Copolymerization of Glycolide and ε-Caprolactone Using 12-Aminolauric Acid Modified Montmorillonite

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Abstract. Poly(glycolide-co-ε-caprolactone) (PGLYCL) nanocomposites were prepared by copolymerization glycolide (GLY) and ε-caprolactone (ε-CL) in the presence of varying loadings 12-aminolauric acid (12-ALA)-modified montmorillonite. Copolymerization was successfully achieved based on the increase in polymer molecular weight after the reaction determined by gel permeation chromatography (GPC). The amount of the poly(glycolide) block and poly(ε-caprolactone) block units in the copolymer, identified by proton nuclear magnetic resonance (¹H-NMR) spectroscopy, suggested that the increase in organo-clay loading cause a reduction GLYL:ε-CLL ratio. The arrangement of the monomers in the polymer products was elucidated to have an ABA triblock structure, where PCL block is the central block and the PGLY is found at both end of the copolymer. The presence of intercalated and exfoliated silicates in the nanocomposites were observed by x-ray diffraction (XRD) analysis. The biocompatibility of the nanocomposites with NCTC 292 mouse normal fibroblast was high relative to untreated cell cultures using tetrazolium bromide (MTT)-dye reduction assay.

1. Introduction

Synthetic biodegradable polymers are designed to perform first their function and then later on be degraded by the body without exhibiting any biocompatibility and excretion issues. Because of this along with their tunable properties, they have gained wide interest not only in the fields of drug delivery applications but also in smart biomaterials, medical devices and tissue engineering applications [1]. Biodegradable polymers commonly used as biomaterials can be prepared from a major class of synthetic polymers, the poly(α-hydroxy acids) or commonly known as polyesters. One example of a polymer biomaterial is polyglycolide (PGLY); which is produced from ring-opening polymerization of glycolide (GLY). PGLY is insoluble in most organic solvent due to its high degree of molecular arrangement, making it difficult to process, unlike the copolymers of PGLY such as the popular poly(lactide-co-glycolide) (PLGA). Poly(caprolactone) (PCL) is known to be useful as polymer for long-term controlled drug delivery and implantable devices because of its relatively low rate of degradation [1]. The copolymerization of GLY with other cyclic esters were previously exhibited the the research group of Díaz-celorio [2] where they synthesized a copolymer of poly(glycolide-co-methylene carbonate) copolymers via ring opening polymerization (ROP) using organometallic stannous (II) octoate (SnOct₂) as the catalyst were conducted by some research group. While SnOct₂ is the most popular catalyst for this reaction, few studies on using alternative eco-
friendly catalysts have been carried out [3]. Montmorillonite clays have been found to be effective catalysts for many organic reactions, including polymerization reactions [4]. Thus, they also contribute a great deal in the shift towards making clays as the most promising/alternative green catalyst [5]. The present study explored the ability of the organo-modified clay to catalyze a ring-opening polymerization of GLY with ε-CL. The functional groups for aliphatic polyesters were determined by Fourier Transform Infrared (FTIR) and Proton Nuclear Magnetic Resonance (1H-NMR) spectrometry. In addition to that, from the 1H-NMR spectra, the terminal polymer chain was determined through end group analysis and the relative amount of GLY and ε-CL in the samples were calculated. Though the relationship of organo-modified clay loading and molecular weight of the polymers are not yet established, the present study also aimed to relate these two factors using Gel Permeation Chromatography (GPC). Finally, the extent of polymer intercalation within the clay silicate layers was also evaluated using X-ray Diffraction (XRD).

2. Methodology

2.1. In situ intercalative polymerization of glycolide and caprolactone with 12-aminolauric acid

Na-montmorillonite (Nanofill 116) with 1.16 meq/g cation exchange capacity (CEC) was loaded with 90% 12-aminolauric acid (12-ALA) to produce an organically modified clay by exchanging the the Na+ counter ions of montmorillonite with 12-ALA in aqueous media. Then, polyester-clay nanocomposites were prepared by in situ intercalative polymerization of cyclic esters, namely ε-CL and GLY, with AMMT. Briefly, 50:50 wt % of GLY:ε-CL, with varying loading of AMMT (1, 3, 5 10 and 15 wt. %), were placed in a reaction flask. The mixture was reacted at 150°C for 24 hours with continuous Nitrogen gas purging. The products were dissolved in chloroform and then precipitated in methanol. As a control, polyesters, from each monomer, were synthesized using the conventional SnOct2 catalyst with 1, 4-butanediol (BDO) as the initiator.

