Effect of Crystallinity of Condensation Polymers on Platelet Adhesion

NAOYA OGATA, KOHEI SANUI, HOZUMI TANAKA, YOICHI TAKAHASHI, and EICHI KITAMURA, *Department of Chemistry, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, Tokyo 102, Japan*, YASUHISA SAKURAI,*† TERUO OKANO,† and KAZUNORI KATAOKA,* *Tokyo Women's Medical College, 10 Kawada-cho, Ichigaya, Shinjuku-ku, Tokyo 162, Japan*, and TOSHIHIRO AKAIKE, *Faculty of Engineering, Tokyo University of Agriculture and Technology, 2-24 Nakamachi, Koganei-shi 184, Japan*

Synopsis

Various polyamides and polyesters having different numbers of methylene groups in their repeating units have been synthesized and their blood compatibilities have been evaluated in terms of the adhesion behavior of blood platelets on the surface of these condensation polymers by a microsphere-column method. The number of methylene groups in the repeating units of polyamides and polyesters influenced the adhesion behavior of platelets and there was an optimum number of methylene groups each in polyamides and polyesters. Poly(hexamethylene terephthalamide) and poly(pentamethylene terephthalate) adsorbed platelets in the smallest number in the polyamide and polyester series, respectively. Blood platelets were adsorbed on polyamides in smaller numbers than on corresponding polyesters. It was found that the platelet adhesion on the surface of polyamides and polyesters was closely related to their crystallinities and the number of the adsorbed platelets decreased linearly with increasing their relative crystallinity.

INTRODUCTION

Biocompatibility and durability of polymeric materials *in vivo* are very important factors for their medical applications. Therefore, lots of efforts have been made in order to evaluate the biocompatibility, especially the nonthrombogenecity of polymeric materials.[1–3] Recently, a systematic investigation of the relationship between polymer structures and their biocompatibilities is required for the molecular design of polymeric materials which may have a good blood compatibility in the use of artificial organs.

Condensation polymers including polyamide and polyester are preferable to polyolefins for the investigation mentioned above, because chemical structures of condensation polymers can be varied systematically by a combination of monomers and they are easily prepared. Polyamides are characterized by a combination of high strength, elasticity, toughness, and abrasion resistance and for these reasons, polyamides are attractive for biomedical application. Though there have been some attempts to use commercial polyamides (Nylon 6 and Nylon 66) as biomaterials, few efforts have been made to investigate systematically the biocompatibility of polyamides for medical application. It is well

* Heart Institute of Japan.
† Institute of Medical Engineering.

Journal of Applied Polymer Science, Vol. 26, 4207–4216 (1981)
 CCC 0021-8995/81/124207-10$01.00

known that a polyamide shows thrombogenicity in contact with blood even though a polyamide has essentially the same basic structure as a polypeptide in the way that amide linkages form long polymer chains.

It was reported in a previous paper[4] that polyamides having various functional groups were synthesized and their blood compatibilities were investigated in terms of the adhesion of blood platelets on these polyamides.

Adhesion, aggregation, and shape change (deformation) of platelets onto the material surface play an important role in the process of thrombus formation.[5] Therefore, the surface characteristics of the material itself, as well as the reaction at the blood–material interface, are important.[6] Nevertheless, relationships between physical or chemical structures of polymeric materials and their blood compatibilities are not yet well understood.

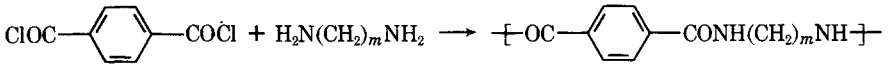
This paper aimed at the systematic investigation of blood platelet adhesion on the surface of various polyamides and polyesters of the same basic structure having a different number of methylene groups in the repeating units. A microsphere-column method[7] was used to evaluate their blood compatibilities in terms of the adhesion behavior of blood platelets on them.

