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REVIEW ARTICLE

Pegylated Polyester Polymeric Micelles as a Nano-carrier: Synthesis, Characterization, Degradation, and Biodistribution

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Lactone monomers with different chain lengths are copolymerized with poly(ethylene glycol) or methoxypoly(ethylene glycol) to form triblock and diblock pegylated polyester copolymers. The pegylated polyester copolymers have an amphiphilic character because they are comprised of both hydrophobic and hydrophilic domains, which spontaneously assemble in aqueous media to form nano-sized micelles at concentrations above the critical micelle concentration. The type of lactone monomer, the chain lengths of the hydrophobic as well as the hydrophilic blocks, the molecular weight of poly(ethylene glycol), and the formation of triblock or diblock polymeric micelles all play important roles in micelle performance. The triblock and diblock pegylated poly(δ-valerolactone) copolymers show higher *in vitro* cytotoxicity than the others. Triblock PCL-$PEG_{10,000}$-PCL micelles possess high drug loading, low *in vitro* cytotoxicity, proper *in vitro* sustained release performance and prolonged mean residence time of drug in blood circulation. Diblock PCL-MPEG micelles have lower critical micelle concentration, higher biocompatibility, and higher drug loading than PVL-MPEG micelles. The enzyme-catalyzed chain cleavage of labile ester linkages of pegylated polyester copolymer occurs in rat plasma, and the changes in mass, molecular weight and morphology of the copolymers are associated with changes in its composition.

1. Introduction

Biodegradable amphiphilic copolymers are an attractive subject in both fundamental research and in the development of drug delivery systems. Diblock and triblock amphiphilic copolymers comprise both hydrophobic and hydrophilic domains, which spontaneously assemble in aqueous media to form micelles at concentrations above the critical micelle concentration (CMC). The size of polymer micelles are measured in nanometers, and they have a hydrophobic core and hydrophilic shell conformation as shown in Figure 1.[1] The CMC plays an important role to affect the stability of micelles. In other words, micelles with low CMC are able to retain their core-shell structure after dilution with a bulk volume of blood in the body. The amphiphilic copolymers usually have low CMC and receive much attention as potential drug carriers.[2] The possible structure of micelles formed by pegylated triblock copolymers has been proposed by Maiti and Chatterji, where the poly(ethylene glycol) (PEG) chain is folded back and inserts the hydrophobic terminals into the micellar core

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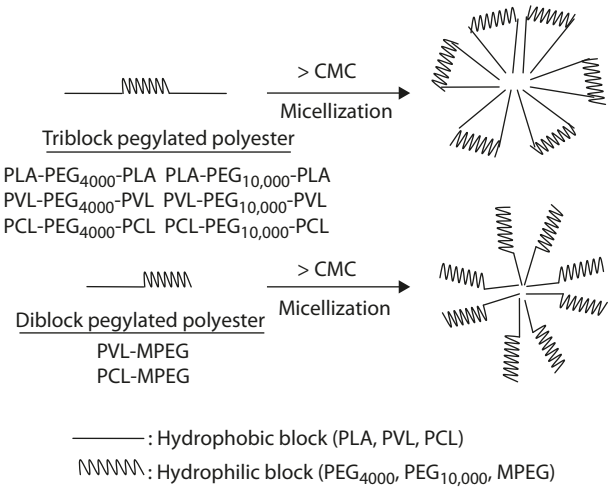

Figure 1 Proposed conformation of polymeric micelles made by triblock and diblock amphiphilic pegylated polyesters. PLA=poly(L-lactide); PVL=poly(δ-valerolactone); PCL=poly (ε-caprolactone); PEG_{4000}=poly(ethylene glycol) with molecular weight ~4000 Da; $PEG_{10,000}$=poly(ethylene glycol) with molecular weight ~10,000 Da; MPEG=methoxypoly(ethylene glycol).

