*Transactions of the Materials Research Society of Japan* 30[4] 1037-1040 (2005)

# Preparation and Biocompatibility of Aromatic Polyamides Containing Phosphorylcholine Moiety

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The phosphorylcholine (PC) group is an important component of cell membrane, and it is well known that 2-methacryloxyethyl phosphorylcholine (MPC) polymer has been reported as an ideal biocompatible material. In this study, in order to develop the durability of MPC polymer, the synthesis of a novel aromatic diamine compound containing PC group was carried out to prepare aromatic polyamides with PC moiety. The polycondensation of the diamine monomer containing PC group and 4,4'-diamino-3,3'-dimethyldiphenylmethane with isophthaloyl chloride gave the desired copolyamides with different PC contents. The obtained copolyamides exhibited the excellent biocompatibility, even though the PC content was around 10 mol%. From the results of contact angle of water and XPS analysis on the surface of the copolyamide films, it was found that PC units were concentrated on the surface after the treatment with water. Therefore, the biocompatibility of the copolyamide containing PC group would be due to the surface property covered with PC units.

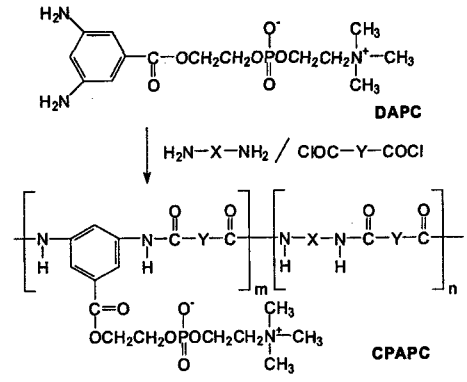
**Key words:** biomaterial / biocompatibility / MPC polymer / phosphorylcholine / aromatic polyamide

## 1. INTRODUCTION

Recently, various polymeric materials are widely used in biomedical fields such as artificial organs and medical devices[1]. However, traditional polymeric materials do not possess an enough biocompatibility including blood compatibility, therefore, the infusion of an anticoagulant is required to repress the clot formation. Moreover, the side effect of anticoagulant has been concerned when the employment of the materials extends over a long period of contact. Thus, biocompatibility of polymeric materials is the most important factor in the development of biomedical devices[1].

According to the fact that the phospholipids are important components of biomembrane, a new monomer compound, 2-methacryloyloxyethyl phosphorylcholine (MPC), was designed based on the inspiration from the chemical structure of phospholipid polar group in biomembrane[2)-3)]. The MPC could be copolymerized with various alkyl methacrylates such as n-butyl methacrylate or styrene. In addition, when the polymer was contacted with human platelet-rich plasma, the MPC copolymers suppressed the adhesion and the activation of blood cell even in the absence of an anticoagulant. Therefore, MPC copolymers are now widely applied in the biomaterial fields, such as artificial organs, contact lens, and cosmetics[4)-5)]. However, most of MPC copolymers do not have an enough durability to the solvents, the heat resistance and the mechanical strength, because of the flexible main chain structure of MPC copolymers. Then, if these physical properties of MPC copolymers are improved satisfactorily while maintaining the excellent biocompatibility, a novel biocompatible polymer material could be developed.

In the present study, the synthesis of a novel aromatic diamine monomer with PC group (DAPC) was selected to prepare the aromatic polyamide containing PC group (CPAPC), as shown in Scheme 1, the backbone component of which was durable as compared with that of MPC copolymers. Furthermore, the general properties such as solubility, thermal property, blood compatibility, and surface property of CPAPC were investigated to reveal the possibility of a new stable biocompatible polymer material.