Since these polymers have different crystallinities, we have investigated how the crystallinity of polyamides and polyesters affects the adhesion behavior of blood platelets on the surfaces of these condensation polymers.

EXPERIMENTAL

Preparation of Polyamides Having a Different Number of Methylene Groups

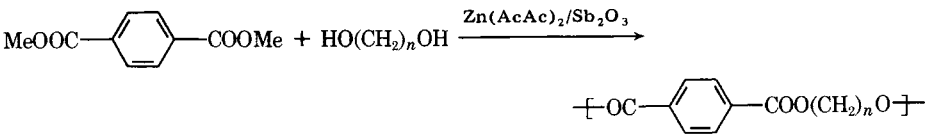
Various polyamides were prepared by conventional interfacial polycondensation of terephthaloyl chloride with diamines, including 1,2-ethylenediamine, 1,3-propanediamine, 1,4-butanediamine, 1,5-pentanediamine, 1,6-hexanediamine, 1,8-octanediamine, 1,10-decanediamine, and 1,12-dodecanediamine by using benzene as an organic solvent[8]:



$m = 2\text{–}12$

Preparation of Polyesters Having a Different Number of Methylene Groups

Various polyesters were prepared by conventional melt polycondensation of dimethyl terephthalate with diols, including ethyleneglycol, 1,3-propanediol, 1,4-butanediol, 1,5-pentanediol and 1,6-hexanediol by using $Zn(AcAc)_2/Sb_2O_3$ as a catalyst:



$n = 2\text{–}6$

The polyesters were purified by reprecipitation from phenol/tetrachloroethane (1/1) solution into an excess of methanol and then dried at 40°C in vacuo for 48 h.

Solution viscosities of the polyamides and polyesters were determined at 30°C in sulfuric acid and those of the polyesters in a mixed solvent of phenol and tetrachloroethane (1/1), respectively.

Melting and decomposition temperatures of the polymers were measured by differential thermal analysis using the Rigakudenki thermoflex 8001 in all cases. Physical properties of the prepared polyamides and polyesters and their abbreviation are summarized in Table I.

Determination of Relative Crystallinity

Polymer films suitable for X-ray diffraction measurements were prepared by casting from polymer solution under the same conditions as were used to give the coating of the corresponding polymers on glass beads for the test of platelet adhesion.

These films and the coated glass beads were annealed under the same conditions. The X-ray diffraction patterns of these films were measured by using a Rigakudenki Geigerflex diffractometer with nickel-filtered CuKα radiation. Relative crystallinities of these polymers were determined from the relative ratios of the integrated intensities of the X-ray diffraction patterns to those of the commercial films of Nylon 66 and poly(ethylene terephthalate) (PET) which were used as standards. The annealing conditions used and the relative crystallinities are summarized in Table II.

Evaluation of Platelet Adhesion[4,7]

Coating of various polyamides and polyesters on glass beads was carried out by using the following solvent evaporation method:

A 30-cm^3 beaker was charged with 20 g of glass beads with a size of 40–60 mesh.

TABLE I
Properties of Polyamides and Polyesters

Polymer		η(sp/C[a])	T_m (°C)	T_d (°C)
Polyamide	m = 2	0.47[b]	—	350
	3	0.51[b]	—	325
	4	0.49[b]	—	320
	5	0.79[b]	—	310
	6	0.60[b]	—	300
	8	0.62[b]	—	295
	10	0.81[b]	—	280
	12	1.23[b]	—	270
Polyester	n = 2	0.44[c]	260	375
	3	0.49[c]	220	370
	4	0.58[c]	225	320
	5	0.50[c]	130	305
	6	0.57[c]	160	310

[a] 0.1 g/10 cm^3 at 30°C.
[b] In dichloroacetic acid.
[c] In phenol and tetrachloroethane (1/1).

