



Synthesis of genistein-containing star-shaped homo- and copolyesters by the ring-opening polymerization

Dr.A.madhu ¹,S.Jyothirmayi ²,

Professor¹, Associate Professor ²,

Department of Chemistry,

BRILLIANT GRAMMAR SCHOOL EDUCATIONAL SOCIETY'S GROUP OF INSTITUTIONS-

INTEGRATED CAMPUS Abdullapurmet (V), Hayath Nagar (M), R.R.Dt. Hyderabad.

Abstract

To facilitate the direct synthesis of aliphatic star-shaped homo- and copolyesters, a new initiator has been created; this initiator may be found in soy. Genistein, one of numerous well-studied isoflavones, has been used as a catalyst for the homopolymerization of poly (L-Lactide) and the co-initiator of the copolymerization of ϵ -caprolactone and L-Lactide with Sn (Oct) 2. Biocompatible functionalized polymers, useful in the medical and pharmaceutical fields, may be easily synthesized at minimal cost using chemicals that are safe for human consumption. Analysis using ¹H NMR, ¹³C NMR, FT-IR, SEC-MALLS, and MALDI-TOF MS was used to characterize the produced polymers.

Introduction

Biomedical applications such as controlled drug delivery systems, surgical sutures,

and internal bone fixation have piqued interest in the production of aliphatic homo- and copolyesters [1-4]. The biodegradability, biocompatibility, and permeability of poly(L-Lactide) (PLA) and poly(ϵ -caprolactone) (PCL), as well as their copolymers, make them ideal candidates for application as biomedical materials [5, 6]. In order to alter the topologies and physical characteristics of PLA and PCL (such as their high degree of crystallinity), researchers are interested in polyesters with well-defined architectures, such as star-shaped, comb-like, and dendritic polymers with regulated chain numbers and lengths [7, 8]. L-Lactide (LLA) or ϵ -caprolactone (CL) are often used to synthesis these homo- and copolymers using ring-opening polymerization (ROP) and copolymerization in the presence of multifunctional initiators having hydroxyl or amine groups. Numerous strategies have been developed in recent years to enhance



the bioactivity and biocompatibility of the resulting polymers to better suit their intended biomedical uses. Hormones, peptides, amino acids, lipids, and other natural substances that are bioactive or biocompatible may be included into the polymer chain [9–12]. Using bulk dipentaerythritol as an initiator in the ROP of CL, Dong and colleagues have recently studied the synthesis, crystallization, and morphology of a six-arm star-shaped PCL. They have also effectively synthesized and studied a novel class of linear-dendron-like PCL-bpoly(ethylene oxide) copolymers [14, 15] and polypseudorotaxanes made of star-shaped porphyrincored PCL and α -cyclodextrin. Cui et al. [17] reported the synthesis and characterization of copolymers of CL and LLA using a cyclotriphosphazene core, and Cho et al. [16] investigated the synthesis of star-shaped, amphiphilic block copolymers composed of fully degradable PCL by initiation with pentaerythritol. Our group has also effectively synthesized and characterized star-shaped PCLs with a PAMAM dendrimer core [18]. Soybeans include a group of compounds called isoflavones, one of which is genistein (Gns) [19]. Recent studies have shown that this chemical is an antioxidant, inhibits topoisomerase II and angiogenesis, and stimulates cell differentiation [19]. It also possesses modest estrogenic and antiestrogenic effects. In addition, Gns has

been shown to inhibit the progression of breast cancer by promoting cell differentiation, which leads to a reduced epidermal growth factor signaling pathway in adulthood [20]. The presence of three hydroxyl groups in the molecule makes it a favorable material for initiating ROP of LLA or CL, making it possible to synthesize star-shaped polymers. Since the Gns molecule possesses the necessary biological characteristics, it makes sense to create homo- and copolymers containing them. In this continuing research, natural Gns were used to manufacture and analyze biodegradable and biocompatible PLA homopolymers and random copolymers of LLA and CL. Next, we will describe how the Gns molecule affects the thermal characteristics and hydrolysis stability of the resultant polyesters, as well as the release profile of the covalently linked medication.

