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Synthesis and characterization of valproic acid ester pro-drug micelles via an amphiphilic polycaprolactone block copolymer design

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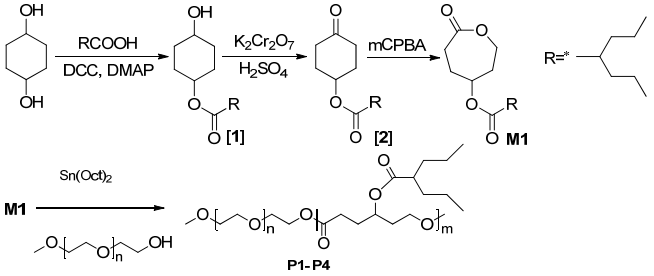
The attachment of Histone deacetylase (HDAC) inhibitors via covalent bonds to biocompatible and biodegradable block copolymers provides a new research direction for cancer treatment. Herein, we report the synthesis and characterization of valproic acid ester pro-drug micelles of amphiphilic polycaprolactone block copolymers and their potential applications in drug delivery.

Biocompatible and biodegradable aliphatic polyesters such as poly(caprolactones), poly(lactides), and poly(glycolides) have been extensively studied due to their applications in biomedical and pharmaceutical fields.[1-4] Chemical and physical properties of the aliphatic polyesters can be tuned by introduction of functional groups to the polymer backbone.[5] Incorporating conjugate drugs, targeting agents or stimuli-responsive molecules into the polymers can be used to improve the efficiency of the target release of the currently available anticancer drugs.[6]

Hydrophobic γ-substituted polycaprolactone blocks can be attached to hydrophilic polymer to generate block copolymers that will self-assemble to form pro-drug micelles in water. The micellar cores conjugated with functional groups or drug molecules enhance the drug loading capacity, micelle stability,[7] which allows the controlled release of the hydrophobic anticancer and histone deacetylase (HDAC) inhibitors. Histone deacetylases (HDAC) are considered to be among the most promising targets in drug development for cancer therapy.[8] Short chain fatty acid valproic acid (VPA) has been used for decades to treat seizure disorders. Recently VPA has demonstrated anticancer activity and the anti-inflammatory activity as a HDAC inhibitor.[9-11] Food and Drug Administration (FDA) approved, water soluble, poly(ethylene glycol) (PEG) is most widely employed as the hydrophilic block. γ-Alkoxy substituted ε-caprolactone monomers can generate the hydrophobic block and a few reports showed their synthesis and properties.[6,12-16] Amphiphilic block copolymers containing HDAC inhibitors can self-assemble to form micelles with hydrophobic cores that contain the masked HDAC inhibitors.

Here for the first time we report the synthesis of γ-valproate ester substituted caprolactone monomer, its ring-opening polymerization, self-assembly in water, and their degradation. HDAC inhibitor, valproic acid (VPA) was attached to the ε-caprolactone ring through a ester linkage. This new monomer has been combined with PEG to generate amphiphilic block copolymers that self-assemble into pro-drug micelles which can be employed to deliver the anticancer drugs.

γ-Valproate-ε-caprolactone monomer (**M1**) was synthesized according to the procedure described in Scheme I. First 1-4, cyclohexanediol reacted with valproic acid in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to obtained 4-valproatecyclohexanol [**1**].[17] Oxidation with chromic acid yielded 4-valproatecyclohexanone [**2**] and the Baeyer-Villiger oxidation with m-chloroperoxybenzoic acid generated γ-valproate-ε-caprolactone monomer **M1** in ~20% overall yield. ^{1}H NMR, ^{13}C NMR and C/H elemental analysis confirmed the formation of the monomer (Supporting Information).



