Synthesis and antimicrobial study of copolymer resins derived from p-hydroxybenzoic acid, semicarbazide and formaldehyde

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Abstract. The Copolymer p-HBSF-I resin was synthesized from p-Hydroxybenzoic acid, semicarbazide and formaldehyde with hydrochloric acid as a catalyst in 1:1:2 molar ratio by polycondensation technique. The copolymer characterization was done with IR and ¹H NMR Spectra. The antibacterial and antifungal screening of copolymer p-HBSF-I was done by using a well-known agar diffusion method. The bacterial strains used were Staphylococcus Aures, Bacillus Subtilillus, Escherchia Coli, Salmonella Typhi and the fungal strains used were Aspergillus Niger, Candida Albicans. The present study includes the antimicrobial investigation of copolymer resins in order to know the effectivity of semicarbazide group.

Keywords: Copolymer, Synthesis, Antimicrobial, Bacterial strain, Fungal strain.

1. Introduction

Microbial infection is a serious problem in food storage, drugs, surgery equipment, packaging, textile, health care, surface coating. To overcome microbial infections, antimicrobial polymers have been designed and synthesized by the researcher. Antimicrobial polymers are also known as polymeric biocides as it has an ability to inhibit the growth of microorganisms. In most of the cases, the killing of the pathogens takes place via electrostatic interaction followed by disruption of the cell membranes [1].

Polymeric biocides contain active functional groups as cationic biocides, like phosphonium, tertiary sulfonium, quaternary ammonium, and guanidinium. Microbes generally have a negative charge at the outer membrane of the cell. Cationic polymers can lead to the destabilization of the cell surface and the ultimately induction of bacterial death [2].

Recently the demand of antimicrobial polymers has increased because such a polymers are chemically stable, limited residual toxicity, long-term polymer, non-volatile and do not permeate through skin. In antimicrobial polymer synthesis, roll of function group and molecular weight is very important which decides the quality, efficiency, fungal and bacterial activity of polymer by introducing functional group. The variety applications of antimicrobial polymers is in the field of surface and material coatings technology, textile, metallurgy, surgical instruments. High molecular weight antimicrobial polymers have reducing leaching out of bioactive substances. To
overcome the associated problem, high molecular weight antimicrobial agent has been synthesized by various researcher and such polymers can maintain environmental balance and are ecofriendly in nature. It reduces the diffusion properties which affects skin, also reducing the skin irritation and toxicity, with lower the rate of corrosion of metals and plastics. However antimicrobial polymers with respect to low molecular weight antimicrobial agents shows several disvantage such as toxicity in environment, short-term antimicrobial activity [3].

Bakaruddin et. al [4] have reported the synthesis of copolymer from anthranilic acid-salicylic acid-formaldehyde(ASF) and the copolymer resin shows an inhibitory action against bacteria, and fungal strain. Tansir Ahmad et. al [5] reported the synthesis of thiourea-formaldehyde polymer and investigated antimicrobial activity. Also P-Chlorophenol-oxime—furfuraldehyde have been synthesized and investigated antimicrobial activity [6]. Patle and Gurnule reported the synthesis of p-Hydroxybenzaldehyde—biuret—formaldehyde (p-HBBF) copolymer resins and studied antimicrobial activity of copolymer using species of E. Coli, Klebsiella species and P. Aeruginosa, and fungi Penicillium species, and C.Albicans [7].

p-HBSF-I copolymer resin has a applications in the field of waste management as it show metal ion uptake capacity and hence used to remove the heavy metals like Cu, Ni, Co, Zn, Pb[8]. Also p-HBSF-I copolymer resin have been studied for TGA, and the decomposition reaction of p-HBSF-I copolymer are found to have ‘slow reaction’ hence concluded that, the copolymer is thermally stable at higher temperature, and can be useful for various applications like packaging, surface coating [9].

On the basis of earlier literature survey it has been observed that, semicarbazide can easily react with aldehydes and ketones and produce semicarbazones by using condensation reaction. Semicarbazide products such as semicarbazones and thiosemicarbazones are known to have an activity of antimicrobial, antiviral, antiinfective and antineoplastic. Semicarbazide-containing copolymer with biological properties, particularly, analogs of antimicrobial nitrofurazone and nitrofurantoin have been studied. [10].

In the present study, the copolymer resin p-HBSF-I have been studied for antimicrobial activity with microorganisms and p-HBSF-I copolymer shows moderate inhibitory activity against some microorganisms.

