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Chemically modified polystyrene co-loaded with antimicrobial agents

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Chemically modified polystyrene co-loaded with antimicrobial agents

Y X Koh[1], H L Choo[1], Y H Wong[2] and C H Yeong[2]

[1]School of Computer Science and Engineering, Faculty of Innovation and Technology, Taylor's University, 47500, Subang Jaya, Selangor, Malaysia
[2]School of Medicine, Faculty of Health and Medical Sciences, Taylor's University, 47500, Subang Jaya, Selangor, Malaysia

Email: HuiLeng.Choo@taylors.edu.my

Abstract. A recent study showed that at least 50% of nosocomial infections are due to medical indwelling devices like surgical guides and prosthetics. This amounts to about 2 million patients affected a year. The reason for such statistics is the growth of microorganisms on the surfaces of the medical devices. There have been many attempts to create antimicrobial materials but most materials are unable to hold more than one antimicrobial agent without a secondary process. The study related to antimicrobial material with more than one type of agent is rarely found in literature. Hence, the objective of this project is to produce an antimicrobial material that can hold more than one antimicrobial agent without the need for a secondary process. The material is produced by sulfonating high impact polystyrene (HIPS) and attaching copper and silver ions. The optimum time of sulfonation of the HIPS was determined by the degree of sulfonation and ion exchange capacity. Then, the sulfonated HIPS were loaded with both copper and silver ions at different ratios. The 6-hour sample yielded the highest degree of sulfonation and ionic exchange capacity of 33.7% and 2.57 meq/g, respectively. In future work, the characterization of the 6-hour sulfonated HIPS sample loaded with copper and silver ions at different concentration ratios will be performed using TGA, DSC and FTIR spectroscopy. Lastly, the efficacy of the antimicrobial properties of the sulfonated HIPS will be tested using different bacterial strains.

1. Introduction

Nosocomial infections are the infections that take place within a medical facility such as a hospital. Despite the helpfulness of medical devices in healthcare, it ironically is one of the main reasons for nosocomial infections. Most of the time the infections happen due to the contamination of indwelling medical devices that are inserted into patients. This introduces microorganisms directly into the bloodstream of the patient. A recent study shows that nearly two million healthcare associated infections are linked to indwelling medical devices per year. This statistic accounts for 50 % of all nosocomial infections. Some of the factors that contribute to such high quantities of infections include increasing rates and types of device utilization, aging of the population, and increasing frequency of comorbidities leading to immune-compromised states [1].

The phenomenon of microorganisms growing on the surface of the surgical devices is known as pathogenesis. During insertion of the devices, the microorganisms enter the body and cause an inflammation reaction. Subsequently, the white blood cells will initiate coagulation cascades as they come into contact with the contaminated material and form biofilms. The biofilms will then stick on the surface of the devices due to physiochemical forces. In many cases, electrostatic attraction via van der Waals forces is formed by the microorganisms of opposite charge with the abiotic surfaces of the implants [1]. Figure 1 shows the electrostatic interaction between the microorganism and the surface of the medical device.

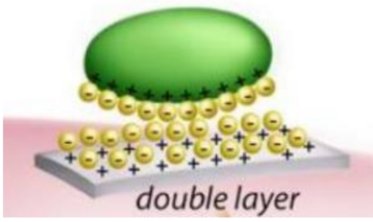


Figure 1. Electrostatic interaction between microorganisms and the surface of the medical device that causes pathogenesis [1].

The use of antimicrobial materials is also trending. However, the main challenges of using antimicrobial materials include the uncertainty of its toxic effects on the human body, and the fact that only one type of antimicrobial agent can be utilized at one time. Studies related to antimicrobial material with more than one type of agent is rarely found in literature.

The term microbial refers to the characteristics of microorganisms while antimicrobial agents are the agents that have the property or ability to inhibit any form of microbial activities [2]. There are many types of antimicrobial agents. Metallic ions such as copper and silver ions are the examples of the oldest antimicrobial agents. Many studies [3-5] have been carried out to study the antimicrobial properties of copper and silver ions. These ions have the antibacterial properties due to oligodynamic effect. Copper and silver ions have the ability to bind to thiol or amine moiety of the cellular proteins to deactivate them and subsequently causes precipitation of proteins and cells death [5]. These ions are known as microbicides or bacteriostatic agents as they kill and inhibit the growth of microorganisms [6]. Furthermore, copper and silver ions have been found to have low toxic effects on the human body [7].