according to the micellization and adsorption energy.[3] Hrkach et al used ^{1}H nuclear magnetic resonance (NMR) spectroscopy to elucidate the structure of diblock poly(lactide-co-glycolide)-poly(ethylene glycol) (PLGA-PEG) nanoparticles in an aqueous environment.[4] They confirmed the formation of a core-corona structure, where the PEG chain mobility was similar to that dissolved in solution. In other words, the PEG domain acts as the hydrophilic outer shell to extend in the aqueous environment, and hydrophobic polyester domain acts as the inner core of the micelles. Riley et al found the molecular weight of poly(lactide) (PLA) block in the range of 3–15 kDa formed highly colloidally stable micelles with a surface complete coverage of PEG.[5] They also mentioned that increase in the chain length of PLA up to 30–110 kDa would decrease surface coverage of PEG.

Allen et al reported that the ideal micellar carrier system should possess several characteristics, including suitable size in the range of 100–200 nm, less than a millimolar range of CMC, slow disassemble rate, being retained in the body for a long period, nontoxic degraded monomer, and ease of excretion from the kidney.[6,7] Polymeric micelles have several advantages that meet the above criteria, including good structural stability, slow dissociation rate, low CMC, easy control of particle size, and good solubilization of hydrophobic drugs.[8] The functionalization of the outer surface of polymeric micelles by PEG or poly(ethylene propylene glycol) can modify their physicochemical and biologic properties. It has been reported that PEG can prevent liposomes and microparticles from uptake by the reticular endothelium system (RES), and prolong the time they are retained in blood circulation.[9,10] A similar phenomenon has been observed from pegylated micelles, where PEG reduces protein opsonization on the micelle surface and subsequent phagocytosis by nonparenchymal cells of the liver. In other words, the avoidance of uptake of micelles by the RES improves its long-circulating characteristics.[10–17]

Polymeric micelles with core-shell conformation can act as a nano-carrier delivery system for drugs. Drugs can be incorporated inside the inner core of micelles via chemical conjugation, hydrophobic interaction, ionic interaction, hydrogen bonding, or physical entrapment. The biodistribution of polymeric micelles is dominated by their physical and biochemical properties, such as particle size, nature of the polymer and drug, and surface biochemical properties.[18] The advanced application of polymeric micelles in the field of passive targeting is due to its unique characteristics in the body including the ability to passively accumulate anticancer drugs in tumor cells, reduce cytotoxicity of anticancer drugs, release drugs for an extended period of time, and prevent rapid clearance by the RES.[7] It has been reported that adriamycin-loaded micelles show dramatically higher antitumor activity *in vivo* than the free drug itself.[6,19] This indicates that the nano-sized micelles relatively easily extravasate into and accumulate within tumor tissue due to enhanced permeation and retention effects, which improve the therapeutic efficacy of the drug and reduce its nonspecific side effects.[20–28] The feasibility of using polymeric micelles as a non-viral gene vector is an important perspective on nano-carrier delivery systems.[23] The pegylated cationic copolymers are able to spontaneously self-assemble with negatively charged plasmid DNA and form a nano-sized complex with a stable nature in blood circulation.[29] The delivery of drugs or active compounds by micelles is generally governed by three mechanisms: the micelles enter the nucleus; the micelles enter the cells; and the micelles remain outside the cells where the drug is released.[20]

2. Synthesis of Amphiphilic Pegylated Polyesters

Polyesters can be synthesized by ring-opening polymerization of lactone monomer through four different mechanisms including anionic, cationic, coordination and radical polymerizations, where the initiators and/or catalysts play an important role in triggering polymer synthesis.[30] However, there is concern about the toxic substance in the resulting polymers. A novel ring-opening copolymerization of pegylated polyester copolymers has been developed. Three different types of lactone monomers [e.g., ε-caprolactone (ε-CL), δ-valerolactone (δ-VL), L-lactide (L-LA)] are copolymerized with PEG (e.g., PEG_{4000} and $PEG_{10,000}$) or methoxypoly (ethylene glycol) (MPEG) in the absence of an external initiator (Figure 2). There are six triblock pegylated polyesters (e.g., PCL-PEG_{4000}-PCL, PCL-$PEG_{10,000}$-PCL, PVL-PEG_{4000}-PVL,

