Preparation of Resins Containing Phenol Derivatives from Chloromethylstyrene–Tetraethyleneglycol Dimethacrylate Copolymer Beads and Antibacterial Activity of Resins

TAKAMASA NONAKA, YASUKO UEMURA, KATSUTO OHSE, KOHKI JYONO, SEIJI KURIHARA

Department of Applied Chemistry and Biochemistry, Kumamoto University, 2-39-1 Kurokami, Kumamoto-shi 860, Japan

Received 1 November 1996; accepted 9 February 1997

ABSTRACT: Copolymer beads (RCCS-4G) with many chloromethyl groups were prepared by treating macroreticular chloromethylstyrene–tetraethyleneglycol dimethacrylate (4G) copolymer beads with chloromethylether. Copolymer beads (RAAS-4G) with benzylamino groups were prepared by treating RCCS-4G with potassium phthalimide. Then the copolymer beads containing phenol derivatives were prepared by treating RAAS-4G with *p*-hydroxybenzoic acid (*p*HBA), 2,4-dihydroxybenzoic acid (DHBA), and 3,4,5-trihydroxybenzoic acid (gallic acid, GA) in *N*,*N*-dimethylformamide. The antibacterial activity of the obtained resins was examined against *Escherichia coli* and *Staphylococcus aureus.* Resins containing phenolic hydroxy groups of 2.3–7.7 mequiv/g were obtained. Antibacterial activity of the resins containing various phenol derivatives against *E. coli* or *S. aureus* increased in the order of RAAS-4G-GA > RAAS-4G-DHBA > RAAS-4G-pHBA. The resins containing phenol derivatives exhibited higher antibacterial activity against *E. coli* than against *S. aureus* and high activity even against bacteria in NaCl solution. Scanning electron micrographs showed that high antibacterial activity was brought about by the phenolic hydroxyl groups in the resin. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **66:** 1621–1630, 1997

Key words: antibacterial activity; chloromethylstyrene–tetraethyleneglycol; dimethacrylate copolymer beads; resins; phenol derivatives; *Escherichia coli; Staphylococcus aureus*

INTRODUCTION

Chlorine or water-soluble disinfectants are used for sterilizing water. However, soluble disinfectants have the problem of residual toxicity of the agents, even when suitable amounts of the agents are used.

To prevent such a residual toxicity of the agents, recently insolubilized agents with antibacterial activity were developed.[1–7] Insolubilized agents with quaternary ammonium groups or phosphonium groups were reported to have high antibacterial activity.[8–10] On the other hand, Kawada et al. showed that hydroxybenzylalcohol immobilized on porous glass beads using silane coupling agents also had antibacterial activity.[11] However, the effect of the structure on the antibacterial activity was not discussed in detail.

We can make resins containing phenol derivatives by the reaction between phenol derivatives with carboxyl groups and macroreticular copolymer beads with benzylamino groups. In this article we describe in detail the preparation of the resins containing phenol derivatives and their antibacterial activity against bacteria such as *Escherichia coli* or *Staphylococcus aureus.*

Correspondence to: T. Nonaka (nonaka@gpo.kumamoto-u.ac.jp)

Journal of Applied Polymer Science, Vol. 66, 1621–1630 (1997)
 CCC 0021-8995/97/081621-10

EXPERIMENTAL

Materials

4-Chloromethylstyrene was kindly provided by Seimi Chemical Co. and was purified by elimination of inhibitors by treating with sodium hydroxide solution and distillation under reduced pressure (82–83°C/7 mmHg). Tetraethyleneglycol dimethacrylate was kindly provided by Nihon Yusi Co. and was purified by elimination of inhibitors by treating with sodium hydroxide solution and drying over anhydrous sodium sulfate. *N*,*N*-Dimethylformamide (DMF) was purified by distillation under reduced pressure (50°C/12 mmHg) after drying it overnight over a molecular sieve (Wako Chemical Co.).

α,α'-Azobisisobutylonitrile was purified by recrystallization with ethanol. Other chemical compounds were of reagent grade and were used as received.

Preparation of Macroreticular Copolymer Beads

Macroreticular 4-chloromethylstyrene–teteraethyleneglycol dimethacrylate (4G) copolymer beads (RCS-4G) were synthesized by suspension copolymerization in the presence of cyclohexane as a diluent. A solution of 4-chloromethylstyrene (48.5 cm^3) and 4G (11.5 cm^3), cyclohexane (120 vol %/monomer), and azobisisobutylonitrile (0.870) as an initiator were combined in an autoclave with a solution of 0.2% (w/v) hydroxyethyl cellulose in 500 mL of water. The mixture was stirred until the monomers were dispersed as fine droplets. The suspension was heated to 70°C. Polymerization was carried out at 70°C for 4.5 h under stirring at 416 rpm. After polymerization, the product was filtered off and washed with hot water (80°C) and soaked in methanol overnight. After drying, copolymer beads with the desired diameter (32–60 mesh) were selected.

Chloromethylation of RCS-4G

RCS-4G (5 g) was chloromethylated with chloromethylmethylether (31.4 mL) at 0–5°C for 2 h in the presence of 1,1,2,2-tetrachloroethane (3.1 mL) as a swelling agent and aluminum chloride (13.5 g) as catalyst. The product was poured into water, filtered off, washed several times with propanone, and soaked in propanone overnight. After drying, the chlorine content of the copolymer beads (RCCS-4G) was determined by the flask combustion method.[12]

Introduction of Benzylamino Groups into RCCS-4G

RCCS-4G (10 g) and potassium phthalimide (10 g) (2.5 mol ratio/chloromethyl groups in copolymer beads) were stirred in anhydrous DMF at 100°C for 5 h. The intermediate product was filtered off and washed with deionized water and ethanol. After drying, the copolymer beads were soaked in a mixture of ethanol and hydrazine monohydrate (ethanol/hydrazine monohydrate = 4 vol ratio) and the mixture was refluxed at 100°C for 6 h to hydrolyze the intermediate product. After reflux, the product, RAAS-4G, was alternately washed 3 times with ethanol and deionized water and then purified by Soxhlet extraction with methanol for 12 h. The introduction of benzylamino groups was confirmed by infrared spectra.