The objective of this project is to develop an antimicrobial high impact polystyrene (HIPS) by sulfonation followed by attachment of two antimicrobial agents. The material was co-loaded with copper and silver ions due to their low toxicity as well as the extensive knowledge about their antimicrobial properties in this field. In addition, the sulfonation parameters to achieve the highest percentage of sulfonation and ion exchange capacity (IEC) were determined.

2. Methodology

2.1. Materials
The HIPS powder was purchased from Sinopec Corporation (Beijing, China). The silver nitrate ($AgNO_3$), copper (II) sulfate ($CuSO_4$), sodium chloride (NaCl), sulfuric acid (H_2SO_4) was procured from Merck & Co., Inc. (Darmstadt, Germany). All the chemicals were used as obtained without any further purification and distilled water was used in all experiments unless otherwise stated.

2.2. Methods

2.2.1. Sulfonation of High Impact Polystyrene. In the sulfonation process, sulfonate groups aim to substitute the hydrogen atoms that are present on the aromatic ring of the HIPS. During the sulfonation process, the sulfonate groups substituted the hydrogen atom and formed a covalent bond with the carbon atoms on the aromatic ring of the styrene monomer within the HIPS [8]. The presence of the sulfonate group causes the polystyrene to have a charged group that can bound the cations. Figure 2 shows the chemical reaction that takes place during the sulfonation of polystyrene.

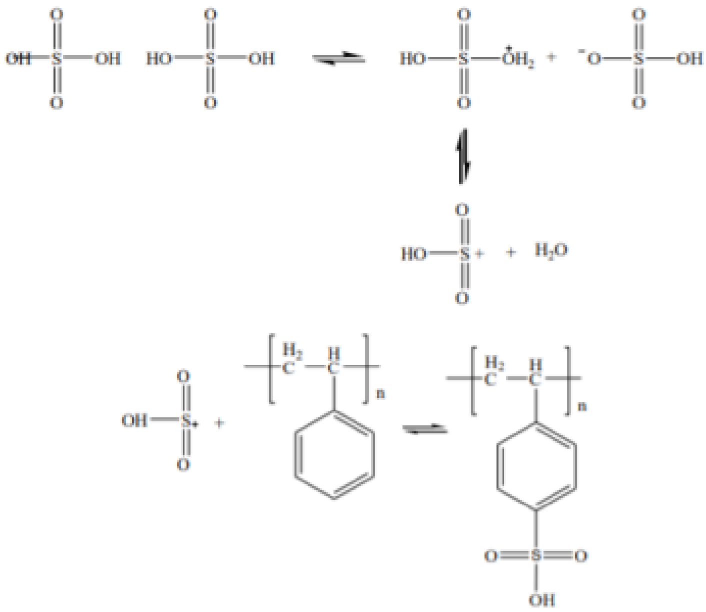


Figure 2. Sulfonation reaction that occurs in polystyrene [8].

Sulfonated HIPS was prepared using the concentrated sulfuric acid, stirring rate of 500 rpm and sulfonation temperature of 60 °C while varying the sulfonation time. In brief, 100 mL of 96% sulfuric acid was added to 50 g of HIPS powder. The ratio of HIPS powder (g) to sulfuric acid (mL) was 1:2 to ensure that all the HIPS powder is suspended in the acid solution. The mixture of sulfuric acid and HIPS powder was then stirred at 500 rpm in the water bath at 60 °C for a predetermined time. A total of four samples were made with different sulfonation time of 1 hour, 2 hours, 3 hours and 6 hours.

Once reaching the desired sulfonation time, cold distilled water was added into the sample gradually to stop the sulfonation reaction. The mixture was then stirred overnight at 500 rpm. The samples were filtered and washed with distilled water until the pH of the filtrate reached neutral pH. The samples produced at different sulfonation time were then dried in an oven at 70 °C for at least 24 hours.

2.2.2. Ionic Exchange Capacity (IEC) and Degree of Sulfonation (DS). In order to determine the amount of sulfonate groups that are attached to the styrene monomer and the ability to exchange for cations, the DS and IEC must be determined [9]. In order to determine the IEC, the sulfonated HIPS were suspended in NaCl solution for at least 48 hours to achieve equilibrium between sodium ions and sulfonated groups. Briefly, 1 g of each sulfonated HIPS sample was suspended in 100 mL of 0.1 M NaCl solution for 48 hours [9]. The mixtures were then filtered and the concentration of the sodium ions before and after the addition of sulfonated HIPS were determined using an atomic absorption spectrometer (PinAAcle 900T, Perkin Elmer). Prior to the measurements, calibration was performed using the known concentration of NaCl solutions. The concentration of the sodium ions in the samples were determined and the difference in concentration of sodium ions were used to determine the IEC and DS using Equation (1) [10] and Equation (2) [9], respectively.

