Synthesis and Characterization of Degradable Anhydride-*co*-imide Terpolymers Containing Trimellitylimido-L-tyrosine: Novel Polymers for Drug Delivery

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ABSTRACT: The goal of this work is to synthesize a polymer specifically designed to deliver vaccine antigens. To accomplish this goal, a series of anhydride-*co*-imide terpolymers based on trimellitylimido-L-tyrosine (TMA-Tyr), sebacic acid (SA), and 1,3-bis(carboxyphenoxy)propane (CPP) was synthesized by melt condensation polymerization. It is desirable to incorporate tyrosine into the backbone of the polymer system due to its inherent ability to enhance the immune response to vaccine antigens. CPP and SA were copolymerized with the tyrosine derivative, TMA-Tyr, in order to develop a polymer with suitable material properties for drug delivery (e.g., high molecular weight, amorphous, and good solubility in low-boiling organic solvents), as well as to provide a series of polymers capable of a wide range of degradation and antigen release properties. To our knowledge, this paper represents the first report of the synthesis and characterization of terpolyanhydrides designed specifically to deliver drugs such as vaccine antigens. A systematic series of studies was performed to evaluate and optimize the influence of monomer ratio, reaction time and temperature, reaction catalysts, and catalyst concentration on polymer molecular weight, percent TMA-Tyr incorporation, and crystallinity. Terpolymers were synthesized with weight-average molecular weights in excess of 80 000 by using heterogenic catalysts and highly purified monomers with low degrees of oligomerization. In addition, the terpolymers had no crystalline regions, the only exception being polymers with >60% SA in their backbone. Monomers and polymers were characterized by ^{1}H NMR and IR spectroscopy, elemental analysis, thermal transition temperature analysis, and gel permeation chromatography. The stability of these polymers in the solid state and in chloroform at various temperatures is also reported.

Introduction

Biodegradable polymers are currently being studied or used clinically for a variety of medical applications, such as surgical sutures, orthopedic implants, scaffolds for cells in tissue engineering, and controlled release depots for drugs.[1−3] Of all biodegradable polymers, the polyesters based on lactide and glycolide have been the most widely studied for drug delivery applications.[4] These polymers have the advantage of years of safe use in humans as surgical sutures[5] and, more recently, as drug delivery depots for chemotherapeutic drugs (e.g., the Lupron Depot and Zoladex).[6,7] Although these polymers may be acceptable for use in many medical applications, they are not always the most suitable. For example, their poor immunostimulating properties may make them suboptimal depots for vaccine antigen delivery. As a result, antigens delivered via lactide/glycolide polymers often require the addition of an adjuvant to be maximally effective in initiating a protective immune response.[8,9]

The goal of this study was to synthesize and characterize a family of biodegradable polymers that could be used as a depot to deliver drugs and, more specifically, vaccine antigens. Initial studies in our group focused on the use of degradable polyiminocarbonates based on *N*-benzyloxycarbonyl-L-tyrosyl-L-tyrosine hexyl ester (CTTH),[10] a dityrosine derivative, due to the inherent ability of L-tyrosine and many of its derivatives to stimulate a potent immune response to adsorbed antigens.[11−18] It was shown that vaccine release from poly(CTTH iminocarbonate) implants leads to enhanced levels of immunity compared with release from a similar polyiminocarbonate that is not based on a tyrosine derivative. However, these poly(CTTH iminocarbonates) were low molecular weight, brittle polymers that could not be used to fabricate micrometer-sized spheres (i.e., microspheres) suitable for *in vivo* injection in a reproducible manner. On the other hand, polyanhydride copolymers based on sebacic acid (SA) and 1,3-bis(carboxyphenoxy)propane (CPP) are versatile drug delivery vehicles, having been used for years to deliver a variety of drugs from proteins to low molecular weight chemotherapeutic agents.[19,20] CPP:SA copolymers are currently used clinically to deliver BCNU locally within the brain to treat patients with brain tumors.[19] They have a history of safe use in humans and animals,[19,21] and their erosion rate can be varied from hours to years depending on the ratio of SA to CPP in their backbone.[22] However, despite their usefulness for drug delivery applications, conventional polyanhydrides are expected to have limited inherent immunostimulatory properties. As a result, they may make only marginal delivery systems for vaccine antigens.

To improve the immunostimulatory effect of polyanhydrides, vaccine adjuvants such as tyrosine may be incorporated directly into their backbone. A class of polyanhydrides capable of incorporating tyrosine into their backbone is the poly(anhydride-*co*-imides).[23] Such a polymer may be ideally suited for vaccine delivery by combining the adjuvanticity of tyrosine with the desirable controlled release properties of polyanhydrides. To achieve this goal, we had two objectives: to incorporate a large percentage of tyrosine into the backbone of a biodegradable anhydride polymer that could be used to deliver vaccine antigens and to produce high molecular

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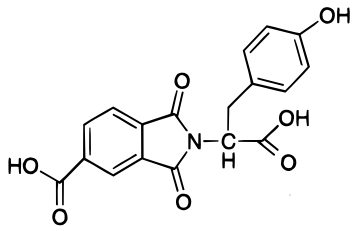
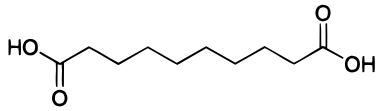
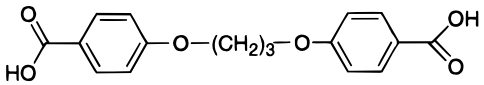


Trimellitylimido-L-tyrosine (TMA-Tyr)



Sebacic Acid (SA)



1,3-bis(carboxyphenoxy)propane (CPP)

Figure 1. Chemical structure of monomer units before acylation.

weight, amorphous polymers (crystallinity can lead to heterogeneous degradation and irreproducible drug loading and release) capable of delivering protein antigens for days to months. To achieve these objectives, we systematically optimized the synthesis and characterized a series of anhydride-*co*-imide terpolymers based on the mixed anhydrides of acetic acid and trimellitylimido-L-tyrosine (TMA-Tyr), SA, and CPP (Figure 1). For the first time, high molecular weight polymers (M_w > 80 000) with appreciable TMA-Tyr content have been synthesized. Polymers were synthesized with a balance of CPP to allow for long-term release kinetics, SA to improve polymer solubility and processability, and tyrosine monomer (TMA-Tyr) for its possible adjuvanticity. Careful selection of the three monomers allowed us to produce a series of amorphous polymers with a variety of monomer compositions that should allow a wide range of polymer erosion and drug release kinetics. Here, we report the effect of various reaction conditions, as well as the effect of the various monomers and monomer ratios, on polymer molecular weight, polydispersity, glass transition temperatures, and crystallinity. This paper represents the first synthesis and characterization of poly(anhydride-*co*-imides) consisting of more than two monomers and discusses their potential usefulness as drug carrier systems.

