

## **SURVEY ON ANTIBACTERIAL ACTIVITY OF ACRYLATE – STYRENE COPOLYMER CONTAINING QUATERNARY NITROGEN ATOMS**

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Received: 30 December 2016; Accepted for publication: 3 March 2017

### **ABSTRACT**

In this research acrylate – styrene copolymer containing quaternary nitrogen atoms was synthesized by a bulk copolymerization method according to free radical mechanism. Antibacterial activity of this resin was examined by changing parameter values such as: types and amount of the neutralized agents, temperature of the neutralization reaction, etc. The antibacterial properties of the acrylate – styrene copolymer were evaluated via the agar diffusion test. In addition, the molecular structure of the copolymer was performed in Fourier transformation infrared (FTIR) spectroscopy. Besides that, the hydrogels from the acrylate – styrene copolymer were also examined on their antibacterial properties. As results, the copolymer showed the highest antibacterial activities with the ratio of ammonia to acrylic acid about 0.8:1 and the neutralizing temperatures at 60 °C.

*Keywords:* acrylate – styrene copolymer, quaternary nitrogen atoms, antibacterial.

### **1. INTRODUCTION**

The rise of the modern industrial revolution has led to the habitat degradation. The suitable climatic would create an appropriate environment for allowing the bacteria to live and grow that cause the risk of a pandemics, such as cholera, malaria, typhoid and so on. Besides, the evolutionary process of the biota has contributed to the bacteria that are resistant to increasing global threats to human life. Therefore, further study on antimicrobial resistance to protect the health has been focusing worldwide [1].

Besides focusing on studying improvement of antibiotics, research and manufacture products capable of killing bacteria are very necessary [2]. In recent years, researchers have discovered the antimicrobial activity of new bis quaternary ammonium compounds and demonstrated a lot of advantages in using them in the life [3]. Thus, the antimicrobial polymers containing quaternary nitrogen atoms have been studying for applications in the world [4].

In this study, acrylic – styrene copolymers were modified with compounds containing nitrogen such as ammonia, urea for manufacturing resin and evaluating their antibacterial

activity. Besides that, amount of neutralized agents, temperature of neutralization reactions and water – swollen properties of the hydrogel from this resin were also assessed. In addition, FTIR spectroscopy was also used to evaluate the relationship between molecular structure and antibacterial activity of acrylic – styrene copolymers.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Acrylic acid (GRM3108–500ML, India), nutrient agar (M001–500G, India), urea (Vietnam), styrene monomer (Vietnam), benzoyl peroxide (China), ammonia solution 25% (China), potassium peroxydisulfate (China) and distilled water were used for all experiments.

### 2.2. Methods

To reduce the possibility of infections spreading between colonies in the laboratory, the instruments were sterilized before carrying out the experiment. Firstly, 100 g acrylic acid, 5.26 g styrene and 0.1 g benzoyl peroxide were charged into a three – neck flask. A series of polymerizations were performed at 85 °C for 150 minutes with mechanical stirrer blade of teflon. Then, acrylate was made by the neutralization reaction with ammonia solution or urea at various temperatures for 45 °C, 60 °C and 75 °C. The mole ratio of neutralizing agent and acrylic acid were changed from 0.6:1, 0.8:1 to 1:1, respectively. After being neutralized the copolymer was diluted to 100ml with water and stored in a brown bottle. The effects of potassium peroxydisulfate used to create hydrogels were surveyed with 0.2%, 0.5% and 0.8% by resin weight with drying temperature at 85 °C for 3 h.

### 2.3. Analytical methods

#### 2.2.1. Agar diffusion method

The bacteria will be implanted on the surface of nutrient agar in petri dishes to prepare before testing. After the resins are transferred to 1 cm diameter paper wafers, these samples are placed on agar surface. Petri dishes must be stored in a clean and sheltered place for the 24 h before the observation. On the surface of agar, if the antibiotic can deactivate or kill the bacteria, there will appear a colourless area around the wafer. This is called an inhibition zone where the bacteria have not largely grown enough to be visible.

With the measuring results from agar diffusion method at Faculty of Materials Technology in Ho Chi Minh City University of Technology (Vietnam National University–HCMC), the size of inhibition zone varies depending on the antibacterial ability of the antibiotic. Diameter of inhibition zone was calculated using Ultimate Paint software by the formula:  $D_z = (D_p \times D_z')/D_p'$ . Where,  $D_p$  (mm) is the diameter of the petri dish,  $D_p'$  (pixel) is the pixel diameter of the petri dish and  $D_z'$  (pixel) is the average pixel diameter of inhibition zone.

