three decades have been made in the synthesis and applications of polymers to prevent microbial attack and degradation for diverse end uses [29].

One method of achieving antimicrobial polymers is to add an organic or inorganic biocide to the polymers during processing of the material [30]. Another method is to endow a biocidal function to the polymer after processing [31,32]. A different approach for the preparation of polymers bearing groups with antimicrobial activity is the preparation of polymerizable monomer containing biocide moieties and then polymerizing subsequently or copolymerizing with another monomer [33-37]. The grafting of antimicrobial agents into natural occurring or synthetic polymers is yet another approach to the problem of the preparation of bioactive materials with a potential use in various applications.

**5.1.1 Basic requirement of antimicrobial polymers**

The ideal antimicrobial polymer should possess the following characteristics: (1) easily and inexpensively synthesized, (2) stable in long-term usage and storage at the temperature of its intended application, (3) not soluble in water for a water disinfection application, (4) does not decompose to emit toxic products, (5) should not be toxic or irritating to those who are handling it, (6) can be regenerated upon loss of activity and (7) biocidal to a broad spectrum of pathogenic microorganisms in short time of contact.

**5.1.2 Major fields of applications of antimicrobial polymers**

(a) **Water Treatment**

Chlorine or water-soluble disinfectants are used for sterilizing water. However, soluble disinfectants have the problems of residual toxicity of the agents, even if suitable amounts of the agents are used [38]. With the use of these disinfectants or antimicrobial agents, the problem of residues cannot be avoided, bringing about more serious consequences. These drawbacks can be solved by the removal of microorganisms from water with insoluble substances [39-41]. One approach is the use of insoluble contact disinfectants that can inactivate, kill or remove target microorganisms by mere contact without releasing any reactive agents to the bulk phase to be disinfected. Polymeric disinfectants are ideal for applications in handheld water filters, surface coatings and fibrous disinfectants because they can be fabricated
by various techniques and can be made insoluble in water. Tyag and Singh have attempted to produce insoluble polymeric disinfectants for use in water treatment [42] in past few decades.

(b) Medicine and Healthcare Products

Most pharmacologically active agents are low molecular weight compounds, which readily penetrate into all cell types and are often rapidly excreted from the body. Attachment of drugs such as antimicrobial agents to macromolecular carriers alters their rate of excretion from the body and provides the possibility for controlled release over a prolonged period [43]. Recently, concerns have been raised regarding the therapeutic effects exhibited by dental restorative materials [44]. Bacterial contamination of biomedical devices, for example, permanent catheters or implants, is a major problem in those medical disciplines employing biomaterials [45]. The use of long-term catheters can lead to serious implant associated infections [46]. Because the success of treatment in these cases is poor, emphasis has been placed on ways to prevent catheter-related infections.

(c) Food Applications

Microbial contamination reduces the shelf life of foods and increases the risk of food-born illness. The demand for minimally processed, easily prepared, and ready to eat “fresh” food products, as well as globalization of food trade, and distribution from centralized processing, create major challenges for food safety and quality [47]. Antimicrobial substances incorporated into packaging materials can control microbial contamination by reducing the growth rate of the target microorganisms or by inactivating the microorganisms by contact [48]. Antimicrobial polymers can be used in several food-related applications including packaging [49,50].

(d) Textile Products

Antimicrobial treatment is rapidly becoming a standard finish for some categories of textile products such as for medical, institutional and hygienic uses. Recently, it became popular in aesthetic clothing to impart anti-odor or biostatic properties [51,52]. Textiles and fibrous materials are subjected to various finishing techniques to afford (a) protection for the user of textile materials against bacteria, yeast, dermatophytic fungi and other related microorganisms for aesthetic, hygienic, or medical purposes; (b) protection of the textile itself from biodeterioration caused by mold, mildew and rot-producing fungi; and (c) protection for textiles from insects
and other pests [53,54]. Basically, there are three mechanisms by which antimicrobial agents provide protection to textiles and the wearer. The majority of antimicrobial protective finishes function by the controlled release mechanism.