Scheme 1 Synthesis of γ-2-propylpentanoate -ε-caprolactone (**M1**) and poly(ethylene glycol)-*b*-poly(γ-2-propylpentanoate-ε-caprolactone) diblock copolymers (**P1-P4**)

Ring-opening polymerization of **M1** was carried out using $Sn(Oct)_2$ as the catalyst. Methoxy-PEG (mPEG) was used as the initiator for the polymerization of monomer **M1** to generate the diblock copolymers (**P1-P4**) with various compositions. The polymerizations were performed in bulk at 110 °C for all four polymers. The molar ratio [**M1**]: [PEG]: [$Sn(Oct)_2$] = 50:1:2 was used for the synthesis of **P1**. For the synthesis of polymers **P2-P4** the ratio of 62:1:2, 65:1:2 and 75:1:2 were used, respectively.

Table 1. Summary of polymers **P1-P4**

Polymer	M_n [a]/gmol^{-1}	Mol% Valproate[b]	PDI[a] (Mw/Mn)	CMC[c] (gL^{-1})	D_h[d] (nm)
P1	5000	38	1.78	2.00×10^{-3}	39 ± 1.2
P2	5200	42	1.76	6.31×10^{-4}	61 ± 0.8
P3	6600	50	1.48	1.58×10^{-4}	86 ± 2.3
P4	17300	72	1.48	7.08×10^{-4}	96 ± 0.5

[a] Determined by size exclusion chromatography. [b] Valproate content determined by ^{1}H NMR. [c] Determined by fluorescence spectroscopy using pyrene as the fluorescence probe. [d] Determined by dynamic light scattering.

The synthesized poly(ethylene glycol)-*b*-poly(γ-2-propylpentanoate-ε-caprolactone) diblock copolymer (**P1**) contained ~38 mol% valproate and had a molecular weight of 5000 gmol^{-1} (Table 1). Diblock copolymer **P2** contained ~42 mol% of valproate, and copolymer **P4** had the largest content of valproate (72 mol%) (Table 1).

^{1}H NMR was used to determine the composition of the synthesized diblock copolymers (Figure 1). The signal at ~3.38 ppm was assigned to the methoxy end group of the PEG block. The peaks at ~3.64 ppm are due to the methylene protons of the PEG block and the triplet at 0.89 ppm is due the methyl protons of the repeat unit of valproate ester group. The composition of the copolymers was determined by integrating the methyl protons of the valproate (*n* protons in Figure 1) *vs* the methylene protons of the PEG block (*b*,*c* protons in Figure 1). The ^{1}H NMR spectra of diblock copolymers **P1-P3** are given in the Supporting Information.

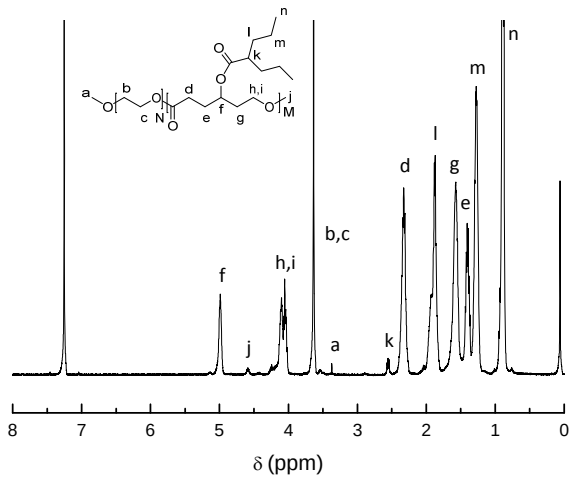
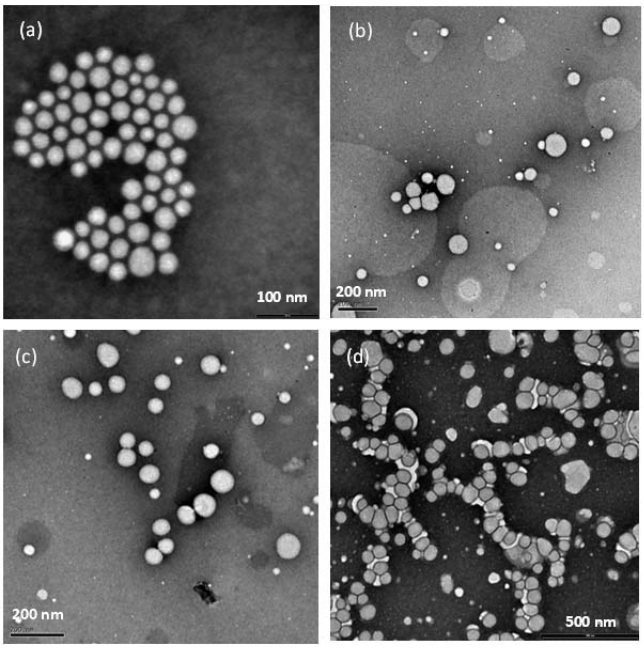