Measurement of Anion Exchange Capacity

In a glass-stoppered 100-mL Erlenmeyer flask was placed 0.25 g of the aminated resin (OH form), followed by 50 mL of 0.1 mol dm^{-3} HCl. The mixture was shaken at 30°C for 15 h. The supernatant (10 mL) was pipetted out and 10 mL of 2 mol dm^{-3} $NaHCO_3$ and 5 mL of 2% (w/v) starch solution were added to the supernatant. Then the anion exchange capacity was determined by titrating the chloride ions in the supernatant with 0.1 mol dm^{-3} $AgNO_3$ solution using fluoresceine as an indicator.

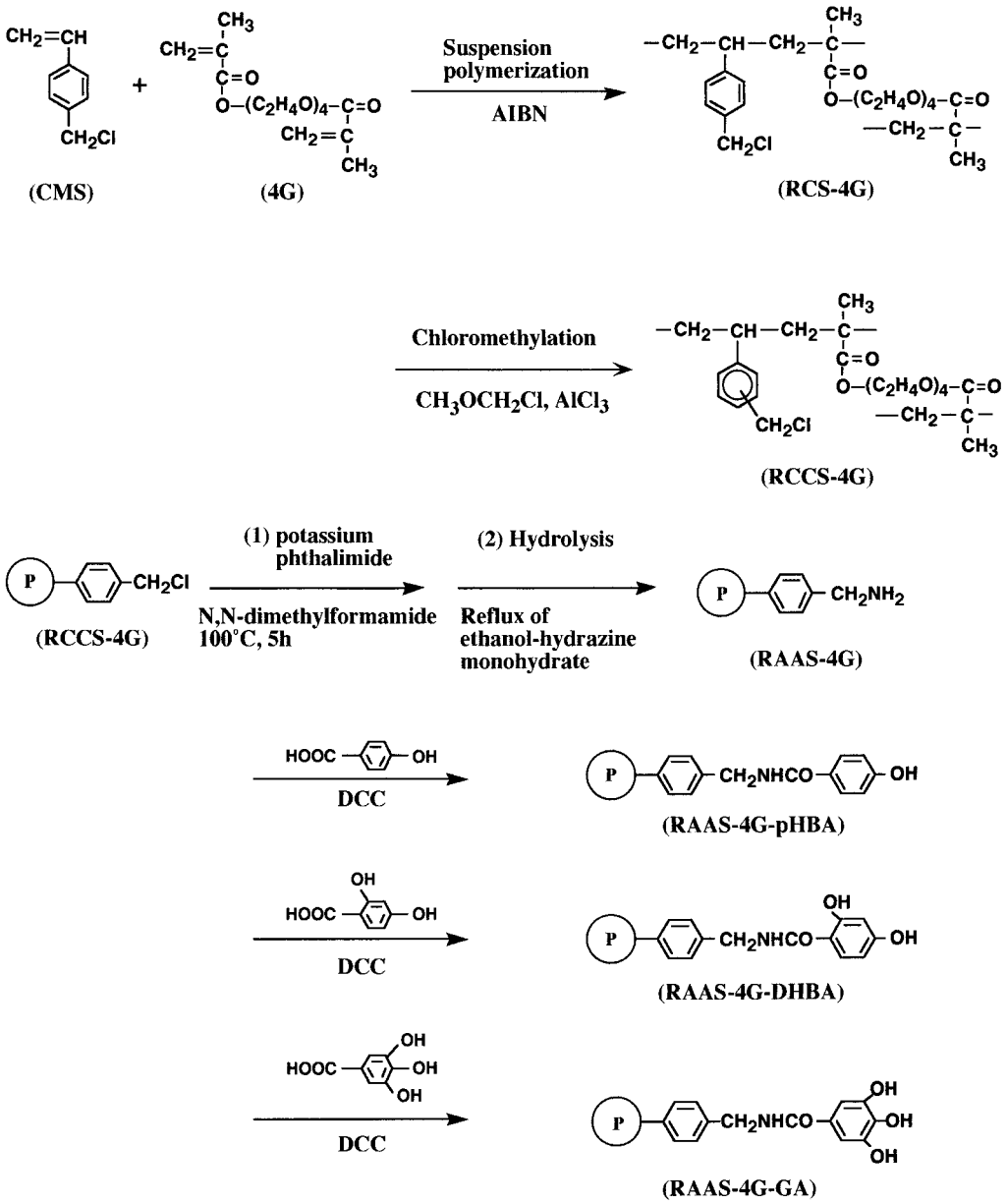
Immobilization of Phenol Derivatives into Copolymer Beads

RAAS-4G (2 g) and phenol derivatives (1.2 mol ratio to the benzylamino groups) were mixed into 30 mL of DMF in a round bottom 300-mL flask and stirred at 0–5°C for about 30 min. An amount of *N*,*N*′-dicyclohexylcarbodiimide equal to that of phenol derivatives dissolved in DMF was added dropwise to the above mixed solution over 30 min. After addition, the mixture was stirred at 0–5°C for 2 h, then at 20°C for 2 h, and further at 60°C for 4 h. With 3,4,5-trihydroxybenzoic acid (GA) the copolymer beads were also stirred at 20 and 40°C in addition to 60°C.