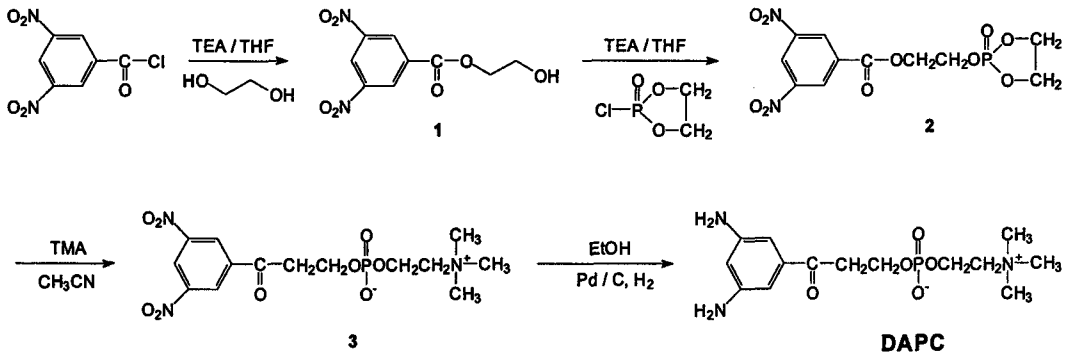



Scheme 1 Copolymerization using DAPC monomer.

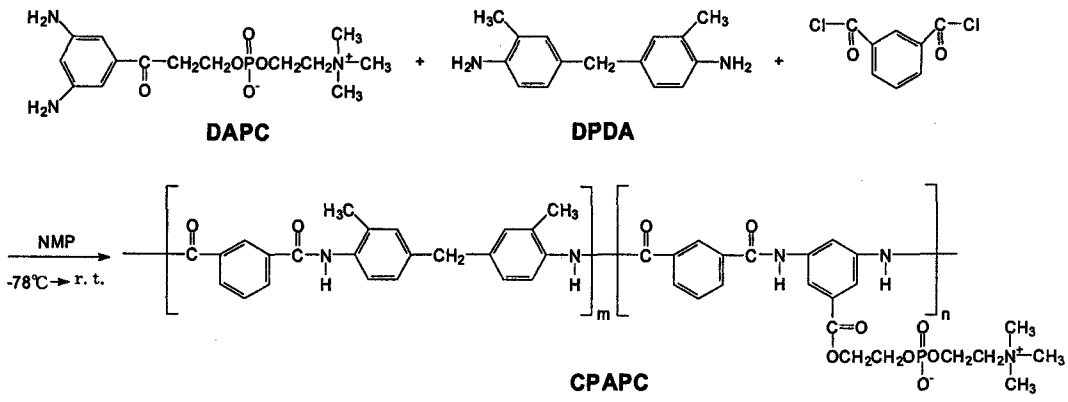
## 2. RESULTS AND DISCUSSION

### 2.1 Preparation of DAPC monomer

The synthetic route of the novel diamine monomer containing PC group, 2-(3,5-diaminophenylcarbonyloxy)ethyl phosphorylcholine



Scheme 2 Preparation of DAPC monomer.



Scheme 3 Preparation of CPAPC using DAPC monomer.

(DAPC), are outlined in Scheme 2. 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 ethylene glycol with 10 equivalents 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**). The purification of **2** with column chromatography was difficult because it was easily hydrolyzed. However, the extraction with chloroform from the crude products and washing with distilled water gave the pure product of **2**. Next, the diamine monomer (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/C. The chemical structure of DAPC was confirmed by IR and $^1$H-NMR spectra. This new diamine compound, DAPC, would be useful for the synthesis of various aromatic polymers which has PC group in the side chain.

2.2 Preparation and characterizations of CPAPC

The preparation of an aromatic polyamide containing PC group (CPAPC) is shown in Scheme 3. CPAPC was prepared by the polycondensation of DAPC and 4,4'-diamino-3,3'-dimethyldiphenylmethane (DPDA) with isophthaloyl chloride in NMP. On the other hand, homo-polyamide (PA) without PC group was prepared from DPDA and isophthaloyl chloride. Table 1 summarizes the results of polymerizations. Three copolyamides with different contents of PC unit were prepared by changing the amount of DAPC in feed of the copolymerization.

