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In vitro degradation behavior of biodegradable 4-star micelles

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Abstract

Drug delivery vectors for sustained release include a variety of polymeric constituents, both natural and synthetic. Among synthetic polymers several linear block copolymer systems have been explored for use as drug delivery vectors. Release of the pharmaceutical agent is affected by the degradation characteristics and/or by the swelling of the polymer. The goal of this study is to evaluate the degradation behavior of branched polyethylene oxide polylactide polyether ester as a drug delivery vector. Three samples of a star polyethylene oxide/polylactide copolymer with differing polylactide chain lengths were evaluated by characterizing the thermal properties of the neat polymer and in vitro degradation behavior.

The thermal and morphological properties were examined by DSC, TGA and XRD. The in vitro polymeric micelle samples were observed over time by UV–vis, TEM and fluorescence. The four star PEO–PLA polymers have exceptional amphiphilic characteristics, which enable their use for a variety of applications. The polymers are thermally stable at biological conditions. In addition, the star polymers have shorter degradation times as compared to previously reported linear PLA and PEG–PLA copolymers, suggesting use as a short-term drug release agent. The four star PEO/PLA copolymer may be an excellent candidate for drug delivery applications.

Keywords: Star-copolymer; Drug delivery; Degradation

1. Introduction

Recently, linear amphiphilic block copolymers have been examined extensively as drug carriers [1–7]. Amphiphilic materials are of particular interest because their microstructure can be modified to possess both hydrophobic and hydrophilic (amphiphilic) properties. Through material design, amphiphilic polymeric systems offer the advantages of biocompatibility, biodegradability, encapsulation, and drug release [1–23]. The bicontinuous nature of amphiphilic polymers enables these synthetics to mimic natural biological systems when subjected to an aqueous or biological environment by forming micelles. Many of these systems incorporate poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO) as the hydrophilic polymer portion [1–21] covalently attached or blended with a hydrophobic polymer. Amphiphilic copolymer systems include, but are not limited to, PEG–Polylactide (PLA), PEG–Polycaprolactone, and PLA–Polyglycolide and blends, all of which have been studied as possible drug delivery systems [1–14,17–23].

Micelles form a core-shell structure consisting of a hydrophobic core surrounded by a hydrophilic shell or corona. This architecture stabilizes the formation of the micelle and prevents excessive hydrophobic aggregation while allowing the micelle to act as a reservoir for hydrophobic substances [1–21]. The hydrophilic shell allows the micelle to remain soluble in hydrophilic solvents such as water and biological serum, creating solubility for insoluble drug constructs.

The concentration of the onset of molecular aggregation is termed the critical micelle concentration (CMC). This parameter is directly influenced by the hydrophobic, insoluble portion of the amphiphilic polymer. In theory hydrophobicity is directly proportional to intramolecular and intermolecular aggregate forces leading to micelle formation. A decrease in hydrophobic interactive forces may in turn affect the stability of the micelle, leading to an increase in the amount of material needed to sustain the micellar structure, i.e. an increased CMC.

The micelle structural geometry allows containment and release of hydrophobic constructs. This structure is achieved by subjecting an amphiphilic polymer such as PEO–PLA to an aqueous environment. The mechanism that provides the containment of hydrophobic drug constructs in the PEO–PLA system is the same hydrophobic aggregate force that initiate

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micelle formation which is driven by the hydrophobic portion of the amphiphilic polymer. The hydrophilic environment contributes to the release of the drug moiety by triggering hydrolytic degradation of the polyester PLA, as well as swelling of the hydrophilic PEO chain. Subsequently, the drug is released during depolymerization of the polyester as the monomers of the polymer are hydrolyzed.

In recent years, linear di- and triblock polymeric systems of PEO–PLA have gained great attention [1–8] due to the aforementioned characteristics as well as its biofriendly nature. Both PEO and PLA are generally regarded as safe and have received Food and Drug Administration approval for human consumption. PEO is quite biocompatible and has been found to create a steric layer and desorbs from particle surfaces in blood circulation [4], a characteristic that is of significance when introducing synthetic materials into biological systems. PLA also offers the desirable characteristics of being both biodegradable and biocompatible because the polymer is composed of lactic acid, a naturally occurring biological chemical. The combination of these materials makes this copolymer ideal for the use as a possible drug carrier. The desorbtion of PEO allows the copolymer to move through the circulation without eliciting an immunoresponse [21,22]. The polyester PLA can maintain release of hydrophobic drug constructs through hydrolytic degradation.

The physical architecture of the polymer is critically important in drug delivery vector design [15]. The polymer type (linear or branched), stoichiometric composition of the polymeric subunits, and polymer morphology (amorphous or crystalline) can influence the aggregation leading to micelle formation, specifically the three-dimensional structure of the micelles [15,24]. This structure is one of the primary determinants of the material micellar degradation [24]. PLA is known to exist in both crystalline and amorphous forms depending on the stereoforms. Polymerization of L-lactide results in a crystalline polymer. When the L-stereoform is copolymerized with D-lactide, the resulting polymer is amorphous. Crystalline materials exhibit a closer, more ordered arrangement of molecules and thereby an increased resistance to degradation. Linear block copolymers of PEG–PLA with short and long PLA subchains have been studied for hydrolytic degradation and reveal slow degradation for samples prepared as thin film casts submerged in phosphate buffer solution at pH 7.4, with no significant change in 48 h [25,26]. Furthermore, linear copolymers with PEO/PLA ratios >4 were soluble in aqueous solution [25]. Crystallinity of these chains favored the sub chain in higher degree of polymerization, with the melting temperature increasing with PLA chain length [25].

