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PREPARATION OF POLYSTYRENE BEADS CONTAINING SULFONAMIDE GROUPS AND THEIR APPLICATION TO POLYMERIC BIOCIDES

Cheoljin Kim, Jee Yeon Kim, Jang-Woong Byun, Jaeeun Kim, Yoon-Sik Lee and Jeyong Yoon†

School of Chemical & biological Eng., Department of Eng., Seoul National University, San 56-1, Sillim-dong, Gwanak-gu, Seoul, 151-742 Korea

Abstract : A novel series of polystyrene (PS) beads containing various sulfonamide groups was prepared, and their chemical stabilities in an aqueous solution were tested in order to determine their ability to inactivate microbes. By reacting aminomethyl polystyrene (AM PS) beads or carboxy polystyrene beads with various benzenesulfonic acid derivatives, the sulfonamide groups were introduced on the PS beads. The characteristics of the product beads were analyzed by elementary analysis after the substitution of various sulfonamide groups. Energy Dispersive Spectroscopy (EDS), and FT-IR analysis were used to analyze the elemental functional group composition, respectively. The hydrolytic stabilities of the PS beads containing various sulfonamide groups along with the relationship between the swelling ratio and their hydrophilicity were investigated. The antibacterial activity of the beads was determined by their ability to inactivate *E. coli*. This study reports that PS beads containing sulfonamide groups had lasting antibacterial efficacy over a satisfactory period, whilst maintaining their chemical stabilities against hydrolysis. The 8 synthesized polymer beads exhibited antibacterial ability.

Key Words : Sulfonamide, Polystyrene bead, Antibacterial activity, Hydrolytic stability

INTRODUCTION

Infection causes significant morbidity and considerable research effort has been directed at solving this problem. One of the best methods to solve this problem is to control the activities of the microorganisms through polymeric biocides. An area of polymer research that is of great current interest and importance, yet one that has received insufficient attention, at least in the published literature, is that of the development of polymeric biocides. However, biocidal polymers have numerous potential applications. Most of the biocidal polymers have been developed till now are usually imparted by the addition of a low molecular weight biocide that inhibits the growth of the microorganisms by slowly diffusing out of the polymer.[1] In some cases, a better control of the rate of leaching may be obtained by linking the toxic group to the matrix through a chemical bond sensitive to hydrolysis. However, the drawbacks are the same in both cases: a loss of activity with time and environmental problems due to the high toxicity of the liberated compounds. Furthermore, this is not a satisfactory solution if a permanent protection of a polymer surface against microorganisms is the goal.[1]

In this study, we focused in the continuous maintenance of antibacterial efficacy. Therefore, we developed novel polymeric biocides containing

† Corresponding author
E-Mail: jeyong@snu.ac.kr
Tel: +82-2-880-8927, Fax: +82-2-876-8911;

sulfonamide groups connected by a covalent bond semi-permanently, which exhibit antibacterial activity. Therefore, antibacterial polymers containing sulfonamide moiety were synthesized through the substitution reactions of eight compounds to polystyrene beads.

It should be noted that the sulfonamides are synthetic bacteriostatic antimicrobials with a wide spectrum against most gram-positive and many gram-negative organisms. Sulfonamides competitively inhibit the incorporation of paraaminobenzoic acid (PABA) into tetrahydropteroic acid resulting in decreased folic acid synthesis of microorganisms.[2] As a result of this study, these materials are not hydrolyzed and have lasting antibacterial ability. Therefore, it can be said that polymer beads themselves have antibacterial ability.

Functionalized polystyrene (PS) beads with sulfonamide groups were prepared by reacting them with various benzenesulfonic acid derivatives. Their antibacterial activity was examined using an *E. coli* inactivation test. In addition, the effect of the polarity of the sulfonamide -loaded PS beads and pH on the antibacterial activity was investigated by examining the hydrolytic stability of the sulfonamide groups on the PS beads.[3]

MATERIALS & METHODS

Materials and Instruments

The aminomethyl polystyrene beads (AM PS, 100-200 mesh, 1.0 mmol/g) were cross-linked particles with divinylbenzene (1%), and were purchased from the Aldrich Chemical Company. The carboxypolystyrene beads (100-200 mesh, 4.5 mmol/g) were also particles cross-linked with divinylbenzene (1%), and were purchased from Beadtech Inc. (Korea). The reaction chemicals, such as oxalyl chloride, benzenesulfonic acid, sufanilic acid, *p*-toluenesulfonic acid, *p* -butylbenzenesulfonic acid, 4-hydroxybenzene sulfonic acid, 2-sulfobenzoic acid, 5-sulfosalicylic acid, sulfadiazine, and silver(I)- sulfadiazine, were purchased from the Aldrich Chemical Company and used without further purification. Dimethylacetamide, which was used as the reaction solvent was purchased from the Sigma Chemical Company.

