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Preparations of Aromatic Diamine Monomers and Copolyamides Containing Phosphorylcholine Moiety and the Biocompatibility of Copolyamides

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ABSTRACT: The synthesis of a novel aromatic diamine compound containing phosphorylcholine (PC) group was carried out to prepare aromatic polyamides with PC moiety, in order to develop durable biocompatible polymer materials. The desired diamine compound, 2-(3,5-diaminophenylcarbonyloxy)ethyl phosphorylcholine (DAPC), was prepared from a reaction of 2-[2-(3,5-dinitrophenylcarbonyloxy)ethyl]-2-oxo-1,3,2-dioxaphospholane with trimethylamine, followed by the reduction of the dinitro group by H_2/Pd. The starting phospholane compound was synthesized by a condensation reaction of 2-hydroxyethyl 3,5-dinitrobenzoate with 2-chloro-2-oxo-1,3,2-dioxaphospholane. The polycondensation of DAPC with 4,4′-diamino-3,3′-dimethyldiphenylmethane and isophthaloyl chloride gave the copolyamides with different PC contents. From the results of contact angle of water and XPS analysis on the surface of the copolyamide coating films, it was found that PC units were concentrated on the surface after immersed in water. In addition, by using 2,2-bis[4-(aminophenyloxy)phenyl]propane or 2,2-bis[4-(aminophenyloxy)phenyl]hexafluoropropane as a diamine comonomer with DAPC, high molecular weight copolyamides containing PC moiety were obtained to prepare homogeneous coating films. The obtained copolyamides exhibited the high thermal stability, and also the excellent biocompatibility even though the content of PC monomer unit in the copolymer was around 20 mol %, which was confirmed by the platelet adhesion test. Therefore, the introduction of PC group in the side chain of aromatic polyamide was effective to develop the biocompatibility, which would be due to the surface property covered with polar PC units. [doi:10.1295/polymj.PJ2006253]

KEY WORDS Diamine Monomer / Aromatic Polyamide / Phosphorylcholine / Polycondensation / Copolymer / Surface Property / Biocompatibility /

The phosphorylcholine (PC) group is an important component of phospholipid molecules in cell membranes,[1] and it is well known that synthetic polymer materials containing PC group have been shown to exhibit excellent biocompatibility including blood compatibility.[2–8] It has been well known that the copolymers consisted of 2-methacryloyloxyethyl phosphorylcholine (MPC) unit, so-called MPC polymers, have been reported as ideal blood compatible and biocompatible materials.[5–11] The MPC was designed based on the inspiration from the outer surface of the cell membrane, *i.e.*, the biomembrane, which is mainly constructed of natural phospholipid molecules. The MPC polymers were synthesized by a conventional radical copolymerization of MPC with various other alkyl methacrylates such as butyl methacrylate.[8,9] Furthermore, the blood compatibility of MPC polymers was investigated in detail, and the applications to medical devices and other uses have been greatly advanced in these years.[12–19] For example, when the surface of MPC polymers was contacted with blood components, the number of platelets adhered on the polymer surface was effectively decreased with an increase of the MPC unit in the copolymers. In particular, the adhesion and the activation of platelets were completely suppressed on the surface of the MPC polymers when the composition of MPC unit was above 30 mol %, and the amount of plasma proteins adsorbed on the surface of MPC polymer film was clearly decreased.[8] Since PC group consists of a zwitterions, MPC polymers behave as an entire neutrality molecule and exhibited no interaction with specific ions in the living organism. Therefore, MPC polymers are very useful polymeric biomaterials not only in the biomedical field but also in the tissue engineering and bioengineering fields. Actually, MPC polymers are now widely applied for development of artificial organs[13–19] and drag delivery systems.[20–22]

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However, most of MPC polymers do not possess the enough durability to several solvents such as alcohols, the thermal stability and the mechanical strength, which were derived from the polymethacrylate type main chain structure. Then, if these physical properties of MPC polymers were improved satisfactorily while maintaining the excellent biocompatibility, novel biocompatible polymer materials could be developed. The purpose of this study is the syntheses of novel polymer compounds, which exhibit the excellent biocompatibility with the processability, the durability to solvents, the thermal stability and the mechanical strength, in order to create the practical biomaterials for several applications.

In the present study, the synthesis of a novel aromatic diamine monomer with PC group was carried out to prepare the aromatic polyamides containing PC group, the backbone component of which was durable as compared with that of MPC polymers. In general, the aromatic polyamides are insoluble in many solvents, thermally stable up to 300 °C and mechanically tough materials,[23,24] which are used in a lot of electric devices and motorcars. Therefore, as the second subject of this paper, we attempted to prepare aromatic polyamides containing PC group in the side chain by using a diamine monomer with PC unit, which would lead to new biocompatible polyamides derived from the characteristics of PC group. In addition, the physical properties such as solubility, thermal property, biological function as blood compatibility, and surface property of the obtained polyamides were investigated to reveal the possibility of a durable biocompatible polymer material.

EXPERIMENTAL

Materials

Tetrahydrofuran (THF) was refluxed with sodium and benzophenone until the color turned blue to remove moisture, and purified by distillation. Triethylamine and acetonitrile was freshly distilled over calcium hydride. Trimethylamine was purified by distillation from its aqueous solution. 2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP) was purchased from Tokyo Kasei Chemical Co. and used as received. 4,4′-Diamino-3,3′-dimethyldiphenylmethane and isophthaloyl chloride was purchased from Tokyo Kasei Chemical Co. and purified by recrystallization from chloroform and hexane, respectively. Other chemicals were used without further purification.

*Synthesis of 2-Hydroxyethyl 3,5-dinitrobenzoate **(1)***

Under an argon atmosphere, a solution of 10.0 g of 3,5-dinitrobenzoyl chloride (43.4 mmol) in 150 mL of THF was gradually added to a solution of 24.0 mL of ethylene glycol (430 mmol) and 60 mL of triethylamine dissolved in 340 mL of THF at 0 °C. The reaction mixture was stirred at room temperature for overnight, and then it was poured into an excess amount of distilled water. The mixture was extracted with chloroform, and the organic layer was dried over sodium sulfate. After the solvent was evaporated under reduced pressure, the product was purified by column chromatography on silica gel with hexane/ethyl acetate (1/2 by vol.) to give **1** as a yellow powder. Yield: 8.95 g (80.6%).