Patel’s group has developed acrylic monomers based on 2,4-dichloro and 4-chloro-3-methylphenol. These 2,4-dichlorophenyl methacrylate and 4-chloro-3-methyl phenyl methacrylate monomers were copolymerized with vinyl acetate (VA), MMA, methyl acrylate or 8-quinolinyl methacrylate.[55-63] All the homo and copolymers were tested against *B. subtilis*, *E. coli*, *S. citreus* bacteria, *A. niger*, *S. pulverulentum*, *T. lignorum* fungi and *C. utilis*, *S. cerevisiae*, *P. stipitis* yeasts. As expected, the chlorine group is the commander in the inhibition process; therefore, the antimicrobial activity decreases as its content in the polymer decreases. Poly(2,4-dichlorophenyl methacrylate) is more effective in inhibiting the growth of microorganisms than that of poly(4-chloro-3-methyl phenyl methacrylate), probably due to the lower chlorine content of the latter. In the case of copolymers with these two monomers, the antimicrobial faculty increases with the chlorine derivative monomer. The same feature occurs for copolymers with 8-quinolinyl methacrylate. Although its homopolymer has inhibitory effect against microorganisms, this is less effective than both chlorine homopolymer derivatives.

Kugel et al.[64] modified triclosan, 2,4,4′-trichloro-2′-hydroxydiphenylether, which is an antibacterial and antifungal agent, with an acrylate functionality and, then, it was copolymerized to form terpolymers with different compositions of hydroxyethyl acrylate and butyl acrylate. Bacterial biofilm retention and algal biofilm growth assay were utilized to assess the antimicrobial activity against *S. epidermidis* and *E. coli* bacteria as well as against other two marine-fouling microorganisms, *C. lytica* bacterium and *Navicula incerta* diatom algae. The data showed that the antimicrobial activity increases with the content of triclosan moieties incorporated and that no triclosan leaching is produced.

Erol and coworkers[65, 66] synthesized two copolymer systems based on 2-(4-acetylphenoxy)-2-oxoethyl-2-methylacrylate (AOEMA) or 2-(4-benzoylphenoxy)-2-oxoethyl-2-methylacrylate (BOEMA) with 2-[(4-fluorophenoxy)-2-oxoethyl-2-methylacrylate (FPEMA) or 2-[(4-fluorophenyl)amino]-2-oxoethyl-2-methylacrylate (FPAMA) with different molar fractions in the feed. In both series the antimicrobial activity was tested against *S. aureus*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, *S. enteriditis*,
and *K. pneumonia* bacteria and *C. albicans* fungus using antibiotic disk assay. The results demonstrated that microorganism inhibition growth increases with the amount of fluorinated fraction in the copolymer. Therefore, the PFPAMA homopolymer is more effective than the copolymers. Authors also claimed that although the fluorine content is undoubtedly the most important factor in the antimicrobial behavior, the polymer conformation also has an effect.

Kanazawa and co-workers [67,68] described the synthesis and polymerization of trialkyl-3-[(and 4-) vinylbenzyl]phosphonium chloride by reaction of 3- and 4-chloromethylstyrene with trialkylphosphine. The polymerization was carried out in toluene at room temperature under an atmosphere of nitrogen. Low molecular weight model compounds of similar structure were synthesized. The results of the antibacterial activity study showed that the polymers, in general, were more active than the corresponding model compounds.

Punyani and Singh [69] described the synthesis of iodine containing quaternary amine methacrylate (QAMA) copolymers with various ratios of QAMA. The antimicrobial activities of the QAMA containing copolymers were evaluated against *E. coli* and *S. aureus*. The results showed that all of the bacteria were killed by the next day after the incubation. In case of 40% content of QAMA in the copolymer, only a contact time of 10 min was required to show 100% kill. It was observed that increasing the QAMA content in the copolymer reduced the contact time required for killing *E. coli*.