Table 1 Polymerization results of CPAPC.

| Code | Composition (mol %) | | $Mn^{b)}$ | $Mw^{b)}$ |
|---|---|---|---|---|
| | DAPC/DPDA | m/n[a)] | ($\times 10^4$) | Mn |
| CPAPC-1 | 10/90 | 6.7/93.3 | 1.98 | 5.57 |
| CPAPC-2 | 20/90 | 12.2/87.8 | 1.00 | 5.24 |
| CPAPC-3 | 30/90 | 41.2/58.8 | 0.53 | 4.36 |
| PA | 0/100 | 0/100 | 1.02 | 3.06 |

[a)] Calculated from $^1$H-NMR spectra.
[b)] Determined by gel permeation chromatography based on polystyrene standards.

Table 2 Tg and contact angle of CPAPC.

| Code | Tg[a)] (℃) | Contact angle of water (degree) | |
|---|---|---|---|
| | | before immersion in water | after immersion in water |
| CPAPC-1 | 210 | 65 | 50 |
| CPAPC-2 | 186 | 60 | 39 |
| CPAPC-3 | 175 | 46 | 44 |
| PA | 187 | 70 | 70 |

[a)] Determined by DSC measurement at a heating rate of 10 ℃/min.

The obtained CPAPC exhibited good solubility 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 specific solvents is advantageous in the processing for medical devices, and the insolubility in other solvents enables the material durable to the solvents. The thermal property of CPAPC was evaluated by differential scanning calorimetry (DSC). As a result, CPAPC was a glassy

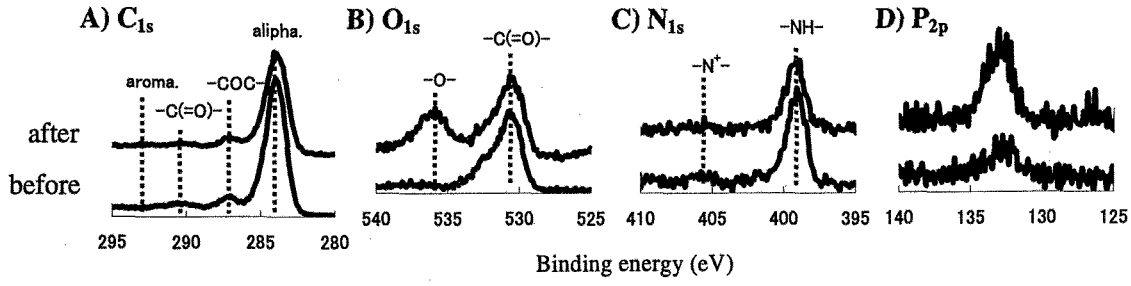



Fig. 1 XPS spectra CPAPC-2 film surfaces before and after immersion in water for a day.

polymer, the glass transition temperature (Tg) of which was in the range of 175 – 210 ℃, as shown in Table 2. Such a thermal stability of CPAPC would be sufficient for the applications to biomaterials and medical devices.

The thin films of CPAPC and PA were prepared by coating from the NMP solutions of CPAPC on poly(ethylene terephthalate) (PET) plates, and the surface analysis of the polymer films was performed. Table 2 also shows the contact angle of water on the surface of polymer films. After the PA film was immersed in water for a day, the contact angle didn't change before immersion. On the contrary, in the case of CPAPC films, the contact angles of the surfaces were decreased with an increase in the composition of the PC unit in CPAPC. Thus, the surface of the polymer membrane became hydrophilic upon the introduction of PC unit into the PA. Moreover, the surface chemical structure of the CPAPC membrane before and after immersion in water for a day was analyzed by X-ray photoelectron spectroscope (XPS) at a take-off angle of 45°, as shown in Fig. 1. The XPS signals observed at 283.9, 287.1, 290.5, and 292.9 eV as $C_{1s}$ binding energy were attributed to the carbon in hydrocarbons ($-CH_3$, $-CH_2-$), ether bond (-C-O-C-), carbonyl group [-C(=O)-], and aromatic carbon, respectively. The peaks which were observed at 530.5, 535.9, 398.9, 405.6, and 133.0 eV were attributed to the oxygen in carbonyl group [-C(=O)-], ether bond (-O-), the nitrogen in amide bond (-NH-), the ammonium group ($-N^+-$), and the phosphorus in phosphate group, respectively. Therefore, the PC unit seems to be concentrated at the CPAPC film surface after immersion in water for a day, because the peaks derived from PC unit were obviously increased after contact with water.