TABLE II
Relative Crystallinity of Polyamides and Polyesters

Polymer		Relative crystallinity[a]	Annealing condition	
			Temp (°C)	Time (h)
Nylon 66		1.00	—	—
Polyamide	m = 2	0.60	60	48
	3	0.56	60	48
	4	0.61	60	48
	5	0.76	60	48
	6	0.87	60	48
	8	0.85	60	48
	10	0.72	60	48
	12	0.73	60	48
PET		1.00	—	—
Polyester	*n* = 2	0.64	60	48
	3	0.63	60	48
	4	0.66	60	48
	5	0.76	60	48
	6	0.53	60	48
Polyamide	*m* = 6	0.70	150	3
		0.53	100	3
		0.28		0
Polyester	*n* = 2	0.75	180	3
		0.52	100	3
		0.35		0

[a] Commercially available Nylon 66 and PET were used as standards of polyamide and polyester series, respectively.

The glass beads were immersed in a 20-cm^3 solution which was prepared by dissolving polyamides in dichloroacetic acid or by dissolving polyesters in a mixture of phenol and tetrachloroethane (1/1) at the concentration of 0.25 wt %. Then the contents were poured into a microfilter mounted in a suction funnel to separate the glass beads from the solution. The glass beads were dried at 40°C for 48 h under reduced pressure. It was confirmed that no influence of difference in coating conditions on the platelet adhesion was observed.

Evaluation: A 1-g portion of the coated glass beads was closely packed in a tubing of poly(vinyl chloride) and was subjected to the following platelet adhesion test.

A 3-cm^3 of fresh blood was collected from the jugular vein of a dog by a disposable syringe without using anticoagulant. This was passed through the column packed with the polymer-precoated beads for 60 s at the flow rate of 1.2 cm^3/min with the use of a Precidol Model 5003 Syringe Pump. The procedure was illustrated in a previous paper.[4] After all blood was passed through the column, the eluted blood was collected together with the primed saline in a sample bottle whose inner surface was covered with EDTA as an anticoagulant. Then the column was washed with saline solution at the flow rate of 1.7 cm^3/min for a period of 120 s. The rinsed column was divided into three parts and the beads situated in the upper part of the column were placed in a saline solution containing 1.25% glutaraldehyde in order to fix adhered platelets on the surface and, then, the beads were freeze-dried, followed by coating with gold.

The surfaces of the beads were observed by a scanning electron microscope (SEM). The scanning electron micrographs were taken on a Hitachi-Akashi S-430 Scanning Electron Microscope. More than 10 views per each sample were

observed by SEM, and typical representative scanning electron micrographs of various samples are shown in Figures 2, 3, and 7.

The number of platelets in the eluted blood was counted according to the method of Brecher and Cronkite.[9]

Dichloroacetic acid solutions of the polyamides were cast on carbon-coated copper grids and thin films were prepared by evaporation at 40°C. After drying under reduced pressure, the films were subjected to osmium tetroxide vapor for 1 h. The microstructures of the film surface were observed by an electron microscope (Hitachi HS-9 type).

RESULTS AND DISCUSSION

Effect of Methylene Length on the Platelet Adhesion

Polyamides and polyesters of the same basic structure having a different number of methylene groups in each repeating unit were synthesized and the adhesion behavior of blood platelets on the surface of these polymers was compared with each other. These results are summarized in Figure 1, where the ordinate indicates the relative number of eluted platelets. There was the optimum number of methylene group in repeating units for the platelet adhesiveness of polyamides and polyesters, respectively. That is, poly(hexamethylene terephthalamide) (m = 6) and poly(pentamethylene terephthalate) (n = 5) adsorbed platelets in the smallest number among polyamide and polyester series, respectively. It was also found that fewer blood platelets adsorbed on polyamides than the corresponding polyesters. Very few adherent platelets were observed on the surfaces of poly(hexamethylene terephthalamide) (m = 6) and poly(octamethylene terephthalamide) (m = 8). On the other hand, aliphatic polyamides such as poly(hexamethylene adipamide) (Nylon 66) adsorbed a large number of platelets which were remarkably deformed. These results suggest that the combination of phenylene group and an appropriate number of methylene groups

