Polymer Bulletin 46, 435–442 (2001)

Polymer Bulletin
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Preparation and properties of poly(dimer acid-sebacic acid) copolymer

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Received: 4 April 2001/Revised version: 10 May 2001/Accepted: 22 May 2001

Summary

Poly (dimer acid-sebacic acid) P(DA-SA) copolymers have been prepared by melt polycondensation of the corresponding mixed anhydride prepolymers. The copolymers were characterized by FT-IR, gel permeation chromatography (GPC), differential scanning calorimetry (DSC), and thermal gravimetric analysis (TGA). *In vitro* studies showed that all the copolymers are degradable in phosphate buffer at 37 °C, and leaving an oily dimer acid residue after hydrolysis for the copolymer with high content of dimer acid. The release profiles of hydrophilic model drug, ciprofloxcin hydrochloride, from the copolymers, follows first order release kinetics. The in vivo biocompatibility of the copolymer in rabbit brain was also evaluated, macroscopic observation and microscopic analysis demonstrated that the copolymer is biocompatible and well tolerated *in vivo*. All the preliminary results suggested that the copolymer might be potentially used as drug delivery devices for brain implantation.

Introduction

Polyanhydrides are one of a few classes of synthetic biodegradable polymers that have received regulatory approval from the U. S. Food and Drug Administration (FDA) for use in humans [1,2]. They have been extensively investigated for use in the controlled delivery of a number of drugs including chemotherapeutics [3,4], antibiotics [4,5], anesthetics [6], and polypeptides [7] in the past twenty years. Recently, novel polyanhydrides designed for use in the fields of orthopedics [8], and polymer drug [9] have been reported. Consequently, polyanhydrides play an increasingly important role in medical application.

Polyanhydrides with desired physico-chemical properties prepared from the derivatives of natural fatty acids, such as erucic acid (C_{22} unsaturated fatty acid) dimer[10], bile acid dimer[11], ricinoleic acid[12,13] and other fat acids[14), have been successfully prepared. They are good candidates for the delivery of hydrophilic drugs due to the desired hydrophobicity of the natural fatty acids in the main chain of the polyanhydrides[3-5,10,11]. Industrial dimer acid (DA) containing C36 aliphatic group is another kind of natural fat acids dimer with good hydrophobicity, which results from the dimerization of refined natural C_{18} unsaturated fatty acids comprised of oleic and linoleic acid [15]. It is an important monomer and widely used in the preparation of copolyamides [15,16] with unique properties, and other functional

materials [17]. Seeking for novel polyanhydride, which may be used for the delivery of hydrophilic drugs, we prepared a new type of polyanhydride derived from dimer acid (DA), and sebacic acid. In this work, we reported some preliminary results on the preparation, characterization, *in vitro* biodegradation, release profiles of hydrophilic ciprofloxcin hydrochloride, and *in vivo* biocompatibility of the copolymers.

Experimental

Materials

Industrial dimer acid (DA) with molecular weight 565, saponification value (mgKOH/g) 193-200, and 95% purity, was supplied by Sichuan Lutianhua Co. Ltd. (China). It is a yellowish transparent liquid. The industrial dimer acid (DA) was purified by dissolving it in CH_2Cl_2 and extracting the impurities with distilled water for twice. The organic phase was dried over 4A molecular sieve, and CH_2Cl_2 in the mixture was evaporated at 40 °C under reduced pressure.

Analytically pure sebacic acid (SA) were purchased from Shanghai Chemical Reagents Factory (Shanghai China), and recrystallized twice from ethanol. Acetic anhydride was refluxed over magnesium strips and distilled, bp139~142 °C before use. All the solvents were dried over 4A molecular sieve and distilled before use.

Ciprofloxcin hydrochloride was purchased from Tianjing Central Pharmaceutical Corp., Ltd. (Tianjing, China).

Instruments

IR spectra were obtained with a Bruker EQUINOX55 FT-IR spectrometer. Polymer samples were prepared in dichloromethane and cast onto NaCl plates for recording IR spectra. Acids and prepolymers were either press into KBr pellets or onto NaCl plates directly.