Results and Discussion

Monomer Preparation. Purified monomers and acylated monomers were prepared and characterized by ^{1}H NMR, IR spectroscopy, elemental analysis, and gel permeation chromatography (GPC). Oligomers consisting of 1.2−1.7 units on average, based on GPC and ^{1}H NMR analysis, respectively, were synthesized from aromatic monomers (TMA-Tyr and CPP), compared with 4.7−5.0 units for the aliphatic monomer (SA) (Table 1). Oligomerization can occur during reflux in acetic anhydride, during subsequent acetic anhydride removal at high temperatures, and during purification by recrystallization. The use of long oligomers in the synthesis of copolymers leads to lower molecular weight polymers[24] and creates large aliphatic regions in the anhydrides (SA-rich regions) that are susceptible to faster degradation, resulting in heterogeneous polymer degradation. To avoid extensive oligomerization during the isolation step, unreacted acetic anhydride was removed at mild temperatures (40 °C) under vacuum. By reducing the reaction time of TMA-Tyr, CPP, or SA in acetic anhydride (3 min under reflux) or lowering the reaction temperature (60 °C for 2 h), followed by filtration of unreacted diacid and evaporation of excess acetic anhydride at room temperature, we were able to produce primarily monomeric acylated monomers. However, as the preparation of monomeric prepolymer is time-consuming and wasteful, short oligomers were used in this study.

Table 1. Characteristics of Acylated Monomers Used in the Synthesis of Poly(TMA-Tyr:SA:CPP)

		GPC analysis			^{1}H NMR
monomer	calcd MW	M_w	M_n	D_p[a]	D_p[b]
acyl-TMA-Tyr	439.4	558	561	558	1.65
acyl-SA	286.3	1513	1415	1513	4.68
acyl-CPP	400.4	460	492	460	1.09

[a] Degree of polymerization based on the M_n of GPC analysis. [b] Degree of polymerization based on ^{1}H NMR analysis.

TMA-Tyr:SA:CPP Terpolymer Synthesis and Structure Confirmation. The reaction scheme used to synthesize the polymers is shown in Figure 2. In the first step, equimolar amounts of L-tyrosine and TMA were reacted at reflux in DMF. As a result of this condensation reaction, the amino terminus of tyrosine is incorporated into a cyclic imide bond. This reaction is performed for two reasons: first, the reactive amino terminus of tyrosine is protected by cyclicization and is therefore not able to participate in side reactions during polymerization; second, the resulting monomer is a diacid suitable for condensation polymerization (after acylation) to make a linear polyanhydride. Once the monomers are made and purified, they are refluxed in acetic anhydride to acylate their carboxylic acid end groups. As confirmed by ^{1}H NMR and IR spectroscopy (see Experimental Section), the phenolic hydroxide of tyrosine also reacts with acetic anhydride to form an acetate ester, thereby protecting it from further reaction during polymerization. Subsequently, the acylated monomer powders are isolated and purified (see Experimental Section) and reacted in bulk at temperatures ranging from 120 to 200 °C under high vacuum, with or without a heterogeneous catalyst.

IR and ^{1}H NMR spectroscopy were used to confirm the polymer composition and integrity. Of the three monomers used, two are symmetric (SA and CPP), and one is asymmetric (TMA-Tyr). As a result, 10 diad sequences are expected in the polymer backbone. In the ^{1}H NMR spectra of poly(TMA-Tyr:SA:CPP) with a molar feed ratio of 20:50:30, multiplets for the SA-SA diad are found at 1.65 and 2.45 ppm, whereas multiplets for the CPP-CPP diad are at 2.35 and 8.08 ppm. Multiplets for the SA-CPP diad are at 1.74 and 2.60 ppm attributed to protons of SA and 6.97 and 7.98 ppm attributed to CPP. Triplets for SA reacted with the tyrosine end of TMA-Tyr (SA-Tyr) and with the TMA end of TMA-Tyr (SA-TMA) are found at 2.48 and 2.65 ppm, respectively. Both triplets are attributed to protons of SA. Finally, multiplets attributed to TMA-Tyr corresponding to overlay signals for the remaining TMA-Tyr diads (TMA-