Since micellar systems of this copolymer reported thus far frequently are composed of linear block copolymer systems [1–7] or branched systems containing other copolymer combinations [27,28], these studies focus on a four arm star shaped PEO/PLA copolymer system. Fig. 1 shows the chemical structure of this polymer. In our previous studies, it was found that the star constructs have enhanced CMCs ($\leq$0.024 mg/ml) which are lower than CMCs of linear systems of comparable molecular weight [29]. This study evaluates the thermal properties of the neat polymers and the in vitro degradation of their micelles.

2. Methods

2.1. Material properties

The investigated polymers differed in the chain length, molecular weight, and stereo form of the PLA subunit. The polymers were batch processed by anionic living polymerization of ethylene oxide, followed by coordination or cationic polymerization of lactide [27]. Polymer Source, Inc. provided all initial material characterization. Table 1 shows a summary of these material physical characteristics.

2.2. Thermal analysis

A TA Instrument DSC Q100 differential scanning calorimeter was used to determine the thermal transitions of each of the star copolymers. The samples were measured in 5–10 mg aliquots. The scans were carried out from -30 to 400 °C at a heating rate of 10 °C/min.

2.3. Thermal stability

A TA Instruments TGA 2950 thermogravimetric analyzer was used to study the thermal stability of the polymers. Scans were conducted in nitrogen with a heat rate of 10 °C/min. The samples were measured in 10–15 mg aliquots and the analysis was performed at a temperature range from ambient temperature to 600 °C.

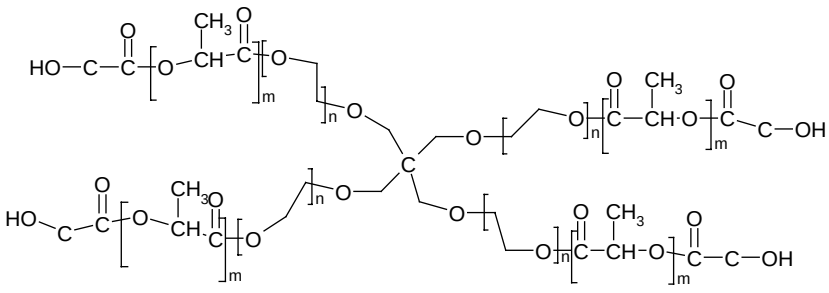

Fig. 1. Four arm star PEO–PLA copolymer.

Table 1
Characterization summary for four star polyethylene oxide polylactide

Polymer	PLA form	PLA content	Total MW (g/mole)	Arm MW (g/mole)	PEO/PLA	M_w/M_n
Sample 1	DL	Low	12,200	3050	2.5/0.55	1.07
Sample 2	L	Medium	13,200	3300	2.5/0.8	1.15
Sample 3	DL	High	16,000	4100	2.5/1.6	1.07

MW, molecular weight; PEO–PLA, polyethylene oxide polylactide; M_w/M_n, polydispersity, where M_w, weight avg MW and M_n, number avg MW.

2.4. X-ray diffraction

A Rigaku RU-200 X-ray diffractometer equipped with Ni filter and Cu Kα source was utilized to evaluate the crystalline nature of the neat polymers. The samples were administered to the goniometer and diffraction was collected in a 2Θ range of 0–31°. The samples were held under a −175 °C nitrogen gas stream while measurements were gathered.

2.5. Micelle sample preparation (coacervation)

The micelle samples were prepared as an approximate 20 mg/ml stock solution in acetone or tetrahydrofuran. A milliliter amount of the stock solution was added drop wise to a given volume of phosphate buffer solution at pH 7.4 to achieve a final polymer concentration of 6.67 mg/ml. The solvated polymer solution was added under agitation in order to coacervate the polymeric solution into micelles. The solvent was allowed to evaporate.

2.6. Lactic acid assay of hydrolytic degradation

A Boehringer D/L lactic acid assay kit from R-Biopharm, Inc. was used to assay the acid content and the chemical stereoform of the solutions. A LKB BIOCHROM Ultrospec II 4050 ultraviolet–visible spectrophotometer (UV–vis) was used at a wavelength of 340 nm to obtain ultraviolet absorbance measurements. The assay was utilized to confirm and quantify hydrolytic degradation of the polyester (PLA) portion of the polymeric micelles as a function of increasing lactic acid concentration. As the PLA chain hydrolyzes lactic acid is formed as the degradation product. The coacervated micelles were incubated at 37 °C, and the acid content was measured in several day intervals over a time spanning 21 days. Sample solutions were diluted by a factor of 10.

