

Synthesis of Macroreticular Copolymer Beads Having Various Phenolic Derivatives Immobilized via Different Bond and their Radical Scavenging Activity

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ABSTRACT: The macroreticular copolymer beads (RCS-4G) were prepared by suspension copolymerization of chloromethylstyrene (CS) and tetraethyleneglycol dimethacrylate (4G) in water, using cyclohexane as a diluent. Then, the copolymer beads carrying phenolic derivatives immobilized via amide, methylene, or ether bond were prepared by subsequent reactions of RCS-4G with various phenolic derivatives. The radical scavenging activity against 2,2-Diphenylpicrylhydrazil (DPPH) of the copolymer beads carrying phenolic derivatives immobilized was investigated in toluene. It was found that the copolymer beads had high radical scavenging activity against DPPH. The order of the radical scavenging activity against DPPH of the copolymer beads carrying phenolic derivatives immobilized did not coincide with the order of inhibition activity against the generation of

1,4-dioxane hydroperoxide. The radical scavenging activity against DPPH of the copolymer beads having phenolic derivatives immobilized via methylene bond or ether bond was higher than that of the copolymer beads having phenolic derivatives immobilized via amide bond. It was found that the radical scavenging activity of the copolymer beads having phenolic derivatives was affected by not only the kind of phenolic derivatives immobilized and bond groups through which phenolic compounds were immobilized, but also the swelling ratio of the copolymer beads in toluene. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 102: 4791–4800, 2006

Key words: antioxidants; functionalization of polymers; radical scavenger; macroporous; polymers; radical polymerization; resins; swelling

INTRODUCTION

Some of natural or synthetic phenolic compounds are known to exert as antioxidation reagents in our body or to have protective action against oxidative degradation of organic materials in our life.^{1–7} For example, 2,6-di-*tert*-butyl-4-methyl phenol (BHT) is used as an antioxidant in food, and Vitamin E (α -Tocopherol) is known to exert as an antioxidant in our body, etc. We have been studied the preparation of the resins containing phenolic derivatives from chloromethylstyrene (CMS)-tetraethyleneglycol dimethacrylate (4G) copolymer beads and their function. And we have previously reported that the macroreticular copolymer beads having phenolic derivatives immobilized via amide bond exhibited an antibacterial activity against bacteria such as *E. coli* or *S. aureus*.⁸ Recently we found that the copolymer beads had also an inhibition activity against the generation of 1,4-dioxane hydroperoxide in the presence of oxygen by UV irradiation and the copolymer beads could be easily re-

used without a decrease of the inhibition activity.⁹ And the relationship between the antibacterial activity or the inhibition activity and the structure of phenolic derivatives immobilized has also been discussed.^{8,9} So far many researchers have studied the antioxidation mechanism by phenolic compounds from the theoretical or experimental point of view.^{10–13} Bolland and Have^{14,15} have reported that the antioxidant properties of phenols are due to their ability to quench the chain carrying peroxy radicals of the substrate by transfer of the hydroxylic hydrogen to form hydroperoxides as described shortly.



where ROO \cdot denotes the peroxy radical and ArOH represents antioxidant phenol.

On the other hand, in 1993, Scott¹⁶ has described that electron-releasing groups in the aromatic rings enhance antioxidant activity, whereas electron-attracting groups suppress it, and substituents delocalizing the electron in the aryloxy or arylaminy radicals also enhance the antioxidant activity. Brigati¹⁷ et al. determined the bond dissociation enthalpies (BDE) of several phenols containing electron-attracting or electron-releasing substituents in the *para* position of 4-substi-

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tuted 2,6-di-*tert*-butyl phenols by means of the EPR radical equilibration technique, and they reported that the electron-attracting substituents such as $-\text{NO}_2$ or $-\text{COOH}$ induce an increase of the BDE value of the O—H bond, thus producing a worsening of the antioxidant activity of phenols, while electron-releasing substituents such as $-\text{CH}_3$ or $-\text{OCH}_3$ in the *para* position show an opposite effect.

Recently, a few researchers have investigated the antioxidation activity of phenolic derivatives by measuring the radical scavenging activity against DPPH radical.^{18–20}

In this study, to enhance the radical scavenging activity of the CMS-4G copolymer beads having phenolic derivatives immobilized via amide bond, we synthesized copolymer beads having various phenolic derivatives immobilized via different bond (methylene bond or ether bond) and investigated the radical scavenging activity of the copolymer beads against DPPH. And the relationship between the radical scavenging activity and the structure of the copolymer beads having phenolic derivatives immobilized via different bonds has also been discussed.

EXPERIMENTAL

Materials

4-Chloromethylstyrene (CMS) was purchased from Tokyo Kasei Co. (Tokyo, Japan) and was purified by elimination of inhibitors by passing through the column filled with Alumina powder. Tetraethyleneglycol dimethacrylate (4G), phenolic compounds, and 2,2-Diphenylpicrylhydrazil (DPPH) were purchased from Aldrich Japan (Tokyo, Japan) and used without further purification. α,α' -Azobisisobutyronitrile (AIBN) was purchased from Tokyo Kasei Co. (Tokyo, Japan) and purified by recrystallization from ethanol. *N,N*-Dimethylformamide (DMF) was purchased from Wako Chemical Co. (Osaka, Japan) and purified by distillation under reduced pressure (50°C/12 mmHg) after drying with molecular sieve 4A (Wako Chemical Co). Toluene of special grade was purchased from Nacalai Tesque Co. (Kyoto, Japan) and used without further purification. Other chemical compounds were of reagent grade and were used as received.