Thermal analyses were performed on a Perkin Elmer system consisting of DSC 7 and TGA 7 analyzers with a TAC7/DX instrument controller. Data were processed using UNIX thermal analysis system software on a DEC computer station. For DSC, an average sample weight of 5~10mg was heated at 10 °C/min under argon atmosphere. The melting point and the heat of fusion were measured by DSC. For TGA, an average sample weight of 10mg was heated at 10 °C/min under a flow of argon. The decomposition temperature was detected by TGA. The molecular weights of the polymers were determined on a Waters GPC system consisting of a Water 600 pump, Water 410 Differential Refractive Index detector. Samples were eluted in dichloromethane through a Varian MicroPak G4000 and G3000 column installed in series at a flow rate of 1.0ml/min. Molecular weight were determined relative to narrow dispersed polystyrene standards with a molecular weight range from 1000 to 200,000. Viscosity of the polymers were measured with a Ubbelohde viscometer at 23 °C.

Synthesis of Prepolymers

The prepolymer of dimer acid(DA) was synthesized by refluxing the purified DA with acetic anhydride(50g in 300ml) for 20~25min. The excess acetic anhydride was

distilled at about 50 °C under reduced pressure (100Pa). The prepolymer of DA was light yellow liquid at room temperature. IR (liquid film on NaCl, cm^{-1}): 2926 and 2854(C-H), 1802, 1720 (C=O, anhydride), 1039(C-O). The strong absorptive peak at 1710cm^{-1} disappeared suggested that all the –COOH groups converted to the corresponding mixed anhydride were insured.
The prepolymer of sebacic acid (SA) was prepared as described by Domb [18]. All the prepolymers were stored in a P_2O_5 desiccator under vacuum at -20 °C until required.

Polymerization

The copolymers of DA and SA were synthesized by melt polycondensation of the corresponding prepolymers. In a typical copolymerization, 5g DA prepolymer and 5g SA prepolymer were added to a glass tube (2 X 20cm) with two side arms with magnetic stirring and was polymerized at 180 °C under high vacuum conditions (about 20~50Pa) for 1 h. During the polymerization, a strong nitrogen sweep with vigorous agitation of the melt was performed for 30 seconds every 15 minutes. The crude polymer was dissolved in CH_2Cl_2 and precipitated by filtering into vigorously stirring dry petroleum ether (60 ~ 90 °C). The precipitate was filtered off, washed with petroleum ether, and dried at 40 °C under vacuum for 48 hours. The yield in this example is greater than 80%.

In vitro degradation and drug release

Samples of polyanhydride in a cylindrical shape (4mm in diameter, 10mm in long, about 145mg) were prepared by melting at 80 °C for 10 min in a homemade polytetrafluoroethylene (PTFE) mould. The mould was then allowed to cool in a desiccator over phosphorous pentaoxide at room temperature for 30 min, after which time the mould was dismantled and the device removed. The drug-loading device (20% ciprofloxcin hydrochloride) in a cylindrical shape (4mm in diameter, 10mm in long, about 150mg) was produced as follows. The copolymer and the drug was sieved to 100~150μm and melted in the above mould as described above.
In vitro degradation and drug release was performed in a reciprocal air bath(60rpm) by placing a device into 50ml of 0.1 M phosphate buffer solutions (pH7.4) at 37 °C. The phosphate buffer solutions were daily changed to maintain sink conditions throughout, and the device was carefully freeze-dried. The degradation rate was measured by the weight loss of dry weight of the polymer sample. The amount of drug released was detected by the absorbance of the release medium at 277nm using a Perkin Elmer UV/Vis spectrometer, Lambda Bio 40. All measurements were carried out in triplicate by analyzing three separate devices.
All the detected data were processed using Microcal Origin 4.5 software on a PC computer.

Results and Discussion

Polyanhydride synthesis

Dimer acid with molecular weight 565, and 95% purity used in our study was

industrially dimerized from refined natural C_{18} unsaturated fatty acids comprised of oleic and linoleic acid. Although it may be a multi-component dimer, its structure commonly expressed as Fig. 1 in previous literature [15,16]. But it may be also simply expressed as HOOC--$C_{34}H_{68}$-COOH according to the molecular weight and carbon atoms numbers of the dimer acid.

For the copolymer synthesis, sebacic acid and dimer acid were converted to the corresponding prepolymer (1) and (2) (Fig. 1) by reacting with acetic anhydride. Poly (dimer acid-sebacic acid) copolymers with different prepolymer weight ratios were successfully prepared by melt condensation of the corresponding dimer acid prepolymer and sebacic acid prepolymer under high vacuum conditions (Fig. 1).

