
Kevin B. Dagbay$^1$ and Florentino C. Sumera$^*$

$^1$Natural Sciences Research Institute, University of the Philippines, Diliman, Quezon City
$^*$Institute of Chemistry, University of the Philippines, Diliman, Quezon City 1101

The fatty acid derived polyanhydrides were synthesized from hydroxylauric acid maleate and sebacic acid (SA). Hydroxylauric acid maleate (HOLAM) diacid half-ester was prepared from the reaction of hydroxylauric acid with maleic anhydride. Copolymers of HOLAM and SA were synthesized by melt polycondensation to yield polymers with molecular weights of 47,000 to 240,000 g/mol and with yields of 55 to 75%. Structural characteristics of the monomers and polyanhydrides were characterized using FT-IR, $^1$H/$^{13}$C-NMR, DSC, GPC, and SEM. Results showed that these polymers have low melting points (51-76$^\circ$C) and are degradable under simulated \textit{in vitro} physiological condition (phosphate buffer, pH 7.4 at 37$^\circ$C). The extent of degradation and crystallinity of these polymers depend mainly on HOLAM content of the copolymers. Phase inversion technique produced poly(SA:HOLAM) microspheres, wherein, particle size (1.33-2.36 $\mu$m) and drug release rates were found dependent on the HOLAM weight ratio. Biphasic \textit{in vitro} drug release profile was observed for ciprofloxacin-loaded poly(SA:HOLAM) microspheres. Kinetic study using models for drug release showed that release by diffusion rather than by erosion was followed.

Key Words: ciprofloxacin, \textit{in vitro} drug release, \textit{in vitro} degradation, hydroxylauric acids, lauric acid, polyanhydride

INTRODUCTION

Polyanhydrides have been used since the 1980’s as polymeric drug delivery system (Rosen et al. 1983, Jain et al. 2005). For a particular application the rate of degradation of polyanhydrides can be manipulated by change in the monomer type and ratio without affecting their attribute of biodegradability. Thus, selection of monomers becomes crucial to obtain the polymer of desired degradability. A case in point are the fatty acids which are found good candidates for polymeric drug delivery system because they can moderate the rate of drug release in biodegradable polymers such as polyanhydrides due to their hydrophobicity (Maniar et al. 1994, Teomin & Domb 2001, Shikanov et al. 2005). Besides affecting the degradation pattern these also affect the physical state of the final polymer, which in turn govern their use either as solid implantable material or directly injectable carrier systems. Owing to the importance of anhydride bond in the controlled drug delivery application, different types of fatty acid components of polyanhydride have been assessed as potential carrier for bioactive agent (Rosen et al. 1983, Maniar et al. 1994). The major limitation...
in synthesizing polymer containing fatty acids is the lack of difunctionality of fatty acids (Conato & Sumera 2012) so that they can be integrated in the polymer chain. In this study, lauric acid was functionalized into a diacid monomer and copolymerized with sebacic acid (SA) to produce an aliphatic polyanhydride system for drug delivery application. Specifically, trifluoroacetic acid/hydrogen peroxide (TFA/H2O2) hydroxylation method was used to synthesize mixtures of hydroxylauric acids and reacted with maleic anhydride to yield a diacid monomer. Polyanhydrides with varying composition of lauric acid maleate and sebacic acid were synthesized by melt-polycondensation. In vitro drug release behavior, in vitro hydrolytic degradation and thermochemical properties of the polyanhydrides were evaluated. In addition, a simple mechanism of the polyanhydrides’ drug release was determined from kinetic models.

MATERIALS AND METHODS
Lauric acid (≥ 99%) was purchased from Fluka, India. Sebacic acid (≥ 95%) and ciprofloxacin (≥ 98%) were obtained from Fluka, China. Analytical reagent (AR) grade trifluoroacetic acid (≥ 99%), sodium sulfate, acetic anhydride, ethanol, 30% hydrogen peroxide, and dichloromethane were all purchased from Merck, Germany. Maleic anhydride (≥ 99%) and polystyrene standards (MW range 3,700-641,340 g/mol) were purchased from Aldrich, Germany. All other reagents used were of analytical grade.