#### 2.2.2. Fourier transformation infrared spectroscopy

The structure of synthesized copolymer matrix was studied by using Fourier transformation infrared spectroscopy (FTIR) at the Institute of Chemical Technology.

### 2.2.3. Swelling and loss method

Hydrogel after being made will be immersed in water for different periods of time to determine the water – swollen ability during the immersed process and the mass loss of this hydrogel after drying at 60 °C by using formula (1):  $\Delta M = (m_t - m_o) / m_o \times 100$  and formula (2):  $Q = (m_o - m_{\text{after dry}}) / m_o \times 100$ , respectively. Where,  $m_o$  (g) is the amount of the original sample,  $m_t$  (g) is total increase amount of sample after t hours and  $m_{\text{after dry}}$  is the amount of the sample which was dried after immersing in water for 1 h.

## 3. RESULTS AND DISCUSSION

### 3.1. Amounts of neutralizing agent

The resin samples were neutralized at 60 °C with the mole ratio of the neutralizing agent to acrylic acid by 1:1. The two types of the neutralizing agents using in this study were ammonia and urea. The petri dish after storing for 24 h was displayed in Figure 1. The result showed that the zone of inhibition was clearly appeared at the sample using ammonia to neutralize.

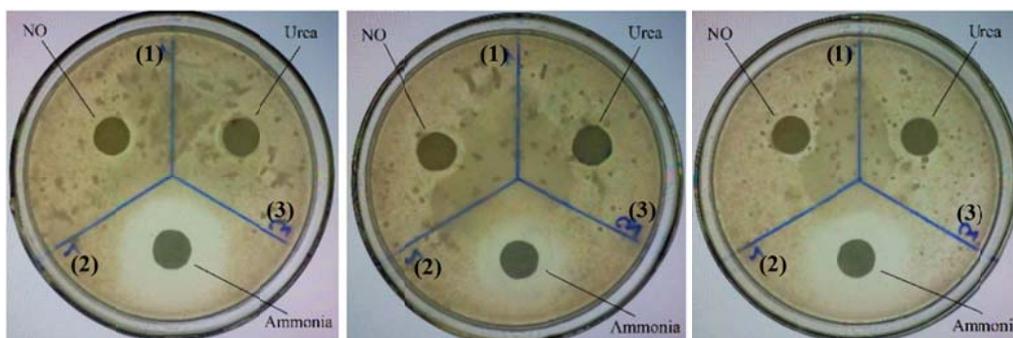


Figure 1. Photos of the three petri dishes of the samples with different neutralizing agents: (1) no neutralizing agent, (2) ammonia neutralizing agent and (3) urea neutralizing agent.

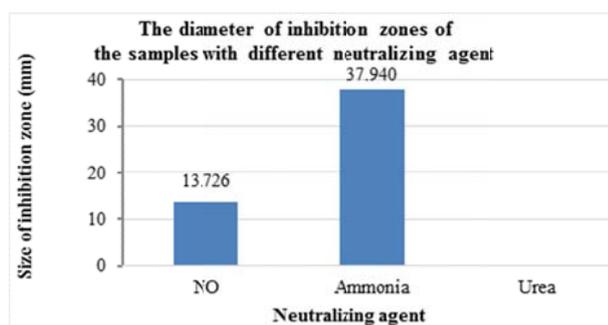


Figure 2. The bar chart displays the diameter of inhibition zones of the samples with different neutralizing agents.

The chart in Figure 2 showed the size of inhibition zone of three samples. The non-neutralizing sample gave a small inhibition zone with the diameter about 13.726 mm. Meanwhile, the sample was neutralized with urea absolutely got no bacterial resistance. In

contrast, the sample was neutralized with ammonia had very good antibacterial activity with the diameter of inhibition zone by approximately 32 mm.

The results showed that the copolymer using ammonia to neutralize gave high antibacterial activity. Because of the quaternary nitrogen atoms with a positive charge, the copolymer could destroy the cell wall of the bacterial when these bacteria exposed to the resin.

### 3.2. Temperature of neutralization reaction

The acrylic acid – styrene copolymer was neutralized by using the mole ratio of the ammonia solution and acrylic acid to 1:1. The temperatures of the neutralization reaction were performed at 45 °C, 60 °C and 75 °C. Antibacterial activity test results were shown in Figure 3.