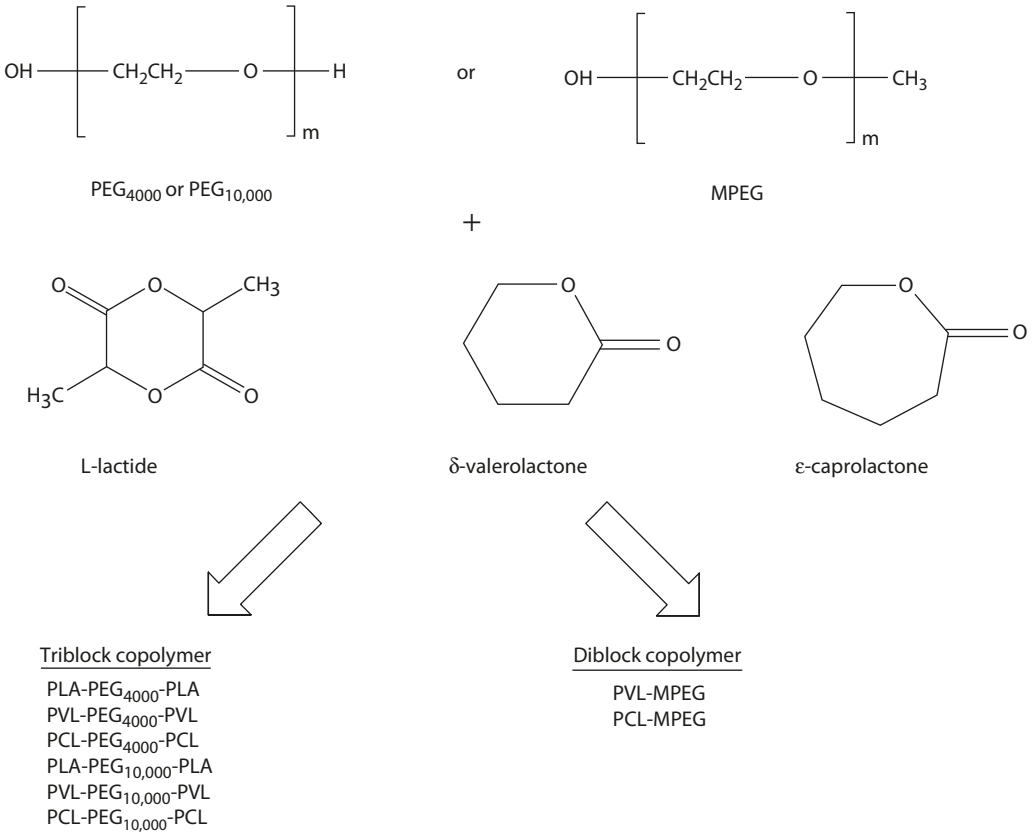

Figure 2 A modified ring-opening copolymerization of three types of lactone monomers [ε-caprolactone (ε-CL), δ-valerolactone (δ-VL), L-lactide (L-LA)] with poly(ethylene glycol) (PEG_{4000} and $PEG_{10,000}$) or methoxypoly(ethylene glycol) (MPEG) in the absence of an external initiator.