Preparation of the RCS-4G copolymer beads⁸

Macroreticular chloromethylstyrene (CMS)-tetraethyleneglycol dimethacrylate (4G) copolymer beads were prepared by suspension copolymerization in water, using cyclohexane as a diluent as follows: A solution of CMS (0.38 mol) and 4G (0.034 mol), cyclohexane (72 cm³) (120 vol % to total monomers), and AIBN (5.3 mmol) as an initiator were poured into an autoclave with 500 cm³ of 0.2% (w/v) hydroxyethyl cellu-

lose aqueous solution. The mixture was stirred until the monomer solutions were dispersed as fine droplets. Then, the suspension was heated to 70°C. Polymerization was carried out at 70°C for 4.5 h under stirring. After polymerization, the product was filtered off and washed with hot water (80°C) several times and immersed in methanol overnight. After drying, copolymer beads with the desired diameter (32–60 mesh) were selected. The macroreticular CMS-4G copolymer bead obtained was abbreviated as RCS-4G. The content of chloromethyl group in the RCS-4G was determined by measuring chlorine content in the copolymer beads by the combustion method.²¹

Measurement of pore structure

Specific surface areas of the copolymer beads obtained were measured on a Yuasa surface area apparatus (BET method). Pore volume and radius of the copolymer beads were determined on a Carlo-Erba mercury porosimeter (Model 220; Carlo-Erba, Italy).

Introduction of benzylamino groups into RCS-4G^{8,22}

RCS-4G (10 g) and potassium phthalimide (0.054 mol) (2.5 molar ratio to 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 intermediate product was immersed in a hydrazine monohydrate ethanol solution (ethanol/hydrazine monohydrate = 4/1 (v/v)) and the mixture was refluxed at 100°C for 6 h to hydrolyze the intermediate product. After reflux, the product was alternately washed several times with ethanol and deionized water, and then purified by Soxhlet extraction with methanol for 12 h. The copolymer bead having benzylamino groups was abbreviated as RCS-4G-BzA. The introduction of benzylamino groups was confirmed by infrared spectra and elemental analysis.

Measurement of anion exchange capacity⁸

In a glass-stoppered Erlenmeyer flask was placed 0.25 g of the copolymer beads, followed by an addition of 50 cm³ of 0.1 mol dm⁻³ HCl solution. The mixture was shaken at 30°C for 15 h and the copolymer bead was filtered off. 2 mol dm⁻³ NaHCO₃ solution and 5 cm³ of 2% (w/v) starch solution were added to 10 cm³ of the supernatant. Then the anion exchange capacity was determined by titrating chloride ions in the supernatant with 0.1 mol dm⁻³ AgNO₃ solution using Fluorescein as an indicator. The value of anion exchange capacity obtained indicates the content of benzylamino groups in the copolymer beads.

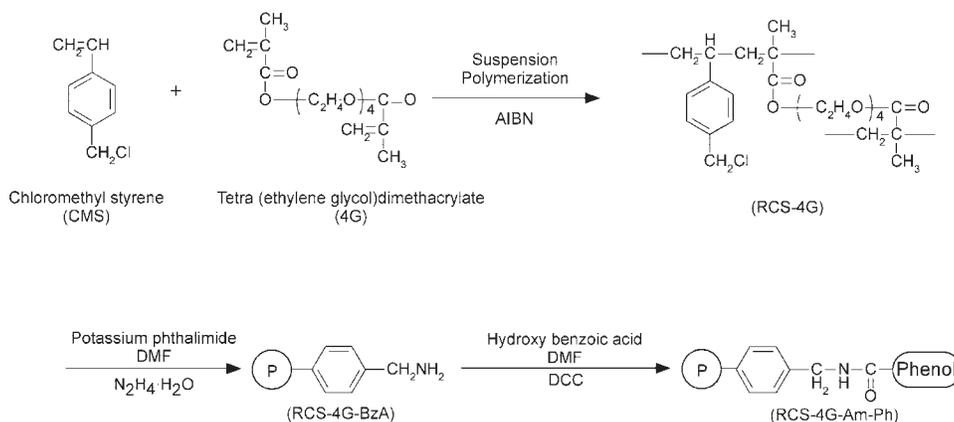


Figure 1. Synthesis of macroreticular copolymer beads containing phenolic derivatives immobilized via amide bond.

Immobilization of phenolic derivatives into copolymer beads

Various phenolic derivatives were immobilized into RCS-4G via various kinds of bonds using the method described shortly.

(1) *via amide bond*:⁸ The scheme of the synthesis of macroreticular copolymer beads containing phenolic derivatives immobilized via amide bond is shown in Figure 1.

RCS-4G-BzA (2 g) and hydroxy benzoic acids (1.2 molar ratio to the benzylamino groups) were added into 30 cm³ of DMF and stirred at 0–5°C for about 30 min. An amount of *N,N'*-dicyclohexylcarbodiimide (DCC) equal to that of the phenolic derivatives dissolved in DMF was added dropwise to the mixed solution over 3 min at 0–5°C. 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. After removal of the urea derivatives formed by decantation, the copolymer beads were filtered off and washed with methanol several times, and then purified by Soxhlet extraction with methanol for 24 h. After drying, the copolymer beads having phenolic derivatives were obtained. The

content of phenolic derivatives immobilized on the copolymer beads could not be determined directly. We decide to determine indirectly the content of phenolic compounds by measuring the decrease of amino groups converted into amide groups, because benzyl amino groups exhibit anion exchange capacity and amide groups do not. That is, the content of phenolic derivatives immobilized on the copolymer beads was calculated by the difference of anion exchange capacity of the copolymer beads before and after the introduction of phenolic derivatives into copolymer beads.