$$IEC = \frac{A(DF)(100\ mL)}{EB(100g)} \text{ ---(1) [10].}$$

$$DS = \frac{(M_{polymer})(IEC)}{1000-(M_{SO_3H})(IEC)} \times 100\% \text{ ---(2) [9].}$$

where,
A = difference in the sodium ions concentration
DF = dilution factor = 10
E = molar mass of sodium × 10 = 229.9
B = dry weight sample = 0.1 g
$M_{polymer}$ = 104 g mol^{-1}
M_{SO_3H} = molar mass of sulfonic group = 81 g mol^{-1}

2.2.3. Loading of antimicrobial agents. The chosen antimicrobial agents for this study are silver and copper ions. These ions will be attached to the polymer via spontaneous ionic exchange reaction. The sulfonated HIPS is suspended in a salt solution containing the desired metal ion concentration. The ions will then displace the hydrogen ions that is present on the sulfonate group. Silver ions as well as cuprous ions, which is one of the two types of copper ions, have a +1 charge. Hence, they will substitute the hydrogen ion in the sulfonate group and bound to the SO_3^- group. Thus, the sulfonated HIPS will obtain the antimicrobial properties through the attachment of the antimicrobial metallic ions via spontaneous ionic exchange reaction. Figure 3 shows the reaction in the spontaneous ionic exchange between the HIPS and the metal ions.

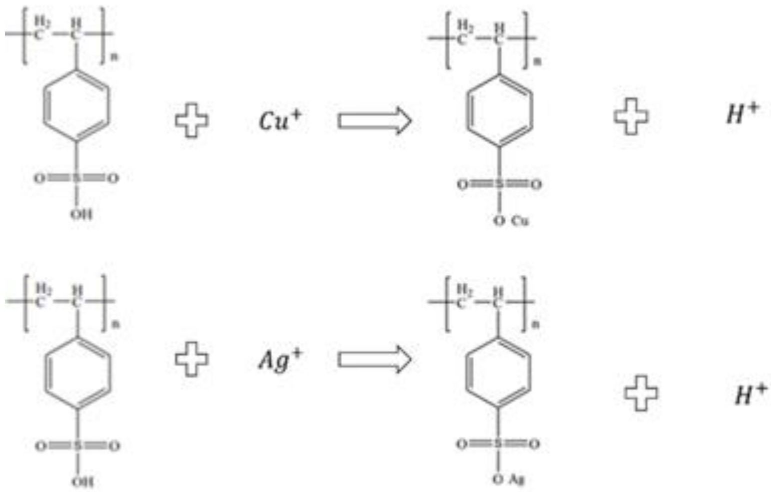


Figure 3. Spontaneous ionic exchange reaction in sulfonated HIPS.

Salt solutions of 0.1 M $CuSO_4$ and 0.1 M $AgNO_3$ were prepared in distilled water. 2 g of sulfonated HIPS was mixed with 100 mL of salt solution. Table 1 summarizes the different ratios of copper and silver salt solutions.

Table 1. Percentage ratios of salt solutions added to each beaker.

Sample	Amount of 0.1 M Copper (II) Sulfate solution (mL)	Amount of 0.1 M Silver Nitrate solution (mL)	Ratio of Copper (II) Sulfate to Silver Nitrate
100 % Copper sample	100	0	1:0
50 % Copper and 50 % Silver sample	50	50	1:1
100 % Silver sample	0	100	0:1

The samples were suspended in the salt solution for 48 hours. The mixtures were then filtered and washed three times with distilled water to obtain the samples loaded with copper and silver ions. After that, the samples were dried in an oven for 24 hours at 70 °C.

3. Results and Discussion

3.1. Sulfonation of High Impact Polystyrene

Figure 4 shows the appearance of the sulfonated HIPS at different sulfonation times of 1 hour, 2 hour, 3 hour and 6 hour.