$PVL\text{-}PEG_{10,000}$-PVL, $PLA\text{-}PEG_{4000}$-PLA, and $PLA\text{-}PEG_{10,000}$-PLA) and two diblock pegylated polyesters (e.g., PCL-MPEG and PVL-MPEG) prepared with different types and compositions of hydrophobic polyester blocks and hydrophilic PEG blocks. The possible reaction mechanism has been proposed by Cerrai et al[11] and Kim et al,[31] where the hydrogen atom of the PEG end groups acts as an initiator to directly induce acyl-oxygen cleavage on the lactone ring. The advantages of this method include the simple reaction condition and the avoidance of using toxic catalysts and organic solvents. Consequently, there are no toxic substances residing in the resulting copolymers, and this is a very important issue for a material that will be used as a drug carrier or as a medical device in the human body.

3. General Characterization of Pegylated Polyesters

The synthesized amphiphilic pegylated polyesters are usually characterized by their physical properties, cytotoxicity, and degradability as shown in Figure 3. The composition and number-average molecular weight (M_n) of copolymers are determined by ^{1}H-NMR. The molecular weight distribution is determined by gel permeation chromatography equipped with a refractive index detector. The CMC of amphiphilic copolymers is determined with a fluorescence spectrophotometer using pyrene as a fluorescence probe. The biocompatibility of synthesized amphiphilic pegylated polyester is evaluated by *in vitro* cytotoxicity testing using normal human fibroblast cells (IMR-90). Normal human fibroblast cells are maintained in minimum essential medium containing 10% fetal bovine serum in an atmosphere of 5% CO_2 at 37°C. Culture medium is plated in the 96-well plates overnight. Cells are then incubated in the various concentrations of polymeric solutions at three 10-fold dilutions ranging from 0.001 up to 1.0 mg/mL for 48 hours. The degree of cell survival is evaluated by the MTT assay and determined with a spectrophotometer at 550 nm. The ratio of cell survival with and without copolymer treatment is calculated.

4. Preparation and Characterization of Polymeric Micelles

The drug-loaded polymeric micelles are prepared via a dialysis method. A model drug, indomethacin, and pegylated polyester are previously dissolved in acetone, and de-ionized water is then added slowly. After

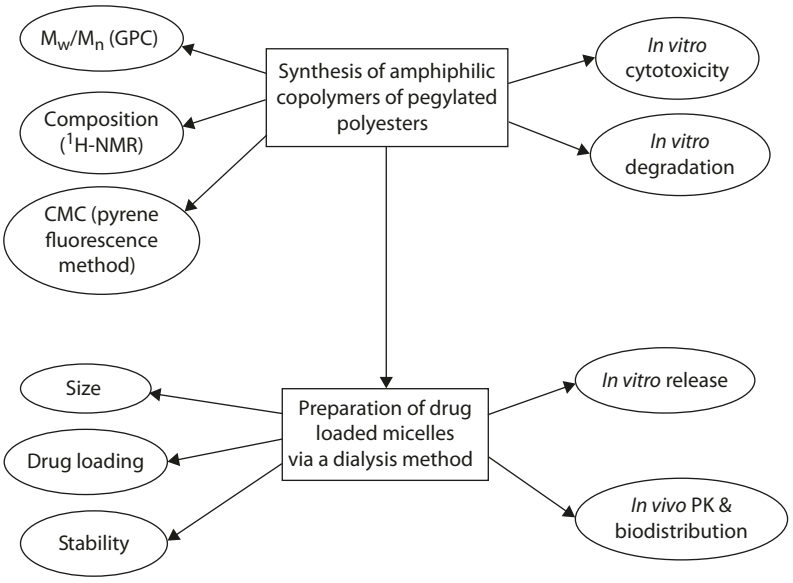

Figure 3 Characterization of synthesized amphiphilic pegylated polyesters and micelles by their physical properties, cytotoxicity, degradability, stability, *in vitro* release and *in vivo* biodistribution.