2.7. Visualization of micellar degradation

A Hitachi 7000 transmission electron microscope (TEM) was used to obtain micrographs of the degrading micelles at an accelerating voltage of 75 kV. Photographs were taken with a Gatan CCD camera. TEM was utilized to observe the degradation of the micelles over time. The coacervated samples were dropped onto a carbon-coated formvar copper grid and tilted 45° to allow the aqueous solution to drain away from the sample. A solution of 2% phosphotungstic acid was applied to the sample to achieve a negative stain.

2.8. Degradation and CMC

A BMG POLARstar fluorescence spectrometer at an emission wavelength of 390 nm was used to obtain the fluorescence intensity [2]. Upon coacervation into micelles, the solutions were diluted to lower concentrations. Pyrene was added to each aliquot to obtain a 1.2×10^{-7} M concentration. Pyrene fluorescence is used as an indication of polymeric aggregation (hence micelle formation). In the presence of polar solvent pyrene fluorescence is quenched. Pyrene undergoes a dequenching effect when micelles are formed, as indicated by an increase in fluorescence intensity. As the hydrophobic portions of the polymers aggregate in the presence of hydrophilic solvent, pyrene is incorporated into the hydrophobic domain of the micelle and is dequenched [2,16]. The sample (pyrene and micelle solution) was allowed to equilibrate at 65 °C for several hours. The samples were allowed to cool to room temperature and were then administered to a Costar 96 well plate. The bulk undiluted samples were incubated at 37 °C. The fluorescence samples were prepared, as described above, and the fluorescence intensity was measured over time.

3. Results and discussion

3.1. Thermal analysis

Fig. 2 shows the DSC thermograms of the star PEO–PLA with differing PLA chain lengths and stereochemistry. The low and medium content PLA DSC thermograms exhibit two distinct transition peaks. These two peaks may indicate phase separation in the polymer. One peak appears for the high content PLA, suggesting an enhanced miscibility of the copolymer system. Blends of PEO and PLLA have been reported to be partially miscible depending on the blend ratio [30]. High miscibility of PEO and PLLA at specific blend ratios has been found to depend on the PEO molecular weight [30].

There is a shift in the T_m of both the PEO and PLA in the low and medium content PLA which may represent distress in the crystallization of the PEO in the presence of PLA and vice versa. When the PLLA content was low and the PEO molecular weight is 3×10^3, the PLLA T_m decreased [30]. The blends were crystallized during solvent evaporation under a constant temperature decrease from the melt [30]. There was a decrease in the T_m with respect to the PLA T_m in the high content PLA sample. No T_g was determined by DSC.

As stated in the section on material properties, the medium content PLA sample contains L-lactide as the stereo monomer. The homoforms of lactide when polymerized result in crystalline material [31]. The enthalpy of fusion of the crystalline homopolymer of PLA and the enthalpy of fusion as determined by the transition peak representing PLA were

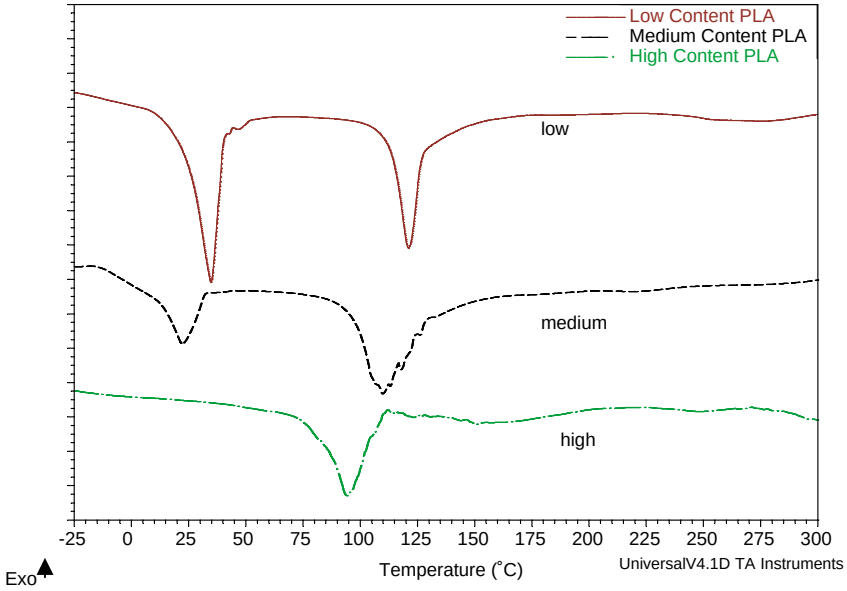

Fig. 2. DSC thermogram overlay of low, medium, and high content polylactide polyethylene oxide polylactide star copolymer. PLA, polylactide.

used to determine the percentage of crystallinity for each of the subunits of the polymers as represented by the following equation:

$$X_c = \frac{H_f}{H_f^0} \times 100\% \quad (1)$$

where X_c is the degree of crystallinity, H_f is the enthalpy of fusion as determined from the melting peak measured by DSC, and H_f^0 is the enthalpy of fusion for crystalline PLA (203 J/g) [31]. The medium content PLA had an average of 51.31% crystallinity.

