SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF NOVEL QUATERNIZED POLY (STYRENE ETHYLENE BUTYLENE POLY STYRENE) 

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Abstract

A new class of biologically potent compound was derived from poly (styrene ethylene butylene poly styrene) via Chloromethylation and Quaternization. Quaternized poly (styrene ethylene butylene poly styrene) [QPSEBS] is a type of anion exchange polymer. The functionalized polymer was characterized by FT-IR and ¹H-NMR spectroscopy. The present investigation is centered on the antimicrobial activity of the QPSEBS polymer. The QPSEBS polymer was found to show antibacterial activity towards the Gram-positive bacteriae (Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa) and Gram-negative bacteria (Escherichia coli, Klebsiella pneumonia) which was determined by agar diffusion method used with tetracycline as a reference control. The minimum inhibitory concentration (MIC) was observed against all the five pathogenic bacteriae.

Keywords: QPSEBS, antimicrobial activity, agar diffusion method, minimum inhibitory concentration

1. INTRODUCTION

An antimicrobial is a substance that kills (microbicidal) or inhibits (microbistatic) the growth of microorganisms [1] such as bacteriae, fungi, viruses or protozoans. Disinfectants are antimicrobial substances used on non-living objects. The history of antimicrobials began with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. It was not known at that time that the reason for the failure of growth of one bacterium was because the other bacterium produced an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that either kill or prevent the growth of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that cures a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.

The basic mechanisms of antibiotic against bacterial cells are: (i) Inhibition of cell wall synthesis (e.g. penicillins); (ii) Inhibition of protein synthesis (e.g. tetracyclines); (iii) Alteration of cell membrane (e.g. polymyxins); (iv) inhibition of nucleic acid synthesis (e.g. quinolones); and (v) antimetabolite activity (e.g. sulfonamides) [2]. Inhibition of cell wall synthesis is the most common mechanism of antibiotic action. All cells, including bacteria, have a cell membrane, which allows movement of substances in and out of the cell in a controlled manner. Synthetic compounds may affect the integrity of the cell membrane leading to cell death [3]. Such molecules may also mimic the complex structures having in vivo antimicrobial activity [4]. Synthetic antibacterial agents are used as antiseptic ingredients in commercial products, such as liquid soap [5] and are used in in vivo systems against bacterial infections [6] which require multiphase clinical trials to be accepted as a commercial drug [7].
Antibiotics from microorganisms have dominated as clinically useful antimicrobial agents. However, several synthetic organic compounds such as methanamide mandelate (Mandelamine®), nitrofurantoin (Furadantin®), metronidazole (Flagyl®), and nalidixic acid (Neggram®) are effective chemotherapeutic agents in man. Members of a new class of synthetic polymers are also active against certain bacterial pathogens [8]. The activities of one of these, poly (styrene ethylene butylene poly styrene), are described.

However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents. Bacteria found in hospitals appear to be especially resilient, and are causing increasing difficulty for the highly susceptible patients–those in the hospital. Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant faster than the rate at which new drugs are being made available; thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means, such as species-specific phages.

Growth of microorganisms must be inhibited at the surface of polymeric materials used in a variety of applications, such as food packaging, antifouling paints [9, 10] and hospital furniture. Nowadays, antimicrobials in plastics are a growing sector of the speciality biocides industry. The effectiveness of an antimicrobial material is related to its ability to limit contact with the microorganisms and to inhibit their growth. Bactericidal and fungicidal properties can be granted to a coating whenever a pre-incorporated biocide is slowly released, which is illustrated by antifouling paints used to protect submerged structures in sea water [11, 12]. Bioactive molecules can be leached from the coating by different mechanisms, including erosion of the binder and by hydrolysis of a chemical bonding. A drawback of the antifouling paints is found, however, in the toxicity of the active molecule and the short-lived protection. An effective way to tackle this problem consists in developing materials with an intrinsic and thus permanent antimicrobial activity. Nowadays, fibres with inherent antimicrobial activity are available in the market [13] and are used to protect clothes, socks, filters and packaging materials. Polymer chains with pendant quaternary ammonium salts (QAS) bonded through non-hydrolysable covalent bonds exhibit bactericidal and fungicidal activities in water and in the solid state, as well [6]. As a rule, polycationic biocides with high positive charge density deserve interest in hygiene and biomedical applications.

In this work, a well-defined quaternized poly (styrene ethylene butylene poly styrene) was prepared via chloromethylation and quaternization. The quaternization reaction was carried out with triethylamine to induce antibacterial property of the poly (styrene ethylene butylene poly styrene) [PSEBS] surface.

