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Hydrolytic and Enzymatic Degradation of Liquid-Crystalline Aromatic/Aliphatic Copolyesters

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Aromatic/aliphatic copolyesters containing hydrophilic moieties in the main chain or side chain were synthesized by bulk polycondensation of aromatic monomers without or with solubilizing substituents and aliphatic monomers. Hydrolytic and enzymatic degradation studies were carried out in vitro at 37 °C in pH 7.4 phosphate buffer and in Tris-HCl buffer containing proteinase K. The results indicate that liquid-crystalline aromatic/aliphatic copolyesters are degradable hydrolytically as well as enzymatically. The change in composition and morphology of the polyester films were monitored by nuclear magnetic resonance and scanning electron microscopy. The results suggested that aromatic species and aliphatic moieties could be released into aqueous solution during hydrolytic degradation of aromatic/aliphatic copolyesters with ethyleneoxy groups on the side chain. Modifying aromatic species with hydrophilic groups in *aromatic/aliphatic* copolyesters was an efficient method to improve degradability and biocompatibility due to improved solubility of degradation products in aqueous solution. Mechanical tests indicated that the copolyesters exhibited good mechanical properties prior to degradation, which can be of relevance for bone tissue engineering.

Biodegradable polymers have gained considerable significance in medical applications, including drug-delivery systems, implant materials, and materials for tissue engineering.[1] An important class of biodegradable polymers are aliphatic polyesters, which are degradable hydrolytically or enzymatically.[2] Biodegradable polymers that exhibit exceptional mechanical properties remain a challenge for particular medical applications such as bone-tissue engineering.[3−6] To optimize biodegradability and mechanical performance, poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymers or blends have been extensively investigated.[7−11] A self-reinforced composite,[12] in which biodegradable reinforcing fibers are embedded in a biodegradable polymer matrix, were designed for use as structural biomaterials with improved mechanical properties. On the other hand, main-chain liquid-crystalline aromatic polyesters are characterized by excellent mechanical properties.[13] However, poor solubility, high melting point, and inadequate degradability under physiological conditions make aromatic liquid-crystalline polyesters of limited apparent interest for biomedical applications such as tissue engineering. The drawbacks of aliphatic polyesters and aromatic copolyesters might be overcome by suitable design of polyesters with aliphatic and aromatic moieties. Previously, we have explored new modified aromatic copolyesters with enhanced degradability under physiological conditions on an acceptable time scale and demonstrated that copolyesters with aromatic moieties and oligolactide moieties in the main chain are liquid-crystalline, degradable, and exhibit excellent mechanical properties.[14] Copolyesters composed of aromatic moieties, lactide moieties, and oligoethylene oxide moieties were also liquid-crystalline and exhibited adequate mechanical properties.[15] For the polymers described, the hydrophilicity of the copolyesters and degradability were significantly enhanced due to the incorporation of oligoethylene oxide moieties in the main chain. However, a remaining disadvantage of these copolyesters for applications such as bone tissue engineering is that the aromatic degradation products would be insoluble under physiological conditions. To improve the solubility, we advanced novel liquid crystalline copolyesters with lateral solubilizing substituents on the aromatic moieties, which retain their liquid-crystallinity and exhibit enhanced hydrophilicity.[16,17] The enzymatic degradation of *aliphatic* polyesters such as polyglycolide (PGA), poly(D,L-lactide) (PDLLA), poly(L-lactide) (PLLA), poly(*β*-hydroxybutyrate) (PHB), and poly(*ϵ*-caprolactone) (PCL) have attracted much attention recently.[18] There have been an increasing number of reports on the enzymatic hydrolysis of polylactide (PLA). Williams et al. reported the enzymatic hydrolysis of PLLA by Pronase, proteinase K, and bromelain.[19] Subsequently, Ashley and

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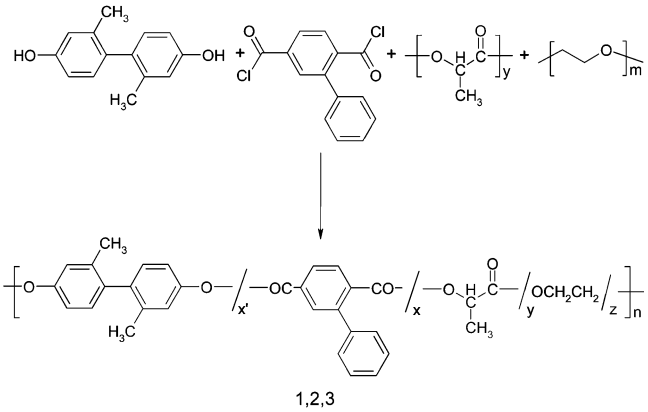
McGinity confirmed the enzymatic degradation of PDLLA by proteinase K.[20]