PDLLA is known to be amorphous; however, PLLA yields crystal structures. The medium content PLA contains, per certificate of analysis, only L-lactide, which is supported by the high percentage of crystallinity as determined by the enthalpy of fusion calculation from DSC thermograms. The medium content PLA may resist degradation due to its crystalline nature. The amorphous nature of the low and high content PLA samples may enhance degradation due to the irregularity in the subchain arrangement which allows small molecules to diffuse into the polymer network.

3.2. Thermal stability

Thermogravimetric analysis (Fig. 3) revealed that the onset of thermal degradation for each of the samples occurs above 100 °C. Thermal degradation continued to the region of 350 °C for each of the polymers. At temperatures of 530 °C or higher, there was a weight loss of at least 98%. The thermal degradation appears to occur in two steps for each of the polymer samples. All samples exhibit significant thermal deterioration in the region from 100 to 300 °C. The two transitions in the polymer samples can be correlated to the physical differences between PEO and PLA chains of the copolymers, as discussed above.

3.3. XRD

XRD was utilized to further investigate the crystalline properties of the neat polymers. Fig. 4 shows the spectrum of each of the samples. All samples were observed under a nitrogen gas stream at −175 °C. The high content PLA exists as a liquid at room temperature. It should be noted that in the difractogram shown in Fig. 4, intensity is plotted against 2Θ. The low and medium content PLA samples both have two distinct 2Θ diffraction peaks. The low content PLA sample 2Θ diffraction peaks occur at 19.34 and 23.6°. The medium content PLA sample 2Θ diffraction peaks occur at 19.19 and 23.3°. The high content PLA sample has one strong diffraction peak, which occurs at 12.19°, and a broadened peak that has a maximum of 19.37°, which reflects a disturbance in the PEO crystallinity. Luo et al. reported 2Θ diffraction peaks for PEG with a molecular weight of 2000 g/mol, with peaks occurring at 19.1 and 23.4° [15]. PLA with a molecular weight ranging from 1000 to 3000 g/mol were all reported to have the same 2Θ diffraction peak of 16.6°. The peaks that are seen in Fig. 4 for the four star PEO–PLA copolymer systems suggests degrees of crystallinity that have influences from both the constituent polymer components, with PEO being more dominant.

3.4. Lactic acid assay of hydrolytic degradation

A lactic acid assay was utilized to confirm and quantify hydrolytic degradation of the polyester (PLA) portion of the polymeric micelles as a function of increasing lactic acid concentration. The lactic acid concentration can be directly correlated to degradation because the degradation product of