2.2 Molecular characterization of the AMMT-catalyzed polyesters

The molecules weight properties of the polyester products were determined by gel permeation chromatography (GPC) with polystyrene standards using a Shimadzu LC-20AD Prominence liquid chromatograph equipped with a Shimadzu RID-10A refractive index detector (RID). Samples were eluted with tetrahydrofuran (THF) through a TSK-GEL G3000HHR at a flow rate of 1 mL/min. The function groups of the copolymer products were determined using Shimadzu IR Prestige-21 Fourier Transform Infrared (FTIR) spectrophotometer and samples were scanned at a rate or 4 cm⁻¹ for 32 times ranging from 4000 to 600 cm⁻¹. To elucidate the molecular structure of the polyester products and their actual GLYL and ε-CLL content, 1H-NMR spectroscopy was performed using a Varian 500MHz spectrometer. The polyester products were dissolved deuterated chloroform (CDCl₃) with trimethylsilane (TMS). The order of oligo-GLY and oligo-ε-CLL in the copolymer was evaluated by based on the presence-methylene group adjacent to hydroxyl (4.67ppm or 3.62ppm) and carboxyl group (4.79ppm or 2.21ppm). The actual amount of GLYL and ε-CLL was derived from the proton NMR spectra using the following equation (Equation 1):

\[
\%mol = \frac{A_{1.66ppm}/n_{1.66ppm}}{A_{1.66ppm}/n_{1.66ppm}+A_{4.2ppm}/n_{4.2ppm}} \times 100\%
\]

where \(A_{4.2ppm}\) and \(n_{4.2ppm}\) is the peak integration and the number of methylene protons giving rise to the chemical shift characteristic of GLYL and the \(A_{1.66ppm}\) and \(n_{1.66ppm}\) is the peak integration and the number of methylene protons giving rise to the chemical shift of ε-CLL. To determine the formation of the nanocomposites, x-ray diffraction analysis using a Shimadzu XRD-700 x-ray diffractometer. The x-ray diffractograms were taken at an accelerating voltage of 40kV using an x-ray source was CuKa1 (1.540598 Å) at a rate of 2.0¹/min from 3° up to 90°. The in vitro cytotoxicity of the clay-catalyzed polyesters was performed using indirect contact MTT-dye reduction assay. Mouse normal fibroblast (NCTC 292, ATCC® TIB-67™) were incubated in MEM supplemented with 10%v/v horse serum, 1%v/v non-essential amino acids, 2mM L-glutamine, 1mM sodium pyruvate and 2%v/v of 7.5%
sodium bicarbonate and 1% v/v antibiotic-antimycotic. A $5.0 \times 10^3$ cells/mL were seeded into 96-well plates and then incubated for 24 hours with the polymer extracts.

3. Results and Discussion

3.1. Synthesis of polyester-clay nanocomposites by in situ intercalative polymerization

Poly(glycolide-co-ε-caprolactone) PGLYCL with different loading amount of the organo-clays (1, 3, 5%, 10%, and 15% by weight) were prepared via in situ intercalative ring-opening polymerization as shown in Figure 1.

![Figure 1. In situ intercalative polymerization of GLY and ε-CL using 12-ALA modified MMT.](image)

3.2. Molecular weight analysis of the copolymers

The molecular weight properties of the SnOct$_2$-catalyzed PGLYCL and PGLYCL nanocomposites are shown in Table 1. The molecular weight of PGLYCL nanocomposites was observed to be increase with increasing clay loading. The presence of the clay’s silicate layers during polymerization may have obstructed the diffusion of the monomers toward the growing polymer chains of the polymer chains. This might also be one of the reasons why molecular weights of PGLYCL polymers in the nanocomposites are much lower than that of SnOct$_2$-catalyzed PGLYCL. The sensitivity of the polymerization reaction to the presence of water molecules should also be considered. While the clay is organically modified, there are still traces of water present in the structure as observed.

| Sample                  | %Recovery | M$_n$  | M$_w$  | PDI  |
|-------------------------|-----------|--------|--------|------|
| PGLYCL-SnOct$_2$        | 96%       | 7691   | 12884  | 1.6752 |
| PGLYCL-1AMMT           | 94%       | 4269   | 4288   | 1.0044 |
| PGLYCL-3AMMT           | 95%       | 3938   | 3956   | 1.0046 |
| PGLYCL-5AMMT           | 92%       | 1462   | 3104   | 2.1231 |
| PGLYCL-10AMMT          | 94%       | 1683   | 3017   | 1.7926 |
| PGLYCL-15AMMT          | 93%       | 1499   | 3076   | 2.0520 |