^{1}H NMR, δ (400 MHz, $CDCl_3$, ppm): 1.92 (1H, t, J = 5.61 Hz), 3.98 (2H, m), 4.54 (2H, m), 9.13 (2H, d, J = 2.20 Hz), 9.18 (1H, t, J = 2.20 Hz).

IR, ν (KBr, cm^{-1}): 3222 (–OH), 3045, 2879 (C–H), 1722 (C=O), 1627, 1595 ($-NO_2$), 1541 (C=C), 1334, 1078, 844, 723.

*Synthesis of 2-[2-(3,5-Dinitrophenylcarbonyloxy)ethyl]-2-oxo-1,3,2-dioxaphospholane **(2)***

Under an argon atmosphere, 2.70 mL of 2-chloro-2-oxo-1,3,2-dioxaphospholane (29.3 mmol) was gradually added to a solution of 5.00 g of **1** (19.5 mmol) and 3 mL of triethylamine dissolved in 90 mL of THF at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was poured into an excess amount of distilled water and then extracted with chloroform. The obtained organic layer was dried over sodium sulfate, and the solvent was evaporated under reduced pressure to obtain **2** as a pink powder. Yield: 6.01 g (85.0%).

^{1}H NMR, δ (400 MHz, $CDCl_3$, ppm): 4.34–4.50 (6H, m), 4.61 (2H, m), 9.17 (1H, t, J = 1.96 Hz), 9.20 (2H, d, J = 2.20 Hz).

IR, ν (KBr, cm^{-1}): 3107, 2974 (C–H), 1720 (C=O), 1587 ($-NO_2$), 1550 (C=C), 1360, 1290 (P=O), 1164, 1060, 931, 721.

*Synthesis of 2-(3,5-Dinitrophenylcarbonyloxy)ethyl phosphorylcholine **(3)***

Under an argon atmosphere, 2.02 mL of trimethylamine (22.4 mmol) was added to a solution of 4.05 g of **2** (11.2 mmol) in 60 mL of acetonitrile at −30 °C, then the reaction vessel was sealed with a glass cap. After stirring at 60 °C for overnight, the reaction mixture was evaporated, and the obtained product was purified by recrystallization from acetonitrile to give **3** as a pink powder. Yield: 4.59 g (97.4%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 3.13 (9H, s), 3.51 (2H, d, J = 4.64 Hz), 4.02 (2H, m), 4.06 (2H, m), 4.51 (2H, t, J = 4.64 Hz), 8.96 (2H, d, J = 2.20 Hz), 9.06 (1H, t, J = 2.20 Hz).

IR, ν (KBr, cm^{-1}): 2893 (C–H), 1718 (C=O), 1602 ($-NO_2$), 1535 (C=C), 1353, 1230 (P=O), 1076 (N–CH_3), 856, 773, 731.

*Synthesis of 2-(3,5-Diaminophenylcarbonyloxy)ethyl phosphorylcholine (**DAPC**)*

5% Pd on charcoal powder (0.25 g, 0.10 mmol by Pd) was suspended in a solution of 2.50 g of **3** (5.95 mmol) dissolved in 60 mL of ethanol. The mixture was degassed under reduced pressure at −78 °C, and the vessel was filled with hydrogen gas. After stirring at room temperature for overnight, the Pd on charcoal was filtered off, and the solvent was distilled off under reduced pressure. Then, the product was purified by recrystallization from ethanol to give **DAPC** as a yellow powder. Yield: 2.15 g (92.1%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 3.15 (9H, s), 3.33 (4H, bs), 3.53 (2H, t, J = 4.64 Hz), 4.00 (2H, m), 4.10 (2H, m), 4.43 (2H, t, J = 4.64 Hz), 7.78 (1H, s), 7.82 (1H, s), 8.03 (1H, s).

IR, ν (KBr, cm^{-1}): 3199 (–NH_2), 2885 (C–H), 1718 (C=O), 1535 (C=C), 1477, 1228 (P=O), 1076, 966, 780, 733.

*Preparations of **CPAPC-1** Series*

The preparation of **CPAPC-1a** listed in Table I is given as a representative example.

Under an argon atmosphere, 0.50 g of **DAPC** (1.38 mmol), 2.81 g of 4,4′-diamino-3,3′-dimethyldiphenylmethane (**DA-1**, 12.4 mmol) and 2.80 g of isophthaloyl chloride (13.8 mmol) were mixed in a flask, and the vessel was cooled to −78 °C. After 20 mL of *N*-methyl-2-pyrrolidinone (NMP) was added slowly, the mixture was stirred for 5 h with increasing temperature from −78 °C to room temperature. Then, pouring the reaction mixture into excess methanol provided the brown precipitate, which was collected by filtration and purified by reprecipitation from its NMP solution to excess methanol. Finally, the product was dried *in vacuo* to give **CPAPC-1a** as a brown powder. Yield: 4.72 g (93.5%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): See Figure 1.

IR, ν (KBr, cm^{-1}): 3270 (N–H), 2860 (C–H), 1652 (C=O), 1508, 1436, 1301, 1228 (P=O), 1076 (N–CH_3), 1050, 813, 725, 696.

CPAPC-1b and **CPAPC-1c** were prepared by the similar procedures of the preparation of **CPAPC-1a** with changing the molar ratio of **DAPC** and **DA-1** as shown in Table I.

*Preparation of **PA-1***

Under an argon atmosphere, 1.00 g of **DA-1** (4.42 mmol) and 0.90 g of isophthaloyl chloride (4.42 mmol) were mixed in a flask, and the vessel was cooled to −78 °C. After 9.0 mL of NMP was added slowly, the mixture was stirred for 4 h with increasing temperature from −78 °C to room temperature. Then, pouring the reaction mixture into excess methanol provided the brown precipitate, which was collected by filtration and purified by reprecipitation from its NMP solution to excess methanol. Finally, the product was dried *in vacuo* to give **PA-1** as a light brown powder. Yield: 1.54 g (97.7%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 2.21 (6H, s), 3.91 (2H, s), 7.08 (2H, d, J = 1.95 Hz), 7.13 (2H, s), 7.28 (2H, d, J = 1.95 Hz), 7.63 (1H, t, J = 7.08 Hz), 8.14 (2H, d, J = 7.08 Hz), 8.55 (1H, s), 9.96 (2H, s).