The elemental analysis of the beads was performed using an elemental analyzer, EA1110 (CE Instrument, Italy). For the spectral measurement of the functionalized beads containing silver(I)- sulfadiazine, energy dispersive spectroscopy (EDS) was carried out on a Super Probe JXA-8900R (JEOL) under the following conditions: The acceleration potential was 15 kV, the beam current was 2 mA, and the beam size was 1 mm. FT-IR analysis was performed using a DA 8 spectrometer (Bomem) with KBr pellets.

Preparation of PS beads containing sulfonamide groups

General procedure for the coupling reaction of the benzenesulfonic acid derivatives to aminomethyl polystyrene beads (Resins 1-6 in Figure 1) was as follows:

In order to activate the sulfonate groups, benzenesulfonic acid (2 mmol, 279.4 mg) and oxalic acid (2 mmol, 253.9 mg) were mixed with dimethylacetamide (20 mL) in a filtered reactor (Libra tube RT-20M, Beadtech Inc., Korea), and heated to 60℃ for 24 h. After the activation step, the aminomethyl polystyrene beads (2.0 g, 1.0 mmol/g) were added to the above mixture, and reacted at 60℃ for 24 h. The product beads were washed vigorously with THF (20 mL) and MeOH (20 mL), and dried to give yellow-colored beads (Resin 2).[4] In case of Resin **1**, p-acetamido benzenesulfonyl chloride was reacted to the aminomethyl polystyrene beads (2.0 g, 1.0 mmol/g), and then the synthesized resin was hydrolyzed in 10% NaOH solution.

A general procedure for the coupling reaction of sulfadiazine derivatives to carboxypolystyrene beads (Resins 7-8 in Figure 2) was as follows:

The carboxyl groups were activated by mixing oxalic acid (9 mmol, 1.14 g) and the carboxy -polystyrene beads (2.0 g, 4.5 mmol/g) with

dimethylacetamide (20 mL) in a filtered reactor (Libra tube RT-20M, Beadtech Inc., Korea), and heated the mixture to 60℃ for 24 h. After the activation step, the beads were filtered and washed with THF (20 mL). Silver-sulfadiazine (9 mmol, 3.21 g) and dimethylacetamide (20 mL) were added to the above mixture, and reacted at 60℃ for 24 hr. The product beads were washed vigorously with THF (20 mL) and MeOH (20 mL), and dried to give white-colored beads (Resin **8**).

Determination of hydrolytic stabilities of sulfonamide groups on PS beads

The hydrolytic stability of the sulfonamide groups on the PS beads was determined using UV-VIS spectroscopy and HPLC. A microtube containing the beads in water was vigorously shaken for 5 days in tumbler (SI-600R, Korea) 25℃. The solution sample was analyzed using a UV-VIS spectrophotometer (Gilson UV/vis -151, USA) at 259 nm for sulfonamide. A solution sample was taken at different time intervals, and the UV-VIS adsorption spectra was determined. The stability of the beads was determined by comparing the result with the spectra of a sulfanilic acid solution (0.1 M).

In addition, the hydrolyzed solution was analyzed by a reverse-phase HPLC on an XTerra RP-18 column (150 mm × 2.1 mm, 5 ㎛, Waters Co., USA) using UV detection (Gilson UV/VIS-151, USA) at a wavelength of 259 nm for sulfonamide. An acetonitrile/water (50%/50%/0.05 M) mixture was used as the mobile phase.[5)]

Determination of the swelling properties of the PS beads containing sulfonamide groups

The swelling volumes of the sulfonamide -loaded PS beads in various solvents (water, methanol, THF, CH_2Cl_2) were measured in a fritted column (ID 0.8 cm, length 20 cm). The beads (1.0 g) was swollen in a solvent at room temperature for 30 min, and washed with a 10-fold volume of each solvent. The volume of the beads was measured after filtering out the solvent.