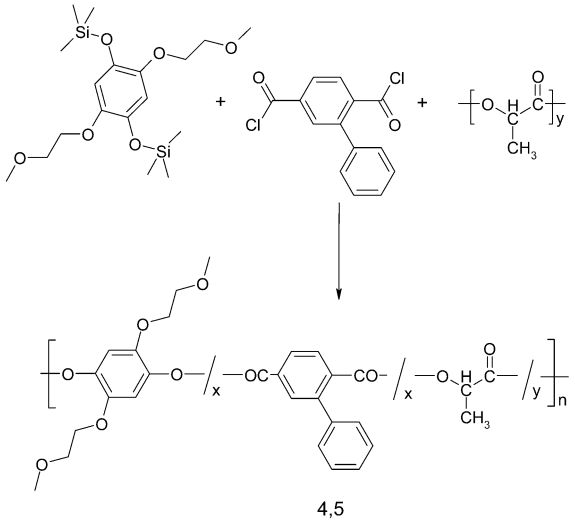
A thorough understanding of structure−property relationships with respect to biodegradability is of fundamental importance for the tailored design of polymeric materials for tissue engineering. Most recently, hydrolytic and enzymatic degradation of *aromatic*/*aliphatic* copolyesters in vitro have been reported by several groups.[21,22] Nagata et al.[21] has presented degradation of thermotropic copolyesters based on terephthalic acid, 3-(4-hydroxyphenyl)propionic acid, and glycol by enzyme. Prasad et al.[22] reported in vitro degradation of liquid-crystalline terpolyesters of 4-hydroxyphenylaliphatic acid with terephthalic acid and 2,6-naphthalene diol. In previous articles, a novel series of thermotropic liquid-crystalline polyesters were prepared from aromatic diacyl chloride, aromatic diol, and lactide with ethyleneoxy groups in main chain or side chain.[14−17] In the present work, we investigated hydrolytic and enzymatic degradation of copolyesters of different chemical architecture under comparable conditions.

The homopolyester poly(2,2′-dimethyl-4,4′-biphenylene phenyl-terephthalate) (**1**) was synthesized as described previously by polycondensation of 2,2′-dimethyl-4,4′-dihydroxybiphenyl and phenylterephthaloyl dichloride.[23] The copolyester poly[(2,2′-dimethyl-4,4′-biphenylene phenylterephthalate)-*co*-lactide] (**2**) was synthesized with a 2:1 molar ratio of aromatic units and lactide moieties in polymer chain by polycondensation of 2,2′-dimethyl-4,4′-dihydroxybiphenyl, phenylterephthaloyl dichloride, and α-hydroxy-*ω*-carboxyoligolactide.[14] A series of copolyesters poly[(2,2′-dimethyl-4,4′-biphenylene phenylterephthalate)-*co*-lactide-*co*-ethyleneoxy]s (**3a**−**e**) were synthesized by polycondensation of 2,2′-dimethyl-4,4′-dihydroxybiphenyl, phenylterephthaloyl dichloride, α-hydroxy-*ω*-carboxyoligolactide, and dihydroxy end-capped polyethyleneoxy 1000 under same conditions.[15] To increase the hydrophilicity and the solubility of degradation products, the homopolyester **4** and copolyesters **5a**−**c** with solubilizing substituent ethyleneoxy groups on aromatic units were synthesized by polycondensation of 2,5-bis-(methoxyethyleneoxy)-1,4-hydroquinone silylate and phenylterephthaloyl dichloride or copolymerization with α-hydroxy-*ω*-carboxyoligolactide, respectively.[16] Accordingly, for further modification, the homopolyester **6** or copolyesters **7a**−**b** were prepared by polycondensation of 2,5-bis-(methoxyethyleneoxy)-1,4-hydroquinone silylate and 2-ethoxyethyleneoxyterephthaloyl chloride or copolymerization with α-hydroxy-*ω*-carboxyoligolactide, respectively.[17] The synthetic route for polyesters **1**−**7** is shown in Schemes 1−3. Detailed synthetic procedures, structural analysis, and molecular characterization were published in previous papers.[14−17] Nevertheless, some of the previously published data of molecular characterization, like molecular weight, molecular weight distribution, and contact angles of solid samples of these series of copolyesters have been summarized in Table 1. The molecular weights of copolyesters **3** ranged between 228 000−65 000 g/mol with polydispersities of 4.5−8.6. The analysis by NMR indicated that the lactide and the ethyleneoxy moieties were chemically incorporated into the polyesters according to randomized molecular structure. The