The content of phenol derivatives introduced into the resins was calculated by the difference of anion exchange capacity of the resins before and after immobilization of phenol derivatives into copolymer beads.

Organism and Growth Conditions

The bacteria used in this study were *E. coli* (IFO 3301) and *S. aureus* (IFO 13276), which were



Scheme 1 Preparation of resins containing phenol derivatives.

obtained commercially from the Institute for Fermentation, Osaka.

One loopfull of bacteria cells on culture medium was inoculated into 100 mL of 0.5 wt % nutrient broth and cultivated at 30°C overnight (ca. 16 h), then 1 mL of the precultivated suspension was placed into 100 mL of fresh 0.5% (w/v) nutrient broth and cultivated at 30°C for 3.5–4 h. Then the cells in the cultured cell suspension were collected by centrifugation at 10,000 rpm for 15 min, washed with sterile deionized water, and then again suspended in water and stabilized at 30°C for 1–2 h under shaking. By diluting the cell suspension with sterile deionized water, cultured cell suspension containing ca. 10^7–10^8 cells/mL was prepared for each strain and used for antibacterial tests.

Contact of Resins with Bacteria

A desired amount of the resins containing phenol derivatives was placed in a 50-mL Erlenmeyer flask, then 10 mL of cell suspension and subsequently 10 mL of water were added into the flask. The flask was shaken at 30°C for the prescribed time.

Table I Content of Cl Introduced

Resin	Cl (mequiv/g R)
RCS-4G	3.22
RCCS-4G	6.77

Measurement of Viable Cell Number after Contacting with Resins

After contacting the resins with a bacteria suspension for the prescribed time, 1 mL of the bacteria suspension was pipetted out from the flask, and 9 mL of sterile water was added to this suspension. The suspension was diluted several times and 0.1 mL of the diluted suspension was spread on an agar plate. The plate was kept at 37°C for 15–24 h and the numbers of viable cells were determined by counting those of the colonies formed on the plate.

RESULTS AND DISCUSSION

Preparation of Resins Containing Phenol Derivatives

The preparation of the resins containing phenol derivatives is shown in Scheme 1. First the content of chlorine in the RCS-4G and the RCCS-4G was measured. The results are shown in Table I. The chlorine content in the RCCS-4G was about 2 times that in the RCS-4G. This result indicates that chloromethyl groups were introduced at the ortho position as well as the para position. Next the RCCS-4G was treated with potassium phthalimide at 100°C for 5 h, followed by reflux at 100°C for 6 h in an ethanol–hydrazine monohydrate mixture. The aminated resins are abbreviated as RAAS-4G. The RAAS-4G was treated with GA at 20–60°C for the prescribed time. The copolymer beads obtained are identified as RAAS-4G-GA. The content of introduced benzylamino groups was calculated from the anion exchange capacity of the resins (Table II). The infrared spectra of

Table II Content of Benzylamino Groups Introduced

Resin	C_a[a] (mequiv/g R)
RAAS-4G	4.81

[a] Anion exchange capacity.

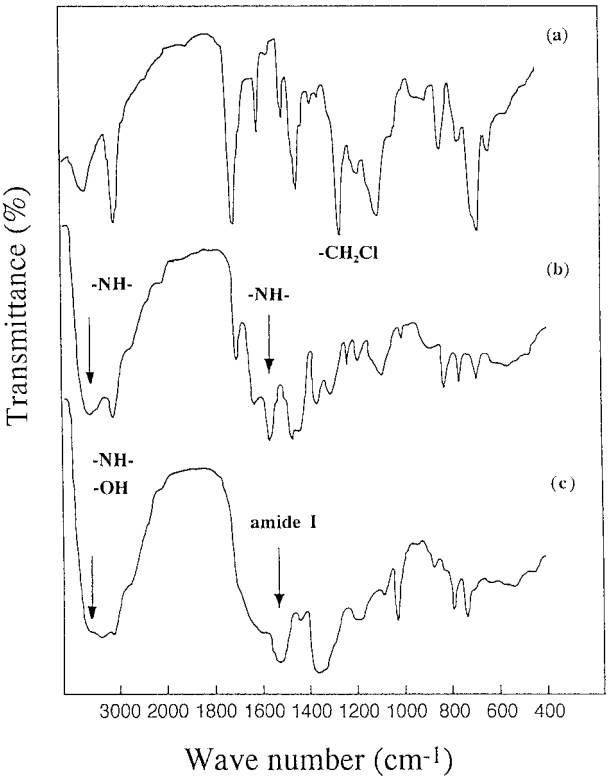


Figure 1 (a) IR spectra of RCCS-4G, (b) RAAS-4G, and (c) RAAS-4G-GA.

the RCCS-4G, RAAS-4G, and RAAS-4G-GA are shown in Figure 1. The absorption band at 1290 cm^{-1} due to reduced $—CH_2Cl$ and new absorption bands at 3400 and 1580 cm^{-1} due to $—NH_2$ groups were observed in the RAAS-4G. The absorption peak at 3400 cm^{-1} became broad because of the introduction of hydroxyl groups and a new absorption peak at 1500–1600 cm^{-1} from amide I appeared in the RAAS-4G-GA. These results indicate the introduction of benzylamino groups into the RCCS-4G and of GA into the RAAS-4G. As shown in Scheme 1, the introduction of GA moiety could be brought about by the reaction between the benzylamino groups in the resins and the carboxyl groups in the phenol derivatives. Therefore, the content of introduced GA was calculated from the difference between the anion exchange capacity of the resins before and after treatment of the copolymer beads with GA (Table III). The content of introduced GA could be controlled by changing the temperature of the treatment of the copolymer beads with GA. The contents of GA introduced in the copolymer beads treated at 20, 40, and 60°C were 1.01, 2.21, and 2.56 mmol/g, respectively. *p*-Hydroxybenzoic acid (pHBA) and 2,4-dihyroxybenzoic acid (DHBA)