It is known that 8-hydroxyquinoline derivatives have antimicrobial activity [70]. Bankova and co-workers [71,72] reported the synthesis of 5-chloro-8-quinolinyl acrylate (AQ) by reacting 5-chloro-8-hydroxyquinoline with the acid chloride of acrylic acid. The monomer AQ was polymerized (PAQ) and copolymerized with acrylic acid P(AA-co-AQ). The antimicrobial activities of AQ, PAQ, and P(AA-co-AQ) were tested against *E. coli* and the results showed that AQ is more active than the homopolymer and copolymer. The antimicrobial activities here are displayed by the release of 8-hydroxyquinoline (HQ) moieties; the release studies showed correlation between the copolymer microstructure and hydrolysis behavior. The lower is the content of the HQ, the higher is the release rate and the higher are the antimicrobial activities. In a subsequent publication [73], the same group reported the synthesis of copolymers of monomer AQ with acrylamide P(AM-co-AQ) and *N*-vinyl-2-
pyrrolidone P(VP-co-AQ). The antimicrobial activities of the monomer AQ, the homopolymer PAQ and the prepared copolymers P(VP-co-AQ), P(AM-co-AQ) and P(AA-co-AQ) were tested against *S. aureus* and *S. tiphimurium*. In general, the antimicrobial activities of the tested materials increased in the order: P(VP-co-AQ) < P(AM-co-AQ) < P(AA-co-AQ). Increasing the acrylic acid content in the copolymer P(AA-co-AQ) to 95% caused an increase in the antibacterial activity of the copolymer to be almost similar to that of the monomer AQ.

Ward *et al.* [74] prepared a series of copolymers derived from 2-(dimethylamino)ethyl methacrylate with four different hydrophobic monomers (ethyl, butyl, cyclohexyl, and octyl methacrylates) via free radical copolymerization. The synthesized copolymers were modified with 1,3-propanesulfone to yield polysulfopropylbetaine derivatives. The antimicrobial activities of the sulfopropylbetaine copolymers were tested against *S. aureus* and *E. coli* using the broth dilution method. The results showed the activities were mainly dependent on the copolymer composition and the test organism. The MIC values for *S. aureus* and *E. coli* were in the range of 1125-2000 µg/mL, which is about 2 orders of magnitude higher than those of the antibiotics ampicillin and erythromycin. Park and co-workers [75] introduced 2-benzimidazolcarbamoyl moiety (CBZ) to poly(ethylene-co-vinyl alcohol). The antifungal activity of the synthesized EVOH-CBZ was evaluated after incubation for 72 h at 28°C against *A. fumigatus* and *P. pinophilum* and showed antifungal activity. The inhibition zone diameter increased with increasing CBZ concentration. The effect here was mainly from CBZ moieties.

Thamizharasi *et al.* [76] synthesized antimicrobial drugs containing monomers (AMBS, MMBS) by reacting acryloyl chloride and methacryloyl chloride with 4-amino-N-(5-methyl-3-isoxazolyl)benzenesulfonamide in the presence of triethyl amine. Another monomer, N-[4-sulfamido-N-(5-methyl-3-isoxazolyl)-phenyl] maleimide (SMPM), was prepared by reacting maleic anhydride with 4-amino-N-(5-methyl-3-isoxazolyl)benzenesulfonamide. The antifungal screenings of these three monomers and their polymers were achieved for two fungi, *A. niger* and *C. albicans*. The authors used a method to measure the zone of inhibition of monomers and polymers. The zone of inhibition of different concentrations of monomers and polymer conjugates reveals that SMPM and its polymer poly(SMPM) have significant
antifungal activities with both C. albicans and A. niger. The polymer-drug conjugate showed superior antimicrobial activity over the monomer at all concentrations.

Kansoh and co-workers [77] synthesized novel binary chelating copolymer of butyl acrylate with itaconic and maleic acid by emulsion polymerization. The antifungal activities of the prepared chelating emulsions and their silver complexes against various fungal strain (A. flavus, A. niger, A. terreus, B. allii, D. oryzae, F. oxysporum, H. turcicum, M. phaseoli and T. reeset) were evaluated by agar diffusion technique. Waschinski et al. [78] studied on design of contact active antimicrobial acrylate using biocidal macromers. Silver-poly(acrylate) cluster have been synthesized in water by reduction of AgNO₃ in presence of poly(acrylate) of different molecular weights by Falletta and co-workers [79]. The nanoparticle dispersions were then used to functionalize cotton, wool and polyester samples in order to obtain antimicrobial textiles for biomedical applications. The antimicrobial activity of samples has been tested against S. aureus, S. epidermidis, P. aeruginosa and C. albicans.