3.3. Structure elucidation of the copolymers

The representative $^1$H-NMR spectra of the SnOct$_2$ and clay-catalyzed polyesters are shown in Figure 2. Chemical shifts observed in the PGLYCL nanocomposites are all consistent with the peaks observed in SnOct$_2$-catalyzed PGLYCL. Signals in the range of δ = 1 ppm to 2.5 ppm are all attributed to methylene protons of PCL while peaks within δ = 4 ppm to 5 ppm are attributed mainly to PGLY methylene protons. The chemical shifts of SnOct$_2$-catalyzed PGLYCL and clay-catalyzed PGLYCL with 1% and 5% wt organo-clay exhibited intense and narrow peaks. End group analysis of the PGLYCL products was conducted to determine the order of the monomers in the polymer chain. The chemical shift of a methylene protons neighboring the hydroxyl and carboxylic acid group for PGLY blocks were found at 4.69 ppm and 4.29 ppm, respectively. The PCL blocks are found in the central part of the molecular structure. In addition, the actual amount of PGLY and PCL content in the copolymer were calculated based on the relative peak intensity that was characteristic for each monomer. Assuming the complete conversion of monomers during polymerization, the expected mole percent of GLY would be 52%, 51%, 52% and 51% for PGLYCL with 1, 5, 15 wt % the organo-clay and PGLYCL-SnOct$_2$, respectively. However, copolymer analyses on the representative peaks of GLY at δ = 4.82 ppm and ε-CLL at δ = 1.66 ppm varied from the theoretical values. The calculated
mole percent of GLYL for PGLYCL with 1, 5, 15 wt % clay and PGLYCL-SnOct$_2$ are 45.57, 40.99, 22.20 and 44.22 %, respectively. There is a decrease in GLYL content with increasing organo-clay content which could be attributed to the initial phase of the monomers. At room temperature, GLY appears to be a white powder while ε-CL is an oily liquid. During the initial stage of polymerization, the more mobile ε-CLs react with one another while GLY independently melts. After melting, only then GLY react with other GLY molecules or with ε-CL monomers. The resulting polymer products were soft and/or appeared like hard waxes. It should also be noted that during the purification stage, the PGLYCL materials were soluble in CHCl$_3$, but there were insoluble white polymer precipitates attributed to the PGLY homopolymer or PGLY-rich polymer. And so, during $^1$H-NMR analyses, it was possible that what were analyzed are only those copolymers that in soluble in CHCl$_3$ while the PGLY-rich polymers were removed or filtered out from the solution. The use of 1 to 5 wt. % of organo-clay allowed the copolymerization of the monomers with relatively similar GLYL and ε-CL composition in PGLYCL-SnOct$_2$. The PGLYCL with 15 wt. % of clay may have a surplus of active initiation sites where GLY and ε-CL polymerized independently, such that their individual polymers did not grow in the same chain.

![Figure 2](image-url)

**Figure 2.** Proposed molecular structure of (a) PGLYCL and the $^1$H-NMR spectra of the (b) PGLYCL-SnOct$_2$ and (c) PGLYCL-5A-MMT.

### 3.4. Infrared investigation of ester bond formation after polymerization

The infrared spectra of the GLY, ε-CL, SnOct$_2$-catalyzed PGLYCL and PGLYCL nanocomposites are shown in Figure 3. A typical cyclic ester has a sharp of C=O stretching vibration at 1715cm$^{-1}$; although the exact position depends on the type of carbonyl structure. For ε-CL, the C=O stretch is observed at 1741cm$^{-1}$ (Figure 3.3a) while it is 1751cm$^{-1}$ for GLY (Figure 3.3b). For the SnOct$_2$-catalyzed PGLYCL and the PGLYCL nanocomposites, the C=O stretch of an aliphatic ester is found at 1735cm$^{-1}$, consistent with all polymer products. This shows that polymerization of GLY with ε-CL has been successful as evidenced by the formation of ester linkages. The peaks observed at 2947 to 2950 cm$^{-1}$ and 2862 to 2870cm$^{-1}$ are attributed to asymmetric and symmetric stretching of CH$_2$ groups of the hydrocarbon chains. The characteristic spectral peaks associated to OAMMT, found at 3600 cm$^{-1}$ and 995cm$^{-1}$ were not detected in the spectra of PGLYCL nanocomposites which is probably due to the small amount of the organo-clay in the polymer matrix that is not detectable by FTIR instrument.
3.5. Formation of polyester-clay nanocomposites