Table III Content of Phenolic Hydroxyl Groups in RAAS-4G-GA Obtained at Various Temperatures

Temperature (°C)	GA Introduced (mmol/g R)	—OH (mequiv/g R)
20	1.01	3.03
40	2.21	6.63
60	2.56	7.68

were also introduced into the RAAS-4G copolymer beads at 60°C. The resins obtained are identified as RAAS-4G-pHBA and RAAS-4G-DHBA, respectively, in Scheme 1. The results are shown in Table IV. They had phenol contents of 2.34 and 3.02 mmol/g, respectively. These results indicate that about 60% of the benzylamino groups in the resins were reacted with each phenol derivative.

Antibacterial Activity of RAAS-4G-GA Against Bacteria

Antibacterial activity of the RAAS-4G-GA and the RAAS-4G containing no phenol derivatives was compared using *E. coli* or *S. aureus.* The results are shown in Figure 2. The RAAS-4G that had benzylamino groups but no phenol derivatives showed low antibacterial activity against *E. coli* and *S. aureus* and the RAAS-4G-GA showed higher antibacterial activity against both bacteria than the RAAS-4G. The pHs of the suspensions after contacting with the resins were 6.84 and 6.23 and the concentrations of total organic carbon in the supernatant liquid which indicates the release of organic compounds, such as GA, DMF, etc. from the resins, were 11.6 and 19.5 mg/L for RAAS-4G and RAAS-4G-GA, respectively. We previously reported that solutions with pHs more than 8.5 or less than 4.5 killed bacteria such as *E. coli* or *S. aureus.*[9] In some cases, a large amount of organic compounds released from the resins also killed bacteria. Therefore, we confirmed that the supernatant liquid with such pHs or the concentrations of total organic carbon had no antibacterial activity against *E. coli* or *S. aureus.* These results indicate that both amino groups and phenol derivatives in the resins participated in the antibacterial activity.

Table IV Content of Various Phenolic Hydroxyl Groups Introduced into RAAS-4G

Resin	Phenol Derivatives Introduced (mmol/g R)	—OH (mequiv/g R)
RAAS-4G-pHBA	2.34	2.34
RAAS-4G-DHBA	3.02	6.04
RAAS-4G-GA	2.56	7.68

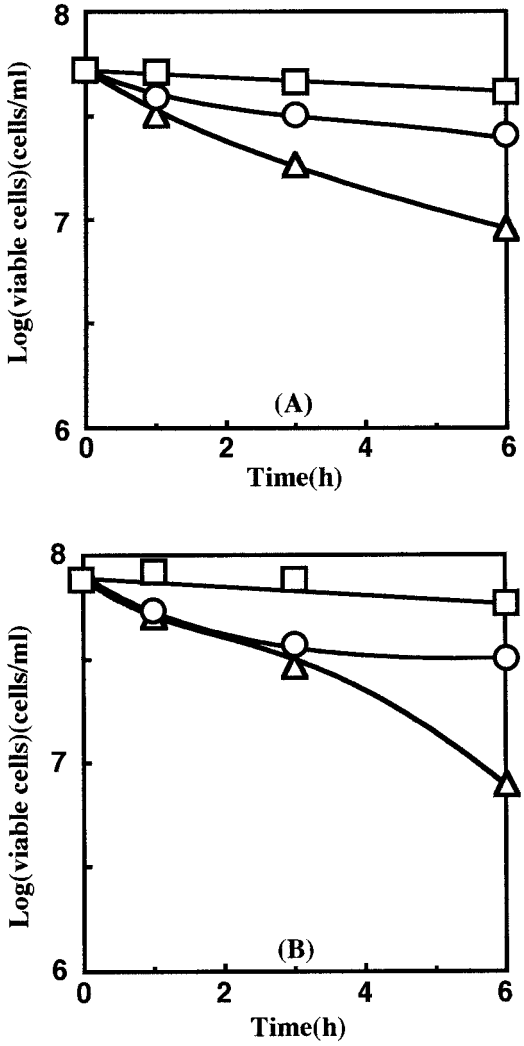


Figure 2 Changes in viable cell number after contacting with RAAS-4G and RAAS-4G-GA. Resins, RAAS-4G, RAAS-4G-GA (—OH, 3.03 mequiv/g R); weight of resins, 0.100 g; shaking at 30°C; suspension of (A) *E. coli* or (B) *S. aureus,* 20 mL (water). (□) Blank resin; final pH (A) 6.79, (B) 5.76; total organic carbon (TOC) (mg/L) none. (○) RAAS-4G; final pH (A) 6.09, (B) 6.84; TOC (mg/L), 11.6. (△) RAAS-4G-GA; final pH (A) 6.19, (B) 6.23; TOC (mg/L), 19.5.