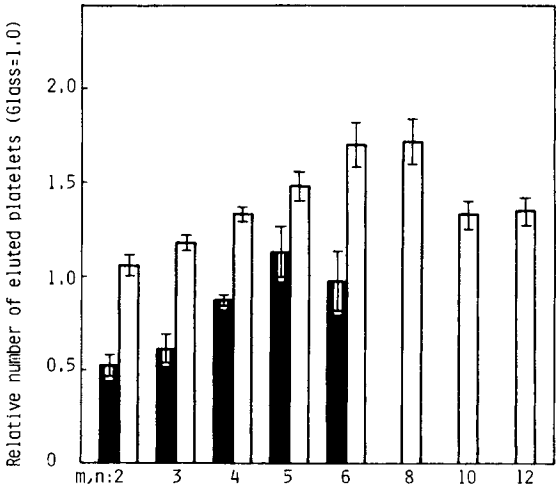
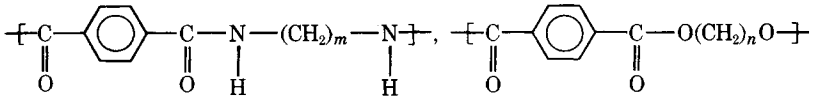


Fig. 1. Platelet adhesion on polyamides and polyesters (±SEM). (□) Polyamides; (■) Polyesters:



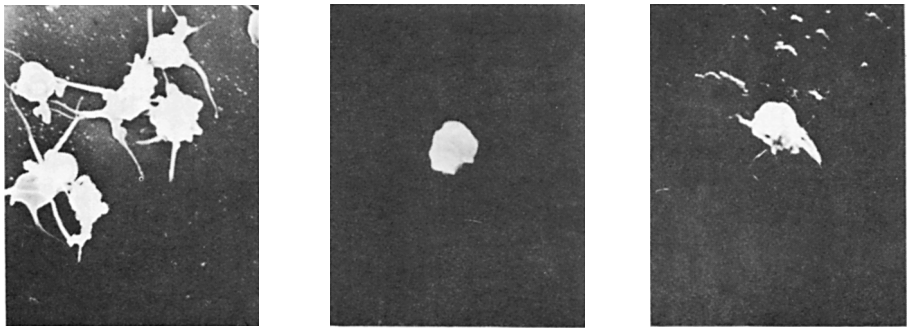


Fig. 2. Scanning electron micrographs of platelets adhered on the surface coated with poly(ethylene terephthalamide) (m = 2), poly(tetramethylene terephthalamide) (m = 4), and poly(pentamethylene terephthalamide) (m = 5): (A) m = 2; (B) m = 4; (C) m = 5; 5μm —.

in the repeating units of polyamides and polyesters was very effective on a decrease in the platelet adhesion on the surface of these polymers.

The scanning electron micrographs of platelets adsorbed on the surface coated with polyamides and polyesters are shown in Figures 2 and 3, respectively.

As seen from Figure 2, adherent platelets on the surface of poly(tetramethylene terephthalamide) (m = 4) and poly(pentamethylene terephthalamide) (m = 5) did not undergo deformation compared to those on that of poly(ethylene terephthalamide) (m = 2).