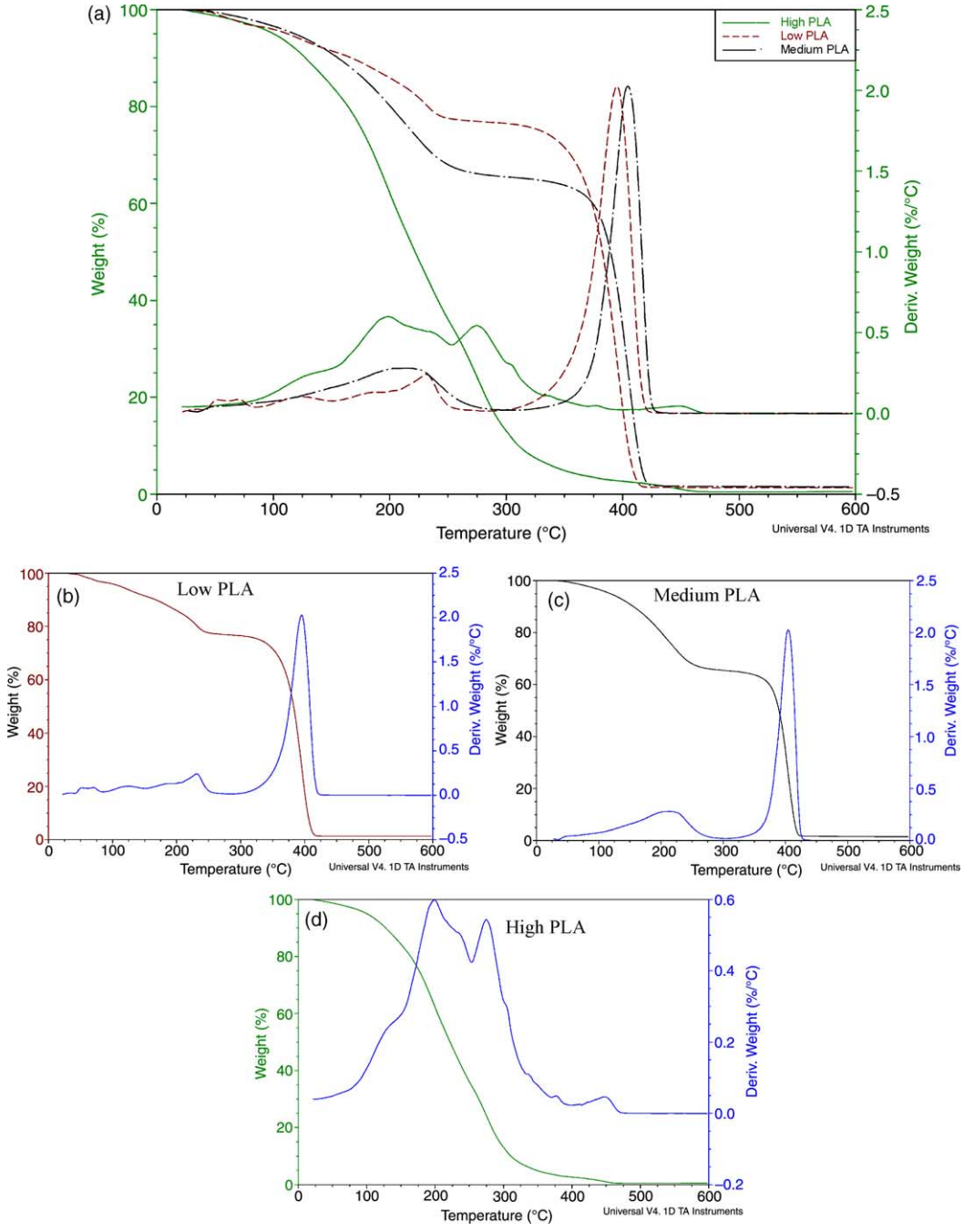

Fig. 3. TGA thermogram overlay (a) and individual thermograms with derivative weight for the low (b), medium (c), and high (d) content thermograms of neat polylactide polyethylene oxide polylactide star copolymer. PLA, polylactide.

PLA is the lactic acid monomer. As the polymer hydrolyzes the lactic acid concentration increases. Lactic acid concentration is directly proportional to and can be a quantified measure of polymeric hydrolytic degradation. Furthermore, lactic acid concentration in the contained micellar solution is inversely proportional to PLA polymeric chain length and molecular weight as the solutions are capped to prevent aqueous evaporation. The acid concentration increased and the PLA chain length and molecular weight indirectly decreased as time progressed. These findings confirmed hydrolytic degradation of the polyester (PLA) portion of the polymer. As the lactic acid concentration increased the PLA chain length decreased. Fig. 5 correlates concentration change with time. The initial degradation for each of the samples has an almost linear increase of lactic acid (loss of mass) with time. The lactic acid concentration of the low and medium content PLA samples began to remain practically constant in acid concentration at day 10, indicating possible depletion of the PLA subchain and therefore depletion of the micelles. The low and medium content PLA samples reach a maximum concentration around day 14 (0.168 mg/ml×10) and day 21 (0.256 mg/ml×10), respectively, suggesting complete degradation of the PLA