Antibacterial Activity of RAAS-4G-GAs Having Different Amounts of Phenol Derivatives

The RAAS-4G-GAs with different amounts of GA moiety were made by treatment of the RAAS-4G

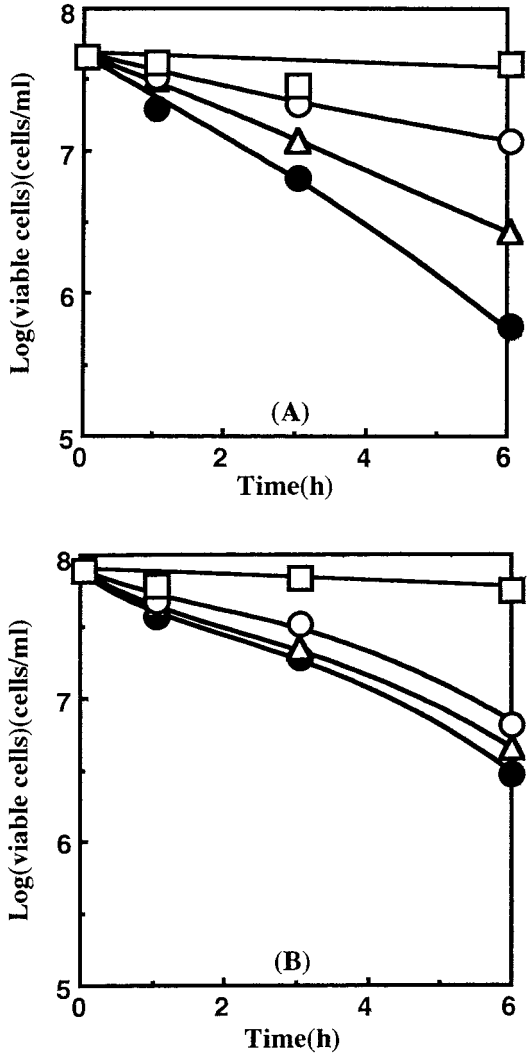


Figure 3 Changes in viable cell number after contacting with RAAS-4G-GA having different amounts of GA introduced. Weight of resins, 0.100 g; shaking at 30°C; suspension of (A) *E. coli* or (B) *S. aureus,* 20 mL (water); (□) —OH (mequiv/g R), none; final pH (A) 6.68, (B) 6.52; TOC (mg/L), none. (○) —OH (mequiv/g R), 3.03; final pH (A) 6.62, (B) 6.50; TOC (mg/L), 19.5. (△) —OH (mequiv/g R), 6.63; final pH (A) 6.10, (B) 5.56; TOC (mg/L), 19.9. (●) —OH (mequiv/g R), 7.68; final pH (A) 5.01, (B) 5.30; TOC (mg/L), 10.2.

with GA at various temperatures (20, 40, and 60°C). The obtained RAAS-4G-GA antibacterial activity against *E. coli* and *S. aureus* was investigated. Figure 3 shows that the antibacterial activity of the RAAS-4G-GAs against both bacteria increased with an increasing amount of GA moiety introduced. In particular, a large difference in the antibacterial activity between those resins was observed against *E. coli.* Furthermore, it was found that the RAAS-4G-GAs showed higher activity against *E. coli* than against *S. aureus.*

Antibacterial Activity of RAAS-4Gs with Various Phenol Derivatives

As phenol derivatives, pHBA and DHBA as well as GA were introduced into the RAAS-4G copolymer beads and the antibacterial activity of the

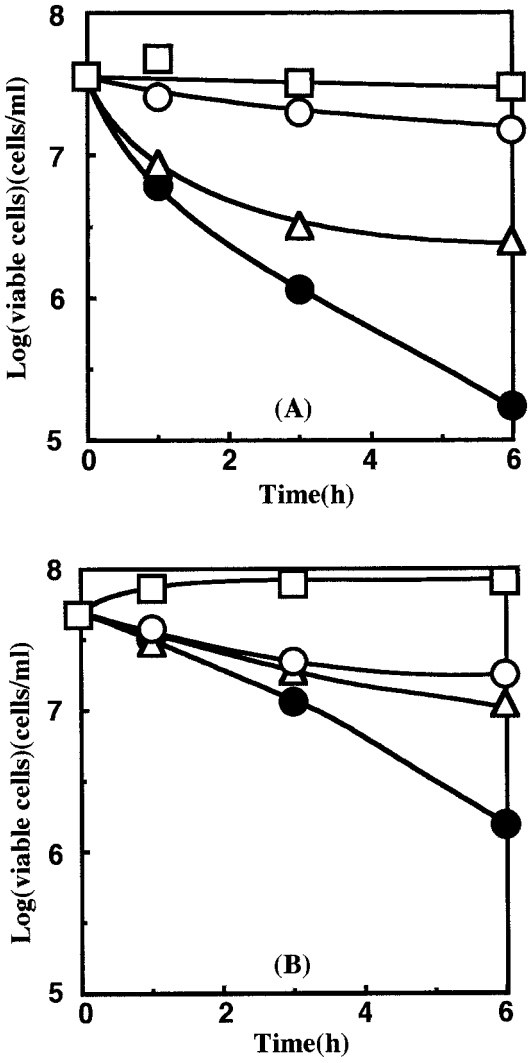


Figure 4 Changes in viable cell number after contacting with resins having various phenol derivatives. Weight of resins, 0.100 g; shaking at 30°C suspension of (A) *E. coli* or (B) *S. aureus* 20 mL (water). (□) Blank resin; —OH (mequiv/g R) none; final pH (A) 6.58, (B) 6.66; TOC (mg/L) none. (○) RAAS-4G-pHBA; phenol derivatives introduced (mmol/g R) 2.34; —OH (mequiv/g R) 2.34; final pH (A) 6.16, (B) 6.07; TOC (mg/L) 13.1. (△) RAAS-4G-DHBA; phenol derivatives introduced (mmol/g R) 3.02; —OH (mequiv/g R) 6.03; final pH (A) 5.59, (B) 5.20; TOC (mg/L) 32.0. (●) RAAS-4G-GA; phenol derivatives introduced (mmol/g R) 2.56; —OH (mequiv/g R) 7.68; final pH (A) 4.82, (B) 5.54; TOC (mg/L) 10.2.