that, the solution is placed in a dialysis bag, immersed in the de-ionized water, and dialyzed for 24 hours. Finally, the micelle solution is sonicated and centrifuged. The average particle size of micelles is measured with a particle sizer. The amount of drug incorporated in the micelles is determined with a UV spectrophotometer at 318 nm. The percentage of drug loaded in the micelles is calculated by dividing the amount of drug in the micelles to the amount of drug added initially. The stability of the micelles is determined by monitoring their change in size while being stored in de-ionized water and 5% dextrose solution, respectively, at 4°C for 12 weeks. The ratio of particle size after and before storage is calculated to evaluate whether the micelles aggregated or dissociated.[15]

5. Important Characteristics of Triblock Pegylated Polyesters and Polymeric Micelles

There are six triblock pegylated polyesters with various types and compositions of hydrophobic polyester and hydrophilic PEG.[32] The conversion rate of different types of lactone monomers into PLA-PEG_{4000}-PLA, PVL-PEG_{4000}-PVL, PCL-PEG_{4000}-PCL, PLA-$PEG_{10,000}$-PLA, PVL-$PEG_{10,000}$-PVL, and PCL-$PEG_{10,000}$-PCL copolymers are 69.0%, 56.0%, 102.5%, 63.5%, 50.0%, and 92.0%, respectively. The conversion efficiency of ε-CL monomer is higher than that of δ-VL and L-LA monomers. The ring-opening of ε-CL monomer is quite efficient under current copolymerization conditions, which results in the final compositions of synthesized PCL-PEG-PCL copolymers very close to their feed molar ratios. The M_n of these copolymers is in the range of several thousand to thirty thousand daltons depending on their compositions. It increases as the feed molar ratio of lactone monomer to PEG increases. The polydispersity of copolymers is affected by the molecular weight of PEG and the type of monomers. Copolymers comprising PEG_{4000} is less broad than copolymers comprising $PEG_{10,000}$, and the polydispersity of PCL-PEG_{4000}-PCL is quite consistent in the range of 1.4–1.9 within the wide molar ratios of CL/PEG_{4000}/CL. All of these copolymers show similar *in vitro* cytotoxicity, and more than 90% of cells are viable after treatment with copolymers 0.001–0.1 mg/mL irrespective of the chain length of the hydrophilic PEG block and the type of polyester block. However, only 80% of cells are viable after treatment with 1.0 mg/mL of PVL-PEG-PVL copolymers. In other words, PCL-PEG-PCL and PLA-PEG-PLA copolymers show less cell cytotoxicity than PVL-PEG-PVL copolymers.

The micellization efficiency of these triblock amphiphilic copolymers with molar ratio of (lactone monomer)/PEG/(lactone monomer) higher than 25/1/25 have similar CMC ~ 10^{-7} M irrespective of the type of hydrophobic polyester block and the molecular weight of the hydrophilic PEG block. The formed micelles have an average particle size around 100–150 nm, which increases with increases in the chain length of the polyester block. In other words, the longer the hydrophobic chain length, the larger the size of the micelles. The loading efficiency of drug in micelles is increased as the hydrophobic polyester chain length increases. The loading efficiencies of drug in PVL-PEG-PVL and PCL-PEG-PCL micelles are similar. However, it is quite low in PLA-PEG-PLA micelles. This could be due to less hydrophobic interaction between drug and the PLA inner core.[7] It has been reported that the alkyl-branch side chain could hinder micellization efficiency due to steric

hindrance.[33] As there is a methyl side group in each PLA monomer, this further hinders drug from being encapsulated inside the inner core of the micelles and results in low drug loading in PLA-PEG-PLA micelles. All of the micelle solutions maintained their size in the range of 1.0 ± 0.3 within 12 weeks irrespective of the molecular weight of PEG and the type of lactone. This result indicates that a stable micellar system can be maintained, and there is no significant aggregation or dissociation during storage.