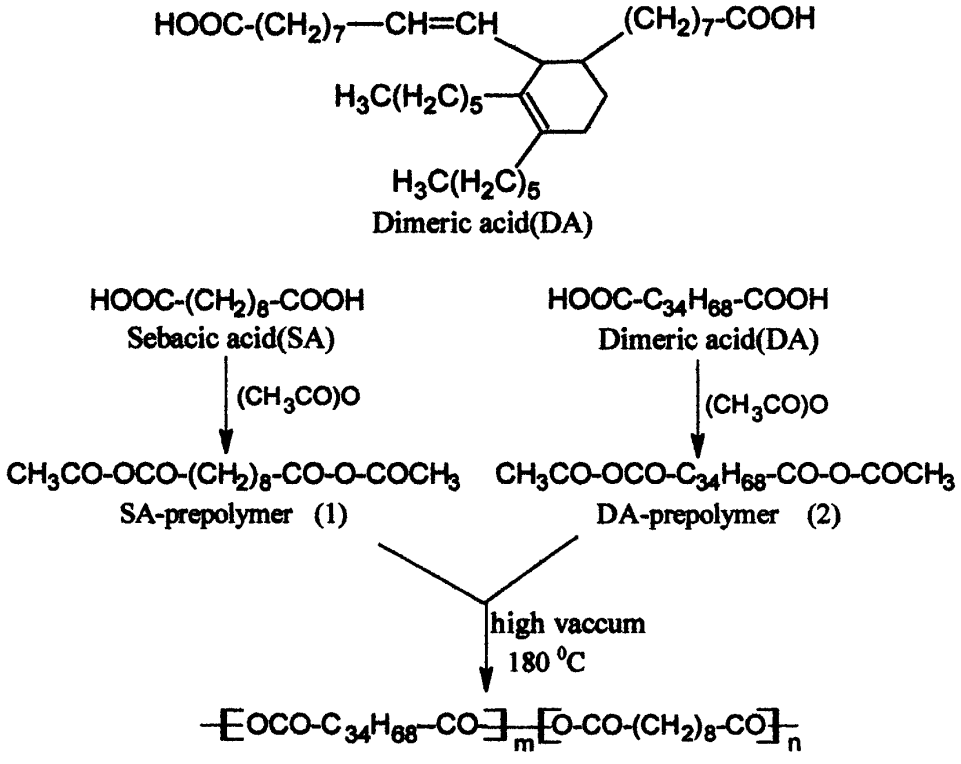

Fig.1. Synthesis of poly (dimer acid-sebacic acid) copolymer [P (DA-SA)]

Product Characterization

All the prepared copolymers were characterized by FT-IR. The IR spectrum of a representative copolymers, poly (dimer acid-sebacic acid) [P (DA-SA) (DA: SA=50:50 by weight)] is shown in Fig.2, strong absorption peaks at ~1810 and 1745 cm^{-1}, which are characteristic absorptions of carbonyl stretching vibrations of aliphatic anhydride groups, occurred in the IR spectra of all the products. The absorption bands at 2900 cm^{-1} and 2800 cm^{-1} are characteristic of C-H stretching vibrations of long aliphatic alkyl, and those at 1050 cm^{-1} are attributed to C-O stretching of anhydride group. No detectable absorption bands over 3000 cm-1 in the IR spectra showed that no –COOH groups occurred in the copolymers.

The dimer acid (DA) content in the copolymer was determined by quantitative analysis of the recovered DA monomer after the copolymer hydrolysis. Briefly, the copolymer was hydrolyzed in distilled water(10g in 100ml) with magnetic stirring at

80 °C for 48h. The oily DA component, which is not dissolved in water, was isolated by extraction with CH_2Cl_2. The excess CH_2Cl_2 was evaporated at about 50 °C under reduced pressure (100Pa), and the DA in the copolymer was determined gravimetrically. All the detected results of the dimer acid contents in the copolymers support the gravimetrical feed ratios.

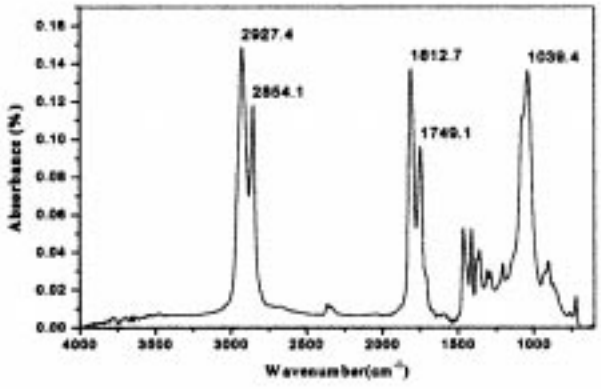

Fig.2. Infrared spectrum of P (DA-SA) (DA /SA =50/50)

The molecular weight, viscosity and DSC, TGA characterizations of the products were summarized in Table 1.