Instrumentation
Molecular weights of the polyanhydrides were estimated on a gel permeation chromatography (GPC) system consisting of a Perkin Elmer (USA) 200 LC pump with UV detection (Applied Bioscience 785a Programmable Absorbance Detector) at 254nm, an injection valve with a 50μL loop, and an integrator. NMR spectra were obtained using a JEOL Lambda FT-NMR spectrometer at room temperature. Deuterated chloroform (CDCl3) was used as solvent and tetramethylsilane (TMS) as reference standard. Samples were analyzed using IRPrestige-21 Fourier Transform Infrared Spectrophotometer. Diffused Reflectance Spectroscopy(DRS) was used for solid samples while a KBr smear technique was used for liquid samples. Thermal analysis of the samples was conducted using TA Differential Scanning Calorimeter (DSC) Q10. Polymer samples (2-5 mg) were sealed in aluminum pans and scanned at a heating rate of 10°C min⁻¹ from 10°C to 150°C. Quantitative determination of ciprofloxacin was done using Shimadzu PharmaSpec UV-1700 at room temperature. Particle size and size distribution of microspheres was determined using Scanning Electron Microscopy (SEM) [JSM 5310 (JEOL, USA)], measuring at least 50 microspheres.

Monomer synthesis
Hydroxylation of Lauric Acid (HOLA)
Hydroxylation of lauric acid was done using H2O2/TFA method as reported by Deno et al. (1977). To 150mL of trifluoroacetic acid, 67 mL 30% aqueous H2O2, and 20g of lauric acid were added. The mixture was then refluxed at 85°C for 2 hours. The product was isolated by adding the resulting mixture to ice-water and extracting with anhydrous diethyl ether. The ether layer was washed three times with water and evaporated to give the product. The product containing mainly trifluoroacetate derivatives of hydroxylauric acid (HOLA) was further treated with 5M NaOH and refluxed at 85°C for 2 hrs, then acidified with 1M HCL to pH~4 and extracted with diethyl ether. The ether layer was washed thrice with water, dried over Na2SO4 and evaporated in vacuo. The product mixture with HOLA was subjected to FT-IR, 1H and 13C-NMR, and GC/MS.

The mixture of ω-1 to ω-6 hydroxylauric acids, ω-1 16.5%, ω-2 19.2%, ω-3 19.3%, ω-4 20.3%, ω-5 and ω-6 with 24.7% (Casalme & Sumera 2013) is an oily yellow liquid. Yield = 80%, FT-IR (KBr, cm⁻¹): 3400 (broad, OH stretch), 2700-2500 (broad, COOH stretch), 1713 (sharp and intense, C=O stretch). 1H-NMR [CDCl3, 400 MHz, δ (ppm)]: 6.16 (s, -COOH), 4.06 (ω-6-CH-OH), 3.81 (ω-5-CH(OH)), 3.63 (ω-4, ω-3, ω-2-CH(OH)), 3.54 (ω-1-CH(OH)), 2.32 (t, -CH2-COOH), 1.61 (m, -CH2-CH-COOH), 1.43 (m, -CH(OH)-CH2-), 1.26-1.40 (m, -CH2-CH-R), 1.22 (d, 3H3-C-CH(OH)-), 0.90 (m, -CH3). 13C-NMR [CDCl3, 100 MHz, δ (ppm)]: 179.38-179.62 (CH2=O of mixture of hydroxy acids), 73.57 (ω-2-CH(OH)), 72.17 (ω-3-3CH(OH)), 72.12 (ω-6-CH(OH)), 71.94 (ω-5-CH(OH)), 71.91 (ω-4-CH(OH)), 68.41 (ω-1-CH(OH)), 39.29 (CH(OH)-CH2-), 34.1 (-CH2-COOH), 25.01-32.17 (-CH2-R), 22.65 (H3-C-CH(OH)-), 9.88 (H3C-CH(OH)-), 14.09 (H3C- of ω-3,4,5 and 6).

Synthesis of Hydroxylauric Acid Maleate (HOLAM)
The synthetic route of lauric acid maleate (HOLAM) was followed according to the procedure reported by Domb and Nudelman (1995) as applied to ricinoleic acid. A solution containing 30 g (0.14 mol) hydroxydodecanoic acid and 27 g (90.28 mol) maleic anhydride in 73 mL toluene was stirred at 85°C for 12 hours. The solution was washed four times with distilled water, dried over MgSO4, and evaporated to dryness. Further, HOLAM was subjected to FT-IR, 1H and 13C-NMR analysis.