Copolymerization of N-((4-amino sulfonyl)phenyl)acrylamide (APA) with 2-hydroxyethyl acrylate (HEA) and acrylic acid (AA) was reported by Reddy et al. [80]. All the monomer and polymers were tested for their antimicrobial activity against five different microorganism strains (E. coli, P. aeruginosa, K. pneumonia, S. typhi and S. aureus). The antimicrobial activity of APA on gram-positive bacteria was enhanced when copolymerized with AA and HEA. The antimicrobial activity on the homo and copolymers of N-(4-bromophenyl)-2-methacrylamide (BrPMAAm) with 2-hydroxyethylmethacrylate (HEMA) was investigated against various bacterias (S. aureus, B. subtilis, E. coli, K. pneumonia, P. aeruginosa) and yeasts (C. tropicalis, C. globrata, C. albicans) by Soykan and co-workers [81]. As the percentage of HEMA in the copolymers increases, the effectiveness of the copolymers to inhibit the growth of the microorganisms increases. Several co-workers have patented on the acrylate polymers containing quartenary ammonium as well as fluorinated amide side groups [82-84].

Kenawy and Mahmoud [85-89] worked intensely on the preparation of antimicrobial linear and crosslinked copolymers with immobilized ammonium or phosphonium salts. Principally they synthesized copolymers based 2-chloroethylvinyl ether and vinylbenzylchloride. The polymers antimicrobial activities were tested
against \textit{C. albicans}, \textit{A. flavus}, \textit{F. oxysporium}, \textit{B. subtilis}, \textit{E. coli}, and \textit{S. aureus} microorganisms, showing good antimicrobial activity. In general, phosphonium-containing polycationic biocides are more effective than the quaternary ammonium salt polymers. Furthermore, the antimicrobial activity increases with an increase of phosphonium units in the polymer.

Various laboratory methods employed for testing of biodegradability and antimicrobial properties of synthetic polymers are listed below:

(i) Agar plate method [90,91]
(ii) Radio tracer [92,93]
(iii) Soil burial method [94]
(iv) Soil enrichment test [95]
(v) In-vitro testing using specific organisms [96-99]

All these methods includes visual inspection of mycelium growth on the polymer surface, quantitative estimation of microbial growth, quantitative estimation of the weight loss of the polymer, measurement of changes in polymer properties and measurement of the metabolic activity of the microorganisms by oxygen uptake or \(\text{CO}_2\) evolution.

Out of these methods in the present investigation in-vitro testing using specific organisms is used for screening of acrylic copolymers for antimicrobial activity.

5.2 EXPERIMENTAL

(a) Materials

Tween-80 solution, 3,5-Dinitrosalicylic acid (DNS) reagent as well as bacterial, fungal and yeast cultures were obtained from Bioscience Department, Sardar Patel University. Nutrient broth (N-broth) medium and Sabouroud’s dextrose broth were used as received. All the microbial cultures were maintained on N-agar, Potato Dextrose Agar (PDA) and YEPD (Yeast Extract Potato Dextrose) slants respectively and were subcultured every fortnight and stored at 0-5°C.

(b) Inoculum preparation

\textit{i. Bacterial Cultures}

A loopful of cell mass from pregrown slants was inoculated into a sterile N-broth tube containing 15 ml medium and incubated at 37°C for 24 hours to get sufficient cell density (i.e. \(1 \times 10^8\) cells.ml\(^{-1}\)).
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ii. Fungal Cultures

Well-sporulated slants of fungal culture were used for preparation of spore suspension. About 5.0 ml of sterile distilled water containing few drops of tween-80 solution was added to the slants and growth was scrapped with sterile nichrome wire loop and collected in sterile tube. Spore suspension thus obtained was inoculated in the inoculum medium as 5% (v/v) and incubated at room temperature on rotary shaker (200 rpm) for 40 hours.

iii. Yeast Cultures

A well-pregrown slant of yeast culture was used for preparation of inoculum. About 5.0 ml of sterile distilled water containing few drops of tween-80 solution was added to the slants and growth was scrapped with sterile nichrome wire-loop and collected in sterile tube. Inoculum thus obtained was inoculated in the test medium as 5% (v/v) and incubated at room temperature on rotary shaker (200 rpm) for 40 hours.