Table 1. Physical properties of poly (dimer acid-sebacic acid) copolymer [P (DA-SA)]

Polymer composition (DA/SA, w/w)	Yields (%)	Molecular weight[a]		Viscosity[b] (dL/g)	T_m[c] (℃)	T_d[d] (℃)	ΔH[c] (k/J)
		M_w	M_n				
P (DA-SA) 10:90	92	56000	25000	0.42	80.0	217.2	160.2
P (DA-SA) 20:80	85	48000	21000	0.26	77.5	214.7	152.6
P (DA-SA) 30:70	87	43000	24000	0.43	73.2	208.9	130.4
P (DA-SA) 40:60	80	34000	17000	0.63	68.7	238.0	76.8
P (DA-SA) 50:50	83	26000	11000	0.31	67.1	223.6	52.1

[a]Determined by GPC using polystyrene as standard; [b]Determined in chloroform using Ubbelohde viscometer at 23℃; [c]Determined by DSC. [d]Determined by TGA.

As shown in Table 1, the weight-average molecular weights of an the prepared copolymers were over 26,000 and high enough to meet the essential requirement for polyanhydride as drug delivery material. The values of T_m determined by DSC for all five P (DA-SA) copolymers are lower than 80 °C. The lower T_m may be advantage for the formulation of thermal unstable drug under mild conditions. Moreover, the molecular weights increase with the sebacic acid content in the copolymers increase,

whereas the T_m values, and heat of fusion (ΔH) decreased. This regularly change trend was also observed in literature [10]. The values of T_d over 200 °C for all the copolymers suggested that the copolymers possess a desired thermal stability.

In vitro degradation

In vitro degradation of the copolymer was studied in 0.1 M phosphate buffer solutions (pH7.4) at 37 °C using a cylindrical device. The degradation rate was determined by measurement the weight loss of the device. The plots of the percentage weight loss of the device vis degradation time were shown in Fig.3. During the tested periods, all the devices did not disintegrate. As shown in Fig.3, the degradation rate of the copolymer decrease with the hydrophobic component (dimer acid) content in the copolymer increases. It suggested that hydrophobicity of dimer acid do much contribution in regulating the degradation rate of the copolymer during the polymer hydrolysis periods. After about 9 days degradation, the P(DA-SA) (DA:SA=50:50) almost remained constant weights, and an oily remnant was remained. The FT-IR spectrum of the oily remnant, which has the same absorptive characteristics of dimer acid, suggests that the remnant was dimer acid.

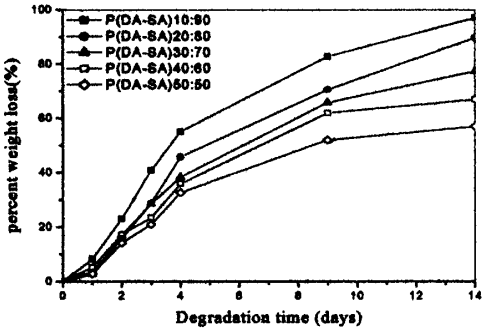

Fig.3. Degradation profile of P(DA-SA) in 0.1M pH7.4 phosphate buffer at 37℃

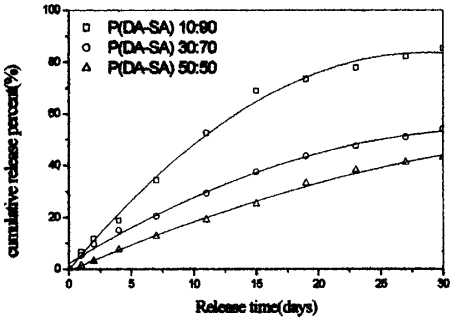

Fig.4.Ciprofloxcin hydrochloride release profile from P(DA-SA) in 0.1M pH7.4 phosphate buffer at 37℃

