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ARTICLE TYPE

New amphiphilic block copolymers from lactic acid and cholinium building units

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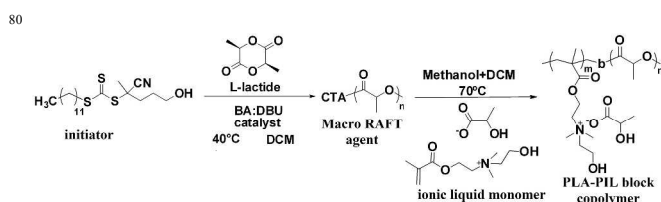
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New polylactide-block-poly(2-cholinium lactate methacrylate) amphiphilic block copolymers were synthesized and characterized. These new block copolymers are composed of chemicals coming from renewable sources such as lactic acid and cholinium. First a polylactide macro chain transfer agent was synthesized and used to conduct block copolymerization of 2-cholinium lactate methacrylate ionic liquid monomer to form an amphiphilic block copolymer. The polymerizations were successfully conducted through ring-opening polymerization and controlled radical RAFT techniques and confirmed by ¹H NMR and GPC experiments. DLS experiments revealed the formation of self assembled micelles in water and TEM studies confirmed the presence of spherical nanostructures in dry state having sizes down to 17 nm. To the best of our knowledge this is the first example of an amphiphilic block copolymer having lactic acid units in both copolymer blocks of potential interest in nanomedicine.

Polylactide (PLA), biocompatible and biodegradable by enzyme and hydrolysis, is hydrophobic aliphatic polyester and probably the most important biobased synthetic polymer.^{1,2} Hydrophobicity of polylactide is a problem for biological and biomedical applications since it will not be applicable in aqueous environment.³ In order to overcome this problem, one of the main approaches is to combine polylactide with water soluble polymers to form amphiphilic block copolymers which can self assemble into different morphologies like micelle or vesicle potentially to be used for drug delivery applications.⁴ The water soluble block to be combined with PLA can be chosen from either synthetic or natural polymers. Among others, poly(acrylic acid) and poly(ethylene oxide) can be given as examples to the important synthetic water soluble polymers that have been utilized for various polylactide based amphiphilic block copolymers.⁵ Natural water soluble polymers are as well used for the synthesis of amphiphilic PLA copolymers. Among those, chitosan,⁶ dextran,⁷ hyaluronic acid,⁸ chondroitin sulfate⁹ can be given as examples. Recently, Fajardo et. al synthesized through click chemistry PLA based amphiphilic block copolymers by using chondroitin sulfate as the water soluble block. Block copolymers self assembled in water and displayed spherical micelles that are in the range of 23-31 nm as revealed by TEM and proposed as new drug delivery platforms.⁹ Robb et. al synthesized drug delivery vehicles based

on polylactide and poly(allyl glycidyl ether).¹⁰ A number of applications can be listed for PLA amphiphilic block copolymers.³ The most widely hypothesized application is the delivery of hydrophobic drugs where drug molecules are encapsulated within the hydrophobic PLA matrix.¹¹ They can also be used as imaging and diagnostic platforms by the use of active agents.¹² Other applications can be the preparation of hydrogels and scaffolds to be used as tissue engineering platforms.^{13, 14}

Cholinium, a quaternary ammonium cation, trimethylethanol ammonium, is an essential micronutrient which supports several biological functions.¹⁵ Due to their particular features, such as biocompatibility, excellent biodegradability, and low toxicity, ionic liquids combining cholinium cation with non-hazardous anions have been used in several applications as diverse as solvents in benign extraction schemes as aqueous biphasic systems, agents for collagen-based materials, or in the pretreatment or the dissolution of biomass.¹⁶⁻²² In a recent study, we have shown the synthesis of cholinium type ionic liquid monomers and their subsequent polymerization.¹⁶ Therefore, we have chosen a bio-ionic liquid monomer which is composed of cholinium cation and lactate as the anion to be combined with PLA for obtaining new block copolymers. As a result, the amphiphilic block copolymer will be carrying moieties coming from lactic acid in block (lactide+lactate) of the copolymer and the biocompatible cholinium moiety in the hydrophilic block. Thus, in this communication we present the synthesis of amphiphilic poly(lactide)-block-poly(2-cholinium lactate methacrylate) and the investigation of its self-assembly properties in water.