About 20% of drug is released from PLA-$PEG_{10,000}$-PLA micelles comprised of different compositions of PLA and PEG in the range of 28/1/28 to 74/1/74 in pH 7.2 buffer solutions in the first day. After that, drug release is strongly dependent on PLA chain length, and slower release is observed from micelles comprised of a higher molar ratio of hydrophobic polyester block. The release of drug from PLA-PEG-PLA micelles is significantly faster than from PVL-PEG-PVL and PCL-PEG-PCL micelles. The weak interaction between drug and the PLA inner core of micelles and the amorphous character of the PLA block contribute to faster drug release from PLA-PEG-PLA micelles.

The drug delivered by micelles shows higher mean residence time (MRT), higher area under the plasma concentration-time curve (AUC), higher apparent volume of distribution (V_D), and a slower elimination rate than subcutaneous injection of drug solution ($p < 0.05$).[34] In other words, micelles have the characteristics of sustained drug release and prolonged circulation time *in vivo*. The total amounts of drug that accumulate in the liver and kidneys within 8 hours (AUC_{tissue}) are significantly different after administration of micelles and drug solution ($p < 0.05$). Decrease of drug uptake by RES-rich liver and the kidneys is correlated with an increase in MRT of drug from 11.99 ± 0.83 to 21.36 ± 4.81 hours after administration of micelles.

6. Important Characteristics of Diblock Pegylated Polyesters and Polymeric Micelles

The diblock amphiphilic pegylated polyester, poly(ε-caprolactone)-co-methoxypoly(ethylene glycol) (PCL/MPEG) and poly(δ-valerolactone)-co-methoxypoly (ethylene glycol) (PVL/MPEG), are synthesized by copolymerization of two types of lactone monomers, ε-CL and δ-VL, with MPEG, respectively, in the absence of external initiators (Figure 2).[35] These diblock pegylated polyesters possess an amphiphilic character, where the polyester block acts as the hydrophobic domain and the MPEG block acts as the hydrophilic domain (Figure 3). The copolymerization efficiency of ε-CL and δ-VL monomers into diblock amphiphilic pegylated polymers are 83.6% and 90.0%, respectively. The opening rate of seven- and six-member lactone rings is similar and efficient under current copolymerization conditions, which results in the final compositions of synthesized PCL-MPEG (CL/MPEG molar ratios 33/1, 65/1, 127/1) and PVL-MPEG (VL/MPEG molar ratios 36/1, 60/1, 134/1) diblock copolymers very close to their feed molar ratios. The M_n values of synthesized PCL-MPEG and PVL-MPEG copolymers are in the range of 8000–20,000 Da, and the polydispersities are in the range of 2.0–2.5 and 1.7–2.1, respectively. All of the amphiphilic pegylated polyesters spontaneously form micelles with a low CMC value in the range of 10^{-7}–10^{-8} M. The micellization efficiency of PCL-MPEG is slightly higher than that of PVL-MPEG, and the CMC is proportionally reduced as the molar ratio of hydrophobic polyester block in copolymers increases. The *in vitro* cytotoxicity of PCL-MPEG (CL/MPEG molar ratio 127/1) and PVL-MPEG (VL/MPEG molar ratio 134/1) in 10^{-3}–1.0 mg/mL is determined by MTT assay. Both types of copolymers show similar *in vitro* cytotoxicity, and more than 93.0% of cells are viable except that 83.8 ± 0.6% of cells survive after treatment with 1.0 mg/mL of PVL-MPEG.

The average particle size of micelles is mostly around 100–200 nm. The loading efficiency of drugs in PCL-MPEG micelles is higher than in PVL-MPEG micelles. There are six and five carbons in each repeated unit of PCL and PVL, respectively. The different hydrophobicity based on their chemical structures could be the reason accounting for differences in the interaction strength between drug and the polyester inner core of micelles. There is no significant burst release of drugs from PCL-MPEG and PVL-MPEG micelles with different compositions in pH 7.2 buffer solutions, and the slowest release is from micelles with a high molar ratio of lactone monomer. This suggests that drug release is dominated by the hydrophobic polyester composition, and the influence of the type of polyester on drug release is insignificant. Since the degradation of PCL-MPEG and PVL-MPEG is very slow, the release of drugs from these micelles is mainly dominated by partition and diffusion process. The stability of micelles in terms of average particle size is maintained after storage in de-ionized water and 5% dextrose solution at 4°C for 12 weeks irrespective of the type of lactone and the composition of copolymers.