Experimental

Materials

Before being used over CaH_2 at low pressure, 2-oxepanone (C99.0%, Aldrich Co., Poland) has to be dried and distilled. Recrystallization of dried ethyl acetate in a dry nitrogen environment followed by complete drying in a vacuum prior to usage yielded L-Lactide ((3S)-cis-3, 6-dimethyl-1, 4-dioxane2, 5-dione, 98.0%, Aldrich Co. Poland). Preparation of



genistein (Gns, 5, 7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, C98%, Sigma Co. Poland) for usage required 4 hours of drying at 40 degrees Celsius under vacuum. As delivered, we made use of stannous octoate (Sn (Oct) 2, tin (II) 2-ethylhexanoate, 2-ethylhexanoic acid tin (II) salt, *95%, Aldrich Co., Poland). Anhydrous dichloromethane (C99.8%, POCh, Gliwice, Poland), methanol (99.8%, POCh, Gliwice, Poland), and diethyl ether (99.8%, POCh, Gliwice, Poland) were all used in their purified forms.

Measurements

In a DMSO-d₆ solution, ¹H and ¹³C NMR methods (Varian 300 MHz) were used to describe the polymerization products. Using a Perkin-Elmer Spectrum 1,000 spectrometer, we collected KBr pellet FT-IR spectra in the region of 400 to 4,000 cm⁻¹. The SEC-MALLS instrument included a 1,100 Agilent isocratic pump, autosampler, degasser, thermostatic box for columns, photometer MALLS DAWN EOS (Wyatt Technology Corporation, Santa Barbara, CA), and differential refractometer Optilab Rex for measuring number-average molecular weights (M_n) and polydispersity indices (M_w/M_n). Wyatt Technology Corporation's ASTRA 4.90.07 software was utilized for the collection and analysis of the data. As a means of separation, we used two 2 X

TSKgel MultiporeHXLcolumns. The samples were dissolved in methylene chloride and injected that way. The injection loop had a capacity of 100 μL. The mobile phase was methylene chloride, and the flow rate was 0.8 mL min⁻¹. The ultrafleXtreme™ MALDI-TOF MS spectrometer (Bruker Daltonics, Fremont, CA) was used to take the mass spectra, and it included a pulsed N₂ laser and a time-delayed extraction ion source. The accelerating voltage was set at 20 kilovolts. The linear mode was used to get the mass spectra. Polymer samples were combined with a matrix solution of 2[(4-hydroxyphenyl)diazetyl] benzoic acid (HABA) dissolved in THF or methylene chloride at a concentration of 10 mg mL⁻¹. Cationic NaI was added to the mix. The concoction was dried on a gold metal target-covered stainless steel plate.

Polymerization procedure

Initiator (genistein) and monomer (ε-caprolactone or L-lactide) molar ratios were varied during polymer material preparation. Received polymers had initiator/monomer feed ratios of 1/25, 1/50, and 1/75 (mol/mol), respectively, for PLA homopolymers and 1/25, 1, 10, and 40 (mol/mol), respectively, for PLA/PCL copolymers (where Gns = genistein; PLA = poly(L-Lactide); PCL = poly(ε-caprolactone)). Dry Gns and LLA were measured out and added to a 50 mL



polymerization tube for homopolymerization. After hooking up the tube to a Schlenk line, the operation of emptying and refilling it was done three times. For 48 hours, the tube was heated in an oil bath at 140 degrees Celsius in an argon environment. The reaction products were cooled, dissolved in dry CH₂Cl₂, precipitated twice from cold diethyl ether, and dried under vacuum for 72 hours after the reaction was complete. ¹H NMR data for Gns/PLA 25 (DMSO-d₆, 300 MHz, dH, ppm): 8.31 (s, H-1), 7.38-7.35 (dd, H-10, 40, J_{10, 20} = 8.5, J_{10, 40} = 2.0), 6.82-6.80 (dd, H-20, 30, J_{10, 20} = 8.5, J_{10, 30} = 2.0), 6.38 (d, H-6, J_{6, 5} = 2.0); ¹³C-NMR (DMSO-d₆, 300 MHz, d, ppm): 181.12 (4), 173.98 (c0), 171.14 (c00), 169.16 (c), 168.11 (40), 158.51 (9), 153.90 (2), 131.15 (20), 124.50 (3), 110, 115.

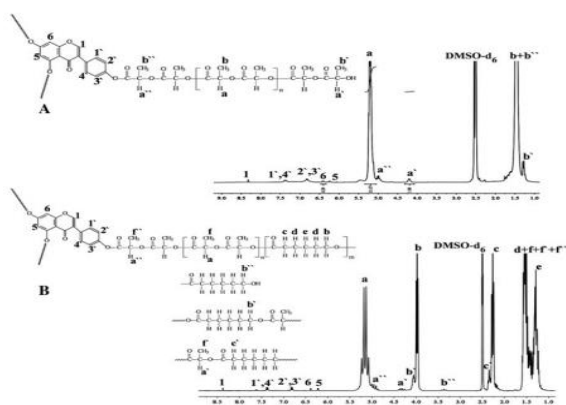


Fig. 1 ¹H NMR spectra of Gns/PLA 25 (a) and Gns/PLA25/PCL25 (b)