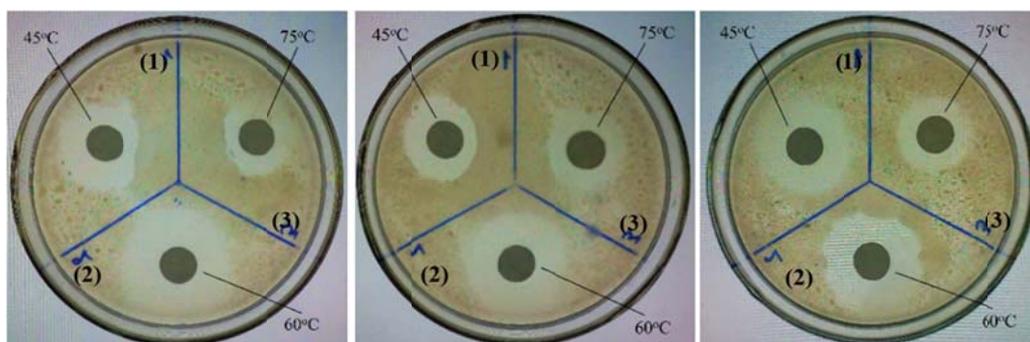


Figure 3. Photos of the three petri dishes of the samples with different temperature: (1) 45 °C, (2) 60 °C and (3) 75 °C.

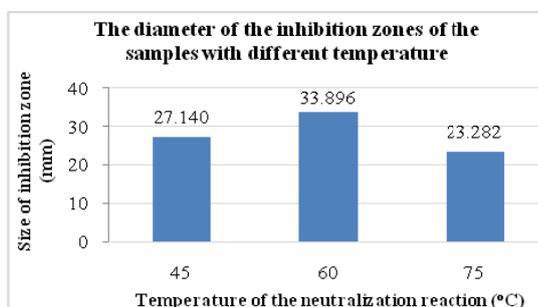


Figure 4. The bar chart displays the diameter of zones of inhibition of the samples with different temperature.

The results in the Figure 4 showed that the resin sample neutralized at 60 °C got the largest inhibition zone with the diameter about 33.896 mm. Two other samples neutralized at 45 °C and 75 °C had smaller zone of inhibition with the diameters of 27.140 mm and 23.282 mm, respectively.

The more acrylate salts were created, the more amounts of water were dissolved and diffused causing the high antibacterial ability. Therefore, not only the low temperature effected to the ability of the neutralized reaction, but also the high temperature reduced the efficiency of neutralization reaction.

### 3.3. Amounts of ammonia

The resin samples were neutralized with ammonia solution at 60 °C. The mole ratio of ammonia to acrylic acid were changed from 1:1 to 0.8:1 and 0.6:1 after storing the test sample in 24 h, the result of antibacterial test was shown in Figure 5.

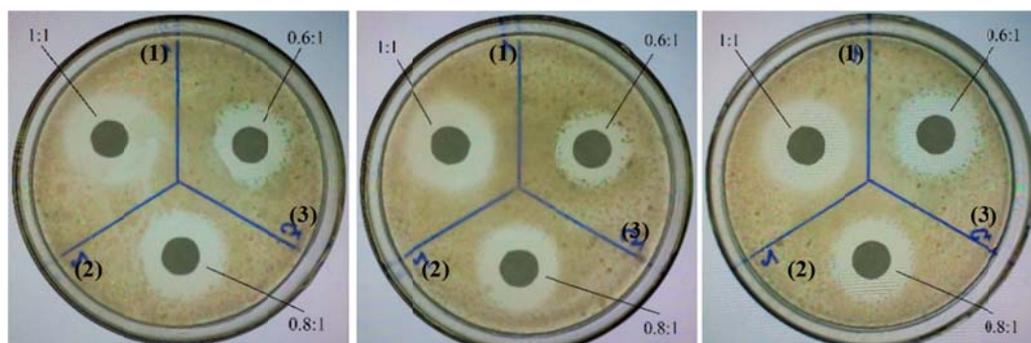


Figure 5. Photos of the three petri dishes of the samples with different amounts of ammonia: (1) 1:1, (2) 0.8:1 and (3) 0.6:1.