(2) *via methylene bond*:^{23,24} The immobilization of phenolic derivatives via methylene group was carried out by two methods (Fig. 2) described as follows:

- (a) RCS-4G (2 g), 1,4-dihydroxybenzene (2.0 molar ratio to chloromethyl group), and solid (powdered) zinc chloride (equal molar ratio to 1,4-dihydroxybenzene) were added into 50 cm³ 1,1,2,2-tetrachloroethane and stirred at 0°C for 1 h, 70°C for 1.5 h, and further 100°C for 6 h. After the reaction, the copolymer beads were filtered off and washed with propanone and deionized water

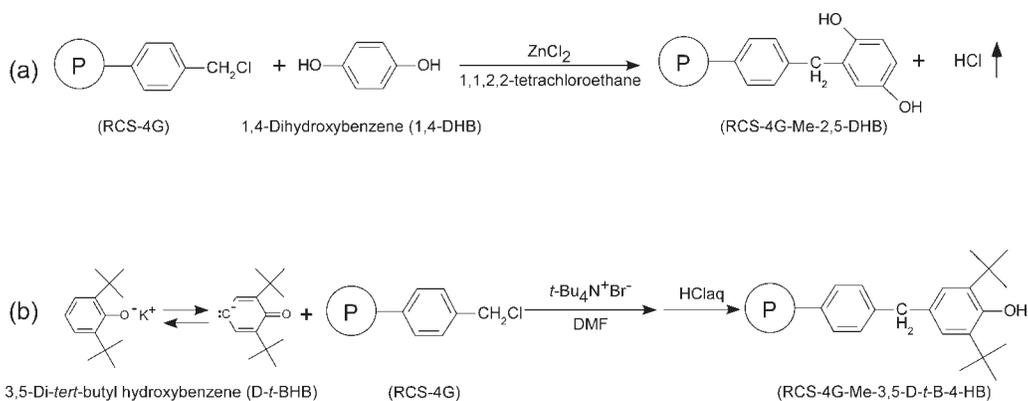


Figure 2. Synthesis of resins containing phenolic derivatives immobilized via methylene bond.

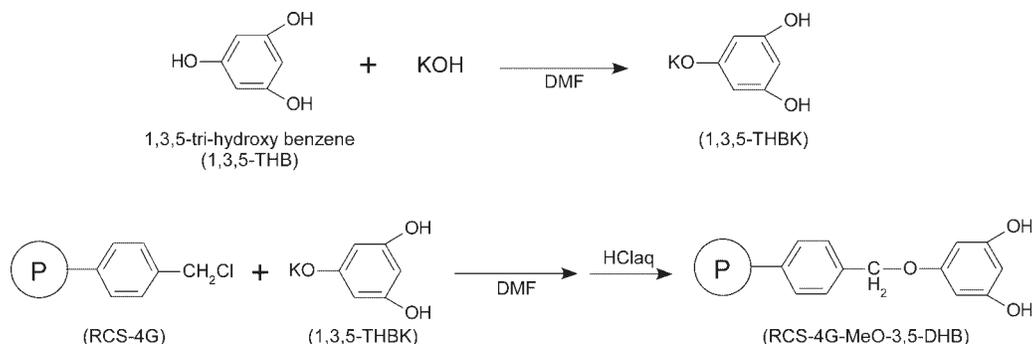


Figure 3. Synthesis of resins containing phenolic derivatives immobilized via ether bond.

several times, and then purified by Soxhlet extraction with propanone for 24 h and dried.

- (b) RCS-4G (2 g), potassium 2,6-di-*tert*-butylphenoxide (1.2 molar ratio to chloromethyl group), and 0.68 g of tetrabutylammonium bromide were added into 30 cm³ of DMF and stirred at 110°C for about 20 h. After that, the copolymer beads were filtered off and washed with methanol and deionized water several times, and then purified by Soxhlet extraction with methanol for 24 h. After drying, the copolymer beads containing phenolic derivatives were obtained. In this case, the content of phenolic derivatives immobilized on the copolymer beads could not be determined directly. Therefore, the content of phenolic derivatives immobilized in the copolymer beads was calculated by the difference of the chlorine content before and after the introduction of phenolic derivatives into the copolymer beads.
- (3) *via ether bond*.²⁵ The scheme of the synthesis of the copolymer bead containing 1,3,5-trihydroxybenzene (1,3,5-THB) immobilized via ether bond is shown in Figure 3. This method is the Williamson synthesis for making ether compounds. First, potassium salt of 1,3,5-THB (1,3,5-THBK) were prepared by treating phenolic derivatives with potassium hydroxide (1/3 molar ratio to 1,3,5-THB) in DMF at room temperature under stirring. Next, RCS-4G (2 g) and 1,3,5-THBK were added into 30 cm³ of DMF and stirred at 110°C for 24 h. After the reaction, the copolymer beads were treated with 1 mol/dm³ HCl solution to convert completely K-type to H-type of phenolic derivatives introduced, and then purified by Soxhlet extraction with methanol for 24 h. After drying, the copolymer beads having phenolic derivatives were obtained. The content of phenolic derivatives immobilized into the copolymer beads was calculated from the difference of the chlorine content before and after the introduction of phenolic derivatives into copolymer beads.