Figure 1. ^{1}H NMR of poly(ethylene glycol)-*b*-poly(γ-2-propylpentanoate-ε-caprolactone) diblock copolymer (**P4**)

The "ideal" pharmaceutical micelle should have the size in the range of 10-100 nm and it should have high thermodynamic stability both *in vitro* and *in vivo*.[18] The hydrodynamic diameter (D^h) of the micelles was determined by dynamic light scattering (DLS) at room temperature. Copolymers **P1-P4** self-assembled in aqueous solution to form micelles. The micelles of amphiphilic diblock copolymers **P1**, **P2**, **P3**, and **P4** showed monodisperse distribution and the mean diameters were 39, 61, 86, and 96 nm respectively (Table 1 and figure 14 in Supporting Information). In general, the micelle size increases as the hydrophobic block becomes larger.[19] The hydrodynamic diameter of the polymers **P1-P4** increased with the increasing the mol% of the hydrophobic valproate block. The size of the micelles measured for copolymers **P1-P4** are larger than the previously reported values for amphiphilic block copolymers containing hydrophobic γ-substituted poly(ε-caprolactones)[13] despite the comparable molecular weights and ratios between the hydrophilic and hydrophobic blocks. The larger hydrodynamic size of micelles of copolymers **P1-P4** is most likely due to the bulkiness of the valproate functional groups which could result in an increase of the volume of the hydrophobic core.

TEM analysis was also employed to characterize copolymers **P1-P4** with negative staining. Cu grid was placed on a drop of the micelle suspension for few seconds and the grid was stained using phosphotungstic acid. All the micelles were spherical in shape and the diameters were determined to be 32 ± 5 nm, 61 ± 22 nm, 64 ± 13 nm and 67 ± 10 nm, respectively (Figure 2). The sizes of micelles in TEM images were slightly smaller than those measured by DLS. During the sample preparation for TEM, the micelles can undergo dehydration which may shrink or collapse the PEG shell.[20] Furthermore, DLS technique reports an intensity-average dimension, whereas TEM reports number-average dimensions. Therefore, TEM can result in smaller sizes relative to DLS.[21]



Fugure 2. TEM images of polymer micelles: (a) **P1**, (b) **P2**, (c) **P3**, (d) **P4**.

The critical micellar concentration (CMC) of the diblock copolymers **P1-P4** were determined by fluorescence spectroscopy using pyrene as a fluorescent probe (Figure 3 and Table 1). In general, the higher molecular weight of the polymer and the higher molecular weight of hydrophobic block will give lower CMC values.[3] The polymer **P1** with the lowest molecular weight and the lowest content of the valproate ester had the highest CMC among the polymers **P1-P4**. Copolymers **P2-P4** had CMC in the range of 10^{-4} g/L. The measured CMC values for copolymers **P1-P4** are one order of magnitude lower than the CMC values previously reported for amphiphilic block copolymers containing hydrophobic γ-substituted poly(ε-caprolactones).[13] The lower measured CMC values indicate better thermodynamic stability for the reported copolymers **P1-P4**.