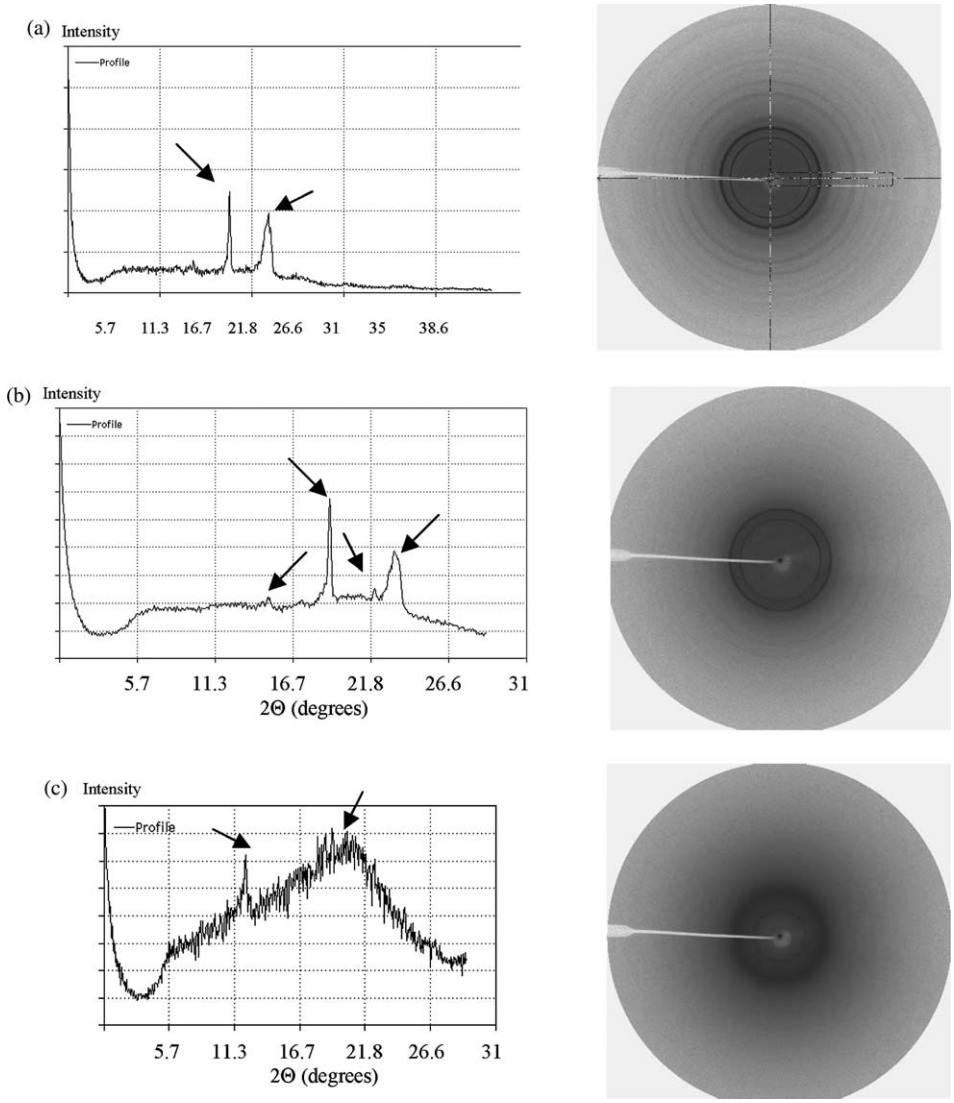

Fig. 4. Two-dimensional diffraction pattern and X-ray difractogram of low (a), medium (b), and high (c) content polylactide polyethylene oxide polylactide star copolymer.

chain. The high content PLA sample did not reach a constant lactic concentration and had a maximum acid concentration (0.449 mg/ml×10) at day 21, suggesting that micelles exist at a degradation time of 21 days. The medium content PLA sample having higher crystallinity and only 250 additional mass units on each arm, degrades comparably to the high content PLA. This finding agrees well with data on the linear PLA, in which hydrolytic degradation was observed to be quicker for amorphous PDLLA; this agreement further confirms the influence that molecular architecture, chain length, and crystallinity have on degradation.

3.5. Visualization of micelles

TEM was utilized to observe the degradation of the micelles over a 21-day time span. Micrographs shown in Fig. 6 were taken at several day increments. Micelles were prominent for the first 7 days for all samples. As time progressed the micelles began to degrade as seen by a decrease in size and a less spherical shapes. By day 11 there were no detectable micelles in the low content PLA sample, seen in Fig. 6(a.3). There were no detectable micelles by day 14 for the medium content PLA, seen in Fig. 6(b.3). At day 21 the high content PLA sample had micelles that lost their spherical shape and are rather irregular, seen in Fig. 6(c.4). This observation can be correlated to the increase in acid concentration as a measure of the hydrolytic degradation of the hydrophobic polyester portion of the polymer as the acid concentration of the low and medium content PLA samples began to remain constant at day 10, reaching a maximum concentration around day 14 (0.168 mg/ml×10) and day 21 (0.256 mg/ml×10), respectively. The high content PLA sample did not reach a constant concentration and had a maximum acid concentration (0.449 mg/ml×10) at day 21. Fig. 6(a)–(c) shows the micellar degradation over the entire 21 day time span.