Table V Decrease Coefficient for Bacteria by Resins Containing Phenol Derivatives

Resin	Amino Groups/Phenol Derivatives in Resin (mmol/g R)	—OH (mequiv/g)	D (mL/g h)	
			E. coli	*S. aureus*
RAAS-4G	4.81/0	0	17.0	11.0
RAAS-4G-BA	2.41/2.40	0	13.7	16.3
RAAS-4G-pHBA	2.31/2.34	2.34	17.7	22.3
RAAS-4G-DHBA	1.53/3.02	6.03	49.0	34.0
RAAS-4G-GA	3.80/1.01	3.03	30.3	24.0
RAAS-4G-GA	2.60/2.21	6.63	42.3	37.5
RAAS-4G-GA	1.83/2.56	7.68	75.1	48.7

$$D = \frac{V}{W \cdot t} \log \frac{N_0}{N_t},$$

where V is the volume of cell suspension (mL), W is the weight of the dry resin (g), t is the contact time (h), N_0 is the initial viable cells (cells/mL), N_t is the viable cells after contact time t (cells/mL).

resins obtained was investigated. The results are shown in Figure 4. The order of the antibacterial activity was as follows:

RAAS-4G-GA > RAAS-4G-DHBA

> RAAS-4G-pHBA

This order corresponds not only to the amount of OH groups in the resins, but also to the kind of phenol derivatives introduced. On the other hand, the supernatant liquid in which the resins were immersed showed no antibacterial activity. These results indicate that the antibacterial activity is responsible for the phenol derivatives introduced into the resins.

Decrease Coefficient for Bacteria with Resins Containing Phenol Derivatives

Decrease coefficients (D) for bacteria with the resins containing various phenol derivatives were calculated using eq. (1).

$$D\ (\text{mL g}^{-1}\ \text{h}^{-1}) = \frac{V}{wt} \log \frac{N_0}{N_t} \qquad (1)$$

where V, W, t, N_0, and N_t are volume of cell suspension, weight of the dry resin, contact time, initial viable cell number, and viable cell number after contact time t, respectively. The results are shown in Table V.

The RAAS-4G with only benzylamino groups and the RAAS-4G-BA with no phenol derivatives, which were obtained by treatment of the RAAS-4G with benzoic acid, had low decrease coefficients. The decrease coefficients of the RAAS-4G-GAs increased with an increasing amount of GA moiety in the resins. The RAAS-4G-GA (— OH, 7.68 meq/g) had decrease coefficients of 75.1 and 48.7 mL g^{-1} h^{-1} against *E. coli* and *S. aureus,*

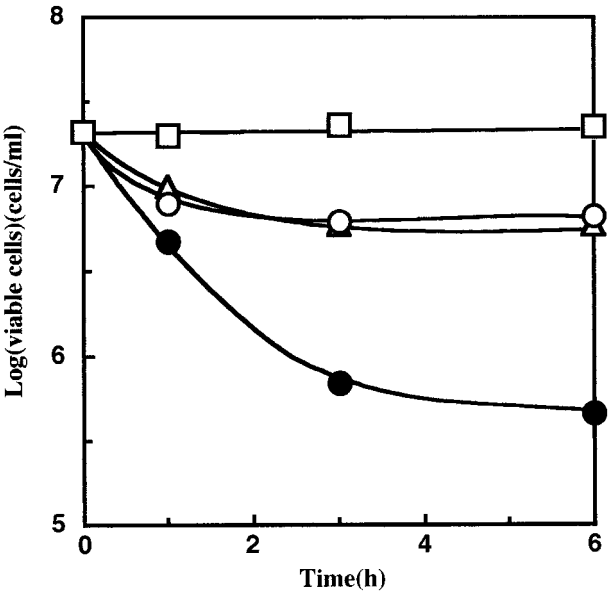


Figure 5 Changes in viable cell number after contacting with various resins having phenol derivatives. Weight of resins, 0.100 g; shaking at 30°C; suspension of *E. coli,* 20 mL (saline). (□) Blank resin; phenol derivatives in the resin (mmol/g R), none; —OH (mequiv/g R), none; final pH, 6.40. (○) RAAS-4G-pHBA; phenol derivatives in the resin (mmol/g R), 2.34; —OH (mequiv/g R), 2.34; final pH, 7.49. (△) RAAS-4G-DHBA; phenol derivatives in the resin (mmol/g R), 3.02; —OH (mequiv/g R), 6.03; final pH, 7.53. (●) RAAS-4G-GA; phenol derivatives in the resin (mmol/g R), 2.56; —OH (mequiv/g R), 7.68; final pH, 7.17.