2.3 Blood compatibility of CPAPC

Fig. 2 shows SEM pictures of the PA and CPAPC 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 membrane surface were observed as large aggregates. On the other hand, the blood cell and platelet were suppressed on the CPAPC film surface. These results clearly indicate that CPAPC have excellent blood compatibility and the PC unit is an important element for the blood compatibility of the polymers. Furthermore, the composition of the PC unit was a dominant factor in the reduction of the blood cell and platelet adhesion. This would be due to the PC unit located at the surface of the polymer film, where the surface is covered with

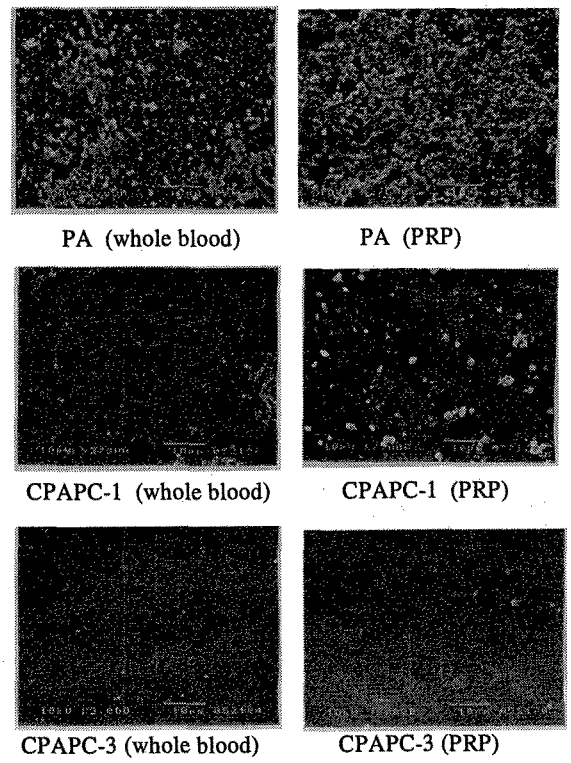



Fig. 2 SEM pictures of polymer film surface after contact with human whole blood and PRP for 60 min.

PC unit, and the interaction between the polymer surface and blood ingredients such as cell and platelet is very weak.

Consequently, it is expected that the aromatic copolyamide containing PC group will be very useful polymeric biomaterial to develop a new generation of biomedical devices because of its durability and excellent biocompatibility.

## 3. EXPERIMENTAL

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

Under an argon atmosphere, a solution of 3,5-dinitrobenzoyl chloride (10.0 g, 43.3 mmol) in 150 ml of THF was gradually added to a solution of ethylene glycol (24.0 ml, 430 mmol) and 60 ml of triethylamine dissolved in 340 ml of THF at 0 ℃. The reaction mixture was stirred at room temperature for 20 h, 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 sulfonate. After the solvent was evaporated under vacuum, the product was purified by column chromatography on silica gel with hexane/ethyl acetate (1/2 by vol.) to afford 8.95 g of **1** as a yellow powder. Yield: 80.6 %

$^1$H-NMR, (400 MHz, $CDCl_3$): δ 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).

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

Under an argon atmosphere, 2-chloro-2-oxo-1,3,2-dioxaphospholane (8.60 ml, 91.4 mmol) was gradually added to a solution of **1** (11.7g, 45.7 mmol) and 12 ml of triethylamine dissolved in 230 ml of THF at 0℃. 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 sulfonate, and the solvent was evaporated under vacuum to afford 8.65 g of **2** as a pink powder. Yield: 52.3 %

$^1$H-NMR, (400 MHz, $CDCl_3$): δ 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).

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

Under an argon atmosphere, trimethylamine (2.02 ml, 22.4 mmol) was added to a solution of **2** (4.05 g, 11.2 mmol) in 60 ml of acetonitrile at -30℃, then the reaction vessel was sealed with a glass cap. After stirring at 60℃ for 20 h, the reaction mixture was evaporated, and the obtained product was purified by recrystallization from acetonitrile to afford 4.59 g of **3** as a pink powder. Yield: 97.4 %

$^1$H-NMR, (400 MHz, DMSO-$d_6$,): δ 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).