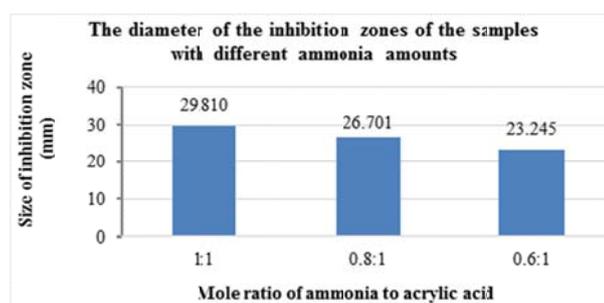


Figure 6. The bar chart displays the diameter of zones of inhibition of the samples with different mole ratio of ammonia to acrylic acid.

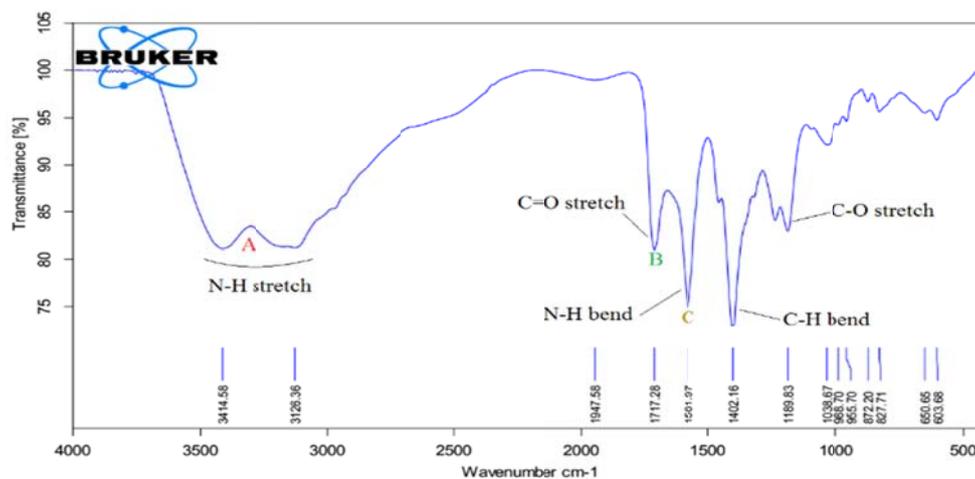


Figure 7. FTIR spectra of 0.8:1 sample.

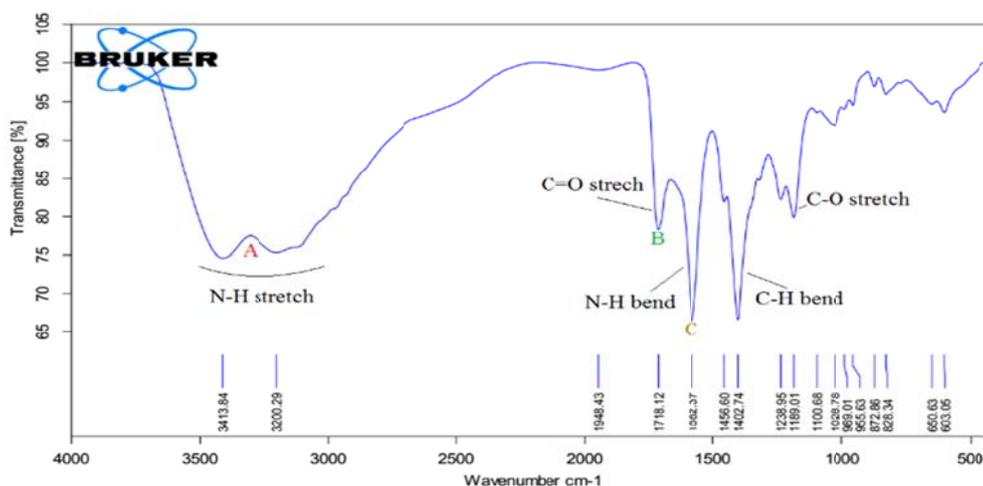


Figure 8. FTIR spectra of 1:1 sample.

The sample with the 1:1 mole ratio of ammonia to acrylic acid showed the highest antibacterial activity with the diameter of inhibition zone of about 29.810 mm and the surface of this zone was very clearly that shown in Figure 6. The size of the inhibition zone decreased from 26.701 mm to 23.245 mm when mole ratios of ammonia to acrylic acid were reduced from 0.8:1 to 0.6:1, respectively.