7. Degradation of Diblock Pegylated Polyester in Rat Plasma

As a drug delivery carrier, the integrity of the core-shell structure of micelles after being diluted in a large volume of body fluid and the stability of the amphiphilic structure of the pegylated copolymer *in vivo* are the two most important issues. The stability and degradation of pegylated polyester has been demonstrated in rat plasma at 37°C for 90 days.[36] There is about 10% weight loss of PCL-MPEG pegylated copolymers during

the first hour in rat plasma, which is due to a quick degradation and solubilization of the short chain copolymer.[37] The further decrease in copolymer mass occurs in two phases with different degradation rates. The degradation in the first phase (1–24 hours) is faster than that in the second phase (1–90 days), with 74.0% and 62.9% of copolymer remaining at the end of 24 hours and 90 days, respectively. The degradation process follows first-order kinetics, and the degradation rate constants calculated from the slopes of the two phases are 1.91×10^{-1} day^{-1} for the first phase and 1.77×10^{-3} day^{-1} for the second phase. The corresponding degradation half lives are 87.1 hours and 391.3, respectively. The slow degradation in the second phase is due to the residual copolymer which contains higher molar ratio of hydrophobic PCL domain. The initial molar ratio of EG/ε-CL of PCL-MPEG pegylated copolymers is 1.30 based on ^{1}H-NMR analysis, which continuously decreases to 0.67 at the end of 90 days. This indicates that the hydrophilic MPEG block is continuously lost in plasma, resulting in higher molar ratio of hydrophobic PCL domain left in the residual copolymer, and accounts for the slow degradation in the second phase. Although the molecular weight distribution of the remaining copolymer is enlarged from 1.55 to 2.24 at the end of 90 days, the corresponding molecular weights (M_w 18,000 Da and M_n 8000 Da) are not very different from the original values (M_w 18,000 Da and M_n 11,000 Da). The presence of partially degraded copolymers in the residuals results in increasing polydispersity of copolymer. PCL and MPEG are highly crystallized polymers, and their enthalpies of fusion are 93.7 J/g and 212.0 J/g, respectively. The prominent reduction of enthalpy of fusion of copolymer after incubation in rat plasma for 90 days is mainly due to the loss of high enthalpy of fusion MPEG. This result is consistent with the ^{1}H-NMR data where the molar ratio of EG/ε-CL decreases with time.

8. Conclusions

A series of triblock and diblock amphiphilic pegylated polyesters were synthesized and found to possess micellization potential to form nano-sized micelles, which are able to incorporate hydrophobic drug and regulate drug release. The hydrophobic polyester block and hydrophilic PEG block play an important role in micelle performance. The triblock and diblock pegylated poly(δ-valerolactone) copolymers show higher *in vitro* cytotoxicity than the others. The PCL-PEG-PCL micelles, especially PCL-$PEG_{10,000}$-PCL micelles, possess high drug loading, low *in vitro* cytotoxicity and proper *in vitro* sustained release performance among these triblock pegylated polyesters. Decrease of drug uptake by RES-rich liver and kidneys via PCL-$PEG_{10,000}$-PCL micelles is in company with increase in MRT of drug in blood circulation. Although PCL-MPEG and PVL-MPEG diblock amphiphilic pegylated polyesters have similar controlled release character, the PCL-MPEG micelles have lower CMC, higher biocompatibility and higher drug loading than PVL-MPEG micelles. The enzyme-catalyzed chain cleavage of labile ester linkage of pegylated polyester copolymer occurs in rat plasma, and the changes in mass, molecular weight and morphology of copolymer are associated with the change in its composition.

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