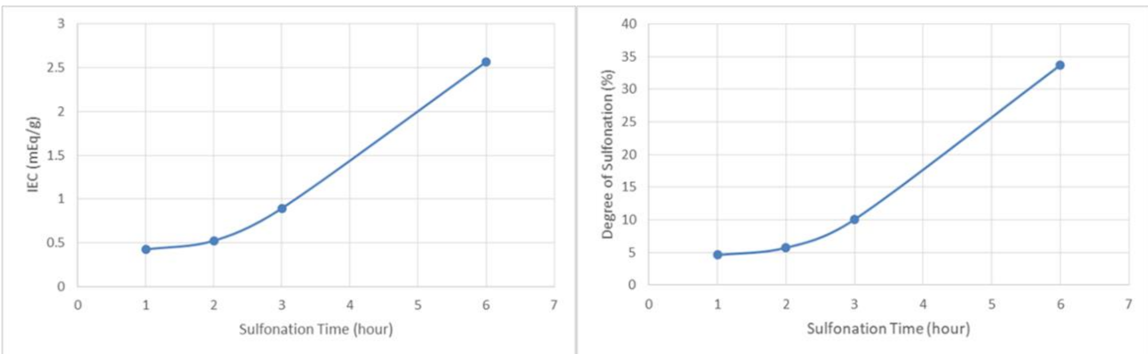
a) b) c) d)

Figure 4. Sulfonated HIPS at a) 1 hour b) 2 hours c) 3 hours d) 6 hours.

The sulfonation reaction is categorized as an aromatic electrophilic reaction substitution reaction which aims to replace the hydrogen atoms in the aromatic ring with sulfonate groups [9]. The presence of these sulfonate groups within the styrene structures allows the polymer to have the ability to bind with cations [8]. During the study, sulfonated HIPS was obtained in the form of a white powder polymer similar to the raw HIPS powder. The 1-hour sample was fine in texture but the 2-hour sample had a more granulated texture. As the sulfonation time increases, the sample produced was more granulated. The granulated appearance of the powder indicates the sulfonation that has occurred [8]. This is due to the fact that the sulfonate groups are hydrophilic and attracts more moisture from the surroundings which results in the granulated appearance. The granulation becomes more obvious as the sulfonation time was increased. Similar results were obtained by Jalal et al. [9]. Thus, the observation obtained was that the reaction was an aromatic electrophilic substitution reaction and the hydrogen atoms in the styrene monomers were replaced by sulfonate groups. Besides that, the longer the sulfonation time, the higher the degree of sulfonation occurred.

3.2. Ionic Exchange Capacity (IEC) and Degree of Sulfonation (DS)

The AAS was used to determine the difference in the sodium ion concentration in the solution before and after the addition of the sulfonated HIPS. The IEC and DS of all samples were calculated using Equations (1) and (2) respectively. Figure 5 shows the graphs plotted for IEC against sulfonation time and DS against sulfonation time.



a) b)

Figure 5. a) Graph of IEC against sulfonation time. b) Graph of DS against sulfonation time.

Based on the results obtained, it can be seen that the IEC and DS of the 6-hour sulfonated HIPS sample is the highest at 2.57 meq/g and 33.7%. This was followed by the 3-hour sample, the 2-hour sample and lastly the 1-hour sample. This shows that a longer sulfonation time resulted in a higher IEC and DS of the sulfonated HIPS. This is so because the longer sulfonation time results in more sulfonate groups attached to the styrene residues of HIPS [9]. Similar studies by Jalal et al. [9] and Ngadiwiyana et al. [8] have also showed results consistent with the one observed in this study.

3.3. Loading of Antimicrobial Agents

There was no change in appearance for samples loaded with different ratios of silver and copper ions. An energy dispersive X-ray (EDX) test will be carried out to determine the amount of copper and silver bound to the sulfonated HIPS. In addition, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy will also be carried out to determine the thermal and physicochemical properties of the samples.

4. Conclusions

To summarize, this project aims to produce a material with antimicrobial properties for the purpose of medical device applications. The material was produced by sulfonating HIPS and co-loading them with copper and silver ions which have been well-known for their antimicrobial properties. The sulfonation of HIPS was done using a one-step sulfonation using concentrated sulfuric acid. Four different sulfonation times were used which were 1 hour, 2 hours, 3 hours and 6 hours. The 6-hour sample produced the highest IEC and DS at 2.57 meq/g and 33.7%, respectively.

In future work, only the 6-hour sample will be analysed using EDX, TGA, DSC and FTIR to determine the thermal and physicochemical properties of the sulfonated HIPS co-loaded with copper and silver ions. The zone of inhibition assay using gram-positive and gram-negative bacteria will also be conducted to determine the antimicrobial efficacy of the sulfonated HIPS co-loaded with two antimicrobial agents. It is expected that the polymer can also be applied to 3D printing applications for manufacturing of medical devices that can benefit from customisation. This will also be investigated in the future.

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