Data for Gns/PLA 25 FT-IR (KBr, cm⁻¹): 2,995 (tasCH₃), 2,947 (tsCH₃), 2,884 (tCH), 1,747 (tC = O), 1,451 (dasCH₃), 1,378 (dsCH₃), 1,368-1,360 (d1CH? dsCH₃), 1,264 (dCH? tCOC), 1,197 (tasCOC? Dry Gns and LLA were measured out and added to a 50 mL polymerization tube for the copolymerization. After hooking up the tube to a Schlenk line, the operation of emptying and refilling it was done three times. For 48 hours, the tube was heated to 140 degrees Celsius in an oil bath with an argon environment. After waiting the proper length of time, the exact quantity of CL and a catalytic amount of Sn(Oct)₂ were added to the molten mixture, and the procedure of depleting and replenishing was repeated. After 24 hours of polymerization, the tube was placed in an oil bath heated to 110 degrees Celsius in a nitrogen environment and allowed to cool to ambient temperature. The byproducts were dried under vacuum for 72 hours after being dissolved in dry CH₂Cl₂, precipitated twice from cold diethyl ether, and dissolved again. Gns, PLA25, and PCL25 NMR Spectra 8.32 (s, H-1) 7.38-7.35 (dd, H-10, 40, J_{10, 20} = 8.5, J_{10, 40} = 2.0) 6.82-6.79 (dd, H-20, 30, J_{10, 20} = 8.5, J_{10, 30} = 2.0) 6.38 (d, H-6, J_{6, 5} = 2.0) 6.21 (d, H-5, J_{6, 5} = 2.0) 5.19 (q, -CH(CH₃)- of PLA (a)) 4.94 ¹³C-NMR (DMSO-d₆, 300 MHz, d, ppm): 181.14 (4), 173.91 (a0), 173.37 (h), 172.04 (h0),



169.65 (a), 168.37 (40 1 5 1 7), 158.61 (9), 153.94 (2), 130.09 (20 1 60), 124.77 (3,10), 115.01 (30 1 50), 106.09 (10), 98.91 (Electronic Supplemental Figures S-2 and S-4). The following are the results of an FT-IR analysis of Gns/PLA25/PCL25 (KBr, cm⁻¹): 2,997 (tasCH₃), 2,949-2,945 (tasCH₂) (tsCH₃), 2,865 (tsCH₂), 1,764 (tC = O), 1,457 (dasCH₃), 1,348-1,387 (dsCH₃), 1,368-1,360 (d1CH₂). Discussion and Results The angiogenesis inhibitor natural isoflavonoid Gns was present during large-scale ROP homopolymerization of LLA [19]. In search of

Table 1 Homopolymerization of LLA and copolymerization of CL and LLA initiated (co-initiated) by Gns; molecular characterization of the resulting polymers

Entry	[LLA]/[CL]/[I]	Yield (%)	DP ^a	DS ^b	M _n (NMR)	M _w (SEC-MALLS)	M _w /M _n
Gns/PLA 25	25/0/1	88	22.30	3.4	5,700	5,500	1.32
Gns/PLA 50	50/0/1	83	36.50	2.9	7,900	7,200	1.40
Gns/PLA 75	75/0/1	90	45.50	2.8	9,500	9,100	1.27
Gns/PLA25/PCL25	25/25/1	93	34.10	3.1	12,400	12,100	1.36
Gns/PLA10/PCL40	10/40/1	98	31.30	2.8	10,300	9,300	1.37
Gns/PLA40/PCL10	40/10/1	96	34.60	2.9	11,700	11,500	1.50

L,L-Lactide (LLA), ε-caprolactone (CL), I initiator—genistein (Gns), and a nitrogen environment (for homopolymerization) or 110 degrees Celsius (for copolymerization) are the reactants. Calculated from the area ratio of the terminal methine proton of PLA (4.18 ppm) or PCL (3.31 ppm) to the area ratio of the internal methine proton of

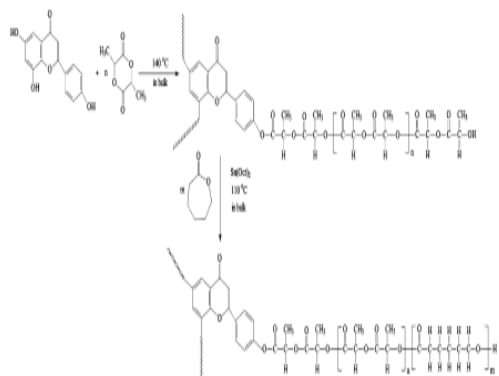
PLA (5.19 ppm) or PCL (3.97 ppm), a DP is an average degree of polymerization as measured by ¹H NMR analysis. b DS peak regions for the terminal methine proton of PLA (4.19 ppm), methylene proton of PCL (3.31 ppm), and benzyl proton of Gns (6.38 ppm) are used to establish an average degree of substitution using ¹H NMR analysis. ¹H NMR spectroscopy was used to provide an estimate of c M_n(NMR). In order to calculate d M_n(SEC-MALLS) and M_w/M_n, an SEC-MALLS instrument was used.