(c) Screening of acrylic copolymers for antimicrobial activity:

Effect of acrylic copolymers on microorganisms was judged by the growth parameter in case of bacterial, fungal and yeast cultures.

i. Bacterial Cultures

5% (v/v) inoculum of bacterial culture was used to inoculated 100 ml N-broth solution [control (without polymer)] and test media (100 ml N-broth + 50 mg polymer) and incubated on rotary shaker (200 rpm) at room temperature. 0.5 ml liquids were withdrawn at specified time intervals (20 to 48 hrs) from test media. After suitable dilution with DNS (1 ml) and distilled water, absorbance was measured at 660 nm and calculated as absorbance per ml (i.e. growth). The method is based on the principle that as the growth proceeds, cell number increases which leads to increase in absorbance of medium. The percentage inhibition for bacteria was calculated by the following formula:

\[
\text{Percentage inhibition} = \frac{100(X - Y)}{X}
\]

Where 
\(X\) = absorbance of bacterial suspension in control set
\(Y\) = absorbance of bacterial suspension in test set.

ii. Fungal Cultures

Since fungal culture shows filamentous growth, optical method cannot be used to monitor the growth, therefore gravimetric analysis was carried out to determine dry
cell mass. 10% (v/v) inoculum was added to the sterile control medium (without polymer) and test medium (100 ml control medium + 50 mg polymer). Flasks were incubated at room temperature on rotary shaker (200 rpm) for 40 hours. Content of flasks were filtered using cheesecloth and cell pellets were dried to constant weight. The percentage inhibition for fungi was calculated after 7 days using the following formula:

\[
\text{Percentage inhibition} = \frac{100(X - Y)}{X}
\]

Where X= Weight of dry fungal cell mass in control set

Y= Weight of dry fungal cell mass in test set.

iii. Yeast Cultures

5% (v/v) inoculum of yeast culture was added to the 100 ml of sterile control medium (without polymer) and test medium (100 ml control medium + 50 mg polymer) and incubated on rotary shaker (200 rpm) at room temperature. Sample contents were withdrawn at specified time intervals (24 to 48 hrs) from test media. After suitable dilution with DNS (1 ml) and distilled water absorbance was measured at 660 nm and calculated as absorbance per ml (i.e. growth). The percentage inhibition for yeast was calculated by the following formula:

\[
\text{Percentage inhibition} = \frac{100(X - Y)}{X}
\]

Where X= absorbance of yeast suspension in control set

Y= absorbance of yeast suspension in test set.
Figure 5.1: Effect of poly(CMA), poly(CMA-co-MMA) and poly(MMA) on growth(%) of bacteria.
Figure 5.2: Effect of poly(CMA), poly(CMA-co-PCPMA) and poly(PCPMA) on growth(%) of bacteria.
Figure 5.3: Effect of poly(CMA), poly(CMA-co-2,4-DCPMA) and poly(2,4-DCPMA) on growth (%) of bacteria.
**Figure 5.4:** Effect of poly(CMA), poly(CMA-co-8-QMA) and poly(8-QMA) on growth(%) of bacteria
Figure 5.5: Effect of poly(CMA), poly(CMA-cc-GMA), and poly(GMA) on growth(%) of bacteria.