Measurement of swelling ratio of the copolymer beads

About 1 cm³ of dried copolymer beads were placed in 10 cm³ of measuring cylinder, and then 5 cm³ of toluene was poured into cylinder. The volume of the copolymer beads in toluene was measured at intervals of several hours at room temperature (24–25°C). The swelling ratio was calculated using the following equation.

$$\text{Swelling ratio} = \frac{\text{Apparent volume of wet copolymer beads (cm}^3\text{)}}{\text{Apparent volume of dry copolymer beads (cm}^3\text{)}} \quad (2)$$

Radical scavenging activity of the copolymer beads having phenolic derivatives

2,2-Diphenylpicrylhydrazil (DPPH) is known as a metastable radical bearing a radical electron stabilized by resonance. A DPPH toluene solution is dark purple because of the maximum absorption peak at 520 nm and this absorption disappears when the radical is lost. Therefore, the radical scavenging activity of the copolymer beads having phenolic derivatives was evaluated by measuring the change in the absorption intensity at 520 nm, after DPPH toluene solution was shaken in the presence of the copolymer beads for a prescribed time.

RESULTS AND DISCUSSION

Preparation of the copolymer beads having phenolic derivatives immobilized

(a) *via amide bond*: Macroreticular copolymer beads (RCS-4G) (CMS:4G = 90:10 molar ratio) were prepared by suspension copolymerization of CMS and 4G using AIBN as an initiator as described already.⁹ The RCS-4G had 1.1 cm³/g of pore volume, 500 Å of average pore radius, and 30 m²/g of specific surface

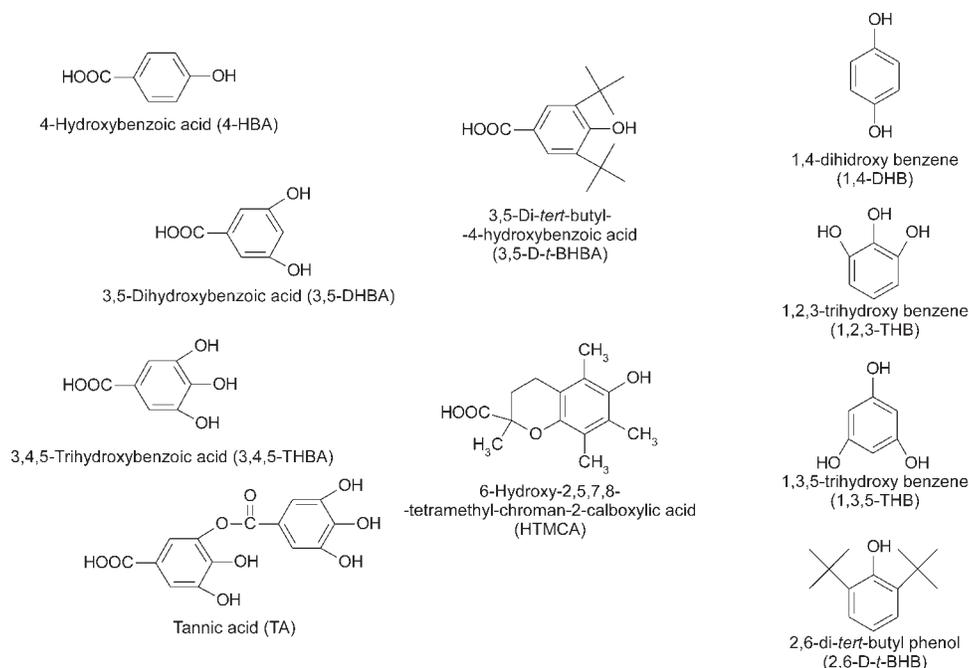


Figure 4. Structures of phenolic derivatives immobilized.

area. The RCS-4G was treated with potassium phthalimide in DMF at 100°C for 5 h, followed by reflux at 100°C for 6 h in an ethanol/hydrazine monohydrate mixture. The RCS-4G had chlorine content of about 5.0 mmol/g and the aminated resin (RCS-4G-BzA) had residual chlorine content of about 0.4 mmol/g. This indicates that about 93% of chloromethyl groups in the RCS-4G were converted to benzyl amino groups. The RCS-4G-BzA had anionic exchange capacity of about 4.8 meq/g. The introduction of phenolic derivatives was brought about by the reaction of benzylamino groups in the RCS-4G-BzA with carboxyl groups in various benzoic acids having phenolic hydroxyl groups. 4-hydroxybenzoic acid (4-HBA), 3,5-dihydroxybenzoic acid (3,5-DHBA), 3,4,5-trihydroxyben-

zoic acid (3,4,5-THBA), 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (3,5-D-*t*-BHBA), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (HTMCA) were used as hydroxy benzoic acids or a carboxylic compound having phenolic hydroxyl groups (Fig. 4).

The amount of phenolic derivatives immobilized is shown in Table I together with abbreviation of the copolymer beads obtained. All phenolic derivatives were immobilized via amide bond into the copolymer beads by the reaction of benzylamino groups more than 80% in the RCS-4G-BzA with various hydroxybenzoic acids or a carboxylic compound having phenolic hydroxyl group. Therefore, a small amount of benzylamino groups still remains in the copolymer beads having phenolic derivatives.