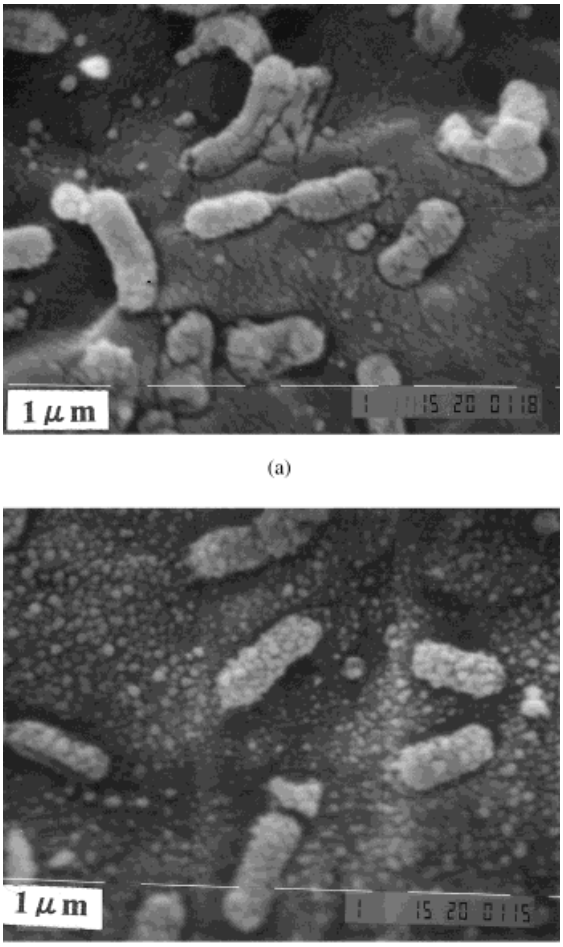


Figure 6 Scanning electron micrograph of the surface of (a) RAAS-4G and (b) RAAS-4G-GA contacted with *E. coli* in deionized water. Original magnification ×20,000.

respectively. We previously reported that the resins containing phosphonium groups, which were obtained by treatment of glycidylmethacrylate–divinylbenzene copolymer beads with trioctylphosphine (P content, 0.25 mequiv/g), had decrease coefficients of 19.6 and 48.6 (mL g^{-1} h^{-1}) against *E. coli* and *S. aureus,* respectively. Thus, these resins containing phenol derivatives had higher decrease coefficients against *E. coli* than *S. aureus,* except for the RAAS-4G-pHBA.

Antibacterial Activity of Resins in Saline Solution

We reported that the resins having quarternary ammonium groups or phosphonium groups had high antibacterial activity against *E. coli* or *S. aureus* in water, but they showed very low activity in saline solution. This is due to the shield of positive charge surrounding the resins in saline solution. The antibacterial activity against *E. coli* of the resins containing phenol derivatives was measured in saline solution. The results are shown in Figure 5. These resins showed high antibacterial activity against *E. coli* even under saline conditions, although the activity became a little lower compared to that in water. This indicates that the mechanism of antibacterial activity of these resins containing phenol derivatives is different from that of the resins having positively charged groups such as quaternary ammonium groups or phosphonium groups.

Scanning Electron Micrography Observation of Surface of Resins after Contacting with Bacteria

Scanning electron micrographs of the surface of the RAAS-4G and RAAS-4G-GA after contacting

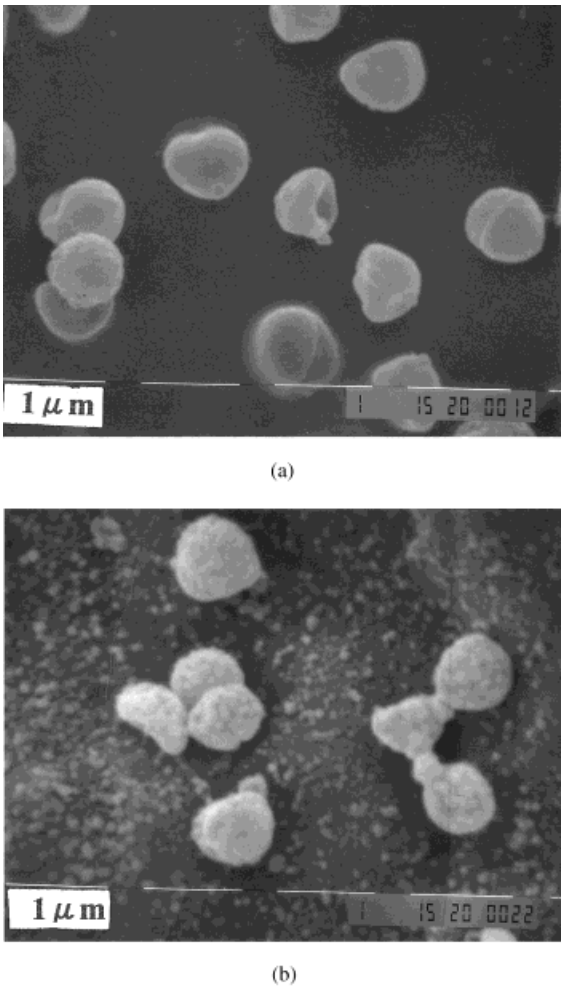


Figure 7 Scanning electron micrograph of the surface of (a) RAAS-4G and (b) RAAS-4G-GA contacted with *S. aureus* in deionized water. Original magnification ×20,000.