Scheme 1 General reaction pathway for the synthesis of polylactide-block-poly(2-cholinium lactate methacrylate) amphiphilic block copolymers

In the first step, ring opening polymerization of L-lactide was conducted by organocatalyzed ring-opening polymerization (ROP) using hydroxyl terminated chain transfer agent (CTA) as the initiator and benzoic acid:1,8-Diazabicyclo [5.4.0]undec-7-ene (1:1) salt as the catalyst in the presence of dichloromethane as the solvent at 40 °C. The experimental details are provided in the supporting information. Conversion of the monomer to polymer was followed by ¹H NMR. Two polymers with different molecular weights were prepared, molecular weights being 3500 and 7100 g/mol as calculated from ¹H NMR (see supporting information). These PLA macro RAFT agents were used for the polymerization of 2-cholinium lactate methacrylate ionic liquid monomer. The reaction pathway is provided in scheme 1 to obtain the desired block copolymer.

Four different block copolymers were synthesized which are PLA3.5-PIL50, PLA3.5-PIL100, PLA7.0-PIL50 and PLA7.0-PIL100 where the coding that is used to designate the block copolymer indicates first the PLA that is used for block copolymerization and the second PILxx designation refers to the PIL block, numbers indicating the degree of polymerization. Molecular properties of the starting polymers and the final block copolymers are provided in Table 1.

Table 1 Molecular weights of the PLA macro RAFT agents and block copolymers measured from GPC

Polymer code	M _n ^a	M _n ^b	M _n ^c	M _w ^d	PDI ^e
PLA 3.5	3800	3500	3900	4500	1.15
PLA 7.0	7300	7100	5500	6600	1.18
PLA3.5-PIL50	18300	-	6200	8100	1.30
PLA3.5-PIL100	32900	-	9250	11500	1.25
PLA7.0-PIL50	21800	-	8900	11800	1.32
PLA7.0-PIL100	36400	-	10300	13300	1.29

^a Theoretical molecular weights ^b Molecular weights calculated from ¹H NMR ^{c,d,e} GPC results based on PS standards in THF containing 10 mM of LiTFSI.

Ring opening polymerization (ROP) of L-lactide was carried out by using a dual RAFT chain transfer agent containing hydroxyl functionality. It is known from literature that benzoic acid:1,8-Diazabicyclo[5.4.0]undec-7-ene (1:1) (BA: DBU) salt has the capacity to polymerize lactide and can be used for controlled ROP of this monomer to produce polymers with narrow polydispersities.²³ ROP was conducted at mild conditions by using dichloromethane as the reaction solvent. The conversion of the monomer to polymer was monitored by ¹H NMR. ¹H NMR spectra obtained during the course of the reaction are provided in the supporting information.

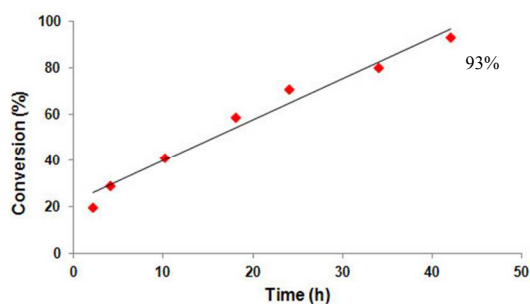


Fig.1 % Conversion plotted against time for the ring opening polymerization of lactide

The methyl proton (-C=OC(-CH₃)O-) signal originating from the lactide monomer at 1.68 ppm disappears as the polymerization reaction proceeds and the methyl signal arising from the polymeric repeat unit appears at 1.61 ppm at the final PLA. The integral ratio of these two peaks during the reaction allows us to determine the conversion of the monomer to polymer. It was calculated by determining the ratio of the integrals of the methyl proton signals of the -C(-CH₃)C(=O)O repeating units in the polymer chains to the corresponding lactide monomer (1.68 ppm and 1.61 ppm). The calculated conversion values were plotted against time and given in Figure 1. As seen from the figure given, the conversion of the monomer proceeds linearly as the reaction time passes; indicating that the polymerization was first-order in monomer concentration. In addition, the linearity of this plot points to a rapid initiation and an absence of chain termination, suggesting a living ROP upto 93% of conversion. This methyl signal of the final clean polymer at 1.61 ppm and the methyl proton signal at 0.88 ppm arising from the methyl unit (CH₃(CH₂)₁₁-) of the RAFT agent were used to estimate the calculated molecular weight of the PLA. The calculated molecular weights determined from ¹H NMR signals correlate well with the expected theoretical molecular weights of the PLA macro RAFT agents. The ¹H NMR signals used to determine the calculated molecular weights of the polymers are provided in supporting information. In order to determine the molecular weights and polydispersities of the macro RAFT agents, GPC was performed. The GPC signals obtained are displayed in Figure 2a.