IR, ν (KBr, cm^{-1}): 3282 (N–H), 2860 (C–H), 1662 (C=O), 1506, 1303, 1234, 815, 696.

*Synthesis of 2,2-Bis[4-(nitrophenyloxy)phenyl]propane (**4a**)*

To a solution of 5.00 g of 2,2-bis(4-hydroxyphenyl)propane (21.9 mmol) in 65 mL of dimethylsulfoxide (DMSO), 6.18 g of 4-fluoronitrobenzene (43.8 mmol) and 6.05 g of potassium carbonate (43.8 mmol) and were added. After the mixture was stirred at r.t. for overnight, the reaction mixture was poured into excess water to precipitate the product. Then, the product was purified by recrystallization with chloroform/hexane to afford **4a** as a pale yellow powder. Yield: 9.64 g (87.7%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 1.70 (6H, s), 7.11 (8H, m), 7.36 (4H, m), 8.25 (4H, m).

*Synthesis of 2,2-Bis[4-(aminophenyloxy)phenyl]propane (**DA-2**)*

5% Pd on charcoal powder (0.50 g, 0.21 mmol by Pd) was suspended in a solution of 5.00 g of **4a** (10.6 mmol) dissolved in 50 mL of ethanol and 50 mL of THF. The mixture was degassed under reduced pressure at −78 °C, and the vessel was filled with hydrogen gas at over 760 mmHg. After stirring at room temperature for overnight, the Pd on charcoal was filtered off, and the solvents were distilled off under reduced pressure. Then, the product was purified by recrystallization from ethanol to give **DA-2** as a pale yellow powder. Yield: 3.80 g (86.8%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 1.52 (6H, s), 5.03 (4H, bs), 6.53 (4H, m), 6.67 (8H, m), 7.05 (4H, m).

*Synthesis of 2,2-Bis[4-(nitrophenyloxy)phenyl]hexafluoropropane (**4b**)*

4b was prepared by the similar procedure of the preparation of **4a** by using 2,2-bis(4-hydroxyphenyl)-hexafluoropropane instead of 2,2-bis(4-hydroxyphenyl)propane. Yield: 91.1%.

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 7.25 (8H, m), 7.47 (4H, m), 8.28 (4H, m).

*Synthesis of 2,2-Bis[4-(aminophenyloxy)phenyl]hexafluoropropane (**DA-3**)*

DA-3 was prepared by the similar procedure of the

preparation of **DA-2** by using **4b** instead of **4a**. Yield: 77.2%.
^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 4.95 (4H, bs), 6.62 (4H, d, $J = 7.81$ Hz), 6.78 (4H, m), 6.88 (4H, m), 7.23 (4H, m).

*Preparations of **CPAPC-2** and **CPAPC-3** Series*

The preparations of **CPAPC-2a**, **CPAPC-2b**, **CPAPC-2c**, **CPAPC-3a**, **CPAPC-3b** and **CPAPC-3c** listed in Table II were carried out as the same procedure as the preparation of **CPAPC-1a** by using **DA-2** or **DA-3** instead of **DA-1** with changing the molar ratio of **DAPC** and **DA-2** or **DA-3**.
^{1}H NMR of **CPAPC-2a**, δ (400 MHz, DMSO-d_6, ppm): 1.65 (s, –CH_3), 3.11 (s, N–CH_3), 3.60 (m, –CH_2–), 4.24 (m, –CH_2–), 4.52 (m, –CH_2–), 6.89 (m, –Ph–), 6.99 (m, –Ph–), 7.21 (m, –Ph–), 7.64 (m, –Ph–), 7.81 (m, –Ph–), 7.95 (m, –Ph–), 8.13 (m, –Ph–), 8.39 (m, –Ph–), 8.49 (m, –Ph–), 8.56 (m, –Ph–), 8.74 (m, –Ph–), 8.87 (m, –Ph–), 10.4 (s, –NH–).
^{1}H NMR of **CPAPC-3a**, δ (400 MHz, DMSO-d_6, ppm): 3.12 (s, N–CH_3), 3.58 (m, –CH_2–), 4.20 (m, –CH_2–), 4.51 (m, –CH_2–), 7.05 (m, –Ph–), 7.34 (m, –Ph–), 7.67 (m, –Ph–), 7.88 (m, –Ph–), 7.96 (m, –Ph–), 8.15 (m, –Ph–), 8.39 (m, –Ph–), 8.48 (m, –Ph–), 8.57 (m, –Ph–), 8.73 (m, –Ph–), 8.87 (m, –Ph–), 10.51 (s, –NH–).

*Preparation of **PA-2** and **PA-3***

The preparations of **PA-2** and **PA-3** were carried out as the same procedure as the preparation of **PA-1** by using **DA-2** or **DA-3** instead of **DA-1**.
^{1}H NMR of **PA-2**, δ (400 MHz, DMSO-d_6, ppm): 1.62 (6H, s), 6.89 (4H, d, $J = 8.54$ Hz), 7.01 (4H, d, $J = 8.79$ Hz), 7.21 (4H, d, $J = 8.55$ Hz), 7.66 (1H, t, $J = 7.82$ Hz), 7.79 (4H, d, $J = 9.03$ Hz), 8.12 (2H, d, $J = 7.32$ Hz), 8.52 (1H, m), 10.4 (2H, s).
^{1}H NMR of **PA-3**, δ (400 MHz, DMSO-d_6, ppm): 7.05 (4H, d, $J = 8.30$ Hz), 7.13 (4H, d, $J = 9.03$ Hz), 7.33 (5H, m), 7.87 (4H, d, $J = 8.30$ Hz), 8.14 (2H, m), 8.55 (1H, m), 8.52 (1H, m), 10.4 (2H, s).

Characterizations

^{1}H NMR spectra were conducted with a JEOL NM-TH5SK 400 MHz FT-NMR spectrometer, and the chemical shifts were estimated in ppm units with tetramethylsilane (TMS) as an internal standard. Infrared (IR) spectra were recorded with a Shimadzu FT/IR-8400 spectrometer. The molecular weights of polymers were estimated by Tosoh gel permeation chromatography (GPC) system equipped with a pump of CCPD, three columns of TSK gels Multipore HXL-M, a column oven of CO-8010 and RI detector of RI-8010 in DMF eluent at 40 °C. Average molecular weights were evaluated by polystyrene standards. Differential scanning calorimetry (DSC) was carried out on a Seiko Instruments DSC-6200 under a nitrogen flow rate of 30 mL/min and a heating rate of 10 °C/min.