Biocidal Assessment

E. coli (ATCC strain 8739) was inoculated on 50 mL of a Tryptic Soy Broth medium in a 200 mL flask and was grown at 37℃ for 18 hr. The bacteria were harvested by centrifugation at 1000 × g for 10 min and washed twice with 50 mL phosphate buffered saline (PBS, pH 7.2). The *E. coli* stock solution was prepared by re-suspending the final pellets in 50 mL of a phosphate buffered saline solution. The initial populations of the microorganism were approximately 1 × 106 CFU/mL. The number of cells was determined using the spread plate method, which involved plating the *E. coli* cells on nutrient agar incubated at 37°C for 24 hr and counting the number of viable colonies. 0.1 mL of the solution was withdrawn at each sampling time and diluted 1/1, 1/10 and 1/100. Finally, 0.1ml of each diluted and undiluted samples were spread on a plate and the number of *E. coli* was counted. Triplicate plates were used at each diluted solution and showed reproducibility was shown with a 10% standard deviation.[6)]

The *E. coli*, which a representative indicator microorganism of vegetative bacteria, was selected to evaluate the biocidal effect of synthesized polystyrene beads containing sulfonamide. The biocidal activity of synthesized polystyrene beads was tested at various pHs (5.6, 7.1 and 8.2) using a phosphate buffer. Potassium dihydrogenphosphate (KH_2PO_4) was used to set the pH (pH 5.6, 7.1, and 8.2) of the buffer. First, the stock solution was prepared by adding 0.2 mL of a buffer solution to a 15mL Falcon tube containing 9.7 mL of distilled water and 0.1 mL of a *E. coli* stock solution adjusted to an initial concentration to 1 × 106 CFU/mL.

In addition, one milliliter of the prepared stock solution was added to a 1.6 mL ependorf tube containing 200 mg of the synthesized polystyrene beads. The contents were mixed thoroughly using a rotator. After centrifuging for two seconds to settle the beads, 0.1 mL of

the supernatant was withdrawn from the ependorf tube containing 0.9 ml of PBS at each sampling point.

RESULTS AND DISCUSSION

Preparation and characterization of PS beads containing sulfonamide groups

Eight types of PS beads containing the sulfonamide derivatives (Resins **1-8**) were prepared in a two-step synthesis using functionalized polystyrene beads, such as AM PS and carboxy PS beads. Figures 1 and 2 show details of the synthetic procedures.

Table 1 summarizes the conversion details of the functionalized product beads, which attempted to introduce the sulfonamide groups. In the case of resins **1-6**, six types of benzenesulfonyl chloride, which were prepared in advance by a reaction between oxalic chloride and the benzensulfonic acid derivatives, were reacted with the AM PS beads. The final loadings of the sulfonamide groups on the PS beads, which ranged 0.5-0.6 mmol/g, were determined by measuring the sulfur content using elementary analysis. For resins **1-6**, which used AM PS beads, the conversion of sulfonamide-loaded PS beads ranged from 53% - 61%.

Table 1. Preparation of the modified PS resin

Entries	Theoretical loading of sulfonamide derivatives on resin (mmol/g)	Final loading of sulfonamide derivatives on resin (mmol/g)[a]	Conversion (%)[b]
Resin 1	1.0	0.60	59.6
Resin 2	1.0	0.61	61.4
Resin 3	1.0	0.61	61.0
Resin 4	1.0	0.60	60.1
Resin 5	1.0	0.56	56.1
Resin 6	1.0	0.53	52.7
Resin 7	4.5	0.36	7.9
Resin 8	4.5	0.26	5.8

[a] The loading of sulfonamide was determined by means of the sulfur content obtained from elementary analysis.
[b] It was compared with theoretical conversion.