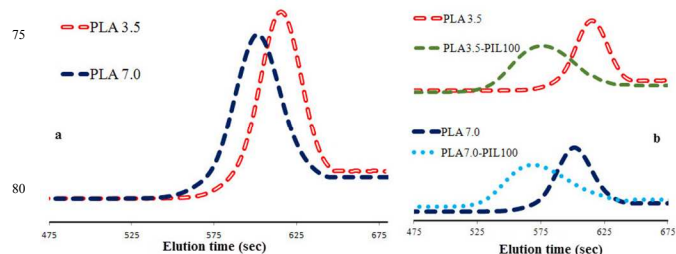


Fig. 2 a) GPC signals of PLA macro RAFT agents b) GPC signals of block copolymers synthesized by using two different PLA macro RAFT agents

As seen from the figure, monomodal GPC signals are obtained with low polydispersities which represent good control over the polymerization. The GPC signals shifts to lower retention times indicating an increase in the molecular weight as expected as the degree of polymerization increases. The theoretical, calculated and measured molecular weights of the PLA macro RAFT agents are provided in Table 1.

The two PLA macro RAFT agents were used to conduct the RAFT polymerization of 2-cholinium lactate methacrylate ionic liquid monomer. All the reactions were performed in methanol/dichloromethane (5/1 v/v) solvent mixture for 24 hours. The final products were analyzed by ¹H NMR to follow the conversion of the monomer to polymer. The crude product was washed sequentially with acetone and dichloromethane to get rid of any unreacted starting material. ¹H NMR revealed that the

conversion was over 99% after each polymerization (see supporting information). All the block copolymers were analyzed by GPC to follow the formation of the block copolymers. All the polymers displayed monomodal GPC signals with reasonably low polydispersities indicating good control over the polymerization by using PLA macro RAFT agent, showing shifts to lower retention volumes as the molecular weight increases with the formation of the block copolymer. To illustrate this, GPC signals of two different block copolymers are provided in Figure 2b.

In order to study the self assembly of the amphiphilic block copolymers in aqueous environment, block copolymers were dissolved in deionized water to have a concentration of 5 mg/ml. Simply, 50 mg of block copolymer was dissolved in 10 ml deionized water and remained under mechanical stirring for 24 hours at room temperature until all material was completely dissolved. The solutions were then poured into dialysis membranes having 3500 kDa cut-off molecular weight and dialyzed against excess deionized water for 72 hours at room temperature under strong agitation. Dialyzed solutions were poured into vials and diluted dropwise to 1 mg/ml and analyzed by dynamic light scattering and TEM. Before the analysis, the solutions were filtered through 0.45 μm regenerated cellulose syringe filters.

The block copolymers were analyzed further for their self assembly in water. Owing to its amphiphilic nature, PLA-PIL block copolymer can self assemble in aqueous medium. The constituent block which is composed of 2-cholinium lactate methacrylate units is hydrophilic and polylactide block is hydrophobic. Therefore, the hydrophilic block stabilizes the hydrophobic part in water, forming micelles having PLA core surrounded by PIL outer shell. The sizes of self assembled nanostructures (1 mg·mL⁻¹) in water were determined by DLS measurements (Table 2).

Table 2 Mean sizes of the self assembled block copolymers from DLS and TEM experiments

Polymer	Intensity avg. (nm)		Number avg. (nm)	TEM (nm)
	DLS			
	1 st peak	2 nd peak	DLS	
PLA3.5-PIL50	22	140	22	17
PLA3.5-PIL100	26	176	26	22
PLA7.0-PIL50	30	200	30	29
PLA7.0-PIL100	37	230	37	35

The DLS revealed for all copolymers a bimodal intensity average size distribution (Figure 3a) and considering the fully stretched polymer chain, the smallest peaks are attributed to the primary micelles, while the second peaks are considered from the aggregation of the individual micelles since the intensity averaged particle size distributions are extremely sensitive to aggregation. Therefore, it can be stated that the number of primary micelles are predominant in the system. This statement can be further verified by analyzing the number averaged size distributions (Figure 3b) of the self assembled solutions.