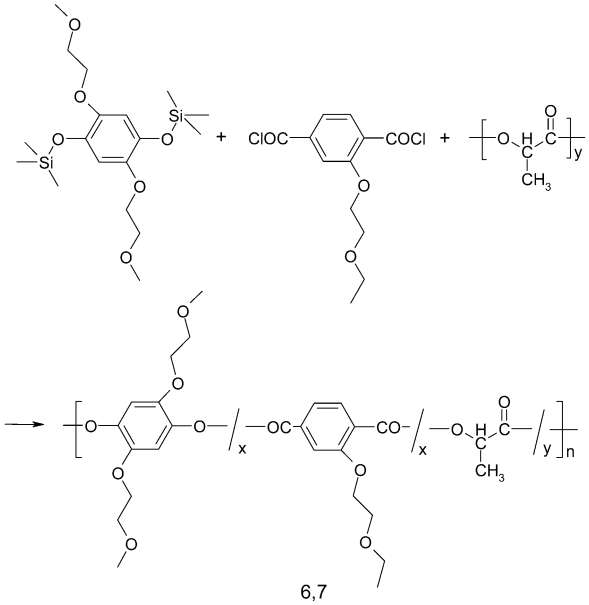
Scheme 1. Synthetic Route and Chemical Structure of Polyesters 1 ($y = 0$, $z = 0$), 2 ($y > 0$, $z = 0$), and 3 ($y > 0$, $z > 0$).



Scheme 2. Synthetic Route and Chemical Structure of Polyesters 4 ($y = 0$) and 5 ($y > 0$).



Scheme 3. Synthetic Route and Chemical Structure of Polyesters 6 ($y = 0$) and 7 ($y > 0$).



decrease of the contact angles of the polyesters against water represents their enhanced hydrophilicity. The values of $\overline{M}_w$ of copolyesters **5** were in the range of 74 000−36 000 g/mol with polydispersities of 3.8−5.10, whereas those of copoly-

Table 1. Molecular Characteristics and Contact Angles against Water of Polyesters **1**−**7**

	x/*y*/*z*[a]	$\overline{M_w}$[b]	$\overline{M_n}$[b]	$\overline{M_w}/\overline{M_n}$[b]	[η] (dL/g)[c]	contact angle (°)
1	1/0/0	544900	59200	9.20	5.80	93
2	2/1/0	254300	28900	8.80	2.15	84
3a	2/1/1.8	227800	26200	8.60	1.34	76
3b	2/1/1.6	163700	24200	6.70	0.89	77
3c	2/1/1.3	94600	20000	4.70	0.75	79
3d	2/1/0.8	83800	10100	8.20	0.65	80
3e	2/1/0.6	65300	14300	4.50	0.62	82
4	1/0	164400	22100	7.40	1.85	82
5a	1/0.1	73900	14500	5.10	1.45	81
5b	1/0.3	49400	11100	4.40	1.25	78
5c	1/0.5	35800	9300	3.80	0.80	76
6	1/0	33200	9800	3.40	0.95	75
7a	1/0.3	27100	8600	3.20	0.67	72
7b	1/0.5	25500	7800	3.30	0.54	70

[a] *x*/*y*/*z* is molar ratio of the repeating units in polyesters which is labled in the structure, values from **4** to **7b** are molar ratio *x*/*y*. [b] Data obtained by gel permeation chromatography with chloroform as eluent. [c] Intrinsic viscosity was determined in chloroform at 25 °C.

esters **7** were about 26 000 g/mol with a polydispersity of about 3.2. Randomized molecular structures of copolyesters rather than block structures or mixtures have been verified by chemical and physical characterization techniques such as NMR, X-ray diffraction (XRD), and differential scanning calorimetry (DSC). Increased hydrophilicity of copolyesters with more lactide moieties or more ethyleneoxy side groups on the aromatic rings was evidenced by contact-angle measurements against water.