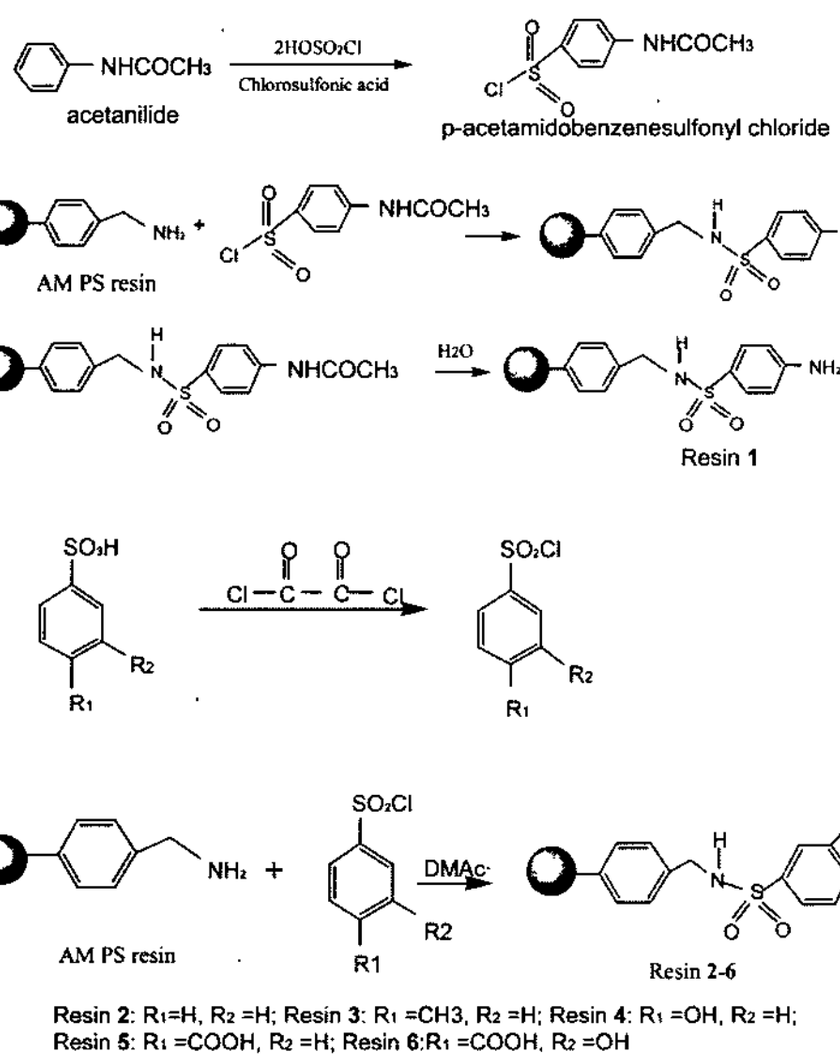

Figure 1. Reaction scheme for preparing the sulfonamide groups on the PS beads based on AM PS beads. (Resins **1-6**)

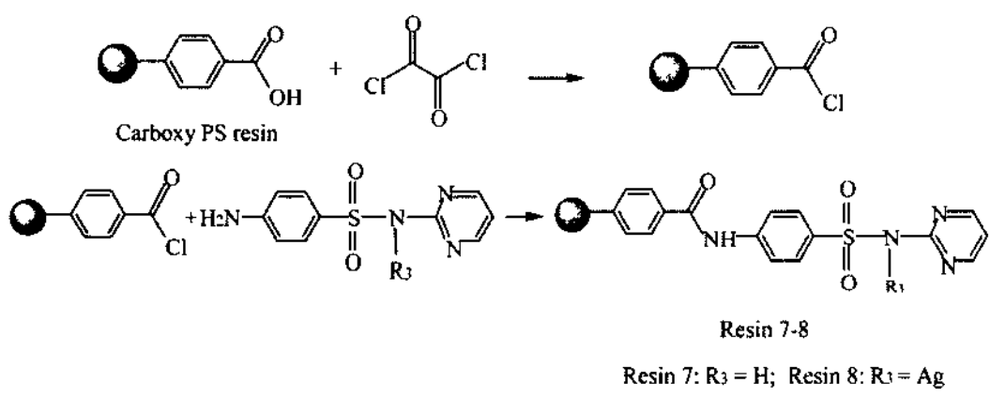

Figure 2. Reaction scheme for preparing the sulfonamide groups on the PS beads based on carboxy PS beads. (Resins **7-8**)

In the case of resins **7-8**, the carboxy PS beads were converted to benzoyl chloride PS beads in advance using oxalic chloride followed by reacting them with two types of sulfazine. Although the initial loading of the carboxy PS beads was high (4.5 mmol/g), the final loading of sulfazine groups showed approximately 0.3 mmol/g, which showed that there was low conversion, < 10%. The low substitution ratio of sulfadiazine into the carboxy PS beads was interpreted as being caused by the low reactivity of aromatic amines in sulfadiazine derivatives. Amines are generally electron donors, but the amine group in aniline gives the benzene ring unshared electrons.

The loading of sulfonamide groups on the PS beads was confirmed by EDS and FT-IR. Figure 3 shows the EDS spectrum of resin **8** showing the presence of the sulfur and silver (2.04-2.73 KeV, 2.34-4.04 KeV) (Figure 3).