2. Experimental

2.1 Copolymer p-HBSF-I Synthesis

p-HBSF-I copolymer was synthesized from p-hydroxybenzoic acid (0.1 mol), semicarbazide (0.1 mol) and formaldehyde (0.2 mol) . The reaction was started with the above starting material using a mol ratio of 1:1:2. with 2M HCl (200 ml ) as a catalyst. The whole reaction mixture is heated with temperature 126°C ± 2°C for 5hours, in oil bath [9]. After completion of heating process the yellowish resinous solid removed from the flask and cleansed with distilled water and dried in the air. The p-HBSF-I copolymer purification was done with solvent diethyl ether and petroleum ether to avoid further contamination with newly synthesized resin. Further the copolymer was cleansed by dissolving 8% NaOH and recovered by Conc. HCl. At last the copolymer ground and kept in vaccum over a silica gel [11-14].
2.2 Copolymer Characterization

The FT-IR spectral characterization of p-HBSF-I copolymers was done by utilizing Shimadzu – 1800S spectrometer on KBr pellets (400-4000 cm\(^{-1}\)). The proton \(^1\)H NMR spectral characterization was done by using Brucker Advance-II,400 spectrometer. All the spectral characterization was carried out at Saphisticated Analytical Instrumentation Facility, Punjab University Chandigarh. The DMSO and tetramethylsilane (TMS) were used as an internal standard. For spectral analysis of copolymer p-HBSF-I [15].

2.3 Antimicrobial Activity Test

An agar well diffusion method was used for antibacterial and antifungal study of p-HBSF-I copolymer. In the present study four bacterial strains and two fungal strains were used. The Staphylococcus Aureus, and Bacillus subtilus, Escherichia coli, Salmonella typhi, bacterial strain were used and Aspergillus niger, Candida albicans as the fungal strains were used [16].

2.3.1 Methodology Used for Anti-bacterial analysis

Initially the inoculation of stock cultures of bacteria in broth media were carried out. It was then grown at 37\(^{\circ}\)C for 18 hrs. The wells in the agar plates were developed then each plate was inoculated with 18 hrs old cultures (100 μl, 10\(^4\) cfu) and spread evenly on the plate. After 20 min, different concentrations of sample solutions filled in the wells. The process of incubation conducted at 37\(^{\circ}\)C for 24 hrs and the diameter of inhibition zones were noted. The control wells were filled with Gentamycin [17].
Table 1. Anti-bacterial analysis of p-HBSF-I Copolymer Resin

| Sample Codes | p-HBSF-I |
|--------------|----------|
| Solvent Used | DMSO     |
| Standard Antibiotic Used | Gentamycin |
| Concentrations Used | 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 mg |
| Sample Preparation | 20 mg sample/ml solvent |
| Concentration of Stock Sample | 20 mg/ml |
| Method Used | Agar Diffusion Method |
| Bacteria Analyzed | S.Aureus, B.Subtilis, E.Coli (ETEC), S. Typhi. |
| Nutrient broth | Peptone :- 10 g, NaCl-10g, Yeast extract :- 5g, Agar 20g in 1000 ml of distilled water |

2.3.2 Methodology Used for Anti-fungal analysis

Initially the inoculation of stock cultures of fungi in broth media were carried out. It was then grown at 27°C for 48 hrs. The wells were made in agar plates having above media. Each plate was inoculated for 48 hrs with old cultures (100 μl 10^4 CFU) and spread evenly on the plate. After 20 min, different concentrations of sample solutions filled in the wells. The process of incubation conducted at 27°C and for 48 hrs. The diameter of inhibition zone were noted. The control wells were filled with antibiotic [18-19].

Table 2. Anti-fungal analysis p-HBSF-I Copolymer Resin

| Sample Codes | p-HBSF-I |
|--------------|----------|
| Solvent Used | DMSO     |
| Standard Antibiotic Used | Amphotericin |
| Concentrations Used | 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 mg |
| Sample Preparation | 20 mg sample was dissolved in 1 ml of solvent |
| Concentration of Stock Sample | 20 mg/ml |
| Method Used | Agar Diffusion Method |
| Bacteria Analyzed | Aspergillus niger, Candida albicans |
| Nutrient broth | Czapek-Dox Agar: Composition (g/l) Sucrose-30.0; Sodium nitrate-2.0; K₂HPO₄-1.0, MgSO₄. 7H₂O-0.5; KCl-0.5; FeSO₄-0.01; Agar-20 |

3. Results and Discussion

The p-HBSF-I copolymer resin is yellowish in color and its molecular weight is found to be 248 g/mol. The copolymer is soluble in solvents such as DMF, DMSO and THF while insoluble in almost all other organic solvents. and the yield of the copolymer resin was found to be 87 %.
3.1 Solubility Behaviour

The solubility of p-HBSF-I copolymer was tested in various solvents to know the solubility behaviour of copolymer resin and are tabulated in Table 3.