Lauric acid maleate (HOLAM) is an oil orange product. Yield = 79%. FT-IR (KBr, cm⁻¹): 2500-3650 (broad, COOH stretch), 1730 (strong C=O stretch of ester), 1712 (strong, C=O stretch of COOH) 1645 (weak, >C=C< stretch). 1H-NMR [CDCl3, 400 MHz, δ (ppm)]:

δ (ppm) [CDCl3, 400 MHz, δ (ppm)]:
Polymer Synthesis

Prepolymer Synthesis
The prepolymer of sebacic acid (SA) and HOLAM was prepared as described previously (Domb & Langer 1987). Briefly each monomer (SA) and HOLAM) were reacted with excess acetic acid anhydride (1:5 w/v) at 120 °C in reflux condition for 30 min. Excess acetic anhydride was removed in vacuo at 70 °C. The crude prepolymer was then recrystallized from dry toluene. The crystal was immersed in a 1:1 mixture of dry petroleum ether and ethyl ether overnight to extract traces of acetic anhydride and toluene. The crystals were dried in a vacuum desiccator and stored in −40 °C until use. The prepolymer were subjected to FT-IR, 1H and 13C-NMR, and GPC. Below are their spectroscopic characteristics.

For polyHOLAM (PHOLAM), FT-IR(KBr, cm−1): 1823 and 1755 (strong and sharp, C=O stretch of anhydride), 1750 (strong, C=O stretch of anhydride), 1639 (weak, >C=C< stretch). 1H-NMR [CDCl3, 400 MHz, δ (ppm)]: 6.88 (m, HOOC-CH=CH-COO-), 6.29 (m, HOOC-CH=CH-COO-), 4.93-4.03 (m, ω-1 to ω-6), 3.57 (m, -CH2-OH), 3.19 (-CH2-CH2-COOH), 2.94-2.64 (-CH2-CH2-COOH), 1.72 (q, -CH2-CH2-COOH), 1.33 (q, -CH2-midde); 13C-NMR [CDCl3, 100MHz, δ (ppm)]: 169.79(-COO-sebacic), 166.76(-COO-acetyl), 161.61, 146.5 (-COO-maleic), 131.15, 129.15 (-CH=C CH2- maleic), 65.94, 73.0, 76.13, 76.3, 77.6 and 80.76 (-CHO, ω-n carbons), 22.2 (-CH2-CO-), 18.51, 14.01, 13.96, and 9.5 (CH3, hydroxylauric mixt.). For sebacic acid prepolymer, FT-IR(KBr.cm−1): 1809, 1750 (strong and sharp, C=O stretch of anhydride), 1639 (weak, >C=C< stretch); 1H-NMR [CDCl3, 400MHz, δ (ppm)]: 2.45 (t, -CH2COO-), 2.07-2.54 (m, -CH2-OH), 1.63 (m, -CH2-CH2-COOH), 1.26 (m, -CH3-CH2-R), 0.88 (m, -CH3).

Polyanhydride Synthesis
Polyanhydrides were prepared by melt polycondensation of the acetylated prepolymers (Domb and Langer 1987). Varying ratios of SA and HOLAM were placed in a test tube equipped with nitrogen and vacuum line ports. Typically, 10 g of the prepolymers of SA and HOLAM at different ratios (80:20, 60:40, 50:50, 40:60, 20:40) were placed in a test tube equipped with nitrogen and vacuum line ports. The tube was immersed in an oil bath at 180 °C. The prepolymers were allowed to melt for 1 min before vacuum was applied through the side arm. The polymerization was continued for 120 minutes and the condensation by-products, acetic acid and acetic anhydride were collected in a trap. Polyamides were synthesized by reacting acetic anhydride and acetic acid with vigorous agitation of the melt was performed for 30 s every 15 min. The crude polymer was dissolved in dichloromethane (1:5 w/v) and was precipitated with dry petroleum ether. The precipitate was collected by vacuum filtration and dried in a vacuum desiccator. The polymers were stored in −40 °C until use. The polyanhydrides were characterized using GPC, FT-IR, 1H and 13C-NMR.

Copolymers of SA and HOLAM, poly(SA:HOLAM) are light to dark brown solids. Yields range from 55-75%.

FT-IR (KBr, cm−1): 1740 (strong, ester C=O stretch), 1813 and 1750 (strong, C=O stretch of anhydride), 1639 (weak, >C=C< stretch). 1H-NMR [CDCl3, 400 MHz, δ (ppm)]: 6.88 (m, HOOC-CH=CH-COO-), 6.29 (m, HOOC-CH=CH-COO-), 4.93-4.03 (m, ω-1 to ω-6), 2.07-2.54 (m, -CH2-OH), 1.63 (m, -CH2-CH2-COOH), 1.26 (m, -CH3-CH2-R), 0.88 (m, -CH3).