The FT-IR spectrum of resin 1 showed typical

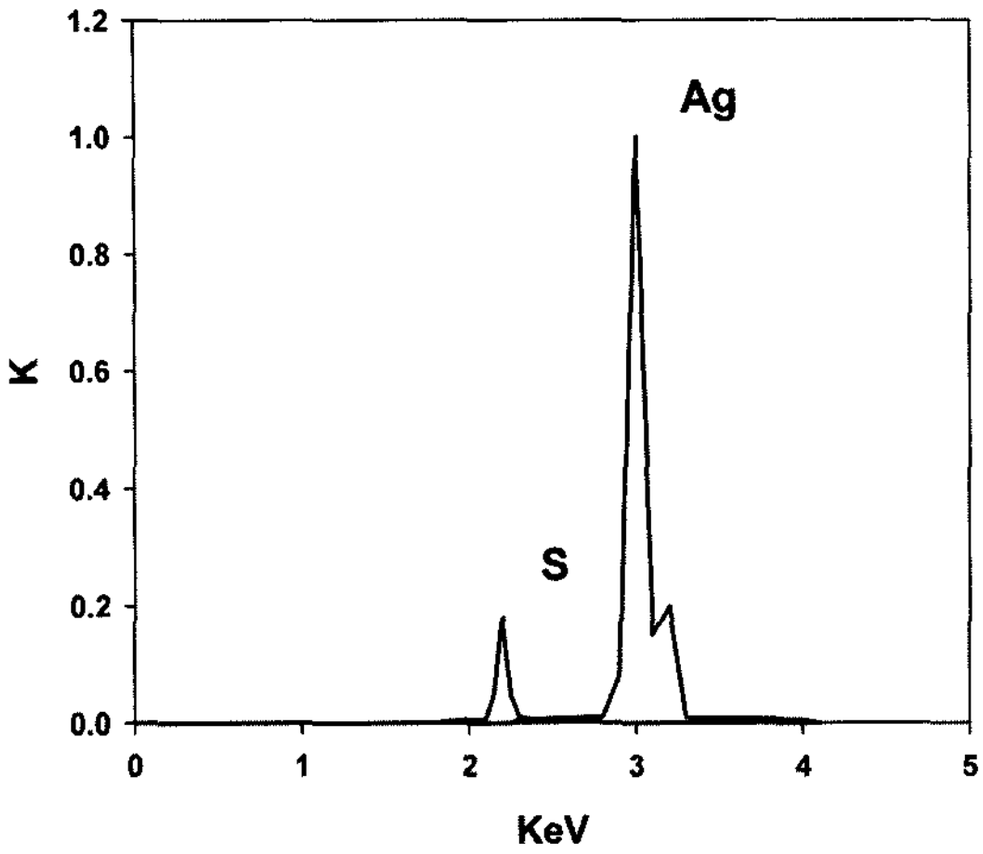

Figure 3. EDS spectra of Resin **8**.