- *B. subtilis*
- *E. coli*
- *S. citreus*
Figure 5.6: Effect of poly(CMA), poly(CMA-co-PAPM) and poly(PAPM) on growth(%) of bacteria
**Figure 5.7:** Effect of poly(CMA), poly(CMA-co-MMA) and poly(MMA) on growth(%) of fungi
Figure 5.8: Effect of poly(CMA), poly(CMA-co-PCPMA) and poly(PCPMA) on growth(%) of fungi
Figure 5.9: Effect of poly(CMA), poly(CMA-co-2,4-DCPMA) and poly(2,4-DCPMA) on growth(%) of fungi
Figure 5.10: Effect of poly(CMA), poly(CMA-co-8-QMA) and poly(8-QMA) on growth(%) of fungi
Figure 5.11: Effect of poly(CMA), poly(CMA-co-GMA) and poly(GMA) on growth(%) of fungi.
Figure 5.12: Effect of poly(CMA), poly(CMA-co-PAPM) and poly(PAPM) on growth(%) of fungi
Figure 5.13: Effect of poly(CMA), poly(CMA-co-MMA) and poly(MMA) on growth(%) of yeast.
Figure 5.14: Effect of poly(CMA), poly(CMA-co-PCPMA) and poly(PCPMA) on growth(%) of yeast
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Figure 5.15: Effect of poly(CMA), poly(CMA-co-2,4-DCPMA), and poly(2,4-DCPMA) on growth(%) of yeast.
Figure 5.16: Effect of poly(CMA), poly(CMA-co-8-QMA) and poly(8-QMA) on growth(%) of yeast
Figure 5.17: Effect of poly(CMA), poly(CMA-co-GMA) and poly(GMA) on growth(%) of yeast
Figure 5.18: Effect of poly(CMA), poly(CMA-co-PAPM) and poly(PAPM) on growth(%) of yeast
5.3. RESULTS AND DISCUSSION

Interaction of acrylic polymers in an environment may either result into biodegradation/bioconversion or it may be inhibitory to the growth of microorganisms (antimicrobial nature). In view of the above facts, acrylic copolymers prepared by changing the sequences of addition of reactants were tested with respective microorganisms, which are commonly employed for microbial studies.

Growth pattern of bacteria, fungi and yeast were studied in presence and absence of acrylic polymers to gain better understanding of the process (biodegradation/bioconversion or inhibition) and comparative data analysis were carried out.

(a) Effect of acrylic copolymers on the growth of bacteria:

*B. subtilis, E. coli, and S. citreus* are the three bacterial strains taken for present study. *B. subtilis* being a most common soil bacterium was selected for this study. It is rod-shaped, gram-positive organism, which undergo sporalation when an adverse condition prevails in environment. Since *B. subtilis* effectively degrade a series of biopolymer which are assumed to play an important role in the biological cycling of carbon and nitrogen. *B. subtilis* species are well known as food spoilage organism.

*B. subtilis* when grown in presence of acrylic copolymers, prepared using different comonomers (MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM) with CMA monomer in different composition, shows differential response with respect to experimental conditions. From the results it is apparent that the acrylic copolymers prepared using CMA shows significant growth inhibition after 48 hrs. The homopolymer of CMA shows 44% growth of *B. subtilis*. Different copolymer compositions of poly(CMA-co-MMA), poly(CMA-co-PCPMA), poly(CMA-co-2,4-DCPMA), poly(CMA-co-8-QMA), poly(CMA-co-GMA) and poly(CMA-co-PAPM) allows 52-85%, 42-37%, 42-28%, 42-36%, 48-74% and 46-49% growth of *B. subtilis* respectively. The homopolymers of MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM registered 91%, 35%, 26%, 34%, 79% and 51% growth of *B. subtilis* respectively. However, during this period control culture (without resin) exhibited maximum growth (100%). The main source of *E. coli* is in human stool. They are gram-positive and short rod shaped. They are aerobic in nature.
They are nonsporing bacteria. During fermentation of various carbohydrates it produces acid and gas, e.g. glucose, lactose, maltose, xylose etc. except sucrose.

Poly(CMA) shows 40% growth of *E. coli*. The copolymers of CMA with MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM having different feed ratio exhibits 47-82%, 38-32%, 38-24%, 38-34%, 46-71% and 42-47% growth of *E. coli* respectively while homopolymer of each comonomer (MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM) shows 88%, 30%, 22%, 32%, 76% and 49% growth respectively.

*S. citreus* is lemon yellow colored and obtained from lemon tree and constantly present on the skin and in upper respiratory tract. The *S. citreus* are ever present pathogenic microorganisms which are the commonest cause of localized suppurative infections and among the longest recognized pathogenic bacteria. Colonies are smooth, low convex, glistening, butyrous and with an entire edge.