Hydrolytic degradation experiments of the polyesters were carried out at 37 °C in a phosphate buffer solution (Fluka, pH 7.413 at 25 °C). Square samples with dimensions of 10 × 10 × 0.3 mm were cut from the various films and placed in vials containing 20 mL of buffer solution. At predetermined degradation time intervals, the specimens were removed from the medium, rinsed with distilled water, dried under vacuum at room temperature for 1 week and weighed. Before continuing the experiment, the buffer solution was renewed. Weight loss percentages of the copolyesters were obtained according to the relationship (weight loss % = $(W_0 - W_t)/(W_0)$, where W_0 is the initial weight, and W_t the dry weight of the specimens after degradation. Weight loss data of the copolyesters **2**, **3a**−**e**, **4**, **5a**−**c**, **6**, and **7a**,**b** are shown in Figure 1. Homopolyesters **1**, **4**, and **6** barely displayed degradation under physiological conditions. In contrast, a weight loss of 5.8%, 6.4%, 4.8%, 3.8%, 3.3%, and 3.0% were observed for copolyesters **3a**, **3b**, **3c**, **3d**, **3e**, and **2**, respectively, after immersion phosphate buffer solution at 37 °C for 100 days. The copolyesters degraded more rapidly with increasing content of ethyleneoxy moieties. The chemical compositional changes of the copolyesters were monitored by ^{1}H NMR spectroscopy. After 100 days in phosphate buffer, the molar ratio of ethyleneoxy and lactide moieties relative to aromatic moieties in the copolyesters **3** decreased slightly from the initial value. The copolyesters with hydrophilic ethyleneoxy side groups on the aromatic rings (**5**,**7**) were observed to degrade faster than those polyesters with ethyleneoxy and lactide moieties in the main chain (**3**). Moreover, the presence of more ethyleneoxy side groups led to increased weight loss during hydrolytic degradation, which