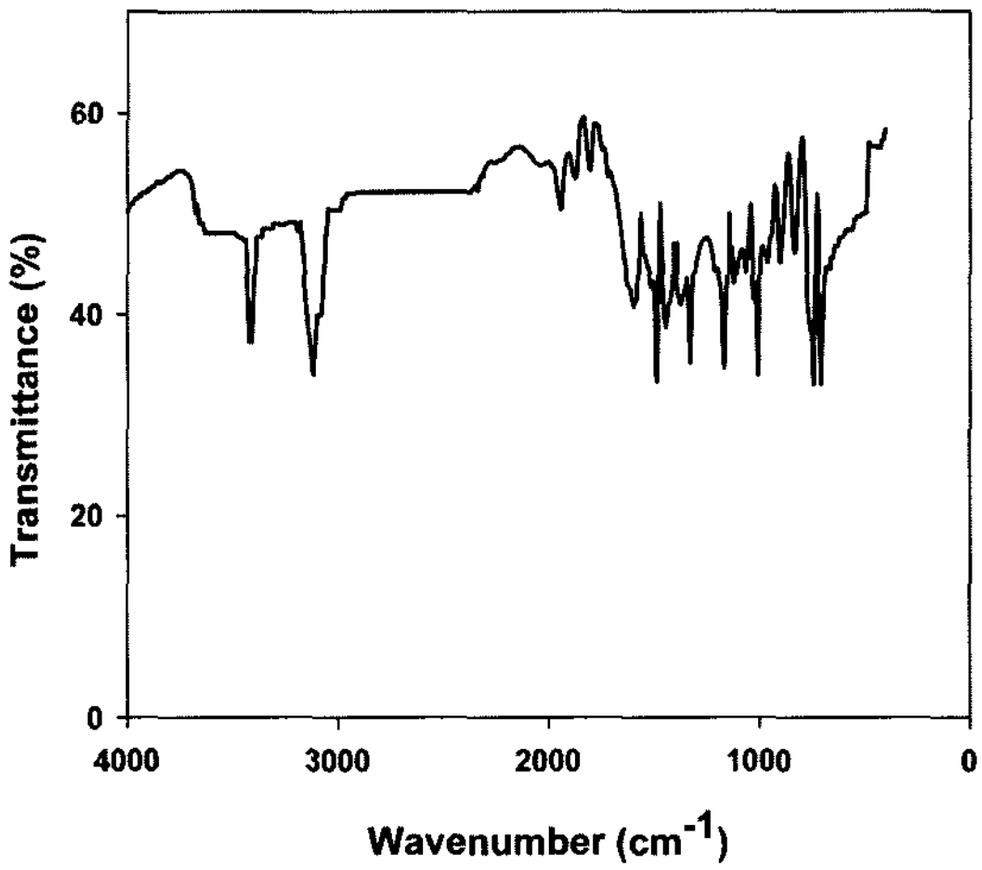

Figure 4. FT-IR spectrum of the sulfonamide containing PS beads. (Resin 1)

absorbance bands that can be attributed to the sulfonamide groups. After the substitution reaction, there were two SO_2 peaks at 1165 cm^{-1} and 1330 cm^{-1}, which were assigned to the symmetric and asymmetric vibrations, respectively (Figure 4).

Chemical and physical properties of PS beads containing sulfonamide groups

The hydrolytic stability of the sulfonamide groups on the PS beads is an important factor for the continuous biocidal activity of sulfonamide loaded PS beads in the form of the polymeric biocides. In order to determine their hydrolytic stability, resin **1** was suspended in an aqueous solution (pH 7.0) at 25℃ for 5 days. The solution was then examined by UV-VIS and HPLC analysis to determine the concentration of hydrolyzed species. There was no compelling evidence for the presence of hydrolyzed species from resin **1** compared with the authentic sample of sulfanilic acid, which showed UV absorption at 259 nm. HPLC analysis also showed similar results, and the peak patterns of the solution samples barely changed by the time interval. This suggests that the sulfonamide groups of the product beads (resin **1-8**) were stable in the aqueous solutions under ambient conditions.

The swelling properties of the product beads (resin **1-6**) were measured in various solvents ((water, methanol, THF, CH_2Cl_2) and compared with that of the AM PS beads (Table 2).

Table 2. Swelling volume(mL/g resin)

Resin	Water	Methanol	THF	CH_2Cl_2
AM PS resin	3.2	3.9	11.8	14
Resin 1	4.4	8.0	8.8	6.4
Resin 2	4.0	4.7	8.6	7.4
Resin 3	4.0	4.8	8.8	7.2
Resin 4	4.0	8.0	6.8	6.0
Resin 5	3.6	6.0	7.6	6.4
Resin 6	4.0	8.2	6.8	5.8

No significant swelling properties were observed in the polar solvents, even though the AM PS resins showed good swelling properties in the nonpolar solvents such as DCM and THF, and MeOH. On the other hand, the product beads showed relatively good swelling properties in the polar solvents due to the hydrophilicity of sulfonamide derivatives, and provided a lower swelling volume in the nonpolar solvents. Generally, conventional PS beads have critical obstacles to overcome for aqueous phase applications on account of their poor water-compatibility and strong hydrophobicity. However, these results suggest that the polarity of the PS beads increased, and their compatibility with water improved after introducing the sulfonamide groups. Therefore, the PS beads containing sulfonamide groups can be used in aqueous solutions.