TABLE I
Characterization of the Copolymer Beads Containing Phenolic Derivatives Immobilized via Various Bond Groups

Abbreviation of copolymer beads	Bond type	Phenolic derivatives in a phenol derivative unit (mmol/g-R)	Number of phenolic OH groups
RCS-4G-Am-4-HB	Amide	4.2	1
RCS-4G-Am-3,5-DHB		4.0	2
RCS-4G-Am-3,4,5-THB		4.1	3
RCS-4G-Am-TA		3.8	5
RCS-4G-Am-3,5-D-t-B-4-HB		4.1	1
RCS-4G-Am-HTMCA		4.3	4
RCS-4G-Me-3,5-D-t-B-4-HB	Methylene	3.8	1
RCS-4G-Me-2,5-DHB		4.5	2
RCS-4G-Me-3,4,5-THB		4.4	3
RCS-4G-MeO-4-HB	Ether	4.5	1
RCS-4G-MeO-3,5-DHB		3.7	2

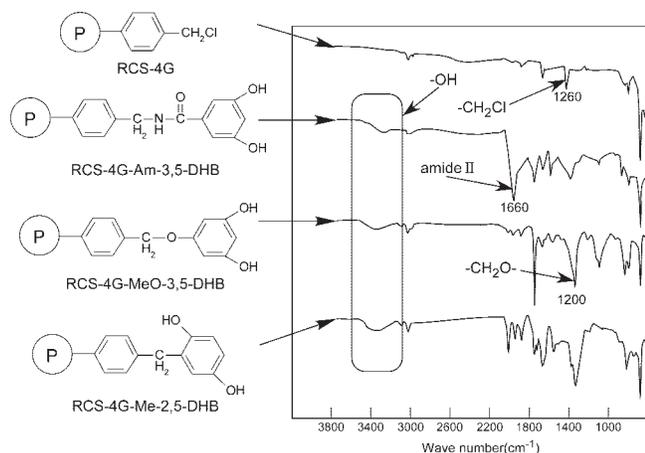


Figure 5. IR spectra of RCS-4G and the copolymer beads having phenolic derivatives immobilized via different bond.

(b) *via methylene bond*: 2,6-di-*tert*-butylhydroxybenzene (2,6-D-*t*-BHB), 1,4-dihydroxybenzene (1,4-DHB), and 1,2,3-trihydroxybenzene (1,2,3-THB) (Fig. 4) were immobilized via methylene bond on the RCS-4G copolymer beads. The amount of phenolic derivatives immobilized via methylene bond, together with the abbreviation of the copolymer beads obtained, is also shown in Table I. All phenolic derivatives were immobilized via methylene bond on the copolymer beads by the reaction of more than 80% chloromethyl groups in the RCS-4G with potassium phenoxide of 1,4-DHB (method (a)) or phenolic derivatives such as 1,2,3-THB or 2,6-D-*t*-BHB (method (b)).

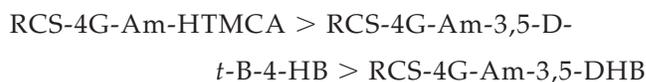
(c) *via ether bond*: 1,4-dihydroxybenzene (1,4-DHB) and 1,3,5-trihydroxybenzene (1,3,5-THB) (Fig. 4) were immobilized via ether bond on the RCS-4G copolymer beads. The amount of phenolic derivatives immobilized via ether bond, together with the abbreviation of the copolymer beads obtained, is also shown in Table I. All phenolic derivatives were immobilized via ether bond on the copolymer beads by the reaction of more than 80% chloromethyl groups in the RCS-4G with potassium phenoxides.

The infrared spectra of the RCS-4G, RCS-4G-BzA, and the copolymer beads (RCS-4G-Am-3,5-DHBA, RCS-4G-MeO-3,5-DHBA, and RCS-4G-Me-2,5-DHBA) having phenol compound (DHB) immobilized via different bond, are shown in Figure 5. The reduced absorption band at 1270 cm^{-1} is due to $-\text{CH}_2\text{Cl}$ and new absorption bands at 3400 and 1600 cm^{-1} due to $-\text{NH}_2$ groups were observed in the RCS-4G-BzA. The absorption peak at $3300\text{--}3500\text{ cm}^{-1}$ is due to OH groups becoming broad because of the introduction of phenolic hydroxy groups and new absorption peaks at 1670 cm^{-1} (amide I) and 1530 cm^{-1} (amide II) appeared in the RCS-4G-Am-3,5-DHB. New absorption peak at 1470 cm^{-1} due to $-\text{CH}_2\text{O}-$ and $3300\text{--}3500\text{ cm}^{-1}$ due to OH groups was observed with RCS-4G-

MeO-3,5-DHB. The reduced absorption band at 1270 cm^{-1} is due to $-\text{CH}_2\text{Cl}$ and new absorption bands at $3300\text{--}3500\text{ cm}^{-1}$ due to OH groups were observed in the RCS-4G-Me-2,5-DHB. Absorption peaks at 1700 cm^{-1} in the all copolymer beads is due to $\text{C}=\text{O}$ groups in 4G. These results indicate the immobilization of phenolic compound (DHB) on the RCS-4G via the different bonds.