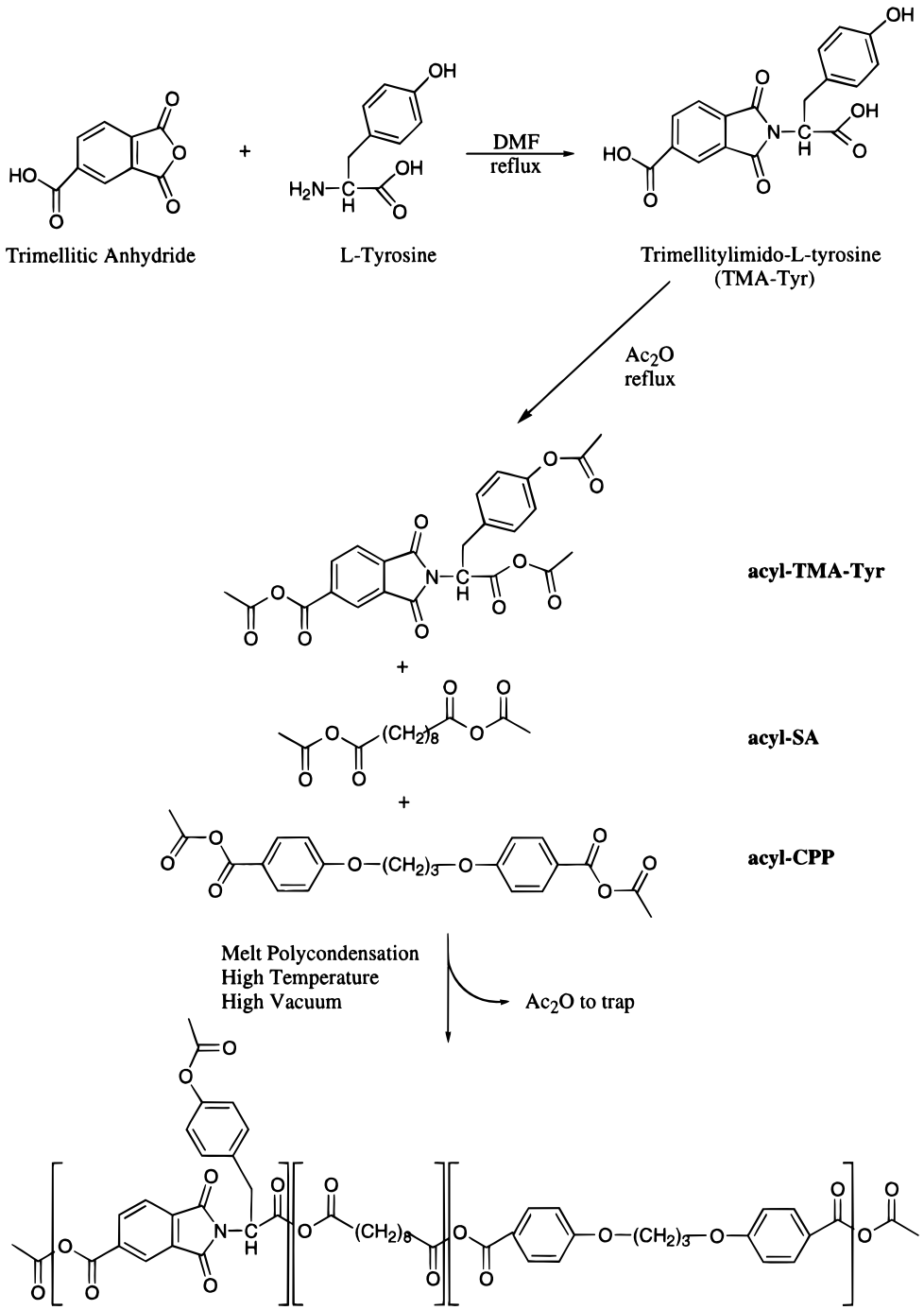


Figure 2. Synthesis scheme of poly(TMA-Tyr:SA:CPP).

CPP, Tyr-CPP, TMA-TMA, Tyr-TMA, and Tyr-Tyr), as well as for SA-Tyr and SA-TMA, are found at 3.59, 3.70, 5.25, 5.43, 7.18, 7.91, 8.42, and 8.52 ppm. Due to the complex overlapping in the ^{1}H NMR spectra when several monomers are used, especially in the regions of asymmetric monomers such as TMA-Tyr, it is difficult to assign the individual diads involving TMA-Tyr. Multiplets for the aliphatic protons of SA and CPP are found at 1.34 and 4.25 ppm, respectively. At 2.22 ppm, there is a multiplet representing a clear overlapping of the terminal CH_3 signals of SA and TMA-Tyr. The signal for the terminal CH_3 of CPP is contained in a multiplet at 2.35 ppm, which is overlapped by signals for the CPP-CPP and CPP-SA diads. It is already well established that copolymers of CPP and SA have a random distribution of the two monomers throughout their polymer backbone.[25] The fact that all three monomers of the terpolymers react with one another suggests that they are random copolymers as well.

For infrared analysis, in general, aliphatic carbonyls of anhydride polymers absorb around 1740 and 1810 cm^{-1}, and those of aromatic polyanhydrides absorb around 1720 and 1780 cm^{-1}. When the polymer contains both aliphatic and aromatic monomers (e.g., SA and CPP), the peaks at 1720−1740 cm^{-1} overlap. Our results agree with these generalities for polyanhydrides, showing an aliphatic carbonyl peak at 1817 cm^{-1} and shoulders of low intensity around 1785 cm^{-1} for the

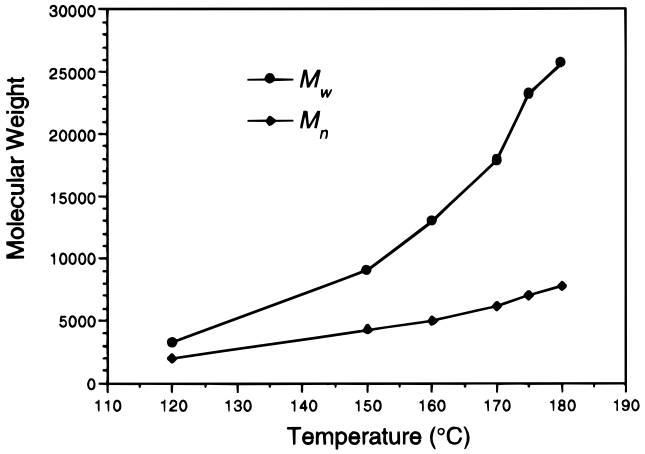


Figure 3. Molecular weight of poly(TMA-Tyr:SA:CPP) (molar feed ratio of 16:42:42) as a function of reaction temperature. The terpolymers were melt polymerized for 1.5 h without catalyst.

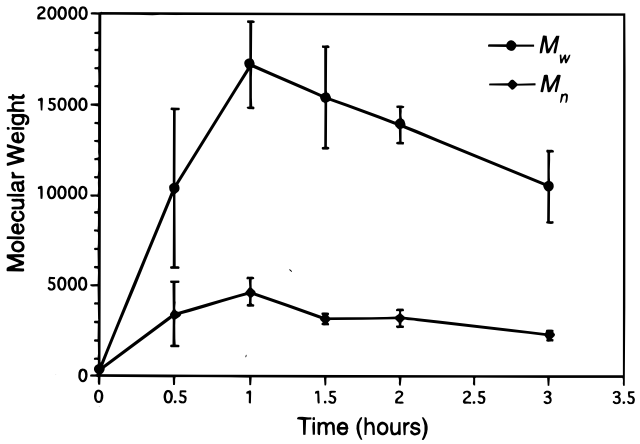


Figure 4. Molecular weight of poly(TMA-Tyr:SA:CPP) (molar feed ratio of 20:50:30) as a function of reaction time. The terpolymers were melt polymerized at 180 °C without catalyst.

aromatic carbonyl and 1730 cm^{-1} for the overlap of aromatic and aliphatic carbonyls. These shoulders partially overlap distinct peaks for the imide carbonyls at 1784 and 1727 cm^{-1}. Extensive overlapping of anhydride and imide carbonyls in the 1700−1800 cm^{-1} region makes it difficult to readily assign some of the anhydride peaks. Characteristic peaks for aromatic C=C bonds (TMA-Tyr and CPP) are found at 1604 and 1582 cm^{-1}, those for aliphatic C−H stretches at 2932 and 2852 cm^{-1}, and a peak for the C−N stretch at 1380 cm^{-1}. The OH band for TMA-Tyr at 3370 cm^{-1} is not present in the polymer spectra, confirming the acylation of the phenol hydroxyl in the polymer structure.