Microbial inactivation test

Figure 5 shows the inactivation *E. coli* in the solution containing eight individual sulfonamide loaded beads at a neutral pH. The microbial inactivation efficacy of the beads containing amine, hydroxyl, and carboxylic groups (resins **1, 4, 5**) was much higher, showing 1.97-log, 2.02-log, and 1.97-log inactivation respectively for 180 min, respectively. On the other hand, 1.67-log, 0.97-log, and 1.07-log inactivation was observed for 180 min in the case of resins **2, 3,** and **6**, respectively, which are less hydrophilic than resins **1, 4,** and **5**. This suggests that the sulfonamide loaded polymer, which has a better hydrophilicity, had a better antibacterial effect. This is because the hydrophobicity of the polymer backbone increases the probability of contact between the biocidal polymer and the microorganism. On the other hand, the inactivation efficacy for *E. coli* of resins **7** and **8**

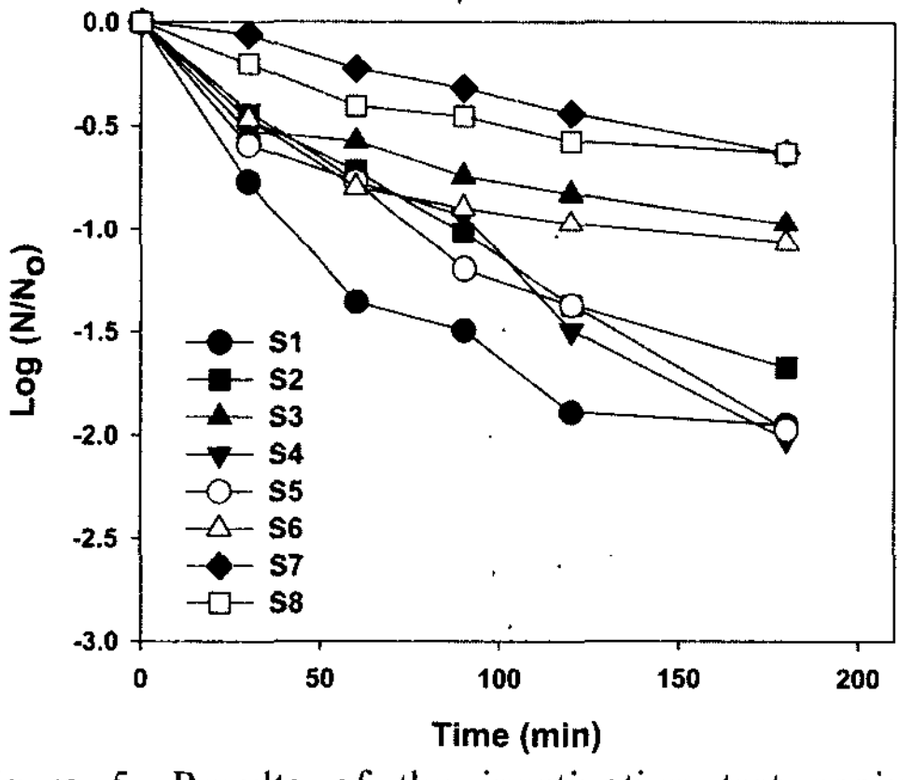

Figure 5. Results of the inactivation test against E. coli using sulfonamide containing the PS beads at 25℃, pH 7.1.(Resins **1-8**)