To create LLA prepolymers of varying molecular weights, we altered the LLA: Gns molar ratio ([LLA]/ [I] = 25, 50, and 75). Fig. 1a displays the ¹H NMR spectra for Gns/ PLA 25. The PLA chain was responsible for the a and b major resonance signals, whereas the hydroxyl group at the end of the methine proton signal was responsible for the a₀ signal. Methine proton signal of PLA unit coupled to initiator molecule was also identified as a₀₀. Importantly, high-resolution detection of the proton signals of the phenyl and benzopyran-4-one rings of Gns (Fig. S-1, electronic supplementary material) [21] indicates that Gns has been incorporated into the macromolecule. The study of ¹H NMR spectroscopy confirmed that ROP of LLA was effectively triggered by naturally occurring Gns. Comparison of the ¹³C NMR spectra of the resultant



homopolymer and the pure initiator (Figs. S-2 and S-3, electronic supplementary material) provides further support of the existence of Gns in the macromolecule. Number-average molecular weights obtained by ^1H NMR spectroscopy ($M_n(\text{NMR})$) were derived from the average chain length (DP) and average degree of substitution (DS) of the generated homopolymers based on the well accepted conclusion [22]. Table 1 displays the calculated findings and SEC-MALLS information. As shown by the data, the three PLA arms may be linked to the Gns initiator molecule, proving that the three hydroxyl groups of Gns served as efficient starting sites of ROP of LLA. In addition, it was shown that the amounts of M_n acquired using SEC-MALLS were consistent with those determined via ^1H NMR analysis.

Using the natural initiator, polymer materials were synthesized with a high yield and a moderate molecular weight dispersion. As shown in Scheme 1, hydroxyl end-capped PLAs containing a Gns core were used as macroinitiators in the ROP of CL. To prevent transesterification, the copolymerization was conducted in large quantities at temperatures of roughly 110 C [23, 24]. The PLA macroinitiator and final copolymer were analyzed by SEC-MALLS. The obtained products' typical SEC-MALLS curves showed an elution peak that was symmetrical and had low polydispersity (M_w/M_n , Table 1). The PLA-rPCL copolymer also showed a trend toward greater molecular weights than PLA macroinitiator (Fig. 2; Table 1). Fig. 1b displays the ^1H NMR spectra of Gns/PLA25/PCL25. The phenyl and benzopyran-4-one proton signals in Gns are easily discernible [for reference, check out the ^1H NMR spectrum of the pure initiator (Fig. S-1, bottom)].



Scheme 1 Synthesis of homo- and copolymers

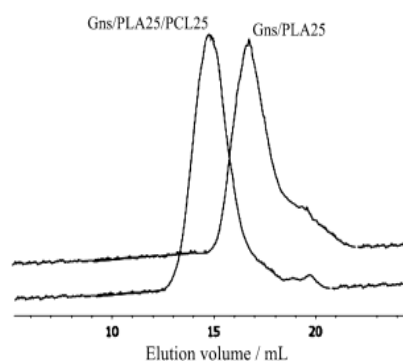




Fig. 2 SEC traces of the PLA macroinitiator and the corresponding PLA-r-PCL copolymer