The extent of the clay’s silicate dispersion and delamination within the polymer matrix was assessed using XRD. The x-ray diffractogram of the organo-clay, PGLYCL-SnOct₂ and PGLYCL nanocomposites are shown in Figure 4. First, organo-modification of Na⁺-MMT with 12-ALA was done prior to polymerization to achieve greater compatibility between the hydrophilic clay and hydrophobic polymer. A shift of peak at 2θ = 4.5º relative to the 2θ position of Na⁺-MMT found at 7.12º, shifting of peak towards lower 2θ is a result of an increase in the basal spacing at (001) plane of organo-clay’s silicate galleries from 1.24 nm (Na⁺-MMT) to 1.82 nm (AMMT). The (001) peak were absent for PGLYCL-1AMMT and PGLYCL-5AMMT, suggesting that the clay silicates are dispersed in the polymer matrix. The diffraction peak of the clay’s (001) plane in the PGLYCL-10AMMT nanocomposite (Figure 3.4e) is 5.1° that corresponds to 1.73 nm and PGLYCL-15AMMT nanocomposite (Figure 3.4f) exhibited the (001) peak at 5.15° that corresponds to and 1.7 nm. Again, the d₀₀₁-spacing of the unmodified Na⁺-MMT is 1.25 nm. Assuming that the surfactant in the clay undergoes Hoffman elimination (Carrasco et al., 2011) [6] during polymerization, there is a 0.48 nm increase for PGLYCL-10AMMT and 0.46 nm increase for PGLYCL-10AMMT of the clay’s d₀₀₁-spacing due to polymer intercalation. The polymer chains that are intercalated in the clay layers may adopt a coexisting (in transition from one phase to another) monolayered to a bilayered structure.
Figure 4. X-ray diffractograms of (a) organo-clay, (b) PGLYCL-SnOct$_2$, (c) PGLYCL-1AMMT, (d) PGLYCL-5AMMT, (e) PGLYCL-10AMMT and (f) PGLYCL-15AMMT.

3.6. Biocompatibility of the polyester-clay nanocomposites
The biocompatibility of the PGLYCL-SnOct$_2$ and PGLYCL-AMMT to NCTC 929 cells was determined by in vitro non-contact MTT assay. The cell viability and also the mitochondrial integrity, upon exposure to nanocomposite extract, were measured based on the reduction of the MTT-dye into purple formazan. The cytotoxicity profiles of the PGLYCL samples at 100% extraction media concentration are shown in Figure 5. Similar to the PCL and PGCLY samples, the PGLYCL material exhibited high cell viability that are more than 80%. The presence of clay within the polymer matrix did not affect the biocompatibility of the nanocomposites, and even at 15% clay loading, the % cell survival is around 95%. Representative photographs of viable cells after exposure to the polymer and nanocomposite extract are shown Figure 6. The inherent biocompatibility of the copolymers is again due to their biodegradability, allowing them to be processed by the cellular machinery. The doxorubicin (positive control) resulted to 17% cell survival. The photograph of viable cells in culture media (negative control) and non-viable cells after exposure to doxorubicin, a cytotoxic compound (positive control) are shown in Figure 3.5.
Figure 5. Cytotoxicity of the SnOct$_2$-catalyzed and clay-catalyzed PGLYCL materials. The negative (-) control represents the incubation of cells in cell culture media. The positive control (+) represents the incubation of cells with doxorubicin.

4. Conclusion

The copolymerization of GLY and ε-CL was successfully synthesized by ring-opening polymerization using AMMT as catalyst. The molecular structure of the polymer products was observed to have a blocks of GLY (A) and ε-CLL (B) units in ABA order. The present study demonstrated the catalytic activity of the organically modified clay to polymerize the cyclic esters but with relatively low molecular weight. The polymerization, dispersion of the clay catalyst was achieved, forming polymer-clay nanocomposites. The composite materials with exfoliated/intercalated structures were observed. Interestingly, the nanocomposites also exhibited high cellular biocompatibility with NCTC mouse normal fibroblast, which shows the possibility of the materials find applications as a biomedical polymer.

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