Preparation of Microspheres
Phase-inversion microencapsulation technique was used to produce drug-loaded polyanhydride microspheres (Chickering et al. 1997). Briefly, polymer (5% w/v) and ciprofloxacin (20% w/w polymer) were dissolved in dichloromethane (solvent). The solution was stirred rapidly at room temperature until dissolution. The solution was poured into continuously sonicated (Hwashin Tech. Co. PowerSonic 410, Korea) petroleum ether (nonsolvent) for 10 minutes. The resulting microspheres were vacuum-filtered [47mm, 0.45μm (Whatman, USA)], washed twice with petroleum ether and vacuum dried at room temperature for 24 hrs.

In Vitro Hydrolysis
The hydrolysis of polymers was evaluated by placing rectangular samples of polymers (1 x 10 x 12 mm) in 5 mL of 0.10 M phosphate buffer pH 7.4 at 37 °C with agitation [100rpm] (Heidolph PROMAX 1020 incubator 1000, Germany). At each time point, the polymer was vacuum-dried at 37°C overnight and weighed. Hydrolysis of the polymer was monitored by weight loss.

In Vitro Drug Release
Approximately 10 mg of each microsphere formulation were suspended in 1.3 mL of phosphate buffer with 2% Tween 80 (pH 7.4) at 37 °C with agitation [100 rpm] (Heidolph PROMAX 1020 incubator 1000, Germany). At certain time points, samples were centrifuged (~6000 rpm) and 1 mL of supernatant was removed and the medium was replaced with...
fresh buffer. Concentration of the released ciprofloxacin was determined by measuring the absorbance at 272 nm using UV-VIS spectrophotometer. Results were expressed as mean ± standard error of the mean (sem).

RESULTS AND DISCUSSION

Prior to the synthesis of the polyanhydride copolymer (SA:HOLAM) the two monomers, the hydroxylauric acid maleate anhydride (HOLUM) and the sebacic acid anhydride (PSA) were prepared. PHOLAM was prepared by 1) hydroxylation of lauric acid, 2) reaction of the hydroxyl fatty acid with maleic anhydride, and 3) reaction with acetic anhydride. The PSA was prepared by simple reaction with acetic anhydride.

Once the polyanhydride copolymer was prepared, microspheres of the right size were produced for the in vitro hydrolysis and drug release studies.

Hydroxylauric Acid Synthesis

Lauric acid was transformed to several isomers of hydroxylauric acids by a reagent consisting of hydrogen peroxide in trifluoroacetic acid. The reaction involved an electrophilic attack by the peracid on the secondary hydrogens away from the carboxylic acid group due to the repulsion of the protonated peracid and protonated carbonyl group of lauric acid (Deno et al. 1977). After extraction, hydrolysis of the ester of TFA and then concentration (Figure 1), the product was purified by flash column chromatography and analyzed by GC-MS. Before GC-MS, the product was first transformed into methyl esters and then silylated on the hydroxyl group. The GC-MS technique was used to determine the structure of the hydroxylauric acids by providing marker fragment ions for identifying the structures. The analysis of the structures were reported and provided by Casalme and Sumera (2013).

The total percentage of the hydroxylauric acids formed was about 80% from the reaction. From NMR studies the peaks that appeared correspond to the ω-1, ω-2, ω-3, ω-4, ω-5 and ω-6 mostly away from the carboxylic acid group as in a similar study (Deno et al. 1977). The product mixture was further analyzed by FT-IR, 1H and 13C NMR. FT-IR spectra confirmed the presence of the alcohol group shown by the broad peak at 3400 cm⁻¹ (O-H stretch), the carbonyl group at 1713 cm⁻¹ (C=O stretch) and the broad carboxylic acid O-H absorption at 2700 – 2500 cm⁻¹. Proton NMR spectra showed the presence of the protons due to the hydroxyl group at 6.16 ppm which is also the same peak for the proton of –COOH. This combination to a single peak is due to proton exchange between the two functional groups (Silverstein et al. 1981). New peaks in multiplets corresponding to methyne protons of the ω-1, ω-2, ω-3, ω-4, ω-5 and ω-6 hydroxy groups can easily be spotted between 3.5 and 4.1 ppm.