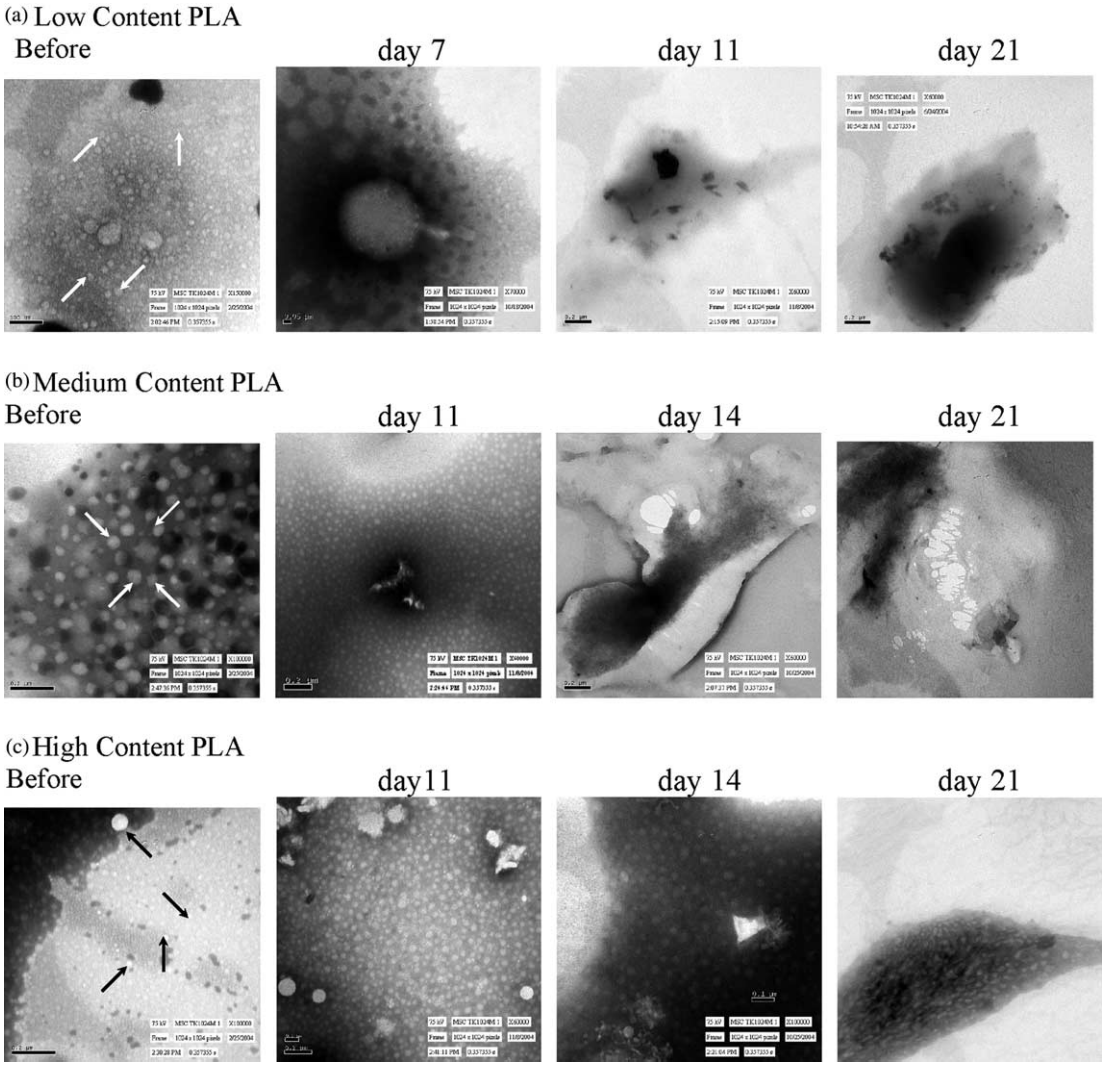

Fig. 6. Micrographs of low (a), medium (b), and high (c) content polylactide polyethyleneoxide polylactide star copolymer polydispersed micelles. Before hydrolysis and at days 7, 11, 14, and 21 days of hydrolysis. Arrows indicate micelles.

3.6. Degradation and CMC

The fluorescence intensity ratio decreased with time but fluctuated within the sample set (as seen in Fig. 7 for the low content PLA). The decrease in fluorescence intensity can be related to the degradation of the PLA portion of the micelles and the exposure of the probe to aqueous solvent, which quenches the intensity of the pyrene probe. The addition of ions through polymer hydrolysis may alter the micellar structure, as seen in Fig. 6(c.4), and/or the fluorophore excitation.

It has been reported that the addition of salt decreases the cloud point of PEO [32]. Gohy et al. measured the effect of pH on the hydrodynamic diameter ($2r_h$) of a linear triblock system of PEO, polystyrene, and polyvinylpyrolidone by cycling the pH with NaOH and HCl [32]. It was found that micelle diameters decreased with increasing pH. The decrease in solubility of the PEO may enhance the micellar aggregates through improved intramolecular association, which may further stabilize the micelle through the PEO steric layer [32]. This decrease in PEO solubility may explain the fluctuation in the intensity ratio, specifically, higher intensity ratio readings at concentrations below the determined CMC. The fluctuations in the intensity ratio as a function of concentration may be due to the probe diffusing into the hydrophobic domains of probable intramolecular aggregates

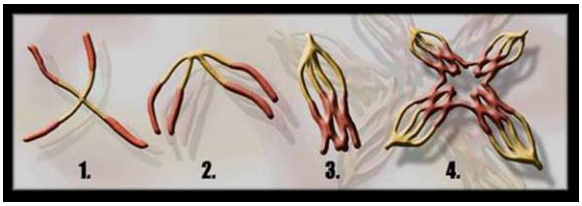

Fig. 8. Proposed micelle formation mechanism of four star polyethylene oxide polylactide. 1. Neat four star polyethylene oxide polylactide. 2. Four star polyethylene oxide polylactide in the presence of aqueous solution. 3. Intramolecular interaction in presence of aqueous solution. 4. A cross section of a micelle formed through intra and intermolecular aggregation of, four molecules of four star polyethylene oxide polylactide in presence of aqueous solution. Yellow, hydrophilic polyethylene oxide block; Red, hydrophobic polylactide block (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