Surface Characterizations of Polymers

Circular pieces of poly(ethylene terephthalate) (PET) plates (diameter: 14 mm, thickness: 0.2 mm) were dipped in 0.5 wt % polymer solutions in NMP for 30 min, and the obtained polymer-coated PET plates were dried slowly at 60 °C for 2 h. This procedure was repeated three times, and then they were dried *in vacuo*. Contact angles of water on the surfaces of the polymer-coated PET plates were measured using an Erma contact-angle microscope at room temperature. On the other hand, X-ray photoelectron spectroscopy (XPS) was conducted on the surface of the polymer-coated PET plates by using ULVAC-PHI Quantum 2000 XPS apparatus. The take-off angle of the photoelectron was 45 degree.

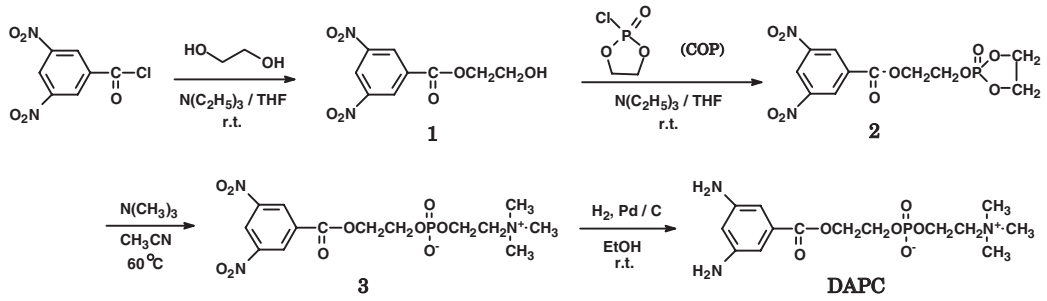
Evaluation of Blood Compatibility

Whole blood was collected from healthy donors. In a polyethylene disposable syringe containing 3 mL of a 3.8 wt % aqueous sodium citrate solution, 30 mL of fresh blood was collected. The citrated whole blood was immediately centrifuged for 15 min at 1200 rpm to obtain citrated platelet-rich plasma (PRP).

The polymer-coated PET plates were contacted with phosphate-buffered solution (PBS, pH = 7.4) at r.t. for overnight to equilibrate the surface, then human whole blood or PRP was poured onto the plates and incubated for 60 min at 37 °C. After the incubation, whole blood and PRP were removed with an aspirator, and the plates were rinsed three times with PBS, and then 0.7 mL of 2.5 vol % glutaraldehyde in PBS was poured onto each plate, and the materials were maintained at room temperature for 2 h in order to fix the blood components on the plates. After the fixation, it had been rinsed five times with distilled water, and then the plate was freeze-dried. The surfaces of the polymer-coated plates were observed with a scanning electron microscope (SEM) by using JEOL JSM-5200 after a gold-sputtering treatment.

Measurement of the Amount of Platelets Adsorbed on the Polymer Surface

After incubating the polymer-coated plates in the above procedure, PRP was removed and the plates were washed 3 times with PBS and transferred into 0.5 wt % aqueous solution of polyethylene glycol mono-*p*-isooctylphenyl ether (Triton X100) to elute the adsorbed platelets. The concentration of platelets in the Triton X100 solution was counted by a lactate



Scheme 1. Preparation of aromatic diamine monomer containing PC group (**DAPC**).

dehydrogenase (LHD) assay using an LDH-Cytotoxic Test Kit (Wako Chemicals, Osaka, Japan). The concentration of platelets in PRP was determined with a Coulter counter (MULTISIZER II, Beckman Coulter, CA) and the number of platelets that adhered on the polymer films was estimated based on the absorbance of the PRP-diluted system.

RESULTS AND DISCUSSION

Synthesis of a Diamine Monomer Containing PC Unit

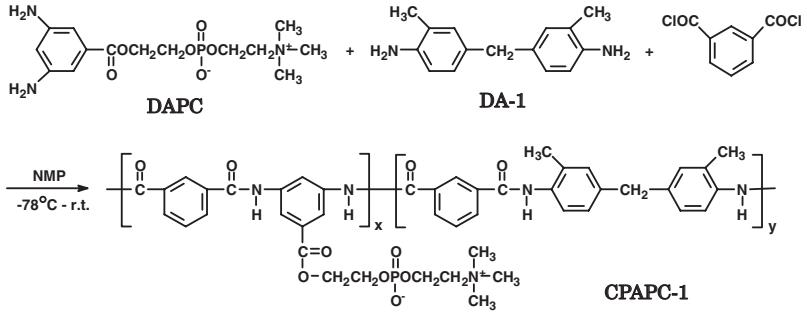
The synthetic route of the novel aromatic diamine monomer containing PC group, 2-(3,5-diaminophenylcarbonyloxy)ethyl phosphorylcholine (**DAPC**), is outlined in Scheme 1. At first, the reaction of ethylene glycol with 3,5-dinitrobenzoyl chloride yielded a dinitro compound (**1**), which was obtained in good yield by using excess amount of ethylene glycol to 3,5-dinitrobenzoyl chloride. Next, the reaction of **1** with 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP) yielded a dinitro-phospholane compound (**2**), which was an intermediate of phosphorylcholine compound. The purification of **2** by a silica-gel column chromatography was difficult because it was easily hydrolyzed. However, the extraction of the crude products with chloroform followed by washing with distilled water gave the pure product of **2**. Next, **DAPC** was obtained by opening the cyclic phosphoric ester moiety of **2** with trimethylamine, followed by the reduction of the nitro groups of **3** with H_2 catalyzed by Pd. The chemical structure of **DAPC** was confirmed by IR and ^{1}H NMR spectra. In the IR spectra of **DAPC**, a broad adsorption peak in the region of 3400–3150 cm^{-1} was observed as the amino groups, and the PC group was identified by the peak at 1228 and 1076 cm^{-1}. This novel aromatic diamine compound, **DAPC**, would be a useful monomer for the syntheses of various aromatic polymers, such as polyamides, polyimides, polyureas and poly(urethane-urea)s that have PC group in the side chain.