Homopolymer of CMA shows 42% growth of *S. citreus*. The copolymer series prepared using MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM with CMA indicates 49-84%, 40-35%, 39-26%, 40-35%, 47-72% and 44-49% growth of *S. citreus* respectively where as the homopolymers of MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM exhibits 90%, 30%, 24%, 33%, 77% and 50% growth of the *S.citreus* bacteria respectively.

Figures 5.1 to 5.6 represent the comparative effect of acrylic copolymers on the growth of bacteria. It is observed from the results that poly(PCPMA), poly(2,4-DCPMA) and poly(8-QMA) shows maximum growth inhibition against all bacterial cultures whereas poly(MMA), poly(GMA) and poly(PAPM) shows minimum growth inhibition. The results represent an overall growth inhibition of bacterial culture by acrylic copolymers, which increase in the order of: MMA<GMA<PAPM<CMA<8-QMA<PCPMA<2,4-DCPMA

Among various bacterial species, *E.coli* suffers maximum inhibition of growth whereas *S.citreus* undergoes normal growth inhibition and *B.subtilis* lead to minimum inhibitory effect on growth by various acrylic copolymers.

(b) Effect of acrylic copolymers on the growth of fungi:

*A. niger*, *S. pulverulentum* and *T. lignorum* are the three fungal strains taken in present study. Figures 5.7 to 5.12 represents the comparative antifungal activity of acrylic copolymers prepared by incorporation of CMA with several vinyl monomers
such as MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM having different monomeric feed ratio.

Microbial degradation of aromatic hydrocarbons by fungi and yeast has been studied to some extent but there are very few reports on detailed studies on biochemistry of degradation [100,101]. Although fungi belong to eukaryotes some of the inducible metabolic characteristics are very similar in prokaryotes and eukaryotes.

The black *A. niger* called black mold is widely distributed from the arctic region to the tropics. *A. niger* often found on exposed foodstuffs causes decay. Several species grow on leather and cloth fabrics, reducing their commercial values and imparting a musty odor to shoes and clothing. These species are animal and human pathogens and cause aspergilloses. It causes ear infection, which may become quite serious. Because of their great enzymatic activities *A. niger* is mainly use in the manufacturing of citric and gluconic acids commercially.

From the results it is observed that poly(CMA) shows 42% growth of *A. niger*. Copolymers of CMA with MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM allows 48-83%, 41-38%, 40-27%, 40-32%, 45-69% and 43-46% growth of *A. niger* respectively, while poly(MMA), poly(PCPMA), poly(2,4-DCPMA), poly(8-QMA), poly(GMA) and poly(PAPM) register 90%, 37%, 24%, 20%, 75% and 47% growth respectively. However, during this period control culture (without polymer) exhibited maximum growth (100%).

*S. pulverulentum* is a white-rot fungus whose parameters are important for the degradation of lignin or synthetic model compounds which have been described [102,103]. It is characterized by simultaneous attack on lignin, cellulose and other polysaccharides in wood [104]. *S. pulverulentum* also produces laccase and peroxides enzymes extracellularly. Therefore, this strain promises to be a good lignocellulolytic biodegradation strain for recycling of wood waste into useful chemicals.

Poly(CMA) shows 39% growth of *S. pulverulentum*. The different copolymers of CMA with MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM exhibits 45-79%, 38-32%, 37-23%, 37-29%, 42-67% and 40-44% growth of this fungi respectively, while homopolymers of respective comonomer shows 87%, 30%, 20%, 27%, 71% and 45% growth of *S. pulverulentum* respectively.

*T. lignorum* is also used as lignocellulolytic biodegradation strain. This genus is common on woody products and in soil. It has become of considerable economic
interest in recent years on account of its strong cellulose decomposing action, both as a spoilage organism and for usage in various schemes for upgrading of cellulose waste.

Homopolymer of CMA shows 41% growth of *T. lignorum*. However the copolymer series prepared using CMA with MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM indicates 47-81%, 40-35%, 39-24%, 39-31%, 43-68% and 42-45% growth of *T. lignorum* respectively, while homopolymers of MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM shows 89%, 33%, 21%, 29%, 73% and 46% growth of *T. lignorum* respectively.