In Figure 7 and 8, the FTIR results of the 0.8:1 and 1:1 mole ratios of ammonia to acrylic acid showed that there was a little difference between the two samples: both the peaks at about 1,717 cm<sup>-1</sup> of C=O stretch (peak B) and the peaks at 1,582 cm<sup>-1</sup> of N-H bend (peak C) were high and the mole ratio of value of peak N-H stretch (peak A) to peak B were equal in two samples. It proved that the amount of salt acrylate in two samples were little difference.

### 3.4. Cross-linking agent concentration

The resin sample that was neutralized with ammonia solution at 60 °C with the mole ratio ammonia to acrylic acid about 0.8:1 was synthesized to create hydrogels with three different amount of potassium peroxydisulfate (0.2 %; 0.5 % and 0.8 %). After the process of soaking the hydrogel in water, the results related to the water – swollen properties were shown in Figure 9.

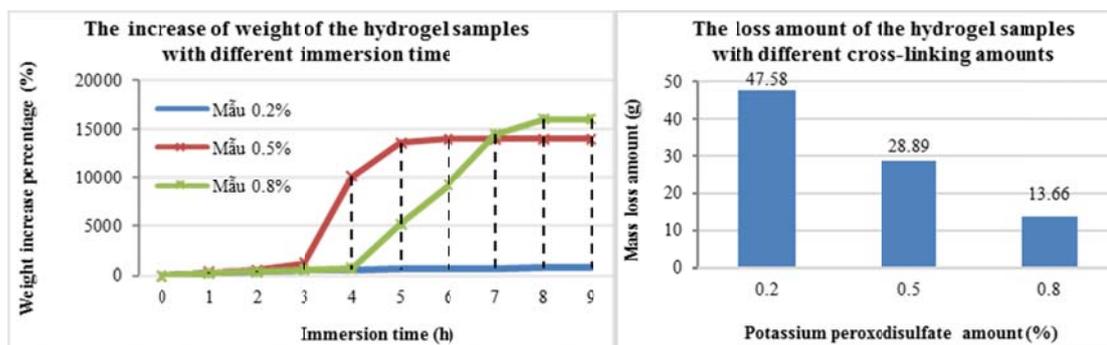


Figure 9. The charts display the increase of hydrogel weight (left) and the loss amount of hydrogel (right).

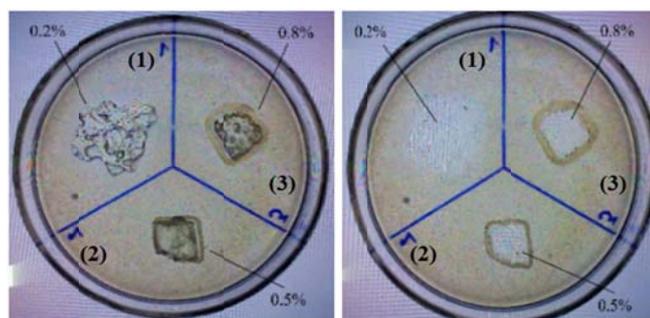


Figure 10. Photos of the petri dishes of the zones of inhibition before (left) and after (right) remove samples with different amounts of potassium peroxodisulfate: (1) 0.2 %, (2) 0.5 % and (3) 0.8 %.

When the potassium peroxodisulfate concentrations were run up, the cross-link were increased led to the high rate of cross-linking in the hydrogel. Therefore, the water – swollen properties of hydrogels will be improved. If the potassium peroxodisulfate concentrations were not guaranteed, the hydrogels with a little of cross-linking would cause of the mass loss [5].

Besides that, the antibacterial activity of the hydrogel was also surveyed with the results were showed in Figure 10. All three samples of hydrogel have just given the high antibacterial properties at the contact surface of the hydrogels and agar. Because of the cross-linking structure of the resin preventing the diffusion process, the inhibition zones have just appeared in the surface contacts of the hydrogels and the agar.

#### 4. CONCLUSIONS

The experimental results have demonstrated that the antibacterial activity of the acrylate – styrene copolymer containing quaternary nitrogen atoms has affected by the type of neutralization chemicals. The resin has reached the best antibacterial ability in the case of using the ammonia solution for neutralized reaction at 60 °C with the mole ratio of ammonia to acrylic acid about 0.8:1. Besides that, the research has also indicated that the hydrogel created from this resin not only has gotten the high water – swollen properties and the less mass loss, but also it has remained the antibacterial activity.

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