Preparation of Polyamides Containing PC Unit

In the case of MPC polymer, which was obtained by copolymerization of MPC monomer and butyl methacrylate, the adhesion and the activation of platelets were suppressed on the surface of the MPC polymer when the composition of MPC unit was above 30 mol %. Then, in this case, the synthesis of copolyamide was carried out, where the polycondensation of **DAPC** with acid chloride by coexisting another diamine comonomer. As the comonomer, 4,4′-diamino-3,3′-dimethyldiphenylmethane (**DA-1**) was used to make the polymer soluble in some solvents. Namely, the aromatic copolyamides containing PC group (**CPAPC-1**) were prepared by the low-temperature polycondensation of **DAPC** and **DA-1** with isophthaloyl chloride in NMP, as shown in Scheme 2. On the other hand, a homopolyamide (**PA-1**) without PC group was prepared from **DA-1** and isophthaloyl chloride to compare the physical properties with **CPAPC-1**.

Table I summarizes the results of polymerizations. Three copolyamides with different contents of PC unit were prepared by changing the amount of **DAPC** in the feed of copolymerization. The number-average molecular weights (M_n) of the obtained polyamides were in the ranges of 5×10^3–2×10^4. Figure 1 shows the ^{1}H NMR spectra of **CPAPC-1a**. The compositions of PC unit in **CPAPC-1** series were determined from the ratio of the peak intensities of the ammonium proton (3.08 ppm) of PC unit and methyl proton (3.89 ppm) of 3,3′-dimethyldiphenylmethane unit. In the IR spectra, the absorption peaks of the amide and ester groups were observed at 3270 cm^{-1} and 1652 cm^{-1}, respectively, and the PC group was identified from the peak at 1228 cm^{-1}.

On the other hand, to obtain the higher molecular weight polyamides with PC unit, different polyamides containing Bisphenol A components were prepared from other comonomers, 2,2-bis[4-(aminophenyloxy)-phenyl]propane (**DA-2**) and 2,2-bis[4-(aminophenyloxy)phenyl]hexafluoropropane (**DA-3**), which were expected to possess the higher reactivity than **DA-1**. These two comonomers, **DA-2** and **DA-3**, were synthesized in high yields by the procedure shown in Scheme 3 and the experimental section. Then, the



Scheme 2. Preparation of polyamide containing PC group (**CPAPC-1**).

Table I. Polymerization results of **CPAPC-1** series

Code	Composition (mol %)		Yield (%)	M_n[b] ($\times 10^4$)	M_w/M_n[b]	T_g[c] (°C)
	DAPC/DA-1	x/y in copolymer[a]				
CPAPC-1a	10/90	5/95	94	1.98	5.57	210
CPAPC-1b	20/80	12/88	73	1.00	5.24	186
CPAPC-1c	30/70	21/79	59	0.53	4.36	175
PA-1	0/100	0/100	98	2.02	3.06	187

a) Calculated from the ratio of peak intensities of ^{1}H NMR spectra. b) Number-average and weight-average molecular weight (M_n and M_w) were determined by GPC based on polystyrene standards. c) Determined by DSC measurement at a heating rate of 10 °C/min.

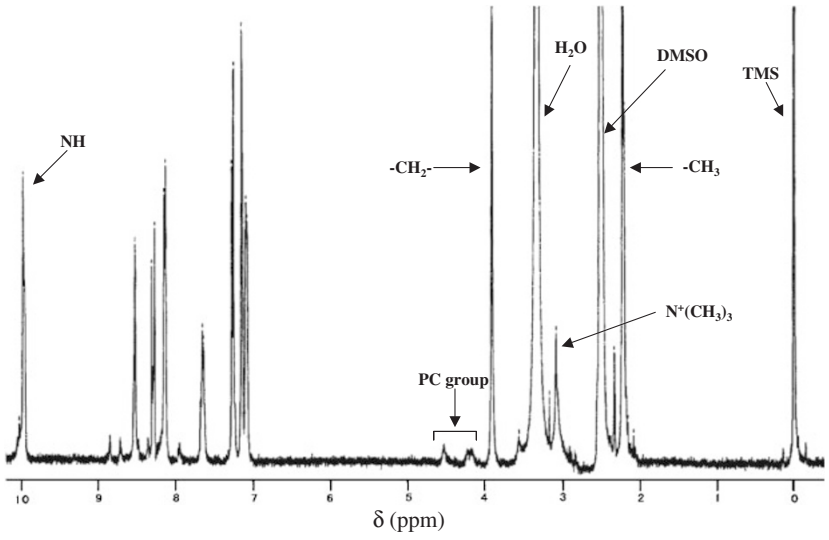
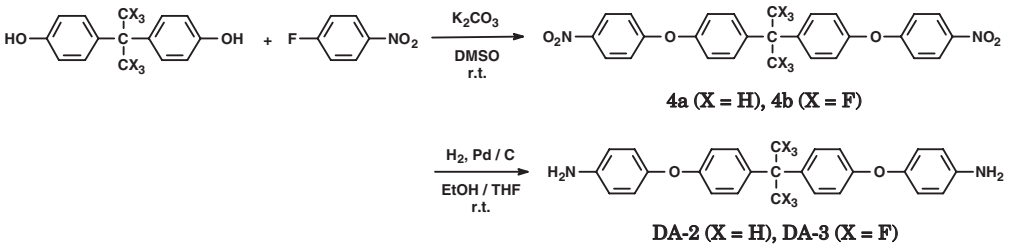


Figure 1. ^{1}H NMR spectrum of **CPAPC-1a**.