(c) Effect of acrylic copolymers on the growth of yeast:

*C. utilis*, *S. cerevisiae* and *P. stipitis* are the three yeast strains taken in present study. The results depicted in Figures 5.13 to 5.18 shows the effect of acrylic copolymers on the growth of above mentioned yeast cultures.

*S. cerevisiae* is gram-positive and is oval or egg-shaped. It is always found in dung, soil and milk. *S. cerevisiae* is known as “backers” and “brewers” yeast and is commonly found on plants, grapes and other fruits. Many scientists have found that *S. cerevisiae* inhibits the growth of various compounds.

It is observed from the results that poly(CMA) shows 37% growth of *C. utilis*. The copolymers of CMA with MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM allows 45-84%, 35-30%, 34-26%, 35-27%, 41-70% and 39-43% growth respectively, while the homopolymers of MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM exhibits 89%, 28%, 25%, 26%, 73% and 41% growth of *C. utilis* respectively. During this period control culture (without polymer) exhibited maximum growth (100%).

The cell *C. utilis* is rich in proteins, fats and vitamins. *C. utilis* appears to be very heterogeneous from genera. It can routinely be isolated from many different sources, including water, soil, plant material, animals and animal feces. It is important yeast used commercially in the production of fodder [105].

Poly(CMA) shows 34% growth of *S. cerevisiae*. 41-80%, 31-22%, 32-21%, 32-25%, 37-67% and 36-40% growth of *S. cerevisiae* were observed for copolymers of CMA with MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM respectively, while poly(MMA), poly(PCPMA), poly(2,4-DCPMA), poly(8-QMA),
poly(GMA) and poly(PAPM) allows 86%, 20%, 19%, 23%, 71% and 42% growth respectively.

*P. stipitis* is yeast of class ascomycetes and family saccharromycetaceae. It has pseudomycelial growth form, cell are void to long cylindrical, vegetative reproduction is by multipolar budding. They have been found in biodegradation of plasticizers in variety of economical products [106]. Members belonging to the genus pichia are shown to utilize various hydrocarbons for their growth and multiplication [107].

Homopolymers of CMA, MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM gives 35%, 87%, 25%, 23%, 24%, 72% and 43% growth of *P. stipitis* respectively. Copolymer series prepared using CMA with MMA, PCPMA, 2,4-DCPMA, 8-QMA, GMA and PAPM shows 43-81%, 33-27%, 34-25%, 33-26%, 39-68% and 37-42% growth of *P. stipitis* respectively. It is manifested from the Figures 6.13 to 6.18 that all the yeast cultures showed less growth after 48 hrs.

The trend of inhibition properties of various microorganisms by acrylic copolymers obtained from various vinyl monomers is as follows:

2,4-DCPMA>PCPMA>8-QMA>CMA>PAPM>GMA>MMA

Figures 5.1 to 5.18 furnish a comparative account of the acrylic copolymers on the growth of bacteria, fungi and yeast. The following important conclusion may be drawn from the investigation.

All copolymers system imparts almost similar antimicrobial properties against bacteria, fungi and yeast. It is observed that copolymers prepared using coumaryl methacrylate shows significant inhibitory effect against tested microorganisms, in comparison with the homo polymers of vinyl monomers. The homopolymers of GMA, and MMA were not much effective against all microorganisms (compared to other homopolymers). This may be due to the unavailability of the bioactive group responsible for biocidal effect in the configuration or site of the polymeric structures. One can conclude for all copolymers system that, as CMA content decreases in the copolymers of GMA, PAPM and MMA the inhibition of the growth of microorganism’s decreases. But in case of QMA, 2,4-DCPMA and PCPMA copolymers, as CMA content in the copolymers decreases the antimicrobial activity increases due to presence of higher content of bioactive groups. Under the toxicity free environmental condition, all the microorganisms exhibited almost identical growth pattern as control. Thus this study indicates the fact that coumaryl methacrylate containing acrylic copolymers may be used as a good antimicrobial agen...
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