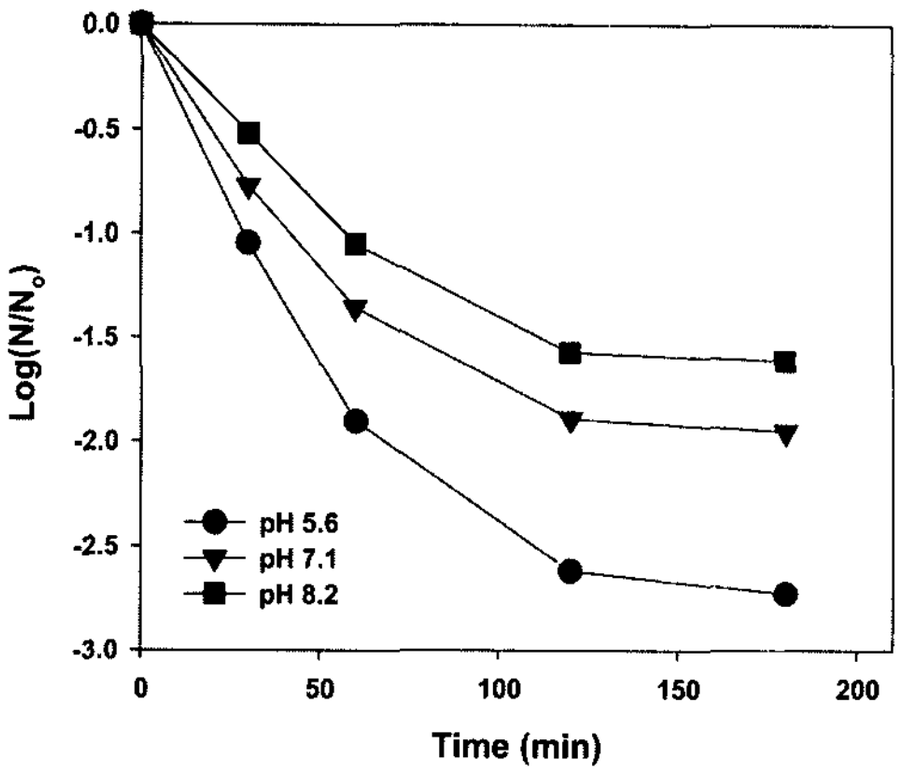

Figure 6. Results of the inactivation test against microorganisms under different using Resin **1**.

was smaller since the substitution ratio of sulfadiazine was only a quarter of the other samples. As shown in Figure 5, 0.63-log, and 0.63-log inactivation were observed for 180 min at 25 ℃ for resins 7 and 8, respectively.

Figure 6 shows that the inactivation efficacy for *E. coli* in the solution containing resins **1** increased with decreasing pH at 25℃. After 180 min, 2.73-log, 1.95-log, and 1.61-log inactivation were observed at pH 5.6, 7.1, and 8.5, respectively. Whereas the inactivation efficacy in the solution containing resin **3** did not change with pH at 25℃. The reason for this was that the amine, which was the functional group of the resin **1** bead, changed to the cationic ammonium as the pH changed. This cationic group (anilinium ion) attracts *E. coli*. Therefore, the antibacterial efficacy of this sulfonamide was increased. Structural changes in the sulfonamide containing amine functional group as a result of

Table 3. Summary of study results

Resins	Substituted functional group	Final loading (mmol/g)	Polarity	Hydrolysis	Structural change by pH
Resin 1	Sulfanilic acid	0.60	○	×	○
Resin 2	Benzenesulfonic acid	0.61	△	×	×
Resin 3	P-toluenesulfonic acid	0.61	△	×	×
Resin 4	Hydroxybenzene sulfonic acid	0.60	◎	×	○
Resin 5	2- sulfobenzoic acid	0.56	○	×	○
Resin 6	5- sulfosalicylic acid	0.53	◎	×	○
Resin 7	Sulfadiazine	0.36	△	×	×
Resin 8	Silver-sulfadiazine	0.26	△	×	×

pH increased the attractive power of the polymer to *E. coli.* However, the molecular structure of the sulfonamide containing a methyl functional group was not changed by pH. Therefore, the antibacterial efficacy of sulfonamide containing methyl functional group was unaffectd by pH. The positively charged beads would be much more effective in inactivating *E. coli* on account of the attract electrostatic interactions with the cell wall. This study found that the structure of the bead was affected by pH. The bead species, which are more effective in a low pH environment might be changed to less effective ones in the neutral conditions although substantial evidence has not been provided. It is possible that pH affects the inactivation efficacy of the bead, which contains some functional groups.

CONCLUSION

Elemental analysis data showed that all the beads were substituted. All the samples showed antimicrobial efficacy against *E. coli.* It was found that the polymer beads, which were substituted with the sulfonamide moiety, had antimicrobial ability, which was similar to the sulfonamide monomer. It was also found that the functional groups in the benzene ring of sulfonamide played an important role in the antimicrobial functions. The microbial inactivation efficacy of the beads containing the amine, hydroxyl, and carboxylic group was much larger (Fig. 5). These beads had a high water-compatibility.

It was also found that structural changes in the functional group on the benzene ring of the sulfonamide altered the antibacterial ability. In view of the results achieved thus far, it is believed that the increase in the water-compatibility or the structural change in the molecule can enhance the antibacterial efficacy. In addition, HPLC and UV adsorption showed that these samples were not hydrolyzed. Therefore, the substituted polymer beads were stable in water and exhibited antibacterial activity.

If this method is applied to other polymers, the use of sulfonamide as a biocide is anticipated in a variety of applications, where an insoluble material would be beneficial. In addition, these antibacterial beads can be used to control bacteria inside the instrument containing water. It is believed that this represents a major innovation in antibacterial technology. Work is continuing with the aim of examining the potential uses of the new antibacterial polymer agents.

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