The presence of the hydroxy groups was further confirmed by 13CNMR at 68.41, 73.57, 72.17, 71.91, 71.94, 72.12 ppm corresponding to the ω-1, ω-2, ω-3, ω-4, ω-5 and ω-6 alcoholic carbons with their accompanying methyl signals

![Figure 1. Synthesis of hydroxylauric acids. Note: The structure ω-1 hydroxylauric acid represents the mixture of ω-1, ω-2, ω-3, ω-4, ω-5 and ω-6 hydroxylauric acids.](image)
at 22.65 (ω-1), 9.88 (ω-2), 14.09 (ω-3, ω-4, ω-5 and ω-6).
The carbonyl peaks between 179.38 – 179.62 ppm show
the signals from a mixture of hydroxylauric acids.

**Hydroxyauric acid maleate (HOLAM) Synthesis**

Hydroxyauric acid maleate was synthesized from the
mixture of hydroxyauric acids (synthesized above)
and maleic acid anhydride at 85°C for 12 hours with a
yield of 79%. The sample illustration below shows ω-1
hydroxyauric acid as an idealized structure representing the
other hydroxyauric acids in the reaction (Figure 2). The
FT-IR spectra showed that the product lost the absorption
due to the O-H stretch at 3400 cm⁻¹ while the absorption
due to the carbonyl stretch of the acid group at 1712 cm⁻¹
remained almost overlapping with the carbonyl stretch
of the ester group at 1730 cm⁻¹. An additional peak due
to to the C=C stretch of fumaric acid was clearly seen at
1645 cm⁻¹. Proton NMR of HOLAM clearly exhibited the
presence of the alkene protons at 6.36, 6.37 (ester side)
and 7.16, 7.18 (acid side) ppm, as well as the methyne and
methylene protons of the ω-1 to ω-6 of the carboxy groups
at 5.03 ppm (multiplet) and 4.26 ppm (triplet) respectively,
accompanied by several low intensity multiplets in the
same region. The position of the chemical shifts of the
methyne and methylene protons here signified that the
esterification was successful. These structures were also
confirmed by ¹³C-NMR where the alkene carbon signals
were found at 128.38 and 129.2, the carbonyl carbons of
the maleic structures at 166.84 and 167.05 ppm, while
the carbonyl carbon of the fatty acids was at 180.54
ppm. The methylene and methyne carbons of the carboxy
group on the fatty alkyl chain were found at 66.80, 80.98,
78.94, 77.72, 76.3, and 74.3 corresponding to the ω-1 to
ω-6 substitution respectively. It should be reiterated
that the product was a mixture of the different carboxy
derivatives with the preponderance of the ω-2, ω-3 and
ω-4 derivatives.

**Synthesis of the Prepolymer of HOLAM**

The reaction between HOLAM and acetic anhydride
at 120°C produced the prepolymer (PHOLAM) where
the two acetyl groups were attached to the two terminal
carboxylic groups to form the prepolymer anhydride
(Figure 3).

A look at the FT-IR spectra of PHOLAM showed the
absorption (stretching band) of the carbonyl due to the
anhydride fragment at 1823 and 1755 cm⁻¹ and the C-O-C
stretch at 1128 cm⁻¹. The absence of the broad O-H stretch
absorption corresponding to the acid group in the 3000
cm⁻¹ region is apparent. The ester carbonyl absorption was
found at 1750 cm⁻¹ while the weak alkene absorption was
found at 1639 cm⁻¹. Proton NMR spectrum exhibited a very
intense peak at 2.2 ppm which could be due to excess acetic
anhydride that was not completely evaporated from the
product. However, a shoulder peak, also at 2.22 ppm could
be the real methyl proton peak from the acetyl group of the
prepolymer. The alkene protons were found at 6.31 and 6.32
ppm while the ω-n methyne protons were found at about
4.99 ppm. The moderately intense peak at 5.32 could be an
impurity due to the dichloromethane solvent (5.29 ppm as
calculated) which was confirmed by the peak at 53.64 ppm.

![Figure 2](image_url)

*Figure 2. Synthesis of hydroxyauric acid maleate (HOLAM). Note: The structure ω-1 hydroxyauric acid represents
the mixture of ω-1, ω-2, ω-3, ω-4, ω-5 and ω-6 hydroxyauric acids.*
in the $^{13}$C NMR spectra. The $^{13}$C NMR clearly displayed all the peaks due to the carbonyl carbons such as 169.69, from the lauric acid structure, 166.92 due to the carbonyl of the acetyl groups, 166.06 and 164.56 due to the maleic structure. The alkene carbon peaks were 131.15 and 129.03 ppm. The $\omega$-1 carbon is at 65.94 ppm while the rest of the carboxy carbons ($\omega$-2 to $\omega$-6) were found at 80.76 77.6, 76.1, 76.3, and 73.0 ppm respectively. The methyl carbons of the acetyl group were represented by the peak at 22.23 ppm while the methyl carbons of the hydroxylaurate skeleton were represented by peaks 18.51 ($\omega$-1), 9.52 ($\omega$-2), 13.96 ($\omega$-3), and 14.01 ($\omega$-4 to $\omega$-6) ppm.