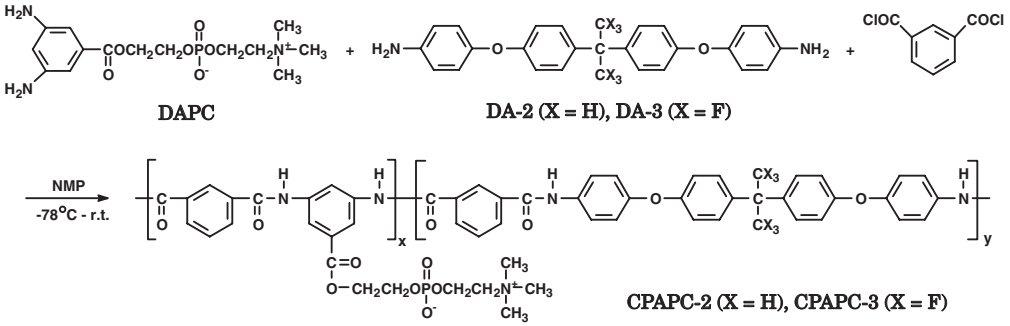
polycondensation of **DAPC** and **DA-2** or **DA-3** with isophthaloyl chloride yielded the desired copolyamides containing PC unit, **CPAPC-2** and **CPAPC-3**, as shown in Scheme 4. The results of polymerizations are summarized in Table II, where the homopolyamides, **PA-2** and **PA-3**, were polymerized of isophthaloyl chloride with **DA-2** and **DA-3**, respectively.

As seen in Tables I and II, the molecular weights of **CPAPC-2** and **CPAPC-3** series were higher than those of **CPAPC-1** series, which would be due to the higher reactivity of **DA-2** and **DA-3** than **DA-1**. However, in all the copolymers, the composition of PC component (x unit of the copolymers shown in Schemes 2 and 4) were lower than the molar composition of **DAPC** against **DA-1**, **DA-2** or **DA-3** in each polymerization, and the polymer yields decreased as the PC content increased. Therefore, the reactivity of **DAPC** in such a polycondensation would be relatively low, and the highly hygroscopic property of **DAPC** would disturb the polycondensation using a moisture-sensitive acid chloride. The molecular design of PC diamine monomer is now in progress to develop the higher reactivity.

The obtained **CPAPC** series exhibited a good solu-



Scheme 3. Preparation of Bisphenol A type diamine monomers (**DA-2** and **DA-3**).



Scheme 4. Preparation of polyamides containing PC group (**CPAPC-2** and **CPAPC-3**).

Table II. Polymerization results of **CPAPC-2** and **CPAPC-3** series

Code	Composition (mol %)		Yield (%)	M_n[b] ($\times 10^4$)	M_w/M_n[b]	T_g[c] (°C)
	DAPC/DA-2 or DA-3	x/y in copolymer[a]				
CPAPC-2a	10/90	4/96	92	8.06	2.84	212
CPAPC-2b	20/80	7/93	82	3.71	4.46	210
CPAPC-2c	30/70	17/83	74	1.76	5.19	191
PA-2	0/100	0/100	99	10.8	2.86	215
CPAPC-3a	10/90	7/93	96	3.48	4.65	215
CPAPC-3b	20/80	10/90	80	2.68	5.98	210
CPAPC-3c	30/70	17/83	63	1.79	4.12	185
PA-3	0/100	0/100	97	19.3	1.92	212

[a]Calculated from the ratio of peak intensities of ^{1}H NMR spectra. [b]Number-average and weight-average molecular weight (M_n and M_w) were determined by GPC based on polystyrene standards. [c]Determined by DSC measurement at a heating rate of 10 °C/min.

bility in aprotic polar solvents such as NMP, DMF and DMSO at room temperature, whereas it was insoluble in several solvents such as methanol, ethanol, acetone, tetrahydrofuran and water. This solubility in the specific solvents is advantageous in the processing for medical devices, and the insolubility in other solvents enables the material durable to these solvents. The thermal property of **CPAPC** was evaluated by differential scanning calorimetry (DSC). As a result, **CPAPC** was a glassy polymer, the glass transition temperature (T_g) of which was in the range of 175–215 °C, as shown in Tables I and II. Such a thermal stability of **CPAPC** would be sufficient for the applications to biomaterials and medical devices, especially in the sterilization process.

Therefore, as compared with MPC polymer, it was found that the physical characteristics of **CPAPC** series were durable to common organic solvents such as alcohols and exhibited the higher softening temperature, whereas the MPC polymer were easily soluble in common organic solvents and softened at T_g of below 100 °C. In addition, the mechanical properties of **CPAPC** and MPC polymer films were quite different, where Young's moduli of **CPAPC-2a**, **PA-2** and MPC polymer were 248, 642 and 15.2 MPa, respectively. These physical properties of **CPAPC** series obviously depended on the aromatic polyamide backbone.

Surface Property of CPAPC

In order to clarify the effect of PC group on the surface of **CPAPC**, the surface analysis of the polymer

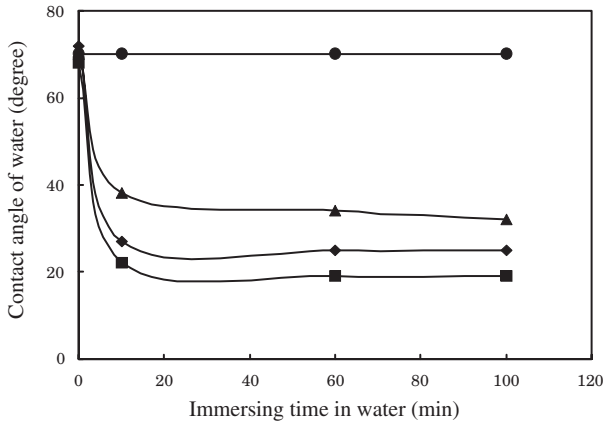


Figure 2. Effect of immersing the polymer coating films in water on the contact angle of water of the film surfaces. ●: **PA-1**, ▲: **CPAPC-1a**, ◆: **CPAPC-1b**, ■: **CPAPC-1c**

films was performed. Figure 2 shows the time-course of contact angle of water on the surface of polymer films after the films were immersed in water. In the case of **PA-1** without PC unit, the contact angle didn't change before and after immersed in water. On the contrary, the contact angles of the **CPAPC-1** films were significantly decreased after the films were immersed in water. Thus, the surface of the polymer membrane became hydrophilic by the introduction of PC unit and by contacting the surface with water. This result indicated that **CPAPC-1** membrane surfaces were largely swelled by water and the PC group of **CPAPC-1** was oriented to the water-side. The similar tendency was observed for **CPAPC-2** and **CPAPC-3** series.