Radical scavenging activity of the copolymer beads having phenolic derivatives immobilized via amide bond

First the radical scavenging activity of the copolymer beads containing various phenolic derivatives immobilized via amide bond, RCS-4G-Am-3,5-DHB, RCS-4G-Am-3,5-D-*t*-B-4-HB, and RCS-4G-Am-HTMCA against DPPH was investigated in toluene. The results are shown in Figure 6. In our previous work,⁹ we have reported that RCS-4G-Am-3,5-DHB exhibited the highest inhibition activity against the generation of 1,4-dioxane hydroperoxide. 3,5-D-*t*-B-4-HB and HTMCA immobilized on the copolymer bead were the phenolic derivatives of low molecular weight model of 2,6-di-*tert*-butyl 4-methylphenol (BHT), which is commonly used as an antioxidant in food, and an important antioxidative moiety in Vitamin E (α -tocopherol), respectively. The solid circles in Figure 6 indicate the results in the absence of the copolymer beads containing phenolic derivatives. The order of radical scavenging activity of the copolymer beads is as follows:



The decrease of DPPH with time was not observed in the absence of the copolymer beads containing phe-

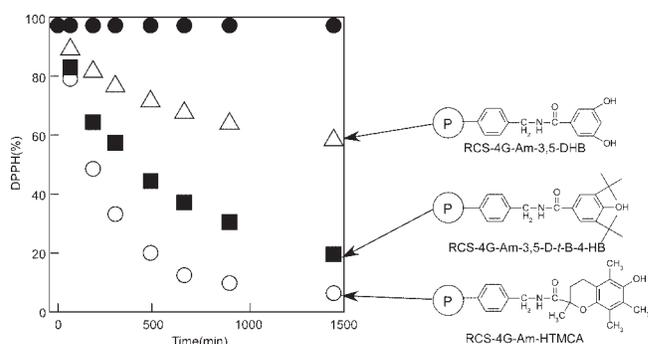


Figure 6. Time dependence of the residual DPPH (%) by the reaction of DPPH with the copolymer beads containing phenolic derivatives Conditions: DPPH:Phenol = 1:200 (molar ratio); DPPH concentration: $1.96 \times 10^{-4}\text{ mol/dm}^3$; Shaking at 30°C ; (●): Blank*; * Blank indicates the results in the absence of the copolymer beads containing phenol derivatives.

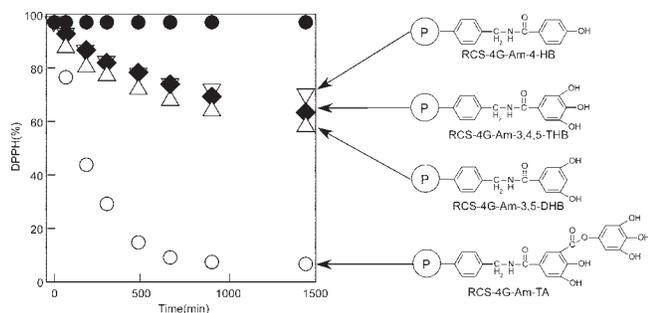
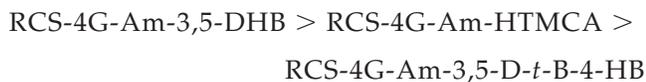


Figure 7. Time dependence of the residual DPPH(%) by the reaction of DPPH with the copolymer beads containing phenolic derivatives having different number of phenolic hydroxyl groups. Conditions: DPPH:Phenol = 1:200 (molar ratio); DPPH concentration: 1.96×10^{-4} mol/dm³; Shaking at 30°C; (●): Blank.

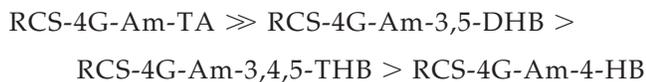
nolic derivatives. In addition, the RCS-4G containing no phenolic derivatives did not have the radical scavenging activity against DPPH.

In our previous work,⁹ we have reported that the order of the inhibition ability against the generation of 1,4-dioxane hydroperoxide of the copolymer beads was as follows:

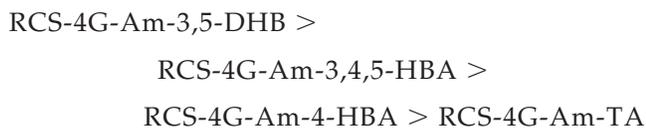


Thus, it was found that the order of radical scavenging activity of the copolymer beads against DPPH was not in accordance with that of the inhibition activity against the generation of 1,4-dioxane hydroperoxide.

Next the radical scavenging activity against DPPH of the copolymer beads (RCS-4G-Am-4-HB, RCS-4G-Am-3,5-DHB, RCS-4G-Am-3,4,5-THB, and RCS-4G-Am-TA) containing phenolic derivatives with different numbers of phenolic hydroxyl groups immobilized via amide bond was investigated in toluene. The results are shown in Figure 7. The order of radical scavenging activity of the copolymer beads was as follows:



However, in a previous work, we have reported that that the order of the inhibition activity against the generation of 1,4-dioxane hydroperoxide of the copolymer beads was as follows:



Thus, the order of radical scavenging activity against DPPH of the copolymer beads containing phenolic derivatives with different numbers of phenolic hydroxyl groups was not also in accordance with that of the inhibition activity against the generation of 1,4-dioxane hydroperoxide. The exact reason for this difference between the order of the radical scavenging activity against DPPH and the order of the inhibition activity against the generation of 1,4-dioxane hydroperoxide is not obvious at present. However, it is considered that this may be due to the difference between ROO radical and DPPH radical or the difference of solvents (1,4-dioxane and toluene) used in each experiment. Further experiments to clarify the reason are under investigation.