TMA-Tyr:SA:CPP Terpolymer Synthesis Optimization. In this part of the study, we systematically determined the factors affecting polymer molecular weight and polydispersity using a fixed TMA-Tyr:SA:CPP feed ratio of 20:50:30, the only exception being the initial reactions run to determine the effect of reaction temperature on the polymerization (Figure 3). The critical factors involved in achieving high molecular weight polymers were monomer purity, temperature of reaction, duration of polymerization, catalyst and catalyst concentration, and the removal of the condensation byproduct, acetic anhydride. Figure 3 shows the effect of reaction temperature on the melt polymerization using a molar feed ratio of TMA-Tyr:SA:CPP of 16:42:42. When the reaction was run for 1.5 h without catalyst, the maximum weight-average molecular weight obtained was 25 600 (at 180 °C). By raising the reaction temperature from 120 °C to 180 °C, higher molecular weight polymers were produced. However, polymer polydispersity also increased with molecular weight. This result is consistent with previous studies on polyanhydrides containing CPP and SA, where 180 °C was also the optimal reaction temperature.[24] Dark-colored, partially insoluble products were observed at reaction temperatures higher than 190 °C, probably as a result of decomposition and/or cross-linking reactions. Polymer cross-linking may be explained by the formation of free phenolic hydroxyl of TMA-Tyr, which is then able to react with anhydride bonds in the polymer backbone. As a result, a reaction temperature of 180 °C was used for subsequent synthesis optimization of the anhydride-*co*-imides in this study.

The effect of reaction time at 180 °C on polymer molecular weight is shown in Figure 4. The results represent an average of 3−5 reactions, except for the 3-h time point, which was run twice. Molecular weight increased with reaction time initially, reached a maximum at 1 h, and decreased thereafter. Increasing the reaction time beyond 2 h at 180 °C (or 30 min at 200 °C) yielded a rubbery gel that swelled extensively in chloroform. This product was partially solubilized after 24 h but had a lower molecular weight than that prior to cross-linking. The decrease in molecular weight with time may be explained by polymer depolymerization, which occurs during excessive heating. It has been proposed that depolymerization of polyanhydrides is due to the formation of low molecular weight cyclic oligomers, which are in equilibrium with the high molecular weight linear polymer.[26,27] Factors such as high viscosity of the polymer melt and steric effects due to chain folding may limit chain mobility and reactive end-group availability after one hour of reaction.

We also studied the effect of catalysts on the melt polymerization. Because the reaction is a transesterification that involves nucleophilic attack of an etheric oxygen on a carbonyl carbon, it was proposed that a catalyst that increases the electron deficiency of the carbonyl carbon should enhance the polymerization rate. Many coordination catalysts were suggested for the transesterification polymerization of polyesters.[28] Similar catalysts have been found to be active in ring-opening polymerization of epoxides due to metal−oxygen complexation.[29] More importantly, coordination catalysts, such as cadmium acetate ($CdAc_2$) and earth metal oxides, have previously been shown to be effective in producing high molecular weight polyanhydrides.[24] In this study, we tested the effect of several coordination catalysts on polymer molecular weight. Two mole percent catalyst was used because it was previously shown to be optimal in the synthesis of SA:CPP copolymers.[24] Figure 5 shows that higher molecular weights in shorter times were achieved when 2 mol % $CdAc_2$ or earth metal oxides was used as catalyst. The weight-average molecular weight reached a maximum of 62 000 in 30 min with $CdAc_2$, compared to 18 000 in 60 min without a catalyst. High molecular weights were also achieved using barium oxide (BaO) after slightly longer times (60 min) than with $CdAc_2$. The use of 2 mol % calcium carbonate ($CaCO_3$) led to significantly higher molecular weights after 30 min compared with polymers synthesized without catalyst. However, after 60 min, the molecular weights were not significantly different between the two groups. In general, the highest molecular weights in the shortest times were achieved

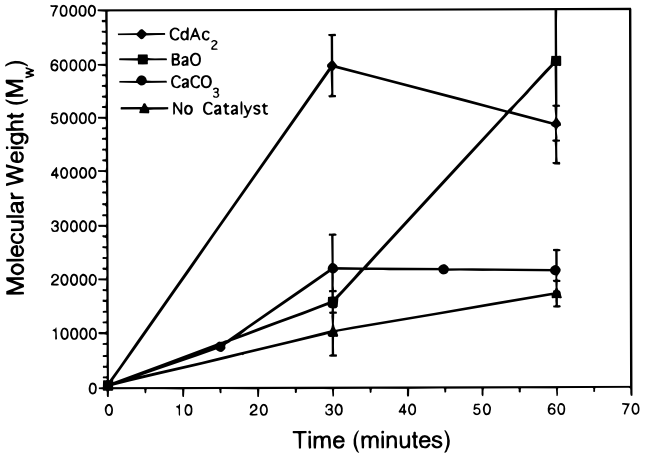


Figure 5. Melt polymerization of poly(TMA-Tyr:SA:CPP) (molar feed ratio of 20:50:30) with 2 mol % of various heterogenic catalysts at 180 °C.

Table 2. Characteristics of Poly(TMA-Tyr:SA:CPP) Synthesized with Constant Acyl-TMA-Tyr (20 mol %) in the Reaction Feed[a]

% TMA-Tyr:SA:CPP (reaction feed)	M_w	PDI	T_m (°C)	% TMA-Tyr:SA:CPP (^{1}H NMR)
20:80:0	77 842	5.82	67.0	10.5:89.6:0.0
20:60:20	80 358	6.80	44.2	13.9:64.5:21.6
20:40:40	45 400	4.13	*b*	13.4:41.7:44.9
20:20:60	21 803	2.93	*b*	17.2:26.6:56.2
20:0:80	4 082	2.02	188.3	*c*

[a] Polymers melt polymerized at 180 °C with 2 mol % cadmium acetate for 30 min. T_g's of these polymers are reported in Figure 7. [b] Not detectable. [c] Not tested.

Table 3. Characteristics of Poly(TMA-Tyr:SA:CPP) Synthesized with Constant SA:CPP Molar Feed Ratio of 1:1

% TMA-Tyr:SA:CPP (reaction feed)	M_w	PDI	T_m (°C)	T_g (°C)	% TMA-Tyr:SA:CPP (^{1}H NMR)
0:50:50	39 506	3.66	185.8	6.4	0.0:59.7:40.3
20:40:40	45 400	4.13	*b*	29.5	13.1:41.5:45.4
40:30:30	38 185	4.29	*b*	46.9	30.8:34.2:35.0
60:20:20	17 589	2.97	*b*	54.4	52.4:23.7:23.9
80:10:10	3 875	1.66	*c*	*c*	*c*
100:0:0	1 988	1.91	*b*	93.5	100:0.0:0.0

[a] Polymers melt polymerized at 180 °C with 2 mol % cadmium acetate for 30 min. [b] Not detectable. [c] Not tested.

using $CdAc_2$. Finally, the best catalysts ($CdAc_2$ and BaO) were less effective in large particle size (300−500 μm) than in small particle size (<50 μm), consistent with the assumption of a heterogenic type of reaction. It may be possible to use a much lower percentage of catalyst if its particle size distribution is very small (e.g., <5 μm). Such particle size distributions may be achieved, for example, using spray drying. However, with very small catalyst particles, it is difficult to ensure their complete removal by filtration after polymerization, a potential problem when toxic catalysts are used.