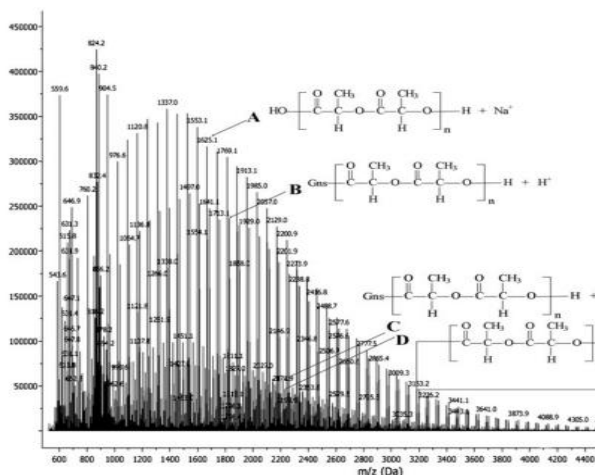


Fig. 3 MALDI-TOF MS spectrum of Gns/PLA 25

added content (in the form of electronic files)]. Polymerization of CL was efficiently kicked off by the terminal hydroxyl groups of the macroinitiators, and the existence of a novel triplet signal ascribed to methylene protons (b00) confirmed that PCL blocks were used as end groups in the resultant copolymers. This event agrees with our earlier research [25]. Additional confirmation of the structure of the resultant copolymers is provided by the ^{13}C NMR spectra (Figure S-4, Electronic Supplementary Material). The degree of substitution, average chain length, and number-average molecular weights were also calculated with the use of ^1H NMR spectroscopy. The calculated trend seems to be consistent with that seen in the PLA system. SEC-MALLS analysis yielded number-average molecular weights

that were consistent with ^1H NMR estimations (Table 1), and the molecular weight dispersion was likewise mild. In Figure 3, we see the MALDI-TOF mass spectrum of PLA that was generated by homopolymerization of LLA started by Gns (Gns/PLA 25) and that had PLA molecules ending with one hydroxyl and one hydrogen end group (residual mass 41, Na^+ adduct, A). The second set of peaks is consistent with PLA that has Gns and hydrogen end groups (H^+ adduct, B; residual mass 271). Third series, weaker in intensity, is compatible with macrocyclic polymers (residual mass 3, Na^+ adduct, D), and fourth series, weaker in intensity, is likewise consistent with PLA ending with Gns and hydrogen end groups, but with Na^+ adduct (residual mass 274, C). Two distinct populations of chains, one with an even number of LA repeating units and the other with an odd number, are seen in the mass spectrum shown in Fig. 3. They had 72 amu between them. The polymerization of LLA and the subsequent intra- and intermolecular transesterification process account for the two populations [25]. The MALDI-TOF mass spectrum shows undeniably that Gns is present in the macromolecule, suggesting that Gns's initiating capabilities are highly potent in the ROP of LLA. Precipitation of the polymer (in this study methylene chloride and diethyl ether/water environment) or with respect to MALDI-TOF MS



measurements as a result of mixing the polymer sample with the matrix materials may explain the formation of polymer chains terminated with hydroxyl and hydrogen end groups (series A, Fig. 3), whereas formation of macrocyclic subunits by an intramolecular transesteriation is the likely explanation for the formation of macrocycl. Conclusions To sum up, to the best of my knowledge, this is the first paper that details the natural isoflavonoid genistein-initiated homo- and copolymerization of cyclic esters. The moderate molecular weight of up to 12,100 [g/mol] and the high yield of 98% were achieved using bulk polymerization. Spectroscopic evidence strongly suggests that genistein has been incorporated into the macromolecule. The findings obtained are encouraging for the synthesis of the star-shaped macromolecules and their use as drug carriers in pharmacy since they were achieved using non-toxic chemicals. Acknowledgments The author would like to express appreciation for the funding provided by the Medical University of Warsaw. Easy Obtainment With proper citation of the author(s) and the source, this article may be used, distributed, and reproduced in any media, according to the conditions of the Creative Commons Attribution License under which it was first published. References A. C. Albertsson & I. K. Varma. Recent progress in biomedically relevant ring-opening

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Four distinct groups of peaks may be seen throughout the spectrum. One hydroxyl and one hydrogen end group (residual mass 41, Na⁺ adduct, A) mark the conclusion of the first series, which is representative of a PLA molecule. The second set of peaks is consistent with PLA that has Gns and hydrogen end groups (H⁺ adduct, B; residual mass 271). Third series, weaker in intensity, is compatible with macrocyclic polymers (residual mass 3, Na⁺ adduct, D), and fourth series, weaker in intensity, is likewise consistent with PLA ending with Gns and hydrogen end groups, but with Na⁺ adduct (residual mass 274, C). Two distinct populations of chains, one with an even number of LA repeating units and the other with an odd number, are seen in the mass spectrum shown in Fig. 3. They had 72 amu between them. The polymerization of LLA and the subsequent intra- and intermolecular transesterification process account for the two populations [25]. The MALDI-TOF mass spectrum shows undeniably that Gns is present in the macromolecule, suggesting that Gns's initiating capabilities are highly potent in the ROP of LLA. Precipitation of the polymer (in this study methylene chloride and diethyl ether/water environment) or with respect to MALDI-TOF MS measurements as a result of mixing the polymer sample with the



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Conclusions

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