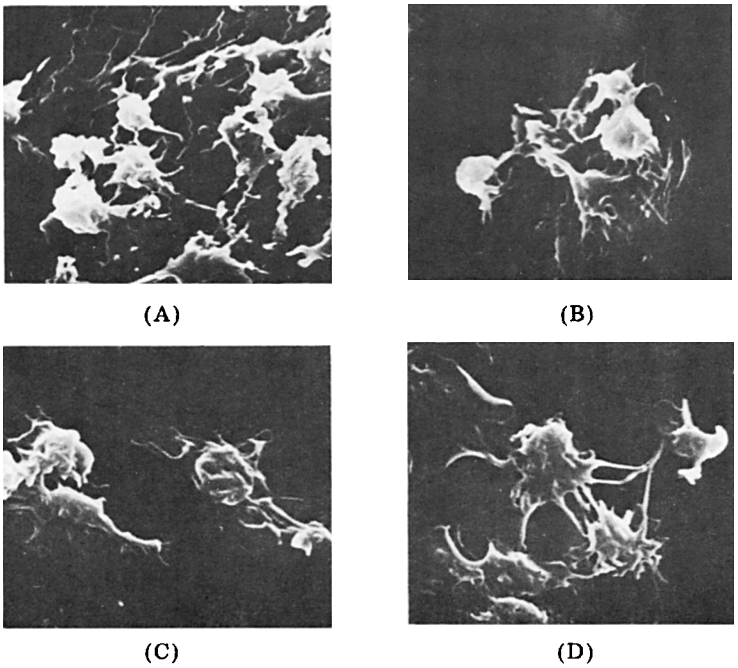


Fig. 3. Scanning electron micrographs of platelets adhered on the surface coated with poly(ethylene terephthalate) (n = 2), poly(trimethylene terephthalate) (n = 3), poly(tetramethylene terephthalate) (n = 4), and poly(pentamethylene terephthalate) (n = 5). (A) n = 2; (B) n = 3; (C) n = 4; (D) n = 5; 5μm —.

On the other hand, the surfaces of polyesters were covered with aggregates of deformed platelets in all cases as shown in Figure 3. It can be seen by comparing Figure 2 with Figure 3 that the deformation and aggregation of the adherent platelets on the surface of polyesters took place to a much greater extent than on polyamides. These results suggest that the structural difference between amide and ester groups in the polymer main chain affects not only the adhesion number but also the morphological changes of the adherent platelets.

Effect of Crystallinity on the Platelet Adhesion

Figures 4 and 5 summarize the dependences of the number of methylene groups in a repeating unit in polyamide and polyester series on the relative number of eluted platelets and on the relative crystallinity. It is seen in Figures 4 and 5 that a good correlation between the relative number of eluted platelets and the relative crystallinity can be observed in both series of polyamide and polyester. The relative crystallinity of these polyamides and polyesters is simply a comparison of the crystallinity among the series of tested polymers, where commercial Nylon 66 and PET were used as standards.

Since both Nylon 66 and PET adsorbed a large number of platelets, the chemical structure of the polymer influenced the adhesion behavior of platelets. In a series of the polymers with similar structures, however, the crystallinity of the polymer affected the platelet adhesion on the polymer surface and the number of the adherent platelets on the surface of polyamides or polyesters decreased with increasing relative crystallinity of these polymers. An electron microscope observation revealed the existence of micro domains of the crystalline phase within the amorphous phase of polyamides.

These results suggest that not only chemical, but physical structures of the

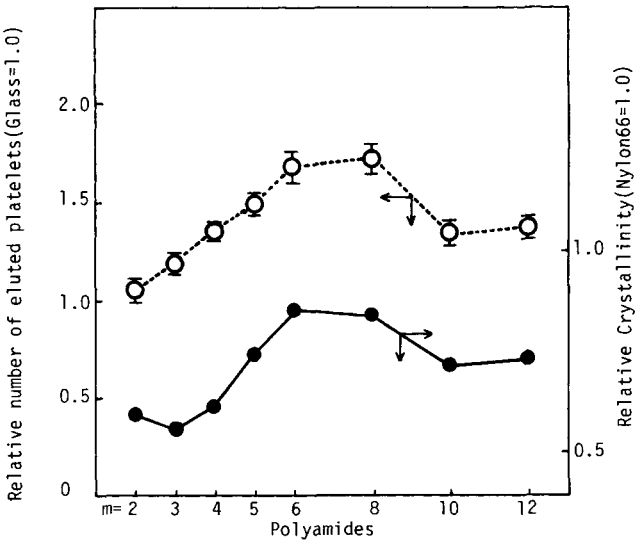
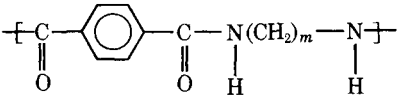


Fig. 4. Platelet adhesion and relative crystallinity of polyamides.