**Prepolymer of Sebacic Acid Synthesis**

The reaction of acetic anhydride with sebacic acid at 120°C for 30 minutes gave a white solid after recrystalization from toluene.

![Prepolymer of Sebacic Acid (PSA)](image)

The product’s FT-IR spectra showed the acetyl anhydride group absorption at 1809 and 1744 cm$^{-1}$ for the C=O stretch and 1043 cm$^{-1}$ for the C-O-C stretch. The absence of the broad acid O-H stretch observed in sebacic acid at 2500 – 3300 cm$^{-1}$ is apparent. Proton NMR showed the intense peak of the methyl protons at 2.22, and the methylene protons at the middle region of the structure at 1.33 ppm. The alpha hydrogens at the alpha carbons are split into triplet at 2.45 ppm and the beta protons of the beta carbons split into quintuplet at 1.65 ppm. The $^{13}$C NMR spectrum showed clearly the following peaks; the acetyl carbonyl carbon peak at 166.86, the carbonyl carbon of sebacic acid at 169.79, the alpha carbons of sebacic acid at 35.25, the beta carbons at 24.15 and the methylene carbons of the middle portion of sebacic acid at 28.95 ppm.

**Polyanhydride Polymer Synthesis**

At first, the optimum reaction time of the melt condensation reaction that would give the highest molecular weight was sought using sebacic anhydride as test compound. From the experiment, higher molecular weight could be obtained by increasing the reaction time even up to three hours or more. However, the time of melt condensation reaction was chosen at 120 min for economy of time and energy. The polyanhydrides were synthesized as previously described (Domb and Langer 1987). Different ratios of the prepolymer of HOLAM and SA were polymerized and the condensation products subjected to GPC to determine the optimum ratio that would give the highest molecular weight as shown on Table 1. As seen on column 2 of Table 1, the ratio 50:50 (SA:HOLAM) gave the optimum molecular weight as determined by GPC.

A scheme of the condensation reaction to the polyanhydride product is shown on the scheme below (Figure 4). The polyanhydride produced from the melt condensation of the anhydrides of HOLAM and sebacic acid gave the following functional group absorptions in its FT-IR spectra; the C=O stretch of the esters (1730 cm$^{-1}$), the C=O of the anhydride (1740 and 1813 cm$^{-1}$), and the C=C
The 1H–NMR spectra showed intense peaks due to the sebacic fragment of the polyanhydride at 1.32, 1.65, and 2.44 ppm, not forgetting the fact that the polymer analyzed was a product of 60:40 combination of the anhydrides of sebacic acid and HOLAM. The HOLAM recognizable signals are shown by weaker peaks at 0.88 (methylprotons of ω-n hydroxylaurate), 4.0 – 5.0 ppm (methyne protons of ω-n hydroxylaurate) and the group of peaks 6.79 – 6.95 ppm (alkene protons of the maleic fragments).

Polymer Properties

Various weight ratios of the diacid HOLAM were copolymerized with sebacic acid (SA) to produce poly(SA:HOLAM) copolymers. Table 1 summarizes the physical properties of poly(SA:HOLAM) copolymers. As one possible targeted applications for this poly(SA:HOLAM) copolymers is drug delivery of bioactive compounds, the lower range of melting temperatures for the copolymers reduces the possibility for heat denaturation of the incorporated bioactive drug compound (protein/drug) in the processing methods, e.g. melt compression. Poly(SA:HOLAM) copolymers are relatively amorphous and an increase in the HOLAM content in the copolymer results to a decrease in crystallinity, melting temperature, and melting enthalpy, ΔH_m, as seen in Table 1.

In vitro Drug Release

Poly(SA:HOLAM) microspheres (Figure 6) were prepared using phase inversion technique. The particle size and % encapsulation efficiency are monomer weight ratio-dependent and ranges from 1.33 - 2.36 μm and 79 - 87%, respectively (Table 2).