Although high molecular weight polymers were obtained using 2 mol % of $CdAc_2$ or BaO for 1 h (M_w = 51 000 and 62 000, respectively), the polydispersity was not reduced (M_w/M_n = 4.2 and 6.3) compared to polymerizations without catalyst. In fact, we found that the polydispersity of the poly(anhydride-*co*-imides) generally increased with increased molecular weight, regardless of the polymer composition (e.g., see Tables 2 and 3). The increase in polydispersity with M_w is consistent with the classical mechanism of condensation reactions.[30]

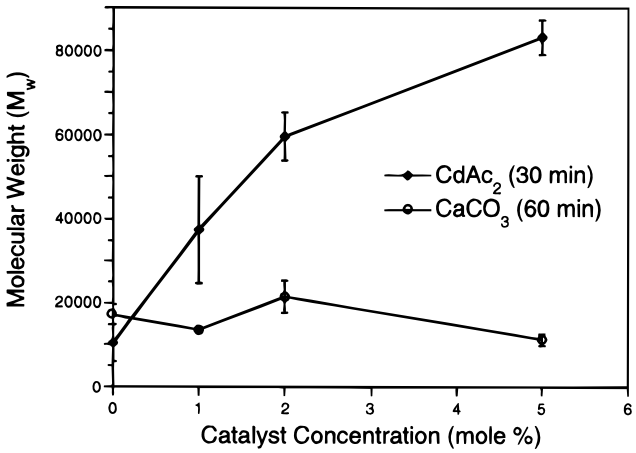


Figure 6. Melt polymerization of poly(TMA-Tyr:SA:CPP) (molar feed ratio of 20:50:30) with various concentrations of cadmium acetate ($CdAc_2$) or calcium carbonate ($CaCO_3$) at 180 °C. Terpolymers synthesized using $CdAc_2$ and $CaCO_3$ were reacted for 30 and 60 min, respectively, according to the optimal reaction conditions of the catalysts.

Finally, the effect of catalyst concentration was determined for $CdAc_2$ and $CaCO_3$. $CdAc_2$ was chosen due to its effectiveness at a concentration of 2 mol %, whereas $CaCO_3$ was selected because it is generally regarded as safe and therefore may be better suited for use with polymers intended for *in vivo* use. Optimal molecular weights for polymers synthesized using $CdAc_2$ and $CaCO_3$ were obtained after 30 and 60 min, respectively, regardless of catalyst concentration (Figure 6). Increasing the $CdAc_2$ concentration from 0 to 5% led to polymers with weight-average molecular weights in excess of 80 000. However, $CaCO_3$ did not appear to be an effective catalyst at any concentration under the conditions tested. Results similar to those shown in Figure 6 were obtained at different reaction times for each catalyst (i.e., 30 min for $CaCO_3$ and 60 min for $CdAc_2$; 1−5 mol % catalyst).

Effect of Monomer Ratio on TMA-Tyr:SA:CPP Terpolymers. The effect of TMA-Tyr:SA:CPP ratios on polymer characteristics was determined by synthesizing a series of polymers with various monomer ratios. For this part of the study, 2 mol % cadmium acetate was used as a catalyst, and the reactions were run for 30 min at 180 °C (optimized conditions). Highly aromatic polyanhydrides, which had >80% TMA-Tyr or CPP content, were generally rigid and brittle and of low molecular weight (M_w < 5000). Higher molecular weights were obtained by copolymerization with SA. Table 2 shows the characteristics of a series of polymers synthesized at a constant 20 mol % acyl-TMA-Tyr and various ratios of SA:CPP in the reaction feed. Molecular weights in excess of 77 000 were obtained for polymers with 0−20% acyl-CPP in the feed. Thereafter, the molecular weight decreases with increasing CPP content. However, polymers with up to 60% CPP (and 20% TMA-Tyr) in the feed with molecular weights in excess of 20 000 were achieved. Similarly, as shown in Table 3, when the SA:CPP feed ratio is kept constant at 1:1, high molecular weight polymers are obtained when the reaction feed contains 0−20 mol % acyl-TMA-Tyr, with lower molecular weights as the amount of acyl-TMA-Tyr is increased. Polymers with molecular weights in excess of 17 000 were synthesized that contained >50% TMA-Tyr in their backbone as determined by ^{1}H NMR spectroscopy. Subsequently, it has been determined that TMA-Tyr:SA:CPP terpolymers with molecular

weights in this range are suitable for the encapsulation of drugs, including vaccine antigens, in injectable microspheres.[31]

The decrease in molecular weight with increased percentages of either CPP or TMA-Tyr can be explained on the basis of the increased rigidity and steric hindrance of these two units compared with the flexible monomer, SA. A polymer chain that ends with an SA unit is able to react more readily with an additional monomer unit than a chain ending in either CPP or TMA-Tyr. Therefore, as the percentage of SA in the reaction feed is increased, the percentage of chains ending with an SA unit at any given time during the polymerization is also increased, and longer polymer chains are produced on average. Despite its effect on polymer molecular weight, CPP is copolymerized with TMA-Tyr and SA to enhance the hydrophobicity of the polymer. As a result, the presence of CPP in the polymer backbone slows down the polymer degradation and erosion process, allowing the release of drugs over longer periods of time compared with a polymer of TMA-Tyr and SA alone.[32] Long-term release is essential to the success of many applications involving the delivery of pharmaceuticals, especially vaccine antigens.[33]

Tables 2 and 3 also show that the percentage of TMA-Tyr incorporated into the polymer backbone is consistently less than the percentage in the reaction feed (by 3−10%). This is in agreement with elemental analysis data (Experimental Section) that show nitrogen levels in the polymers consistently below predicted values based on monomer feed ratios. This result is also consistent with data obtained in the synthesis of poly(anhydride-*co*-imides) containing glycine and alanine.[34]

The solubility of these polymers in organic solvents was predominantly a function of polymer composition. Increasing solubility in common organic solvents, such as chloroform, methylene chloride, and *N*,*N*-dimethyl formamide, was observed with a higher SA content. To encapsulate drugs, including water-soluble vaccines, into microspheres via convenient solvent evaporation processes, polymer solubility in low-boiling organic solvents is a necessity.[33] Therefore, it is difficult to produce microspheres with TMA-Tyr:CPP polymers if SA is not included in the backbone.[31]