3.4 Synthesis of 2-(3,5-diaminophenylcarbonyloxy)ethyl phosphorylcholine (DAPC)

5% Pd on charcoal powder (0.05 g, 0.02 mmol by Pd) was suspended in a solution of **3** (0.50 g, 1.19 mmol) dissolved in 15 ml of ethanol. The mixture was degassed under reduced pressure at -78℃, and the vessel was filled with hydrogen gas at over 760 mmHg. After stirring for 20 h at room temperature, the Pd on charcoal was filtered off washing with THF, and the solvent was distilled off under reduced pressure. Then, the product was purified by recrystallization from ethanol to afford 0.43 g of DAPC as a yellow powder. Yield: 92.1 %

$^1$H-NMR, (400 MHz, DMSO-$d_6$): δ 3.15 (9H, s), 3.53 (2H, t, J=4.64 Hz), 4.00 (2H, m), 4.10 (2H, m), 4.43 (2H, t, J=4.64 Hz), 7.79 (2H, d, J=16.8 Hz), 8.03 (1H, s), 8.98 (2H, s), 9.13 (2H, s).

IR, ν (KBr neat, $cm^{-1}$): 3199 (N-H), 2885 (C-H), 1718 (C=O), 1535 (C=C), 1228 (P=O), 1076 (N-$CH_3$), 733 (-$NH_2$).

3.5 General procedure of polymerization

The preparation of CPAPC-1 listed in Table 1 is given as a representative example. Under an argon atmosphere, DAPC (0.50 g, 1.38 mmol), 4,4'-diamino-3,3'-dimethyldiphenylmethane (2.81 g, 12.4 mmol) and isophthaloyl chloride (2.80 g, 13.8 mmol) were dissolved in 20 ml of NMP, and the solution was cooled to -78℃. The mixture was stirred for 5 h with gradually increasing temperature from -78℃ 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 methanol. Finally, the product was dried *in vacuo* to afford 4.72 g of CPAPC-1 as a brown powder. Yield: 93.5 %

$^1$H-NMR, (400 MHz, DMSO-$d_6$,): δ 2.21 (s, -$CH_3$), 3.08 (s, N-$CH_3$), 3.55 (m, -$CH_2$-), 3.89 (s, -$CH_2$-), 4.17 (m, -$CH_2$-), 4.52 (m, -$CH_2$-), 7.08 (m, -Ph-), 7.15 (m, -Ph-), 7.28 (m, -Ph-), 7.65 (m, -Ph-), 8.13 (m, -Ph-), 8.27 (m, -Ph-), 8.53 (m, -Ph-), 8.72 (m, -Ph-), 8.87 (m, -Ph-), 9.98 (s, -NH-).

3.6 Preparation of polymer coating films

Circular pieces of poly(ethylene telephthalate) (PET) films (diameter: 14 mm, thickness: 0.2 mm) were dipped in 0.5 wt% polymer solutions in NMP for 30 min. Then, the solvent was removed slowly at 60℃ for 2 h, and then dried *in vacuo*.

3.7 Surface characterization of polymers

The surfaces of the polymer films were analyzed with an X-ray photoelectron spectroscope (ULVAC-PHI Quantum 2000 XPS). The take-off angle of photoelectrons was adjusted to be 45 degree. Contact angles of water on the surfaces of the polymer-coated PET films were measured using an Erma contact-angle microscope at room temperature.

3.8 Evaluation of blood compatibility

At first, the polymer coating films were equilibrated in phosphate buffered solution (PBS) for overnight. Then, 0.7 ml of human whole blood and platelet-rich plasma (PRP) prepared from healthy donor were added, and the films were incubated for 60 min at 37℃. After the incubation, whole blood and PRP were removed and the films were washed 3 times with PBS. The surfaces of the polymer films after contact with whole blood and PRP were observed by a scanning electron microscope (SEM, JEOL JSM-5200).

**Acknowledgment:** This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 15550110).

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(Received December 24, 2004; Accepted March 19, 2005)