Radical scavenging activity of the copolymer beads having phenolic derivatives immobilized via methylene bond

As described already, it was found that the copolymer beads having phenolic derivatives immobilized via amide bond had radical scavenging activity. However, the radical scavenging activity was not so high. Scott¹⁶ has described that electron-releasing groups in the aromatic rings increase antioxidant activity, whereas electron-attracting groups decrease it, and substituents delocalizing the electron in the aryloxy or arylaminy radicals increase the antioxidant activity. So, to increase the radical scavenging activity of the copolymer beads containing phenolic derivatives, we prepared the copolymer beads containing phenolic derivatives immobilized via methylene ($-\text{CH}_2-$) bond of electron-releasing group and the radical scavenging activity of the copolymer beads was investigated. Figure 8 shows the radical scavenging activity against DPPH of the copolymer beads (RCS-4G-Me-

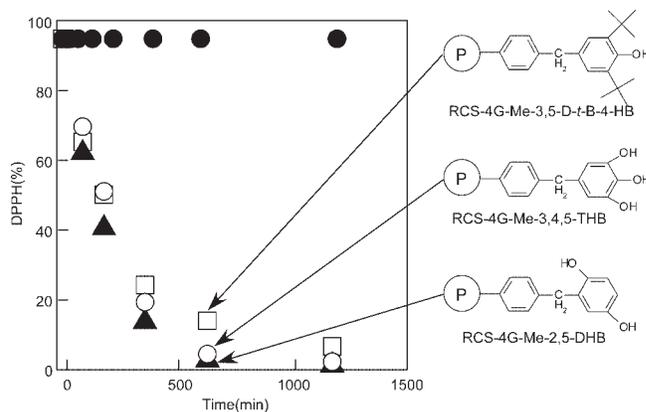


Figure 8. Time dependence of the residual DPPH (%) by the reaction of DPPH with the copolymer beads containing phenolic derivatives. Conditions: DPPH:Phenol = 1:200 (molar ratio); DPPH concentration: 1.96×10^{-4} mol/dm³; Shaking at 30°C. (●): Blank.

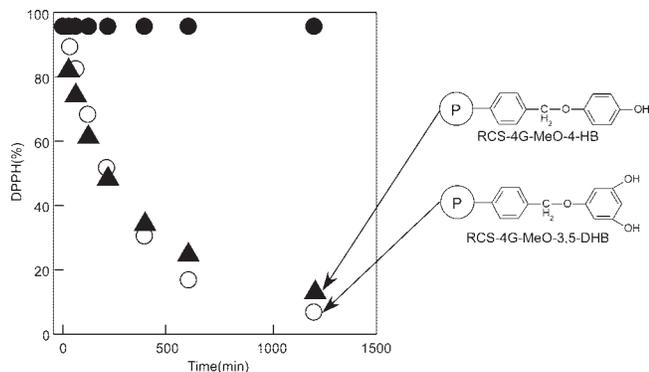
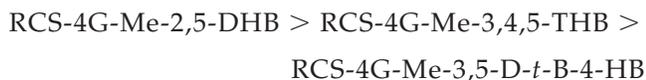


Figure 9. Time dependence of the residual DPPH (%) by the reaction of DPPH with the copolymer beads containing phenolic derivatives. Conditions: DPPH:Phenol = 1:200 (molar ratio); DPPH concentration: 1.96×10^{-4} mol/dm³; Shaking at 30°C; (●): Blank.

3,5-D-*t*-B-4-HB, RCS-4G-Me-3,4,5-THB, and RCS-4G-Me-2,5-DHB) containing phenolic derivatives immobilized via methylene bond. The rate of the radical scavenging of all the copolymer beads having phenolic derivatives immobilized via methylene bond became significantly high when compared with that of the copolymer beads containing phenolic derivatives immobilized via amide bond.

The order of radical scavenging activity of the copolymer beads was as follows:

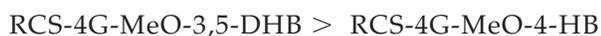


However, the difference of the radical scavenging activity of those copolymer beads was not so large.

Radical scavenging activity of the copolymer beads having phenolic derivatives immobilized via ether bond

Next, we prepared the copolymer beads containing phenolic derivatives immobilized via ether ($-\text{CH}_2\text{O}-$) bond of electron-releasing group and the radical scavenging activity of the copolymer beads was investigated. Figure 9 shows the radical scavenging activity against DPPH of the copolymer beads (RCS-4G-MeO-4-HB and RCS-4G-MeO-3,5-DHB) containing phenolic derivatives immobilized via ether bond.

The rate of the radical scavenging activity of the copolymer beads containing phenolic derivatives immobilized via ether bond became fairly high when compared with that of the copolymer beads containing the same phenolic compound (DHB) immobilized via amide bond. The order of radical scavenging activity of the copolymer beads was as follows:



However, the difference of the radical scavenging activity of the copolymer beads was small. In both copolymer beads having phenolic derivatives immobilized via methylene or ether bond, the copolymer beads containing phenolic compound (DHB) with two phenolic hydroxyl groups immobilized exhibited the highest radical scavenging activity against DPPH.

Comparison of radical scavenging activity of the copolymer beads having phenolic derivatives immobilized via different bonds

The radical scavenging activity against DPPH of the copolymer beads having phenolic compound (DHB) with two phenolic hydroxyl groups immobilized via three different bonds was compared. In three copolymer beads used, only RCS-4G-Me-2,5-DHB had two phenolic hydroxyl groups at different positions from that of RCS-4G-Am-3,5-DHB and RCS-4G-MeO-3,5-DHB. This is because of difficulty of the preparation of RCS-4G-Me-3,5-DHB.