Thermal Analysis. Glass transition temperatures (T_g) of the amorphous polymer fractions and melting temperatures (T_m) of the crystallites were determined by differential scanning calorimetry (DSC). In the case of polyanhydrides made up of three monomers, thermal behavior and extent of crystallization depend on the monomer makeup, polymer molecular weight, and the ability of the repeat units to pack into regular structures. Polymers containing >60% SA exhibit melting transitions (Tables 2 and 3), which suggests the presence of long-range order in these polymers (i.e., regions of crystallinity). Regions of crystallinity were also observed when the polymer contained a high percentage of CPP (i.e., TMA-Tyr:CPP 20:80). However, polymers that contained all three monomers did not show any crystallinity, the only exception being a high molecular weight polymer (>80 000) containing >60% SA in its backbone. This result is expected since the appearance of several melting transitions, corresponding to sebacic acid-rich regions, has previously been observed in poly(anhydride-*co*-imides) with an appreciable SA content.[35] The lack of crystallinity in the terpolymers is primarily due to the presence of three structurally different monomers, especially the asymmetric TMA-Tyr moiety,

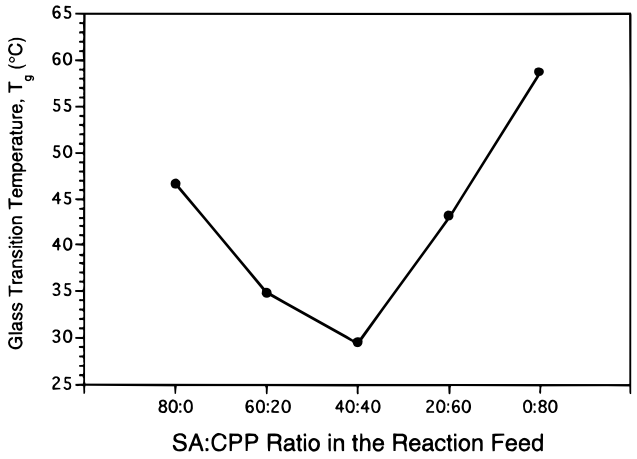


Figure 7. Effect of SA:CPP feed ratio on polymer glass transition temperature. The TMA-Tyr feed was held constant at 20 mol %. Polymers were melt polymerized at 180 °C with 2 mol % cadmium acetate for 30 min.

which does not allow for a regular structure. In Table 2, we report T_m's of 67.0 °C and 188.3 °C for poly(TMA-Tyr:SA) 20:80 and poly(TMA-Tyr:CPP) 20:80, respectively (i.e., copolymers that contained 20% TMA-Tyr and 80% SA or CPP). These values are similar to values reported elsewhere for CPP:SA copolymers that contain 80% SA (66−72 °C) and 80% CPP (205 °C), respectively.[25] This result, combined with the fact that the homopolymer of TMA-Tyr does not exhibit a melting transition, indicates that the crystalline regions in TMA-Tyr:SA and TMA-Tyr:CPP copolymers are due to the excess of SA or CPP monomers in these polymers. The fact that terpolymers with appreciable percentages of all three monomers (Table 3) lack crystallinity is further evidence that the TMA-Tyr monomer is uniformly distributed throughout the polymer backbone, where it serves to disrupt packing. For drug delivery, it is desirable to use amorphous polymers with a uniform monomer distribution in order to achieve (a) homogeneous drug distribution in, and degradation of, the polymer matrix and (b) reproducible drug release kinetics.

Figure 7 shows T_g as a function of SA:CPP ratio in the reaction feed when the acyl-TMA-Tyr feed is held constant at 20 mol %. As expected, polymer T_g goes through a minimum when the SA:CPP ratio is 1 (29.5 °C) and increases as the polymer is enriched in either CPP or SA. In other words, as the polymer goes from a terpolymer with high percentages of all three repeat units (20% TMA-Tyr, 40% SA, 40% CPP) to a copolymer consisting of only two repeat units (20% TMA-Tyr and 80% SA or CPP), the T_g approaches that of the homopolymers of SA or CPP. Poly(SA) has a reported T_g of 50−60 °C,[25,35] compared with 46.6 °C reported here for poly(TMA-Tyr:SA) 20:80. Similarly, poly(CPP) has a reported T_g of around 96 °C,[25] compared with 58.7 °C for poly(TMA-Tyr:CPP) 20:80 reported here. Additionally, the polymer T_g increases steadily as the percent of imide monomer (TMA-Tyr) in the terpolymer backbone increases when the SA:CPP ratio is kept constant at 1 (Table 3). This trend has previously been reported for poly(anhydride-*co*-imides) containing glycine and alanine.[34] The increase in T_g with increased levels of TMA-Tyr is likely due to the high rigidity of the imide-containing monomer unit, which sterically hinders chain movement, even though polymers rich in TMA-Tyr are typically low molecular weight. In general, the thermal transition temperatures of terpolymers were signifi-

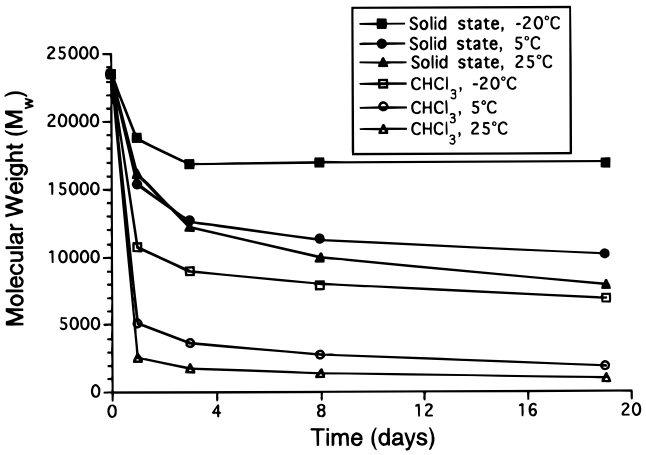


Figure 8. Stability of poly(TMA-Tyr:SA:CPP) (molar feed ratio of 20:50:30) in the solid state and as a 10 mg/mL solution in $CHCl_3$ at various temperatures. The initial terpolymer molecular weight was 23 500. The terpolymer was melt polymerized for 1.5 h at 180 °C without catalyst.

cantly reduced compared to those of the corresponding homopolymers. The reduced transition temperatures are probably a result of disrupted packing of neighboring polymer chains caused by the use of several structurally different monomers. Finally, the appearance of a single T_g for each of the terpolymers (Tables 2 and 3) is further proof that monomers are randomly distributed in the polymer backbone.