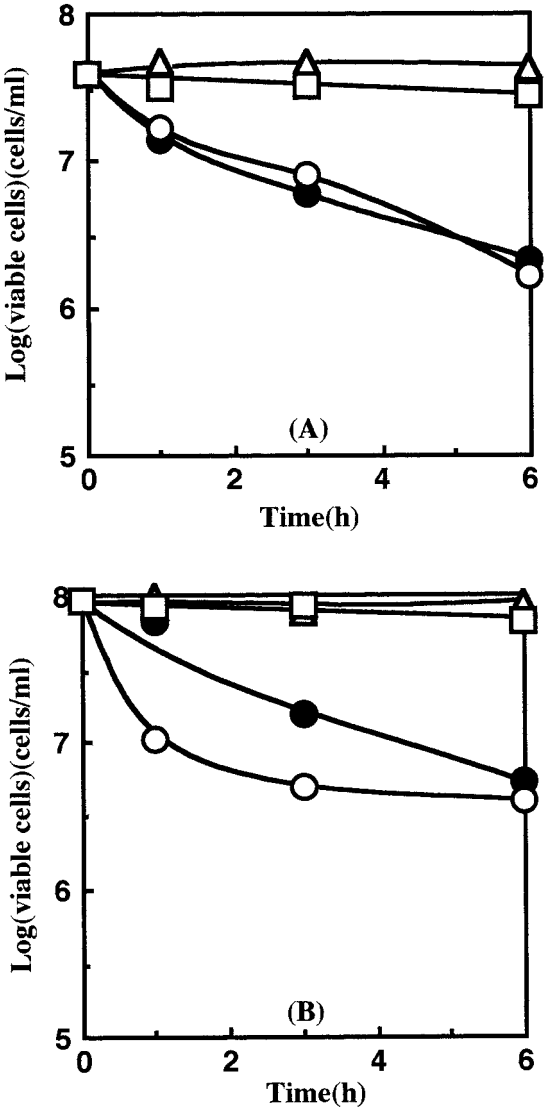


Figure 8 Changes in viable cell number after contacting with RAAS-4G-GA (Na type) and RAAS-4G-GA (OH type). Weight of resins, 0.100 g; shaking at 30°C; suspension of (A) *E. coli* or (B) *S. aureus,* 20 mL (water). (□) Blank resin; final pH (A) 6.40, (B) 6.19. (○) RAAS-4G-GA (— OH: 7.68 mequiv/g R); final pH (A) 4.92, (B) 5.02. (△) (I) RAAS-4G-GA (Na type); final pH (A) 6.55, (B) 5.96. (●) (II) RAAS-4G-GA (OH type); final pH (A) 4.30, (B) 4.31.

with *E. coli* and *S. aureus* are shown in Figure 6 and Figure 7, respectively. It was found that bacteria were adsorbed on both RAAS-4G and RAAS-4G-GA. The same phenomena were observed with the RAAS-4G-pHBA and RAAS-4G-DHBA. Furthermore, the scanning electron micrographs show that the surfaces of *E. coli* and *S. aureus* adsorbed on the RAAS-4G-GA are considerably destroyed and a lot of pieces formed by destruction exist around bacteria on the RAAS-4G-GA. These results indicate that phenol derivatives introduced into the resins participated mainly in the antibacterial activity of the resins.

Mechanism of Antibacterial Activity of Resins Containing Phenol Derivatives

To investigate the mechanism of the antibacterial activity of these resins against bacteria, the sodium salt of RAAS-4G-GA was prepared by treating RAAS-4G-GA with sodium hydroxide solution and then the resins (OH type) were again obtained by treating the RAAS-4G-GA (Na type) with HCl solution. The antibacterial activities of the two types of resins were measured. The results are shown in Figure 8.

A sodium salt of RAAS-4G-GA had no antibacterial activity against *E. coli* and *S. aureus.* However, the resins (OH type), which were again obtained by treating the RAAS-4G-GA (Na type) with HCl solution, recovered the antibacterial activity. This result indicates that phenolic hydroxyl groups are necessary for antibacterial activity. However, the exact mechanism of destruction of the surface of the bacteria cells due to the interaction between phenolic hydroxy groups and the cell wall is not obvious at present and is now under investigation.

CONCLUSION

1. The resins containing phenol derivatives were prepared by amination and were used for treatment of RCCS-4G with the various phenol derivatives such as pHBA, DHBA, or GA.
2. The resins containing phenol derivatives showed high antibacterial activity against *E. coli* and *S. aureus.* The antibacterial activity of the resins increased with increasing phenolic hydroxyl groups in the resins. In particular, RAAS-4G-GA had the highest antibacterial activity.
3. These resins showed higher antibacterial activity against *E. coli* than *S. aureus.*
4. Scanning electron micrographs showed that the antibacterial activity was brought about by phenolic hydroxyl groups in the resin.

REFERENCES

1. S. L. Taylor, L. R. Fina, and J. L. Lambert, *Appl. Microbiol.,* **20,** 720 (1970).

2. I. C. Kao, D. E. Robker, L. T. Fina, A. Snyder, and L. E. Frickson, *J. Ferment. Technol.,* **50,** 438 (1972).
3. I. C. Kao, L. E. Frickson, W. E. Craig, L. T. Fina, and L. T. Fina, *J. Ferment. Technol.,* **51,** 159 (1973).
4. Y. Nakagawa, Y. Yamano, T. Tawaratani, H. Kourai, T. Horie, and I. Shibasaki, *Appl. Environ. Microbiol.,* **43,** 1041 (1984).
5. N. Kawabata, T. Hayashi, and T. Matsumoto, *Appl. Environ. Microbiol.,* **46,** 203 (1983).
6. T. Tashiro, *Angew. Makromol. Chem.,* **206,** 1 (1993).
7. H. Kourai, *Kagaku Seibutsu,* **26,** 834 (1988).
8. Y. Nakagawa, H. Hayashi, T. Tawaratani, H. Kourai, T. Horie, and I. Shibasaki, *Appl. Environ. Microbiol.,* **47,** 513 (1984).
9. T. Nonaka, Y. Uemura, and S. Kurihara, *Nippon Kagaku Kaishi,* **1994,** 1097 (1994).
10. T. Nonaka, T. Ohtsuka, Y. Uemura, and S. Kurihara, *Nippon Kagaku Kaishi,* **1995,** 529 (1995).
11. K. Kawada, T. Uchibori, and S. Watanabe, *J. Antibact. Antifung. Agents,* **11,** 515 (1983).
12. Yuki Biryo Bunseki Kondankai Henshu, Ed., *Yuki Biryo Teiryo Bunseki,* Nankodo, Tokyo, 1969, p. 383.