The order of the radical scavenging activity of the copolymer beads containing phenolic compound (DHB) immobilized via different bond was as follows (Fig. 10):

The order of the radical scavenging activity of the copolymer beads containing DHB immobilized via different bonds was as follows:



Thus, it was found that the radical scavenging activity against DPPH of the copolymer beads having DHB immobilized via methylene or ether bond is fairly high when compared with that of the copolymer beads having DHB immobilized via amide bond. This result indicates that electron-releasing groups in the

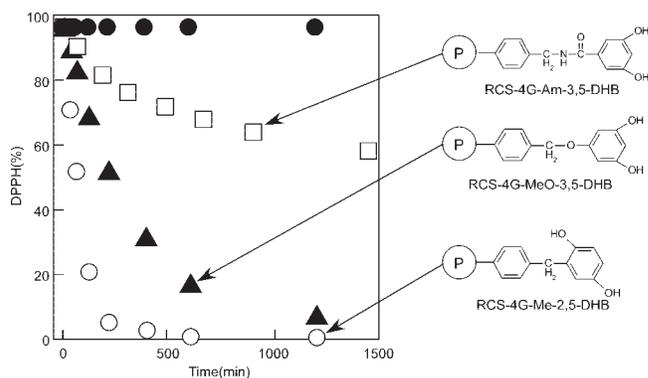


Figure 10. Time dependence of the residual DPPH (%) by the reaction of DPPH with the copolymer beads containing phenolic derivatives. Conditions: DPPH:Phenol = 1:200 (molar ratio); DPPH concentration: 1.96×10^{-4} mol/dm³; Shaking at 30°C; (●): Blank.

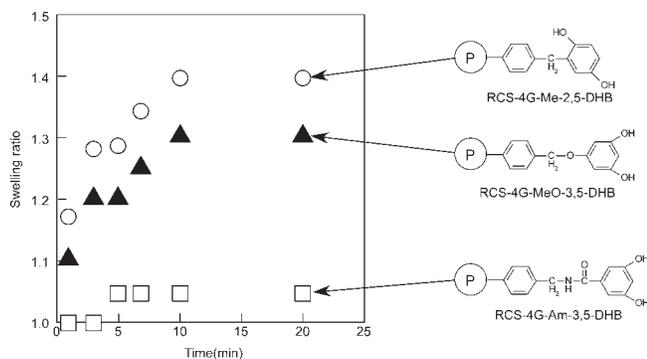
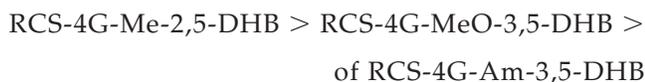


Figure 11. Time dependence of the swelling ratio of the copolymer beads having DHB immobilized via different bond.

aromatic rings enhance antioxidant activity, whereas electron-attracting groups suppress it. However, the radical scavenging activity of the copolymer beads having phenolic derivatives immobilized via methylene is higher than that of the copolymer beads having phenolic compound (DHB) immobilized via ether bond, although it is known that methoxy group ($-\text{OCH}_3$) is higher electron-releasing groups than methyl group ($-\text{CH}_3$).

To understand the reason for the phenomena, the swelling volume of the copolymer beads having phenolic compound (DHB) immobilized via different bond was measured in toluene. The result is shown in Figure 11. The swelling ratio of the copolymer beads increased with time and approached to equilibrium in 20 min, although those copolymer beads were macroreticular type resins. The swelling ratio of the parent macroreticular CMS-4G copolymer beads was about 2.5 at equilibrium in toluene. Therefore, the swelling ratio of the copolymer beads having phenolic derivatives became smaller than that of the parent CMS-4G copolymer. The order of the magnitude of the swelling ratio was as follows:



The lowest swelling ratio of RCS-4G-Am-3,5-DHB in toluene is due to high hydrophilicity of amide groups in the copolymer beads. The order of the magnitude of the swelling ratio coincides with that of the radical scavenging activity of those copolymer beads.

We mentioned earlier that the copolymer beads having different phenolic derivatives immobilized via amide bond exhibited clear different radical scavenging activity as shown in Figures 6 and 7. In this case, the swelling ratios of those copolymer beads were almost same irrespective of the kind of phenolic derivatives immobilized. The copolymer beads having

different phenolic derivatives immobilized via methylene or ether bond, which showed high radical scavenging activity as shown in Figures 8 and 9, also had almost the swelling ratios. Therefore, the earlier-mentioned results indicate that the kind of bond groups, through which phenolic derivatives were immobilized on the copolymer beads, affected greatly the swelling ratio of the copolymer beads in solvent, and this results in the increase or decrease of the radical scavenging activity.

CONCLUSIONS

1. The macroreticular copolymer beads carrying various phenolic derivatives immobilized via amide, methylene, or ether bond could be prepared.
2. The order of the radical scavenging activity against DPPH of the copolymer beads carrying phenolic derivatives immobilized via amide bond did not coincide with the order of inhibition activity against the generation of 1,4-dioxane hydroperoxide.
3. The radical scavenging activity against DPPH of the copolymer beads having phenolic derivatives immobilized via methylene bond or ether bond was higher than that of the copolymer beads having phenolic derivatives immobilized via amide bond.
4. The radical scavenging activity of the copolymer beads having phenolic derivatives was affected by not only the kind of phenolic derivatives immobilized and bond groups via which phenol groups immobilized, but also the swelling ratio of the copolymer beads in toluene.

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