In vitro drug release

Ciprofloxcin hydrochloride was used as hydrophilic model drug to investigate the drug delivery property of the copolymers. The drug release characteristics from P(DA-SA) copolymers(DA:SA=10:90, 30:70, and 50:50, weight ratio)were determined using 20% ciprofloxcin hydrochloride loading devices. All the devices did not crumble The plots of cumulative release percent of the device vis release time is shown in Fig.4 and Table 2. It demonstrated that the drug release rate is decreased with the dimer acid content in the copolymer increased. This result shows that the hydrophobic component, dimer acid (DA), play an important role in adjusting the drug release rate. A similar release characteristics also has been observed for poly(erucic acid dimer-sebacic acid) copolymer in literature [10]. Moreover, the relatively steady drug releases from the copolymers over one month were achieved. The cumulative release percent as a function of the detected release time was simulated using a first order release kinetics equation $Y=a+bt+ct^2$ (Y is the cumulative

release percent and *t* is the corresponding sustained release time). The results demonstrated that the release profiles correlate first-order release kinetics very well. Similar results have been reported for copolymers derived from other natural fat acids [10,11]. All above have shown that the copolymers have desired properties for the delivery of hydrophilic model drug, ciprofloxcin hydrochloride.

Table 2 Fitting data using first-order equation for 20% drug-loading devices

Polymer composition (DA/SA, w/w)	$Y=a+bt+ct^2$			Coefficient (r^2)
	a	*b*	*c*	
P(DA-SA)10：90	0.206	5.852	-0.102	0.996
P(DA-SA)30：70	2.242	2.969	-0.0423	0.996
P(DA-SA)50：50	-0.706	2.109	-0.0206	0.997

Preliminary in vivo biocompatibility

The biocompatibility of the P(DA-SA) (DA;SA=50:50) copolymer was preliminary evaluated in rabbits brain as compared the clinically used biopolymer, absorbable gelatin sponge. Briefly, the tested device and the absorbable gelatin sponge (3mm in diameter, 1mm in thickness) were implanted in two sides of the rabbit's brain, respectively. The evaluation was designed to last for 1 month. At the predetermined time post implantation, rabbits were sacrificed and their brains were removed to assess the microscopic histopathology using hemotoxylin-erosin stained analysis.

Macroscopically, all the experimental rabbits survived healthily and actively to the date of their sacrifice indicated the devices were well tolerated. The implanted sites were clean and the devices were easily retrieved, no obvious damage to the locally implanted tissue was observed. Fig.5 shows the photomicrography of rabbit brain at day 30 after P(DA-SA) (DA:SA=50:50) implantation. Microscopically histopathologic observations show that the cellular and tissue response to the implanted the polymer was essentially equivalent to the absorbable gelatin sponge except for very minimal edema observed. The results presented here suggest that P(DA-SA) copolymer is well tolerated by the brain tissue of rabbit. It maybe used as a material suitable for brain implantation.

Conclusions

The copolymers derived from the dimer of C_{18} unsaturated fatty acids and sebacic acid were successfully prepared by melt polycondensation with high molecular weights and high yields. All the prepared copolymers possess low melting points, desired thermal stability, biodegradability, and relatively steady release rate for hydrophilic model drug, ciprofloxcin hydrochloride. The preliminary result of the *in vivo* biocompatibility of P(DA-SA) (DA:SA=50:50) in rabbit's brain shown that the tested copolymer is well tolerated by the brain tissue of rabbit. All these results shown that the copolymers might be potential candidates for the delivery of hydrophilic drugs locally in brain.