Stability of TMA-Tyr:SA:CPP Terpolymers. The stability of TMA-Tyr:SA:CPP terpolymers, in a molar feed ratio of 20:50:30, was assessed in the solid state and as a 10 mg/mL solution in chloroform at different temperatures. The initial weight-average molecular weight of the polymer used in this study was 23 500. Polymer samples were stored in the solid state and in $CHCl_3$ solution at −20 °C, 4 °C, and 25 °C, and their molecular weights were followed with time by GPC (Figure 8). Whether the polymer was stored as a solid or in solution, increasing depolymerization was observed at elevated temperatures. In addition, polymers were more stable in the solid state than in $CHCl_3$ solution. For example, in $CHCl_3$ at 20 °C, the polymer molecular weight decreases rapidly within a few days; however, polymer samples stored in the solid state at −20 °C showed only a slight decrease in molecular weight initially. The sharp initial decrease in M_w in solution may be explained as due to the increased mobility of the chains, enabling them to interact and react with each other by transesterification to form low molecular weight oligomers.[26] However, the presence of residual amounts of water in the chloroform may also play a role. After an initial drop in M_w, the polymers also show good stability for long times (greater than 20 days) at room temperature in the solid state (Figure 8).

Conclusions

We have reported the synthesis of biodegradable, poly(anhydride-*co*-imides) of high molecular weight and high tyrosine content. The use of three repeat units allows the synthesis of amorphous polymers capable of a wide range of degradation times that may be useful in the delivery of pharmaceuticals, including vaccine antigens. The incorporation of tyrosine is important due to reports of its inherent ability to increase the immune response to adsorbed antigens. ^{1}H NMR spectroscopy and thermal transition temperature analysis suggest that the monomers of terpolymers are randomly distributed throughout the polymer backbone, thus allowing a more uniform degradation of the polymer, a distinct advantage for drug delivery applications. These polymers show good stability in the solid state at room temperature, making them potentially useful as carriers for antigens in regions of the world where refrigeration is difficult or expensive to maintain.

Experimental Section

Materials. Trimellitic anhydride (TMA), L-tyrosine (Tyr), and sebacic acid (SA) were purchased from Aldrich Chemical Co. 1,3-Bis(carboxyphenoxy)propane (CPP) was synthesized according to the method described by Conix.[36] Calcium carbonate (Mallinckrodt), barium oxide (EM science), and cadmium acetate hydrate (Aldrich Chemical Co.) were reduced to <50 μm particle size and dried in an oven at 140 °C for 48 h before use. Organic solvents were analytical grade.

Methods. The molecular weight and polydispersity of the polyanhydrides were determined using a Perkin Elmer gel permeation chromatography (GPC) system equipped with a Series 10 pump, a 3600 data station, and an LC-25 refractive index detector (Perkin Elmer, Norwalk, CT). Samples were filtered and eluted in chloroform through a Phenogel 15 μm column (Phenomenex, Torrance, CA) at a flow rate of 0.90 mL/min. The molecular weights were determined relative to polystyrene standards (Polysciences, Warrington, PA) using CHROM2 and GPC5 computer software (Perkin Elmer).

Thermal analysis was performed using a Perkin Elmer DSC-2 differential scanning calorimeter consisting of a DSC7 analyzer and TAC7/7 instrument controllers. UNIX software was used on a DECpc 433 data station. An average sample weight of 5−10 mg was heated at heating rates ranging from 5 to 10 °C/min under a flow of N_2 (30 psi).

Infrared (IR) spectroscopy was performed using a Nicolet Magna-IR 550 spectrometer and a Nicolet data station with OMNIC 1.20 software (Nicolet, Madison, WI). The samples were either film cast in chloroform onto an NaCl plate or ground and pressed into KBr pellets.

Elemental analysis (C/N/H/O) was performed by Galbraith Laboratories (Knoxville, TN).

^{1}H NMR (360 MHz) spectroscopy was performed by Spectral Data Services, Inc. (Champaign, IL). The composition of poly(TMA-Tyr:SA:CPP) was determined by using the ratio of the average intensities per proton of each of the monomers. Degree of oligomerization of acylated monomers was determined from the average intensity per proton of several representative peaks of the repeating unit and the methyl terminal protons of the acetic mixed anhydride end group.

Stability studies were performed in solid state and in anhydrous chloroform under inert atmosphere (Ar gas replaced) at 25 °C, 4 °C, and −20 °C. The polymer molecular weight was followed by GPC with time.

Monomer Purification. TMA (130 g) was recrystallized from a 1:1 mixture of hot toluene and acetic anhydride (180 mL) three times before use. SA (60 g) was recrystallized three times from ethanol (240 mL). L-Tyrosine was purified by precipitation from a concentrated ammonia solution using glacial acetic acid several times before use. Briefly, tyrosine was dissolved in a large volume of ammonia, followed by slow addition of glacial acetic acid with vigorous stirring until the solution was slightly acidic (pH 6−7). The solution was kept cool using an external ice bath. The precipitated tyrosine was collected by filtration, washed extensively with water and methanol, and dried to constant weight under high vacuum. CPP was purified by extraction with acetone and ether before use.

Preparation of Trimellitylimido-L-tyrosine (TMA-Tyr). TMA-Tyr was prepared using the procedure outlined by Staubli et al.[23] with a minor modification. Briefly, equimolar amounts of TMA and L-tyrosine were reacted at reflux in DMF under argon for 3 h. The solution was cooled, filtered, and concentrated *in vacuo* at 60 °C to obtain a viscous oil. Subsequently, TMA-Tyr was washed with excess warm water to remove impurities, followed by washing with excess 0.5 N

HCl to remove unreacted tyrosine. The product is a white to slightly yellow powder. The yield was, in general, above 80%.

TMA-Tyr. ^{1}H NMR (DMSO-d_6): ∂ 3.17−3.4 (m, 2H, CH_2), 5.04 (m, 1H, CH), 6.53 (d, 2H, ArH), 6.92 (d, 2H, ArH), 7.98 (m, 1H, ArH), 8.20 (s, 1H, ArH), 8.36 (m, 1H, ArH), 9.17 (s, 1H, ArOH), 13.42 (s, 1H, OH), 13.68 (s, 1H, OH). IR (KBr, cm^{-1}): 3370 (OH phenol), 1777, 1723 (C═O imide), 1705 (C═O acid), 1608, 1595 (C═C aromatic), 1380 (C−N stretch). Anal. Calcd: C, 60.9; H, 3.7; N, 3.9; O, 31.5. Found: C, 61.4; H, 4.0; N, 3.9; O, 31.7.