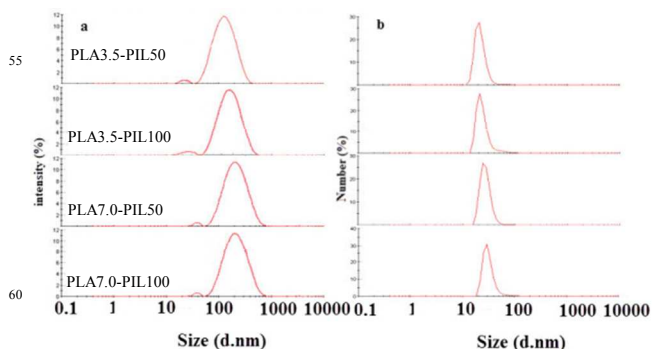


Fig. 3 a) Intensity and b) number average mean size distribution of the block copolymers in deionized water

As it clearly seen, the number averaged DLS data represents that the primary micelles are predominant in the system confirming our statement. In order to further analyze the size, morphology and distribution of the self assembled structures, TEM experiments were conducted. TEM images are displayed in Figure 4 for all the block copolymers that were analyzed.

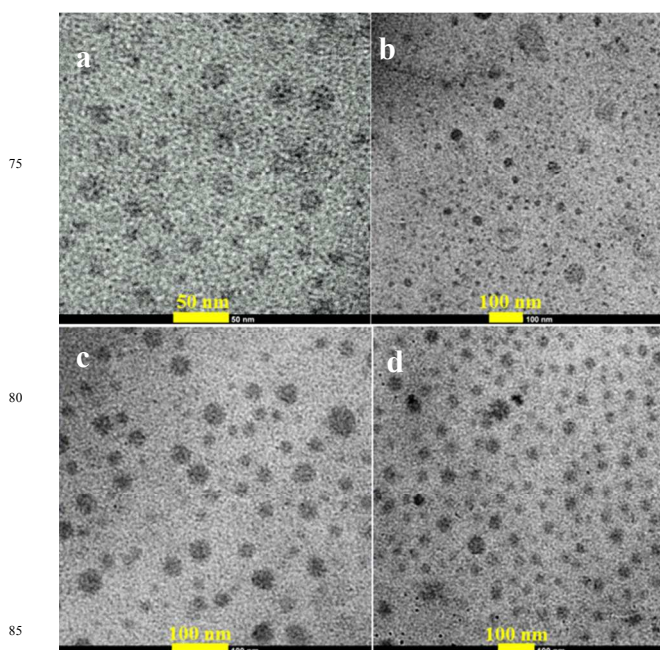


Fig. 4 TEM images of the block copolymers a) PLA3.5-PIL50 b) PLA3.5-PIL100 c) PLA7.0-PIL50 d) PLA7.0-PIL100

TEM images confirmed the predominant presence of primary spherical micelles in dry state and the mean diameter that is obtained from the pictures are smaller than those obtained from DLS in swollen state. The average sizes are 17, 22, 29 and 35 nm for PLA3.5-PIL50, PLA3.5-PIL100, PLA7.0-PIL50, PLA7.0-PIL100, respectively. This may be attributed to the fact that the hydrodynamic volume is measured from DLS whereas the size in TEM refers to the dry state where the micelles are collapsed. The mean diameters that are obtained from DLS and TEM are provided in Table 2. If the mean sizes of the block copolymers

are compared, it is clearly seen that the average mean size increases as the molecular weight of the PLA block increases. For the block copolymer having the same PIL degree of polymerization, the size changes from 17 to 29 nm for PLA3.5 and from 22 to 35 nm for PLA7.0. Although the changes are not that dramatic, the same observation is valid for the block copolymers with increasing PIL degree of polymerization. The values determined from TEM reveals that considerably small size micelles can be prepared, sizes going down to approximately 17 nm.

Conclusions

In summary, new poly(lactide)-block-poly(2-cholinium lactate methacrylate) amphiphilic block copolymers were synthesized by a combination of organocatalyzed ROP and controlled radical RAFT polymerizations. These amphiphilic block copolymers contain units coming from lactic acid in both blocks (hydrophilic and hydrophobic) as well as natural cholinium units in the hydrophilic block. GPC revealed the formation of well defined copolymers with relatively low polydispersities. The block copolymers self assemble into spherical micelles having sizes down to 17 nm as revealed by TEM analysis. The sizes of the micelles varied by playing with either PLA or PIL chain lengths, giving opportunity to produce spherical nanostructures in the range of 17-35 nm which makes them of potential interest in nanomedicine.

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