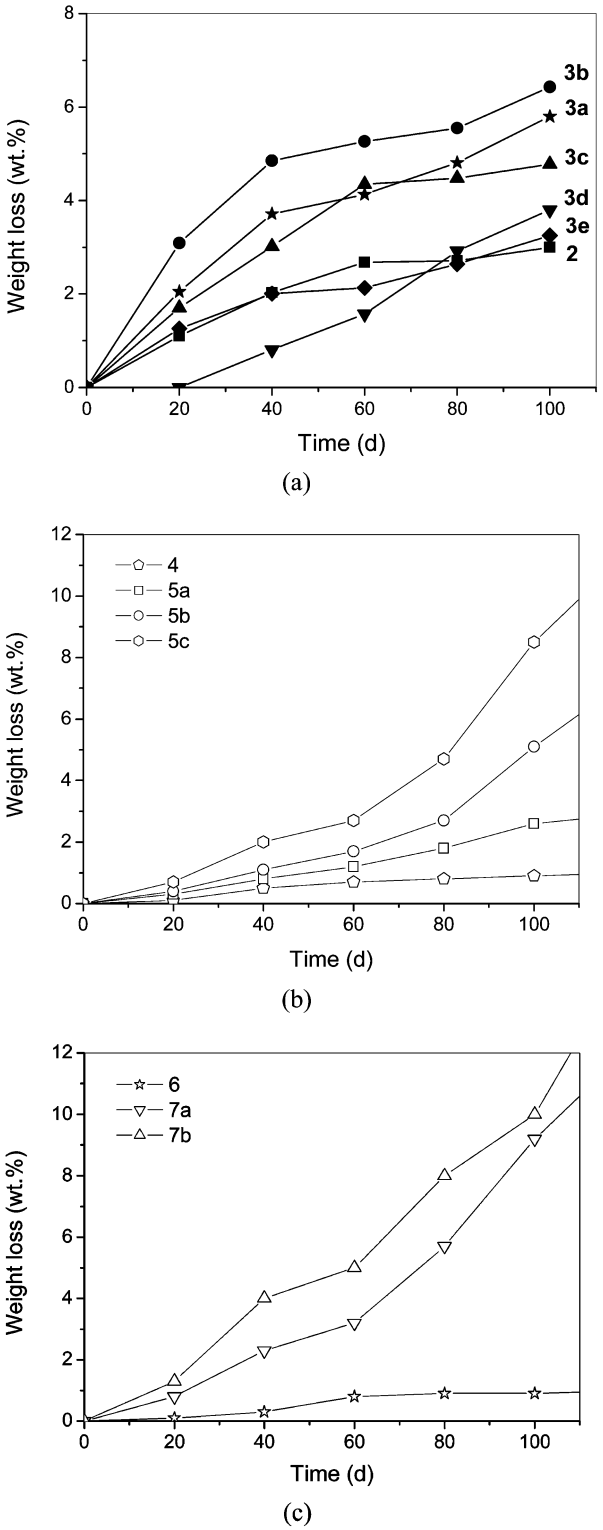


Figure 1. Weight loss versus time during hydrolytic degradation of copolyesters **2** and **3** (a), **4** and **5** (b), and **6** and **7** (c) in a pH 7.4 phosphate buffer (part a has been shown in previous paper[15]).

is likely to be due to enhanced hydrophilicity of the copolyesters. After 100 days, the weight loss of the copolyesters **5a**, **5b**, and **5c** attained 2.6%, 5.1%, and 8.5%, respectively, whereas the weight loss of the copolyesters **7a** and **7b** reached 9.2% and 10%, respectively. A slight decrease of the molar ratio of lactide moieties relative to aromatic moieties was also observed after 100 days in buffer in ^{1}H NMR experiments. The *x*/*y*/*z* ratio of the copolyester

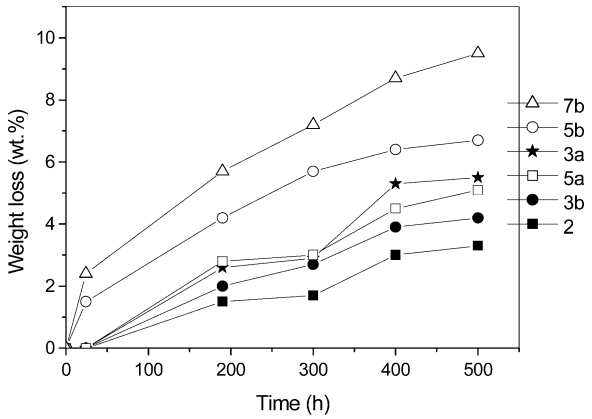


Figure 2. Weight loss profiles of the copolyesters during enzymatic degradation by proteinase K (0.2 mg/mL) in a pH 8.0 Tris-HCl buffer.

3a decreased from the initial 2/1/1.8 to 2/0.7/1.5, whereas the *x*/*y* ratio of the copolyester **5b** and **7b** decreased to about 1/0.2 and 1/0.4 from 1/0.3 and 1/0.5, respectively. Withdrawing **5b** and **7b** films from the buffer solution after 100 days, the components of lactic acid and aromatic derivatives have observed by extracting the buffer with dichloromethane and NMR detection. Nevertheless, only aliphatic moieties such as lactic acid and ethylene glycol were detected from the buffer solution after 100 days of degradation of **3a**. The release of degradation products of **5b** and **7b** in the buffer solution was attributed to the improved solubility of aromatic species with ethyleneoxy groups. GPC was used to follow the changes of the molecular weight during the hydrolytic degradation. The $\overline{M}_w$ of copolyesters **3a**, **5b**, and **7b** after 100 days of degradation slightly changed to 154 700, 38 800, and 18 900 g/mol from the initial values of 227 800, 49 400, and 25 500 g/mol, respectively.

Enzymatic degradation of copolyester thin films was performed at 37 °C in 1 M Tris-HCl buffer (pH = 8.0) including 0.2 mg/mL of proteinase K (both from Sigma-Aldrich Chemie GmbH). Film samples of copolyesters were placed in a small bottle containing 5 mL of a buffer solution. The enzymatic reaction was started by the addition of 1.0 mg of proteinase K and 1.0 mg of sodium azide. The buffer−enzyme system was changed every 24 h. For a given experiment, three replicate samples were withdrawn from the medium and washed with distilled water. After wiping, the specimens were weighed and vacuum-dried at room temperature for 1 week before being subjected to analysis. Weight loss data of the copolyesters **2**, **3a**, **3b**, **5a**, **5b**, and **7b** in function of time periods are collected in Figure 2. The aromatic copolyesters with lactide moieties in the main chain were found to degrade in the presence of proteinase K. More rapid enzymatic degradation was observed for polymers with lactide and ethyleneoxy moieties in the main chain, whereas the fastest enzymatic degradation was observed for copolyesters with ethyleneoxy side groups on the aromatic rings. We attribute the latter observation to the increased hydrophilicity of the copolyesters comprising ethyleneoxy moieties. Within 500 h, the weight loss of the copolyesters **7b**, **5b**, **5a**, **3a**, **3b**, and **2** reached 9.5%, 7%, 5%, 5.5%, 4%, and 3%, respectively. The homopolyesters **1**, **4**, and **6** did not exhibit any change in weight during storage in buffer solution containing proteinase K.