Figure 7 shows the in vitro release profile of ciprofloxacin-loaded microspheres (~10 mg) with maximum dissolution of 14 days. The initial burst release of the drug before the 0.29 day (7h) is clearly noticed. This initial burst release decreases as HOLAM increases in the copolymer. This burst effect phenomenon occurs due to the release of the drug bound at the surface of the microspheres before the erosion process takes place. This initial burst release could be governed by diffusion, where ciprofloxacin appears to be more retained in the presence of more HOLAM. This is probably due to a similarity in polarity between ciprofloxacin and HOLAM, thereby ciprofloxacin partitioned preferably into HOLAM rich copolymer.

### Table 1. Physical properties of Poly(SA:HOLAM) prepared by melt polycondensation at 180°C for 120min.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>M_w (x10^3) g/mol</th>
<th>M_w Range (x10^3) g/mol</th>
<th>T_m (°C)</th>
<th>ΔH_m (J/g)</th>
<th>% X (DSC)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(Sebacic Acid)</td>
<td>167.5</td>
<td>1.00-745</td>
<td>81.5</td>
<td>124.8</td>
<td>57.0</td>
<td>68</td>
</tr>
<tr>
<td>P(SA:HOLAM) 80:20</td>
<td>126.8</td>
<td>1.02-759</td>
<td>76.2</td>
<td>88.3</td>
<td>40.6</td>
<td>70</td>
</tr>
<tr>
<td>P(SA:HOLAM) 60:40</td>
<td>63.70</td>
<td>3.70-779</td>
<td>70.2</td>
<td>87.2</td>
<td>38.5</td>
<td>75</td>
</tr>
<tr>
<td>P(SA:HOLAM) 50:50</td>
<td>242.1</td>
<td>1.19-792</td>
<td>67.5</td>
<td>76.3</td>
<td>32.6</td>
<td>60</td>
</tr>
<tr>
<td>P(SA:HOLAM) 40:60</td>
<td>162.2</td>
<td>1.36-877</td>
<td>62.2</td>
<td>58.3</td>
<td>25.6</td>
<td>55</td>
</tr>
<tr>
<td>P(SA:HOLAM) 20:80</td>
<td>47.69</td>
<td>2.08-882</td>
<td>50.8</td>
<td>18.8</td>
<td>8.66</td>
<td>63</td>
</tr>
<tr>
<td>P(HOLAM)</td>
<td>65.27</td>
<td>0.73-794</td>
<td>semi solid</td>
<td>-</td>
<td>-</td>
<td>52</td>
</tr>
</tbody>
</table>

M_w = molecular weight determined at peak maximum
M_w Range = molecular weight range determined from difference of molecular weights at peak’s intersections with the base (chromatogram)
T_m = melting temperature
ΔH_m = melting enthalpy
% X = percent crystallinity determined from DSC
Figure 4. Synthesis scheme of poly(SA:HOLAM). The ω-1 hydroxylaurate fragment represents the mixture of ω-1, ω-2, ω-3, ω-4, ω-5 and ω-6 hydroxylaurate fragments.

Figure 5. *In Vitro* Hydrolysis of poly (HOLAM:SA) copolymers monitored by weight loss in phosphate buffer pH 7.4 at 37°C. Each data indicate mean ± sem (standard error of the mean) of three replicates marked with a vertical line (⊥).
This burst effect followed by constant drug release rate is important in drug delivery systems to achieve a therapeutic level concentration and maintain the level by compensating for metabolic loss thereafter (Modi et al. 2006).

After the initial burst, the release of the drug entered a phase of constant and similar rate release for all copolymers from 1st day to 6th day. After the 6th day the HOLAM containing polymer entered a slight change in rate of release (slower). The overall result showed complete release only at day 5 for 100:0, day 6 for 80:20, day 14 for 60:40 and 50:50 poly(SA:HOLAM) describing in general a slower complete release as HOLAM increases in the copolymer matrix.

It should be noticed, however, that the drug release did not strictly follow the hydrolysis profile which only showed that the drug release is not erosion driven but perhaps diffusion driven.

A look on Table 3 shows the tabulated drug release’s kinetic parameters of poly(SA:HOLAM) according to different known models. The release profile of the copolymers followed release rates data that linearly fit ($r^2 > 0.95$) with zero-order, Higuchi (Roseman & Higuchi 1970) and power law kinetic models (Ritger & Peppas 1987). This suggests that the drug release profiles from poly(SA:HOLAM) microspheres can be tailored to achieve the desired drug release, as one can vary the monomer weight ratios in the poly(SA:HOLAM) copolymer (Modi et al. 2006). Moreover, from the power law equation, the value of $n$ (obtained by using only the first 60% drug released in the medium) reveals important

Table 2. Particle size and percentage drug loading efficiency of ciprofloxacin-loaded poly(SA:HOLAM) microspheres at 10 wt.% drug loading.