Furthermore, the surface chemical structure of **CPAPC-1b** film before and after immersed in water for a day was analyzed by X-ray photoelectron spectroscopy (XPS), as shown in Figure 3. The XPS signals observed at 284, 288, 291, and 293 eV in C_{1s} region were attributed to carbon in hydrocarbons ($-CH_3$, $-CH_2-$), the ether bond (–C–O–C–), the carbonyl group [–C(=O)–], and aromatic carbon, respectively. The peaks which were observed at 531, 536, 399, 406, and 133 eV were attributed to the carbonyl group [–C(=O)–], oxygen of the ether bond (–O–), the nitrogen atoms in the amide bond (–NH–), the ammonium group ($-N^+(CH_3)_3-$), and phosphorus of the phosphate group, respectively. Therefore, the PC unit seems to be concentrated at the **CPAPC** film surface after immersed in water for a day, because the peaks derived from PC unit were obviously increased after contact with water. The chain rearrangement of the copolymer film surface would result in such a change of elemental distribution. In addition, it is expected that **CPAPC** series show the blood compatibility, because the film surface after immersed in water is very similar to a biomembrane surface which is covered with the polar group of phospholipid.

Biocompatibility of CPAPC

The thin films of **CPAPC-1a**, **CPAPC-1c** and **PA-1** were prepared by coating of the NMP solutions of the polymers on poly(ethylene terephthalate) (PET) plates, and the blood compatibility of the coating films was evaluated by contacting the plates with a human blood. Figure 4 shows SEM pictures of the **PA-1**, **CPAPC-1a** and **CPAPC-1c** film surfaces after contact with human whole blood and platelet-rich plasma (PRP) for 60 min. The numerous adherent blood cells and human platelets on the **PA-1** film surface were observed as large aggregates. In contrast, the blood cells and platelets were significantly suppressed on the **CPAPC-1a** and **CPAPC-1c** film surfaces as shown in Figure 4. These results clearly indicated that **CPAPC-1** series exhibited the excellent blood compatibility and PC unit in the copolyamide was an important element to develop the blood compatibility. Furthermore, the composition of the PC unit was a dominant factor in the reduction of the blood cell and platelet adhesion, which was revealed from the result that the number of adhered platelets was much decreased on **CPAPC-1c** film rather than **CPAPC-1a** film. These results would be due to the PC unit located at the surface of the polymer film, where the surface is covered with PC unit, and the interaction between the polymer surface and blood ingredients such as cells and platelets is very weak.

On the other hand, the homogeneous coating films without defects on PET plates were prepared from

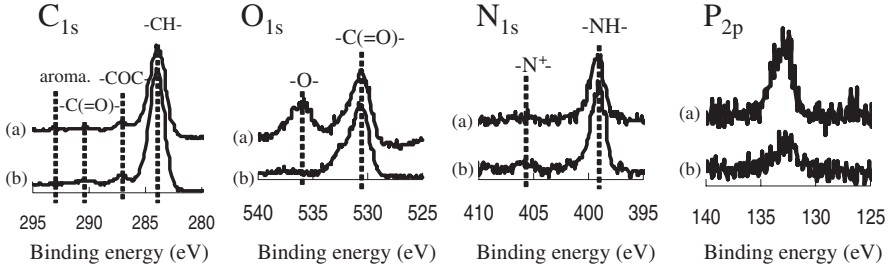


Figure 3. XPS spectra **CPAPC-1b** film surfaces before (b) and after (a) immersed in water for a day.

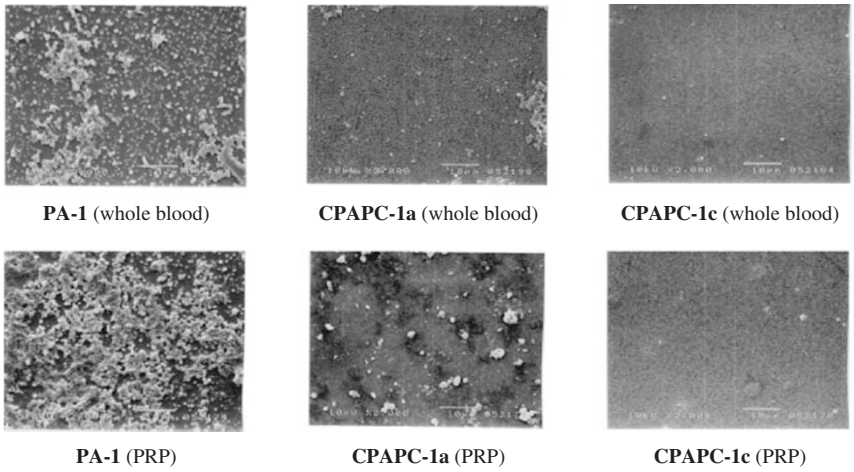


Figure 4. SEM pictures of polymer film surfaces after contact with human whole blood or PRP for 1 h (×1,000).

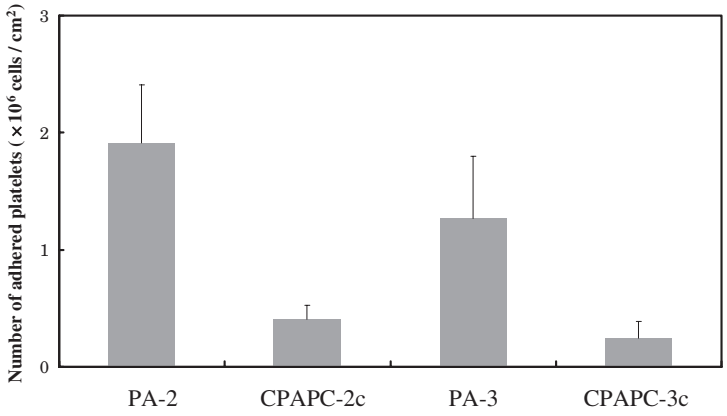


Figure 5. Number of adherent platelet on the polymer surfaces after contact with PRP for 1 h. Each bar represents mean ± S.E. for 4 experiments.