Chemical composition changes of the copolyesters after enzymatic degradation were monitored by ^{1}H NMR. After 500 h in the presence of proteinase K, the *x*/*y*/*z* ratio of the copolyester **3a** decreased from the initial 2/1/1.8 to 2/0.6/1.6, whereas the *x*/*y* ratio of the copolyester **5b** decreased to about 1/0.2 from 1/0.3. The relative changes of the chemical composition during degradation can be explained by the fact that the copolyesters are disintegrated from lactide segments, but on the other hand, ethyleneoxy-rich segments can escape from the bulk and dissolve in the degradation medium due to good solubility in water. GPC was used to follow the changes of the molecular weight during the enzymatic degradation. The $\overline{M}_w$ of copolyesters **3a** and **7b** after 500 h degradation slightly changed to 198 600 and 18 600 g/mol from the initial values of 227 800 and 25 500 g/mol, respectively.

Surface morphology changes were investigated by scanning electron microscopy (SEM). Figure 3 presents scanning electron micrographs of the copolyester films **3a**, **5b**, and **7b** before and after enzymatic degradation in the presence of proteinase K. The surface of the films before degradation was smooth with small holes due to the evaporation of the solvent (Figure 3, parts A, C, and E). After degradation for 500 h, the smooth surface of the copolyester films became cracked or porous (Figure 3, parts B, D, and F). However, images of cross-sections revealed no significant changes after degradation, even though degradation of the copolyesters was most likely to occur also in bulk. These results indicate that the enzymatic degradation of the aromatic/aliphatic copolyester films occurred predominantly on the film surfaces.

Selected mechanical properties were determined with an Instron universal test system 4400 equipped with a temperature-controlled environmental chamber. Dumbbell-shaped samples of 12 mm gauge length and 2 mm width were cut from films cast from 20 wt % solutions in chloroform and re-molten above the glass transition temperature of the copolyesters **3a**, **3b**, **5b**, and **7b**. A set of stress−strain curves of these films were recorded at different temperatures ranging from room temperature to 100 °C, and the Young's modulus, E, nominal tensile strength, σ, and elongation at break, ϵ were determined. The undrawn sample **3b** exhibited the Young's modulus of 1.1 GPa, compared to that of sample **3a** of 0.7 GPa. The undrawn sample **5b** exhibited $E = 2.3$ GPa, $\sigma = 67$ MPa, and $\epsilon = 5\%$. Isotropic sample **7b** showed $E = 1.9$ GPa, $\sigma = 55$ MPa, and $\epsilon = 3.7\%$. Because of ethyleneoxy moieties in main chain, the Young's moduli of copolyesters **3** dropped markedly. Nevertheless, the mechanical characteristics of a tape that was drawn by 40% (draw ratio = final length/initial length = 1.4) at 120 °C improved and exhibited Young's moduli of 3.0 GPa for **3b** and 1.6 GPa for **3a**. The changes of tensile strengths of the copolyesters during enzymatic degradation have been observed. An almost 10% loss of the tensile strength for the copolyesters has been found after 500 h in enzymatic degradation.

In conclusion, a series of liquid-crystalline aromatic/aliphatic copolyesters were prepared by bulk polycondensation. The hydrophilicity of the copolyesters was increased by incorporation of ethyleneoxy moieties in the main or side