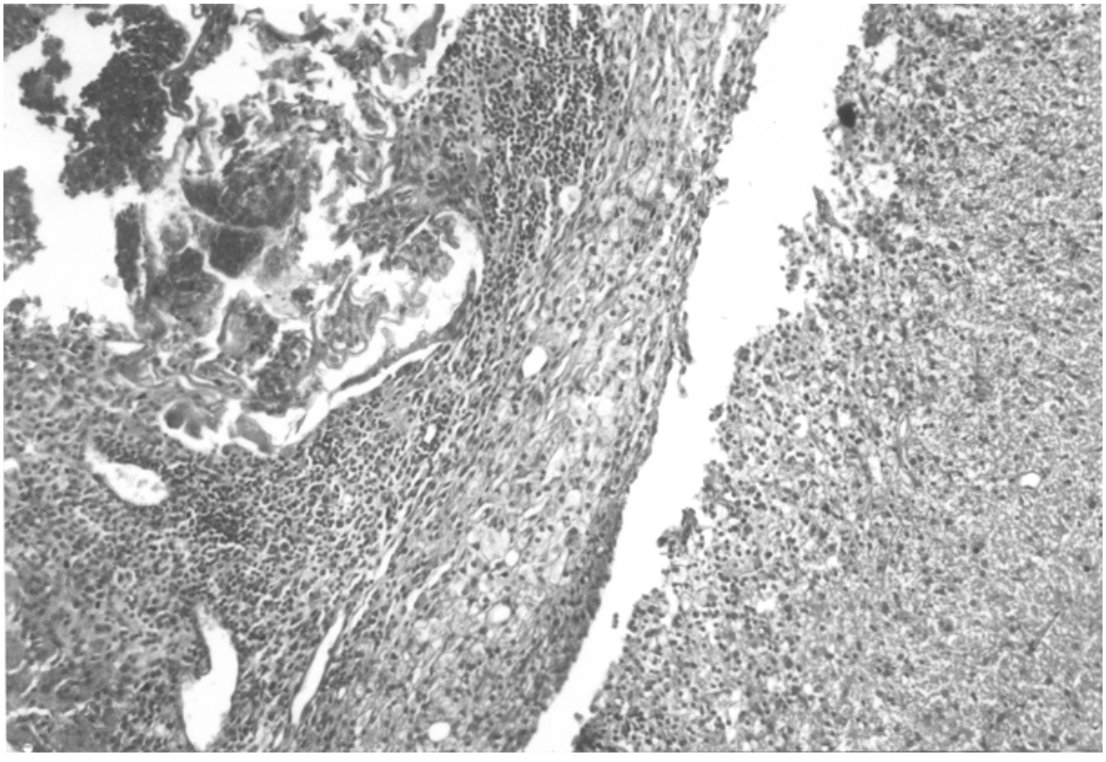

Figure 5. Hematoxylin-eosin stained photomicrograph of rabbit brain at day 30 after P(DA-SA) (DA:SA=50:50) implantation

Acknowledgement
Funding by Natural Science Foundation of Wuhan City is gratefully acknowledged.

Reference

1 Langer R (2000) *Acc. Chem. Res.* **33(2)**: 94
2 Brem H, Piantadosi S, Burger PC, Walker M, Selker R, Vick NA, Black K, Sisti M, Brem S, Mohr G (1995) *Lancet* **345(8956)**: 1008
3 Sampath P, Brem H (1998) *Cancer Control* **5(2)**: 130
4 Park ES, Maniar M, Shah JC (1998) *J. Control Release* **52 (1-2)**: 179
5 Stephens D, Li L, Robinson D, Chen S, Chang H, Liu RM, Tian Y, Ginsberg EJ, Gao X, Stultz T (2000) *J. Control Release* **63**: 305
6 Masters DB, Berde CB, Dutta S, Turek T, Langer R (1993) *Pharm. Res.* **10(10)**: 1527
7 Carino GP, Jacob JS, Mathiowitz E (2000) *J. Control Release*, **65 (1-2)**: 261
8 Muggli DS, Burkoth AK, Anseth KS (1999) *J. Biomed Mater. Res.* **46 (2)**: 271
9 Erdmann L, Macedo B, Uhrich KE (2000) *Biomaterials* **21(24)**: 2507
10 Domb AJ, Maniar M (1993) *J. Polym. Sci., Part A: Polym. Chem.* **31**: 1275
11 Domb AJ, Nudelman R (1995) *J Polym. Sci., Part A: Polym. Chem.* **33**: 717
12 Teomim D, Nyska A, Domb AJ (1999) *J. Biomed Mater. Res.* **45 (3)**: 258
13 Gouin S, Zhu X, Lehnert S (2000) *Macromolecules* 33**(15)**: 5379
14 Teomim D, Domb AJ (1999) *J. Polym. Sci., Part A: Polym. Chem.* **37**: 3337
15 Fan XD, Deng Y, Waterhouse J, Pfromm P, Carr WW (1998) J. Appl. Polym. Sci. **68(2)**: 305
16 Fan XD, Deng Y, Waterhouse J, Pfromm P (1999) J. Appl. Polym. Sci. **74(6)**: 1563
17 Tunca Ü, Koc S (1997) J. Appl. Polym. Sci. **63(10)**: 1275
18 Domb AJ, Langer R (1987) *J. Polym. Sci., Part A: Polym. Chem.* **25**: 3373