CPAPC-2 and **CPAPC-3** series, whereas the coating films from **CPAPC-1** series had some defects. Such a difference of the film forming ability of these PC copolyamides would be due to the difference of their molecular weights. Therefore, by using the coating films prepared from **CPAPC-2c** and **CPAPC-3c**, the platelets adhesion test was carried out as compared with **PA-2** and **PA-3** films. In the case of **CPAPC-1**, the platelets were remarkably adhered on the defects of coating film, thus, the quantitative results of the adhered platelets on **CPAPC-1** films were ambiguous. Figure 5 shows the number of platelets that adhered to polymer coating films after contact with PRP for 1 h. The number of adhered platelets on **CPAPC-2c**, **CPAPC-3c**, **PA-2** and **PA-3** films were 0.41, 0.25, 1.91 and 1.27 ($\times 10^6$ cells/cm^2), respectively. It was obvious that **CPAPC-2c** and **CPAPC-3c** films exhibited fewer adhered platelets than **PA-2** and **PA-3** films. These results indicated that the introduction of PC groups in these polyamides were very effective to enhance the biocompatibility, and the PC content of 17 mol % was enough to reduce the adhered platelets on the polymer surfaces. However, such a reduction of the adhered platelets on the MPC polymer film was more effective than the **CPAPC** series, where the number of adhered platelets on the MPC polymer surface was less than 10^5 cells/cm^2 in the same condition. Probably, the density of PC group on the surface of **CPAPC** series would be lower than that of MPC polymer, because of the rigidity of main chain structure of **CPAPC**.

CONCLUSION

Synthesis of a novel aromatic diamine compound containing PC group, **DAPC**, was carried out to prepare aromatic polyamides with PC moiety. The polycondensation of **DAPC** with other diamine compounds and isophthaloyl chloride gave the desired copolyamides with different PC contents. The obtained copolyamides were glassy polymers with high glass transition temperatures over 150 °C, and soluble in aprotic polar solvents but insoluble in many other solvents, the properties of which were derived from

the main chain rigid structure. Regarding the effects of PC group of these copolyamides on the blood compatibility, the introduction of such a polar group of phospholipid was effective to appear the blood compatibility even in the aromatic polyamide system. From the results of surface analyses of the copolyamide films, it was found that the PC group was easily rearranged by the immersion in water. Consequently, it is expected that the aromatic copolyamides containing PC group will be useful polymeric biomaterials to develop a new generation of biomedical devices, because of the different solubility, the higher thermal stability and the similar biocompatibility as compared to the MPC polymer.

However, the self-standing films were difficult to prepare from the copolyamides described in this paper except **CPAPC-2a**, thus, the mechanical property of the copolyamides could not be estimated in detail. To obtain the self-standing films, the main chain structure and the molecular weight of the copolyamides must be developed, which are now in progress.

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REFERENCES

1. J. A. Hayward and D. Chapman, *Biomaterials*, **5**, 135 (1984).
2. K. Sugiyama, K. Ohga, H. Aoki, and N. Amaya, *Macromol. Chem. Phys.*, **196**, 1907 (1995).
3. T. Ohishi, T. Fukuda, H. Uchiyama, F. Kondou, H. Ohe, and H. Tsutsumi, *Polymer*, **38**, 3109 (1997).
4. Y. K. Gong, L. Luo, A. Petit, D. J. Zukor, O. L. Huk, J. Antoniou, F. M. Winnik, and F. Mwale, *J. Biomed. Mater. Res., Part A*, **72**, 1 (2005).
5. K. Ishihara, R. Aragaki, T. Ueda, A. Watanabe, and N. Nakabayashi, *J. Biomed. Mater. Res.*, **24**, 1069 (1990).
6. K. Ishihara, N. P. Ziats, B. P. Tierney, N. Nakabayashi, and J. M. Anderson, *J. Biomed. Mater. Res.*, **25**, 1397 (1991).
7. T. Ueda, H. Oshida, K. Kurita, K. Ishihara, and N. Nakabayashi, *Polym. J.*, **24**, 1259 (1992).
8. K. Ishihara, T. Ueda, and N. Nakabayashi, *Polym. J.*, **22**, 355 (1990).
9. K. Ishihara, H. Oshida, T. Ueda, Y. Endo, A. Watanabe, and N. Nakabayashi, *J. Biomed. Mater. Res.*, **26**, 1543 (1992).
10. S. Sawada, Y. Iwasaki, N. Nakabayashi, and K. Ishihara, *J. Biomed. Mater. Res., Part A*, **79**, 476 (2006).
11. J. Patel, Y. Iwasaki, K. Ishihara, and J. Anderson, *J. Biomed. Mater. Res., Part A*, **73**, 359 (2005).
12. Y. Iwasaki, A. Mikami, K. Kurita, N. Yui, K. Ishihara, and N. Nakabayashi, *J. Biomed. Mater. Res.*, **36**, 508 (1997).
13. T. Uchiyama, J. Watanabe, and K. Ishihara, *J. Membr. Sci.*, **210**, 423 (2002).
14. T. Moro, Y. Takatori, K. Ishihara, T. Konno, Y. Takigawa, T. Matsushita, U.-I. Chung, K. Nakamura, and H. Kawaguchi, *Nat. Mater.*, **3**, 829 (2004).
15. H. Ueda, J. Watanabe, T. Konno, M. Takai, A. Saito, and K. Ishihara, *J. Biomed. Mater. Res., Part A*, **77**, 19 (2006).
16. S.-H. Ye, J. Watanabe, M. Takai, Y. Iwasaki, and K. Ishihara, *Biomaterials*, **27**, 1955 (2006).
17. T. Goda and K. Ishihara, *Expert Rev. Med. Devices*, **3**, 167 (2006).
18. Y. Okajima, S. Saika, and M. Sawa, *J. Cataract. Refract. Surg.*, **32**, 666 (2006).
19. T. A. Snyder, H. Tsukui, S. Kihara, T. Akimoto, K. N. Litwak, M. V. Kameneva, K. Yamazaki, and W. R. Wagner, *J. Biomed. Mater. Res., Part A*, **81**, 85 (2007).
20. T. Konno, J. Watanabe, and K. Ishihara, *J. Biomed. Mater. Res., Part A*, **65**, 210 (2003).
21. K. W. Nam, J. Watanabe, and K. Ishihara, *Eur. J. Pharmacol.*, **23**, 261 (2004).
22. N. Chiba, M. Ueda, T. Shimada, H. Jinno, J. Watanabe, K. Ishihara, and M. Kitajima, *Eur. Sur. Res.*, **39**, 23 (2007).
23. P. E. Cassidy, "Thermal Stable Polymer," Dekker, New York, 1980, Chapter 4.
24. J. Preston, *Encycl. Polym. Sci. Eng.*, **11**, 381 (1988).