(±SEM)



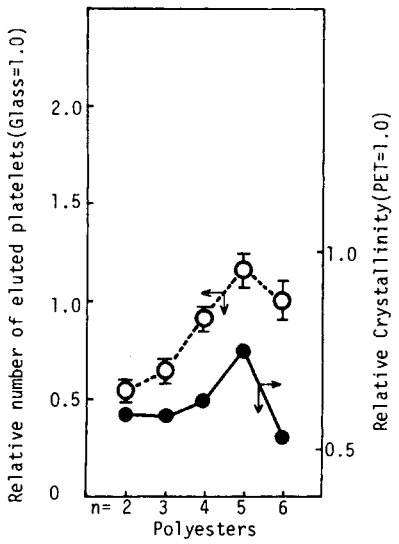
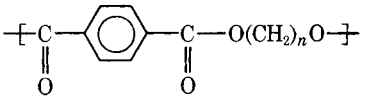


Fig. 5. Platelet adhesion and relative crystallinity of polyesters.

(±SEM)



surface, including the size and distribution of microdomains of crystallites dispersed within the continuous amorphous phase, are very important factors for the adhesion behavior of blood platelets on the surface of polyamides and polyesters. Therefore, the crystallinity of poly(hexamethylene terephthalamide) (m = 6) and poly(ethylene terephthalate) (n = 2) was varied by annealing under different conditions in order to confirm the importance of crystallinity for the adherent behavior of platelets.

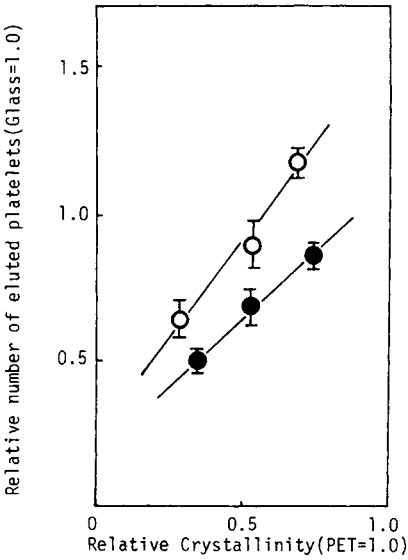


Fig. 6. Relationship between platelet adhesion and relative crystallinity of polyamide and polyester (±SEM). (○) Poly(hexamethylene terephthalamide); (●) poly(ethylene terephthalate).