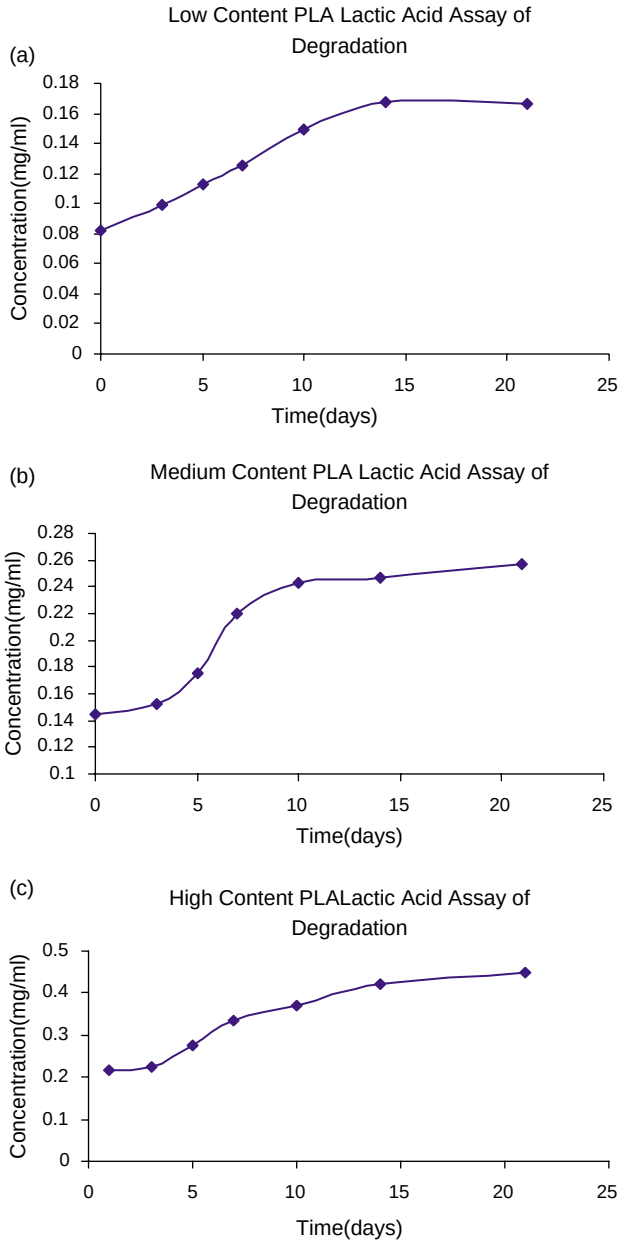

Fig. 5. Loss of mass of low (a), medium (b), and high (c) content PLA measured by lactic acid assay of degrading PEO–PLA star copolymer micelles.

that initiate the micellar association, see Fig. 8. This observation suggests that the micellar structure may be capable of hydrophobic entrapment on the single molecule level.

In contrast, cations such as cesium and those attached to macromolecules are reported quenchers of the pyrene fluorophore [33,34] suggesting possible quenching due to the lactic acid induced cations resulting from the degrading PLA portion of the copolymer chain. The results of the acid assay and TEM indicate that the molecular aggregates undergo continuing hydrolytic cleavage of the lactide chain, which may in turn quench the pyrene fluorophore. In addition, Frank et al. found that branched polymers reduced the quenching rate constant with accessible chromophores as the generations of branching increased [35].

4. Conclusions

The four star PEO–PLA copolymers were investigated as received by using DSC, XRD, TGA, UV–vis, fluorescence spectrophotometry, and TEM. An enhanced miscibility may occur with increasing PLA chain length. The polymers exhibit phase separation and distressed crystallinity, which may enhance degradation due to the irregularity in the polymer arrangement. Diffraction peaks observed by XRD analysis further confirm that the polymers have a degree of crystallinity. Thermogravimetric analysis revealed that the onset of thermal degradation for each of the samples occurs well above the application temperature. Hydrolytic degradation of the polyester (PLA) portion of the polymer was confirmed and quantified by assaying the lactic acid content of the micellar solution. The degradation time for each of the samples was relatively short although remnants of micelles were still detectable at day 21 for the high content PLA sample.

The four star PEO–PLA polymers have exceptional thermal stability, which enable their use for a variety of applications. These copolymers have morphological properties that suggest that they would be an outstanding control release drug delivery system. The polymers have short degradation times, suggesting use as a short-term drug release agent. Furthermore, the polymers could be optimized for specific release times by altering chain length and/or stereochemistry of the PLA

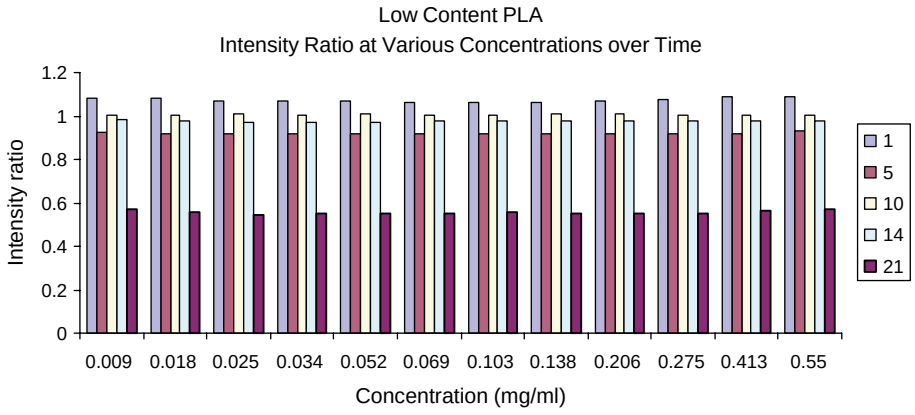

Fig. 7. Effect of degradation on critical micelle concentration at 1, 5, 10, 14 and 21 days of degradation for low content polylactide polyethylene oxide polylactide star copolymer.

polyester, which degrades through hydrolysis to release the pharmaceutical agent.

Acknowledgements

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