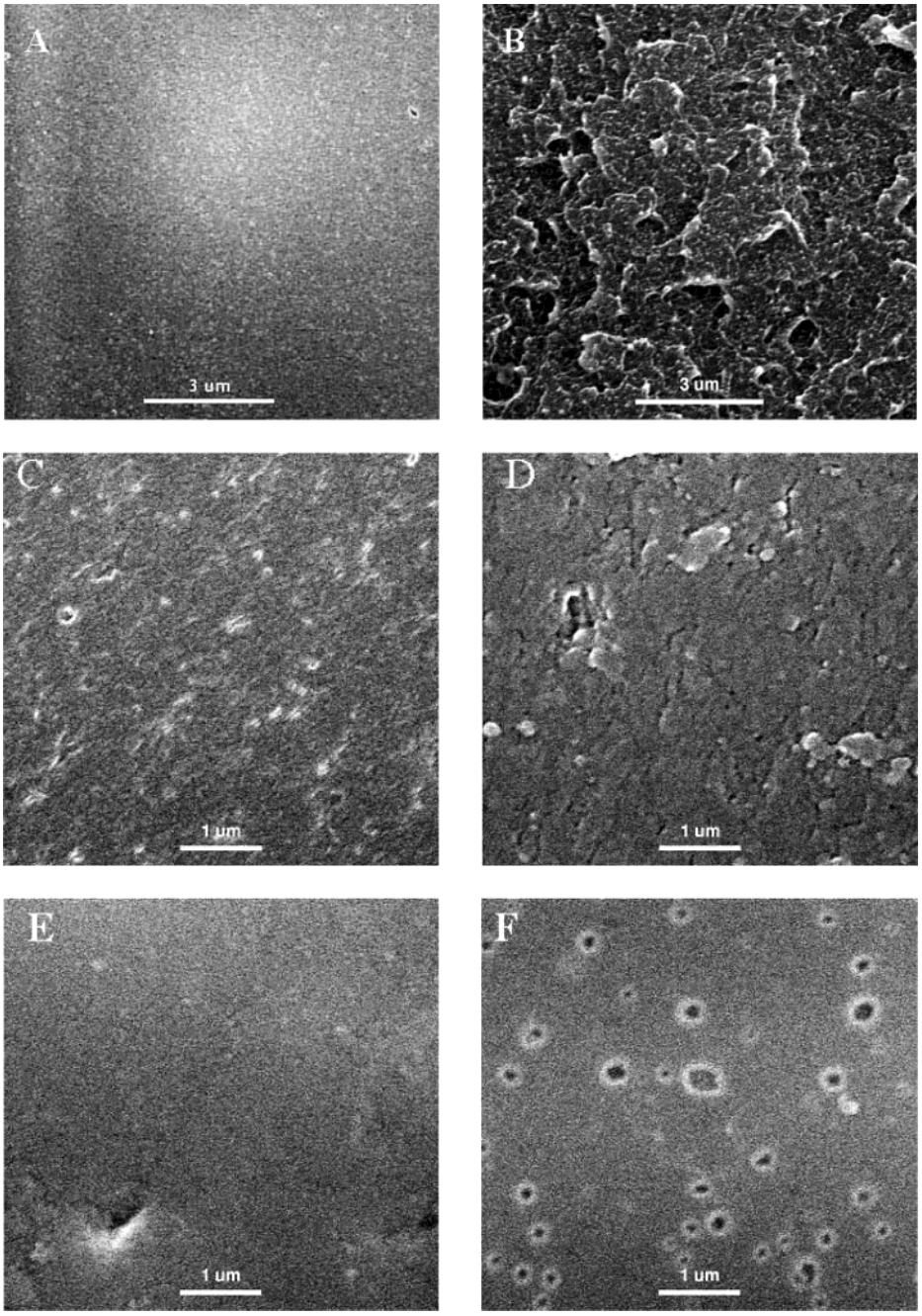


Figure 3. Scanning electron micrographs of the surface of the copolyester films **3a** (A), **5b** (C), and **7b** (E) before enzymatic degradation and **3a** (B), **5b** (D), and **7b** (F) after enzymatic degradation for 500 h.

chain. Degradation of the aromatic/aliphatic copolyesters in buffer solution in the presence or absence of enzyme proteinase K was investigated. Disintegration of the copolyesters occurred during hydrolytic degradation or enzymatic degradation. The copolyesters with an increased content of ethyleneoxy moieties in the main or side chain exhibited more rapid degradation, which probably resulted from and increased hydrophilicity. Enzymatic degradation of the copolyesters resulted in morphological changes principally on the surface. Modifying aromatic species with hydrophilic groups in *aromatic/aliphatic* copolyesters was an efficient method to improve degradability because of the improved solubility of degradation products in aqueous solution. Solution cast/re-molten films of the copolyesters displayed encouragingly good mechanical properties, which might be further enhanced by increasing the molecular weight of the materials.

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