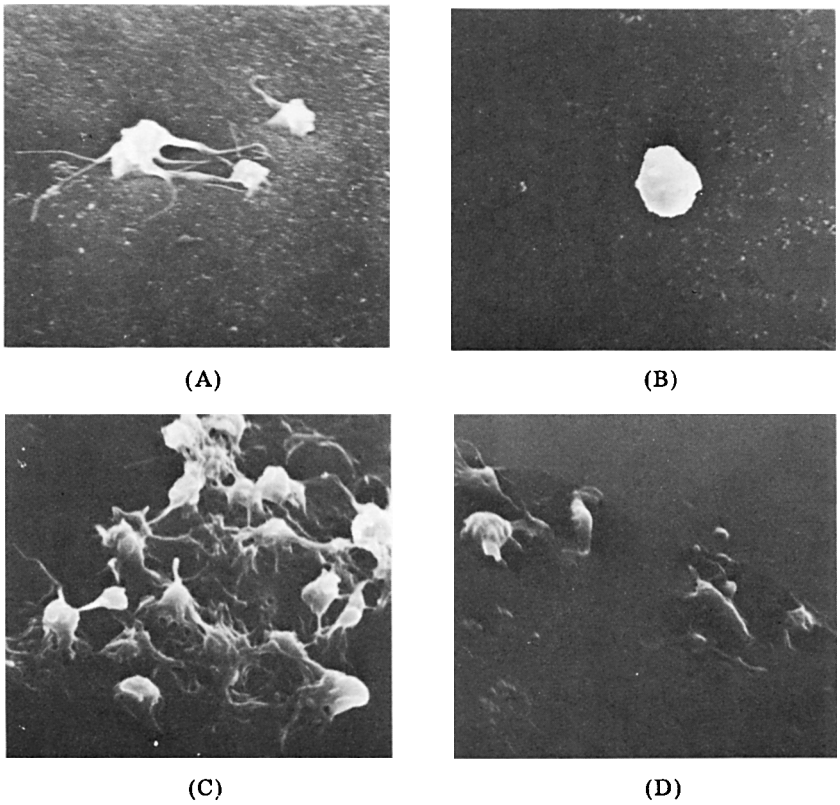


Fig. 7. Scanning electron micrographs of platelets adhered on the surface with annealed or quenched polyamides and polyesters. (A) Quenched polyamide (m = 6); (B) annealed polyamide (m = 6); (C) quenched polyester (n = 2); (D) annealed polyester (n = 2); 5μm —.

Figure 6 shows the relationship between the platelet adhesion and the relative crystallinity of poly(hexamethylene terephthalamide) (m = 6) and poly(ethylene terephthalate) (n = 2). The number of the adherent platelets on the polyamide (m = 6) and polyester (n = 2) decreased linearly as their relative crystallinity increased.

Figure 7 gives the scanning electron micrographs of the platelets adsorbed on the surface of annealed or quenched polyamide (m = 6) and polyester (n = 2). The surface of the annealed polymers, which have higher crystallinity than the quenched polymers, did not significantly cause the deformation and aggregation of the adherent platelets when compared with the surface of the quenched polymers, as shown in Figure 7. In particular, no deformation and aggregation were observed in the case of the annealed polyamide (m = 6). The number of the adherent platelets on the surface of the annealed polymers was also smaller than that of the quenched polymers.

These results indicate that crystallinity is one of the important factors which control the blood compatibility of polyamides and polyesters.

In conclusion, it was found that poly(hexamethylene terephthalamide) (m = 6) with relatively high crystallinity can be used as a nonthrombogenic material for biomedical applications.

References

1. K. Kojima, Y. Imai, and A. Masuhara, *Kobunshi Ronbunshu*, **34,** 267 (1977).
2. D. J. Lyman, K. Knutson, B. Maneil, and K. Shibatani, *Trans. Am. Soc. Artif. Intern. Organs*, **21,** 49 (1975).
3. H. Matsumoto and T. Hasegawa, *Surgery*, **74,** 519 (1973).
4. N. Ogata, K. Sanui, H. Tanaka, Y. Takahashi, Y. Sakurai, T. Akaike, T. Okano, and K. Kataoka, *J. Appl. Polym. Sci.*, **26,** 2293 (1981).
5. T. Okano, S. Nishiyama, I. Shinohara, T. Akaike, and Y. Sakurai, *Polym. J.*, **10,** 239 (1978).
6. Y. Sakurai, T. Akaike, K. Kataoka, and T. Okano, "Biomedical Polymers," *Interfacial Phenomenon in Biomaterials Chemistry*, ACS Advances in Chemistry Series, Academic, New York, 1980, p. 335.
7. K. Kataoka, Y. Akaike, Y. Sakurai, and T. Tsuruta, *Makromol. Chem.*, **179,** 1121 (1978).
8. H. Nakamura, T. Nakahara, K. Sanui, and N. Ogata, *J. Polym. Sci., Polym. Chem. Ed.*, **16,** 3035 (1978).
9. G. Brecher and E. P. Cronkite, *J. Appl. Physiol.* **3,** 365 (1950).

Received March 16, 1981
Accepted June 12, 1981