<table>
<thead>
<tr>
<th>Poly(SA: HOLAM)</th>
<th>Microsphere size (mm ± sem)</th>
<th>% Encapsulation Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>0.767 ± 0.067</td>
<td>100.00 ± 1.94</td>
</tr>
<tr>
<td>80:20</td>
<td>1.33 ± 0.049</td>
<td>84.63 ± 2.52</td>
</tr>
<tr>
<td>60:40</td>
<td>2.36 ± 0.112</td>
<td>87.01 ± 5.05</td>
</tr>
<tr>
<td>50:50</td>
<td>2.26 ± 0.118</td>
<td>78.92 ± 1</td>
</tr>
</tbody>
</table>

sem = standard error of the mean
Figure 7. In vitro drug release of ciprofloxacin-loaded microspheres of poly(SA:HOLAM) copolymers in phosphate buffer of pH 7.4 at 3.7°C with agitation (100 rpm). Each data are indicated by a mean ± sem (standard error of the mean) of three replicates marked with a vertical line (1).

Table 3. Drug release kinetic data obtained from ciprofloxacin-loaded poly(SA:HOLAM) microspheres in phosphate buffer of pH 7.4 at 37°C.

<table>
<thead>
<tr>
<th>Poly(SA: HOLAM)</th>
<th>Zero-order&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Higuchi&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Power Law&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K&lt;sub&gt;0&lt;/sub&gt;</td>
<td>r&lt;sup&gt;2&lt;/sup&gt;</td>
<td>K&lt;sub&gt;H&lt;/sub&gt;</td>
</tr>
<tr>
<td>100:0</td>
<td>0.1799</td>
<td>0.6204</td>
<td>0.3349</td>
</tr>
<tr>
<td>80:20</td>
<td>0.1585</td>
<td>0.9086</td>
<td>0.3220</td>
</tr>
<tr>
<td>60:40</td>
<td>0.1386</td>
<td>0.9861</td>
<td>0.1386</td>
</tr>
<tr>
<td>50:50</td>
<td>0.1094</td>
<td>0.9957</td>
<td>0.2610</td>
</tr>
</tbody>
</table>

<sup>a</sup> Zero Oder Model: M<sub>t</sub> = K<sub>0</sub> t, where M<sub>t</sub> is the amount of drug released at time t

<sup>b</sup> Higuchi Model: M<sub>t</sub> = K<sub>H</sub> t<sup>0.5</sup>

<sup>c</sup> Power Law Model: M<sub>t</sub>/M<sub>∝</sub> = K<sub>PL</sub> t<sup>n</sup>, where M<sub>∝</sub> is the total amount of drug incorporated in the polymer matrix

n is the diffusional coefficient

r<sup>2</sup> is a measure of curve fitting (correlation coefficient)

information about the drug release mechanism. In general, the power law equation describes that for n < 0.5, the release follows Fickian diffusion transport mechanism, for 0.5 < n < 0.9, the release follows a mixed diffusion and erosion mechanism, and for n > 0.9, the release transport mechanism is controlled by erosion. A look on the table shows that all the values of n is less than 0.5, showing that the transport mechanism in general is controlled by Fickian diffusion rather than by erosion or degradation in the drug release of ciprofloxacin from the copolymer (SA:HOLAM).

CONCLUSION

Synthesis of high molecular weight poly(ester-anhydride) based on hydroxylauric acid, maleic anhydride and sebacic acid was achieved by melt polycondensation intended for drug delivery application. Phase inversion technique produced poly(SA:HOLAM) microspheres, wherein, particle size and drug release rates were dependent on HOLAM content of the polyanhydrides. Finally, biphasic in vitro drug release profile was observed for ciprofloxacin-loaded poly(SA:HOLAM) microsphere which released the drug much slower at higher HOLAM content. A study of the kinetics of drug release using the power law equation revealed that ciprofloxacin
was probably released from the polyanhydride copolymer poly(SA:HOLAM) by diffusion and not by erosion. A future study of different drugs with different properties of hydrophobicity and hydrophilicity using the polyanhydride poly(SA:HOLAM) as matrix may be recommended to clarify better the process and mechanism of drug release. The drug ciprofloxacin could be substituted by anticancer drugs such as cis-platin and paclitaxel for more data on its characteristic drug release and future medical benefit.

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