2. EXPERIMENT
2.1 Materials
2.1.1 Polymer and Chemicals
The monodisperse tri-block co-polymer PSEBS having a number-average molecular weight ($M_n$) of 89,000, a molecular weight distribution (weight-average molecular weight/number-average molecular weight)
molecular weight ($M_w/M_n$) of less than 1.06, and 28.6 wt% styrene units and composed of two poly styrene (PS) end blocks and a poly (ethylene-co- butylene) central block, was used as the starting material. Other chemicals like chloroform, methanol, triethyl amine (TEA), para formaldehyde, conc. HCl and zinc chloride were purchased from Sisco Research Laboratory (SRL) Pvt. Ltd. All were of analytical grade and were used as received. Double distilled water was used throughout.

2.1.2 Microorganisms
Cultures of the following microorganism were used in the study: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*.

2.2 METHODS

2.2.1 Chloromethylation and Quaternization
Chloromethylation reaction of aromatic polymers is an electrophilic substitution at aromatic rings that needs a chloromethylating agent and a Lewis acid catalyst. Chloromethylated PSEBS was prepared from PSEBS using concentrated hydrochloric acid and para formaldehyde as chloromethylating agent and zinc chloride as catalyst. In order to obtain soluble chloromethylated polymer, the aromatic tri-block PSEBS (5 g) was dissolved in chloroform; thereafter 180 mmol of para formaldehyde and 540 mmol of conc. HCl mixture was added drop-wise in to the solution with continuous stirring and then 25.3 mmol of zinc chloride was added in a round bottom flask and the reaction was allowed for 48 hours, with continuous stirring. The reaction temperature was maintained at 60°C throughout the reaction time. After 48 hours, the reaction was terminated and chloromethylated PSEBS (CMPSEBS) product was obtained by simply adding the reaction contents into methanol and washed with methanol to remove the excess acid and formaldehyde from the product, after which the product was dried in vacuum dessicator for a day.

The chloromethylated polymer was dissolved in tetrahydrofuran (THF) and then 25 mL of quaternizing agent, triethyl amine were added to this solution with continuous stirring. The quaternizing reaction was carried out in the nitrogen atmosphere at 80°C for 12 hours. The quaternized PSEBS (QAPSEBS) was recovered after removing all the solvent by evaporation. The product was washed with water for several times until the pH became neutral. The obtained ionomer was then dried at room temperature. The schematic representation of the preparation of anion exchange membrane is shown in Fig.1.

2.2.2 Structural confirmation
The chemical structure of the membrane was analyzed using FT-IR. The FT-IR spectra of PSEBS, CMPSEBS and QPSEBS were recorded in transmission mode using Perkin Elmer spectrometer by placing the membranes in KBr windows. Confirmation of chloromethylation and quaternization was also done by $^1$H NMR Spectroscopy. Proton NMR ($^1$H-NMR) spectra were obtained with Bruker NMR
spectrometer using deuterated chloroform as the solvent.

2.2.3 Antibacterial Screening Test
All Petri dishes (diameter 86 mm) and graduated measuring pipettes were dry heat sterilized in a canister at 420 °C for 4 hours. The agar diffusion method (M. W. Jenny, 2006) was used for the determination of antibacterial activity of novel QPS EPS against the microorganism listed above. About 15mL of nutrient agar media were poured into Petri plates (9 cm in diameter) and inoculated with respective test organism. Wells were made with sterile cork borer on solid agar and loaded with 25 - 100μg/ml of the test compound with Tetracycline as control. Petri plates were incubated at 37 °C for 24 h and the average diameter of the inhibition zone surrounding the wells was measured after specified incubation period.

3. RESULTS AND DISCUSSION

3.1 FT-IR

Figure 2(a) shows the IR spectrum of PSEBS. Appearance of peak around 1638 cm\(^{-1}\) was assigned to the aromatic ring C=C. Appearance of peak around 1458 cm\(^{-1}\) and 1366 cm\(^{-1}\) was assigned to the bending vibration which is due to the presence of aromatic ring backbone –CH- bending vibration. The peak at 2924.23 cm\(^{-1}\) is due to the stretching of C-H bond of aromatic hydrocarbon. Appearance of peak around 699 cm\(^{-1}\) and 747 cm\(^{-1}\) was assigned to the aromatic ring out of plane C-H bending vibration. Appearance of these peaks confirmed the structure of PSEBS.

Due to chloromethylation reaction (Figure 2b), the IR bands of C=C in aromatic ring from styrene unit are shifted to a lower frequency in the range of 1601 cm\(^{-1}\). As well as the IR bands of the aromatic ring backbone CH bending vibration, are shifted to a lower frequency in the range of 1404 cm\(^{-1}\) and 1247 cm\(^{-1}\). This confirms that the reaction has occurred in the aromatic ring. Appearance of peak at 1247 cm\(^{-1}\) shows that the CH\(_2\)Cl group is substituted at the phenyl ring.