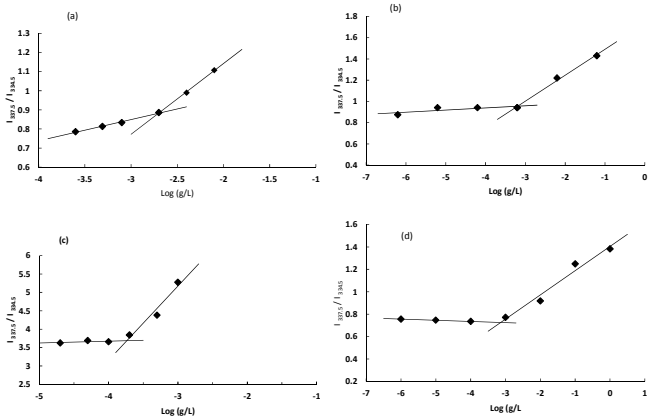


Figure 3. Dependence of ratio $I_{337.5}/I_{334.5}$ from pyrene excitation spectra as a function of polymer concentration: (a) **P1**, (b) **P2**, (c) **P3**, (d) **P4**. [Py] 4.0×10^{-7} M, λ_{em}= 390 nm.

To study the biodegradability, copolymers **P1-P4** were stirred at 37 °C for 5 days at pH 6. The molecular weights were analyzed periodically by size exclusion chromatography. As shown in Figure 4, the molecular weights of polymers **P1-P4** decreased over time due to the acid catalyzed hydrolysis of ester groups.

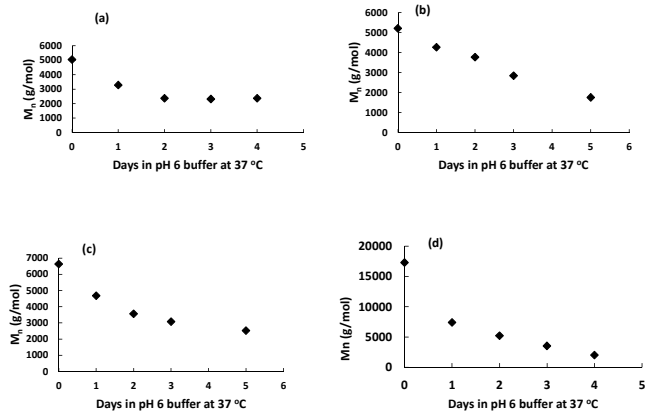


Figure 4. Demonstration of biodegradability of **P1-P4**: number average molecular weight from SEC as a function of time spent in pH 6 phosphate buffer at 37 °C. (a) **P1**, (b) **P2**, (c) **P3**, (d) **P4**.

The release of VAL was analyzed by ^{1}H NMR (Figure 13 in Supporting Information). The polymer P3 was stirred in pH 6 phosphate buffer solution at 37 °C. After 3 days the VAL content has been decreased by 7 mol% while the caprolactone content remaining constant. This suggests the potential release of the VAL upon the hydrolysis of the ester linkage in pH 6 buffer solution. Further structural characterization will be carried out to confirm structure in future.

In summary, a new valproate ester substituted ε-caprolactone monomer (**M1**) and its corresponding amphiphilic diblock copolymers (**P1-P4**) were synthesized. Four poly(ethylene glycol)-*b*-poly(γ-2-propylpentanoate-ε-caprolactone) diblock copolymers were synthesized by varying the valproate content. These valproate substituted block copolymers demonstrated self-assembly into micelles and biodegradation at pH 6. The reported copolymers have the capability of delivering valproic acid HDAC inhibitor in a sustained manner by the cleavage of the valproate ester groups. These polymers are currently being tested in combination therapy with the anticancer drug paclitaxel.

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Notes and references

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Electronic Supplementary Information (ESI) available: ^{1}H NMR, ^{13}C NMR, and HSQC spectra of monomer and compounds [1] and [2], ^{1}H NMR spectra of polymers, and DLS of micelles. See DOI: 10.1039/c000000x/

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