**Table 3. Solubility behaviour of p-HBSF-I copolymer Resin**

| Sr. No | p-HBSF-I copolymer       | Solubility behaviour |
|--------|--------------------------|----------------------|
| 1      | Tetrahydrofuran (THF)    | S                    |
| 2      | Dimethylsulfoxide (DMSO) | S                    |
| 3      | Dimethylformamide (DMF)  | S                    |
| 4      | Conc. H₂SO₄              | S                    |
| 5      | Conc. HCl                | S                    |
| 6      | NaOH (aq.)               | S                    |
| 7      | Isopropylalcohol         | S                    |
| 8      | Pyridine                 | S                    |
| 9      | 1,4-Dioxane              | S                    |
| 10     | Diethyl ether            | I                    |
| 11     | Chloroform               | I                    |
| 12     | Methanol                 | I                    |
| 13     | Chlorobenzene            | I                    |
| 14     | Toluene                  | I                    |
| 15     | Cyclohexane              | I                    |
| 16     | Benzene                  | I                    |
| 17     | Acetone                  | PS                   |
| 18     | Conc. HNO₃              | PS                   |

[Solubility Behaviour - S: Soluble, I: Insoluble, PS: Partially Soluble]

3.2 Characterization of p-HBSF-I Copolymer

3.2.1 Infra-Red Spectra

The IR-Spectra of p-HBSF-I as shown in Fig. 2, gives a very informative data on the basis of stretching, bending, twisting, wagging of the various types of bondings, linkages and functional group present in the given copolymer. Spectral study shows that, in the region 3500 - 3200 cm⁻¹ a broad band is appeared due to phenolic hydroxy groups, which exhibits an intermolecular hydrogen bonding having stretching vibration. The region of 3000-2800 cm⁻¹ suggests that there should be -NH linkage. Also broad intense peak is observed due to hydroxyl group. The presence of carbonyl group is further confirmed due to the strong band displayed at 1900-1650 cm⁻¹ region. The methylene bridges in the copolymer chain was depict at 1500-1450 cm⁻¹. Sharp peak in the IR-spectra is at 1624-1605 cm⁻¹ for breathing modes of aromatic skeletal ring. The recognition of 1, 2, 3, 4, 5 Penta substitution of aromatic ring moiety and bands are ascribed with the bands at 980, 1012, 1080, 1207 and 1281 cm⁻¹ [20-22].
3.2.2 Nuclear Magnetic Resonance

The $^1$H NMR spectra of p-HBSF-I copolymer is shown in Fig.3. The presence of methylene protons of the Ar-CH$_2$-Ar bridge has attributed a singlet signals in region of 2.2 – 2.5 (δ) ppm. In Ar – CO – CH$_3$ group the methyl protons is shown with medium singlet peak and assigned at 2.5 to 3 (δ) ppm. For amino proton of –C–NH–CS- linkage a triplet signal appeared in the region 3.0 to 4.1 (δ) ppm. At 3.5 to 4.5 (δ) ppm an intense signal appeared which assigned to methylene protons (CH$_2$) of polymer chain. An unsaturated pattern with weak signal is at 6.5 to 8.5 (δ) ppm for the aromatic protons. Proton weak signal is found in the region 7.5 to 8.2 (δ) ppm is phenolic –OH proton [23-24].

Figure 2. FTIR Spectra of p-HBSF-I Copolymer Resins

Figure 3. $^1$H NMR Spectra of p-HBSF-I Copolymer Resin
3.3 Anti-Bacterial and Anti-Fungal Screening

A well known agar diffusion method was used to investigate the antibacterial and antifungal activity of p-HBSF-I copolymer resins. The bacterial pathogens used were B.Subtilis, E.Coli, S.Aureus, S. Typhi with A. Niger, C.Albicans fungi for antifungal analysis. In this process agar plates were prepared and wells were made in the plate [25-26]. The test samples were tested with varying concentration to know their efficacy in inhibiting the growth of the human pathogens. The antimicrobials present in the p-HBSF-I copolymer are then diffused in the medium and interact in a plate freshly seeded with the test organisms. There observed a confluent lawn of growth which resulting in uniformly circular zones of inhibition [27]. The inhibition zone (mm) diameters with varying concentration against the test bacteria have been mentioned in Table 4. The standard antibiotic gentamycin and amphotericin were used for antimicrobial activity of the copolymer resin[28-32]. From the Fig-4 and the data obtained the growth of B. subtilis, E. coli, S. aureus were found to be affected.

| Organism     | 1.00 (mg) | 2.00 (mg) | MIC (mg) |
|--------------|-----------|-----------|----------|
| B. Subtilis  | -         | 3         | 2        |
| E. Coli      | -         | 6         | 2        |
| S. Aureus    | 6         | 10        | 1        |
| S. Typhi     | -         | -         | -        |
| A. Niger     | -         | -         | -        |
| C. Albicans  | -         | -         | -        |

Table 4. Bacterial and Fungal inhibitory growth in p-HBSF-I Copolymer Resin

![Escherichia coli](image1)
![Aspergillus niger](image2)
![Candida albicans](image3)
![Salmonella typhi](image4)
4. Conclusion
The condensation polymerization technique was used for the synthesis of copolymer p-HBSF-I. The structure of copolymer has been confirmed using FT-IR and 1H-NMR studies. The moderate inhibitory activity against only B. subtilis, E. coli, S. aureus, is found which due to the presence of semicarbazide group. No fungal activity was observed due to Aspergillus niger, and Candida albicans for p-HBSF-I copolymer used.

5. References
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Figure 4. Antimicrobial Screening of p-HBSF-I Copolymer Resin
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