Due to quaternization reaction, the IR bands of aromatic ring C=C and aromatic ring backbone –CH- bending vibration are shifted to a lower frequency around 1600 cm\(^{-1}\) and 1371 cm\(^{-1}\). In this reaction, triethylamine is used to form the quaternized product. IR bands for tertiary amine cannot be seen in the spectrum. Appearance of peak around 1371 cm\(^{-1}\) was assigned to C-N stretching vibration. Due to the quaternization (Figure 2c), a small intense peak at 2365.72 cm\(^{-1}\) has appeared, which is the characteristic absorption peak of quaternary ammonium groups. From all these evidences, it is clear that chloromethylation and quaternization
reactions have been successfully carried out in the PSEBS unit at phenyl rings.

3.2 Nuclear Magnetic Resonance

Figure 3 shows the NMR spectra of PSEBS and QAPSEBS. By characterizing the polymer samples of PSEBS and QAPSEBS with proton nuclear magnetic resonance spectroscopy, the following conclusions were drawn. The base polymer PSEBS contains 29% of polystyrene and 71% of ethylene and butylene. Since the styrene content is only 29%, it shows a very low intensity in NMR bands. The solvent peak appears at δ= 7.28 ppm. The alkyl proton bands namely CH₃ proton, CH₂ proton and CH proton are appearing at 0.9ppm, 1.3ppm and 1.5ppm respectively. The bands that appear in the region between 6-8.5ppm are due to the aromatic ring protons. Appearance of these proton bands clearly confirms the structure of the PSEBS. Due to quaternization reaction, some structural changes takes place in the reactant PSEBS. Appearance of a new triplet peak at 1.3 δ is assigned to the CH₃ protons from N-CH₂-CH₃ group. The new peak appears at 4.1 δ is assigned to the CH₂-N protons due to the presence of the phenyl group. The quartet band formed at 3.0 δ is assigned to the N-CH₂-CH₃ group. All these confirm the structure of QPSEBS. In summary, the changes in NMR spectra support the chemical modification of PSEBS in to an anion exchange polymer.

3.3 Pharmacological studies

We have subjected all the synthesized compounds for bioactivity studies against micro organisms and the results were recorded.

The bioactivity studies were carried out against the following bacteriae: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *P.aeroginosa*, *Klebsiella pneumonia*.

3.4 Anti microbial activity of QPSEBS (Agar diffusion method)

The anti bacterial activity of the QPS EPS compounds against human bacterial pathogens as determined by agar diffusion method with tetracycline as a reference control is presented in the table 1.

The QPSEPS compounds had a maximum inhibition zone of 28 mm against *Bacillus subtilis* at a concentration of 100 μg/ml compared to other concentrations of 25, 50, 75 μg/ml which showed minimum inhibitory zones of 16, 19 and 22 mm respectively. The least inhibition concentration was observed at the concentration 25 μg/ml.

The maximum zone (23 mm ) was observed against *Staphylococcus aureus*; for concentration of 100 μg/ml compared to other concentrations of 25, 50, 75 μg/ml which showed zones of 15,19 and 22, mm of respectively. The minimum inhibition concentration was observed at the concentration 25 μg/ml.

The maximum zone (17 mm) was observed against *Escherichia coli* ; for concentration 100 μg/ml compared to other concentrations of 25, 50, 75 μg/ml
which showed zones of 10, 12 and 14 mm respectively. The minimum inhibition concentration was observed at the concentration of 25 μg/ml. The maximum zone (22 mm) was observed against *Klebsiella pneumonia*; for concentration 100 μg/ml compared to other concentrations of 25, 50 and 75 μg/mL which showed 13, 15 and 20 mm respectively. The minimum inhibition concentration was observed at the concentration 25 μg/ml.

The bacterium *Pseudomonas aerogenosa* has no activity against the compounds up to 100 μg/ml. The QPSEBS showed more potential antibacterial activity against *Bacillus subtilis* in minimum concentration compared to other pathogenic bacteria like *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumonia*.

### Table 1  Effect of QPSEPS on the growth of human pathogen

<table>
<thead>
<tr>
<th>Compound</th>
<th>Human pathogens</th>
<th>Concentration of the compounds (μg/ml) and their Zone of the inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Bacillus subtilis</em></td>
<td>25</td>
</tr>
<tr>
<td>QPSEPS</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>10</td>
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<tr>
<td></td>
<td><em>Klebsiella pneumonia</em></td>
<td>13</td>
</tr>
</tbody>
</table>

**4. CONCLUSION**

The novel quaternized anion exchange polymer was successfully synthesized from poly (styrene ethylene butylene poly styrene) via chloromethylation and quaternization. The modification of the polymer was confirmed by FT-IR and ¹H-NMR spectroscopy.

The QPSEBS was seen to have good antibacterial properties against some of the most common bacteria found. The study shows that QPSEBS has good potential
to resist attack by bacteria in the environment. This can be exploited for wide industrial and medical applications.

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5. REFERENCES