Preparation of Acylated Monomers. (a) Acylation of TMA-Tyr. TMA-Tyr was added to excess acetic anhydride and heated at reflux under dry nitrogen for 15 min. The solution was cooled in an ice bath, filtered, and concentrated under vacuum at 40 °C. Excess acetic anhydride was extracted by swirling the product (acyl-TMA-Tyr) in anhydrous ethyl ether at room temperature. The product was left in excess anhydrous ethyl ether at −20 °C overnight to allow complete precipitation. The product was then filtered and vacuum dried over calcium chloride to give a white solid (85−90% yield). ^{1}H NMR ($CDCl_3$): ∂ 2.13, 2.28, 2.42 (s, 6H, CH_3), 2.23 (s, 3H, ester CH_3), 3.5−3.65 (m, 2H, CH_2), 5.26 (t, 1H, CH), 6.93 (d, 2H, ArH), 7.19 (dd, 2H, ArH), 7.92 (m, 1H, ArH), 8.42 (m, 1H, ArH), 8.48 (s, 1H, ArH). IR (KBr, cm^{-1}): 1824 (C═O anhydride), 1780, 1728 (C═O imide), 1609 (C═C aromatic), 1386 (C−N stretch). Anal. Calcd: C, 60.1; H, 3.9; N, 3.2; O, 32.8. Found: C, 58.9; H, 4.1; N, 3.1; O, 33.5.

(b) Acylation of SA. Purified SA was stirred into excess acetic anhydride and heated at reflux under dry nitrogen for 15 min. The solution was filtered and cooled in an ice bath immediately. Excess acetic anhydride was removed under vacuum at 40 °C. Crude acylated SA (acyl-SA) was recrystallized from dry toluene. The crystals were then stirred in a 1:1 mixture of dry petroleum ether and ethyl ether to extract traces of acetic anhydride and toluene. The product was dried under vacuum over calcium chloride to give a white crystalline solid (75−88% yield). ^{1}H NMR ($CDCl_3$): ∂ 1.34 (m, 8H, CH_2), 1.66 (m, 4H, CH_2), 2.23 (s, 6H, CH_3), 2.45 (t, 4H, CH_2). Anal. Calcd: C, 63.3; H, 8.5; N, 0.0; O, 28.3. Found: C, 62.9; H, 8.5; N, 0.0; O, 28.5.

(c) Acylation of CPP. CPP was refluxed in excess acetic anhydride for 15 min under nitrogen, followed by removal of the unreacted diacid by filtration. The solution was cooled using an ice bath and concentrated under vacuum at 40 °C. The product was stirred in dry ethyl ether to remove traces of acetic anhydride. Finally, acylated CPP (acyl-CPP) monomers were recrystallized from a 1:9 mixture of dimethylformamide (DMF) and ethyl ether at −20 °C. Purified acyl-CPP was filtered, washed with dry ethyl ether, and dried under vacuum over calcium chloride to give a white solid (40−50% yield). ^{1}H NMR (DMSO-d_6): ∂ 2.25 (m, 2H, CH_2), 2.38 (s, 6H, CH_3), 4.29 (t, 4H, CH_2), 7.14 (d, 4H, ArH), 7.99 (d, 4H, ArH). Anal. Calcd: C, 63.0; H, 5.0; N, 0.0; O, 32.0. Found: C, 63.4; H, 5.0; N, 0.0; O, 32.0.

Melt Polymerization. Poly[trimellitylimido-L-tyrosine-*co*-sebacic acid-*co*-1,3-bis(carboxyphenoxy)propane]. Acyl-TMA-Tyr, acyl-SA, and acyl-CPP were mixed in a defined ratio (with or without 1−5 mol % catalyst) in a Kimax glass sidearm tube (2 cm × 20 cm) with a capillary nitrogen inlet. The melt polycondensation procedure outlined by Domb and Langer[24] was used to synthesize the polymers. Briefly, the tube was immersed in an oil bath at the selected temperature (120−200 °C), and the activated monomers were allowed to melt (approximately 1 min). High vacuum was applied ($\leq 10^{-1}$ Torr), and the condensation byproduct, acetic anhydride, was collected in a liquid nitrogen-chilled trap. Throughout the polymerization, a strong nitrogen sweep with vigorous agitation of the melt was performed for 30 s every 15 min. At the end of the reaction, the crude polymer was removed from the glass tube and dissolved in methylene chloride. The solution was filtered to remove the heterogeneous coordination catalysts and any insoluble fractions and precipitated dropwise into excess stirred dry petroleum ether. The precipitate was collected by filtration, washed several times with anhydrous ethyl ether, and dried under vacuum at room temperature to constant weight. If the polymer was not soluble in methylene chloride, it was purified by stirring in anhydrous ethyl ether for 2 h. ^{1}H NMR ($CDCl_3$): reported in text. IR (KBr, cm^{-1}): 2932, 2852 (C−H aliphatic), 1817 (C═O anhydride), 1784, 1727 (C═O imide), 1604, 1582 (C═C aromatic), 1380 (C−N stretch).

Poly(TMA-Tyr:SA:CPP) 20:80:0. Anal. Calcd: C, 64.52; H, 7.74 ; N, 0.73 ; O, 26.55. Found: C, 62.57; H, 7.96; N, 0.69; O, 27.78.

Poly(TMA-Tyr:SA:CPP) 20:60:20. Anal. Calcd: C, 65.87; H, 6.45; N, 0.84; O, 26.84. Found: C, 61.93; H, 6.47; N, 0.66; O, 30.94.

Poly(TMA-Tyr:SA:CPP) 20:50:30. Anal. Calcd: C, 66.21; H, 5.75; N, 1.07; O, 26.97. Found: C, 64.38; H, 6.57; N, 0.83; O, 28.22.

Poly(TMA-Tyr:SA:CPP) 20:40:40. Anal. Calcd: C, 66.68; H, 5.65; N, 0.73; O, 26.94. Found: C, 64.17; H, 5.74; N, 0.78; O, 29.31.

Poly(TMA-Tyr:SA:CPP) 20:20:60. Anal. Calcd: C, 66.94; H, 5.09; N, 0.87; O, 27.10. Found: C, 65.07; H, 5.44; N, 0.84; O, 28.65.

Poly(TMA-Tyr:SA:CPP) 0:50:50. Anal. Calcd: C, 66.86; H, 6.62; N, 0.0; O, 26.52. Found: C, 65.61; H, 6.64; N, 0.0; O, 27.75.

Poly(TMA-Tyr:SA:CPP) 10:80:10. Anal. Calcd: C, 65.61; H, 7.40; N, 0.46; O, 26.53. Found: C, 64.93; H, 7.47; N, 0.50; O, 27.10.

Poly(TMA-Tyr:SA:CPP) 40:30:30. Anal. Calcd: C, 65.97; H, 5.09; N, 1.59; O, 27.35. Found: C, 63.76; H, 5.47; N, 1.37; O, 29.40.

Poly(TMA-Tyr:SA:CPP) 60:20:20. Anal. Calcd: C, 65.30; H, 4.44; N, 2.51; O, 27.75. Found: C, 62.78; H, 4.97; N, 2.13; O, 30.12.

Poly(TMA-Tyr:SA:CPP) 100:0:0. Anal. Calcd: C, 63.89; H, 4.05; N, 3.36; O, 28.70. Found: C, 61.90; H, 4.02; N, 3.34; O, 30.74.

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