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Mendeleev Commun., 2023, **33**, 404–407

Mendeleev Communications

Metal-free organic catalyst for synthesis of low dispersity poly(ethylene glycol-*block*-polylactide) copolymers with well-defined structure

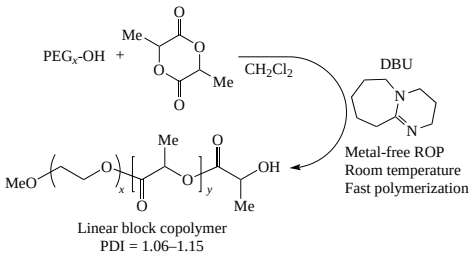
Yulia A. Puchkova,*[a] Nikita G. Sedush,[a,b] Antonina D. Ivanenko,[a] Valentina G. Shuvatova,[a] Galina A. Posypanova[a] and Sergei N. Chvalun[a,b]

[a] *National Research Center 'Kurchatov Institute', 123182 Moscow, Russian Federation. E-mail: puchkova.yuliia@gmail.com*

[b] *N. S. Enikolopov Institute of Synthetic Polymeric Materials, Russian Academy of Sciences, 117393 Moscow, Russian Federation*

DOI: 10.1016/j.mencom.2023.04.033

Ring-opening polymerization of lactide was performed in the presence of 1,8-diazabicyclo[5.4.0]undec-5-ene as an organic catalyst and polyethylene glycol as a hydroxyl-containing macroinitiator. A series of amphiphilic poly(ethylene glycol-*block*-polylactide) copolymers with a low dispersity (PDI = 1.1), different stereoregularity and length of the polylactide block was obtained. Nanoparticles with a diameter of 20–25 nm were produced from selected polymers and were studied by *in vitro* cytotoxicity tests.



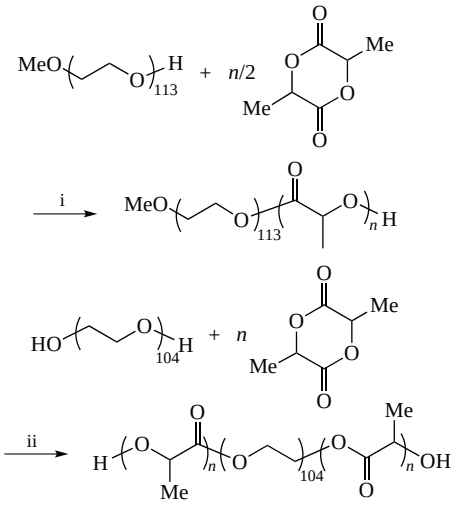
Keywords: metal-free ring opening polymerization, DBU, block copolymers, poly(lactide), poly(ethylene glycol), nanoparticles, drug delivery systems.

Amphiphilic biocompatible poly(ethylene glycol-*block*-polylactide) (PEG_x-PLA_n) polymers are of considerable interest for the development of novel anticancer drug formulations.[1–3] Their effectiveness in design of nanoformulations is well illustrated by several clinically relevant drugs based on PEG-PLA nanoparticles, including the Genexol-PM micelle-based formulation of paclitaxel, which was approved by the U.S. Food and Drug Administration. Another PEG-PLA nanocarrier platform 'Accurins' is currently in clinical trials.[1,4] It is known that the size of nanoparticles is one of the key parameters affecting their long-term blood circulation, ensuring the pass through physical and biological barriers or targeting to specific organs and tissues.[5,6] The control of structure and polydispersity of the initial macromolecules is crucial for the preparation of particles with a predetermined size, narrow size distribution and good reproducibility of the process.[7] Hence, for the successful engineering of tumor-targeted nanoparticles it is extremely important to synthesize PEG_x-PLA_n block copolymers with a low polydispersity and a well-defined structure.

Typically, for the polymerization of lactide, glycolide and other cyclic esters, organometallic catalysts based on Zn, Al, Zr, Ti, Ca, *etc*., are used, with tin(II) 2-ethylhexanoate being the most popular.[7–11] Typical drawbacks of tin-based catalysts are high reaction temperature, which promotes racemization and transesterification reactions leading to an increase of polydispersity, as well as poor control over the end groups and the structure of the main chain.[7,12] These side reactions may cause a deterioration in the reproducibility of characteristics both at the stage of polymer synthesis and during the preparation of biomedical materials, including nanoparticles. In addition, there are certain restrictions on the content of residual tin in biodegradable polyesters for medical applications, since they can cause a toxic effect.[13]

The use of organic catalysts for ring-opening polymerization (ROP) of lactide, for example, of amidine or guanidine derivatives, such as 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD), gains a lot of interest due to mild reaction conditions (room temperature, polar solvents) and more exquisite control of polymer polydispersity and topology.[12] The extreme activity of TBD (time to reach 95% conversion is less than 1 min) provides the synthesis of polylactide with a molecular weight of more than 60 kDa; however, a significant increase in the polydispersity index can be observed due to the transesterification reactions. The polymerization of lactide in the presence of MTBD and DBU proceeds slower and requires the use of an initiator and a higher catalyst concentration.[14] In this research we propose the DBU-catalyzed ROP that provides the synthesis of narrowly dispersed di- and triblock copolymers poly(ethylene glycol-*block*-polylactide) using a lower DBU concentration and a reaction time compared to those previously reported. It was shown that rigorous purification of reagents and solvents was necessary for the synthesis of copolymers with a PLA block length of 14–120 units. This provides complete monomer conversion and significantly reduces the reaction time. Nanoparticles based on synthesized polymers were prepared and their biocompatibility was proved in an *in vitro* experiment.

Amphiphilic linear block copolymers PEG_x-PLA_n were synthesized by ROP of L,L- or D,L-lactide under an inert atmosphere using a Schlenk line (for details, see Online Supplementary Materials). The polymerization was carried out in dichloromethane at 25 °C, using monomethoxy-PEG with a molar mass of 5 kDa (PEG_{113}) or bifunctional PEG with a molar mass of 4.6 kDa (PEG_{104}) as a macroinitiator and DBU as a catalyst (Scheme 1). The degree of polymerization of polylactide



Scheme 1 *Reagents and conditions*: i, PEG_{113}, lactide, DBU, CH_2Cl_2, 25 °C, [OH]/[DBU] = 2.5; ii, PEG_{104}, L-lactide, DBU, CH_2Cl_2, 25 °C, [OH]/[DBU] = 2.5.

in PEG_x-PLA_n was controlled by varying the [OH]/[LA] (the hydroxy group of macroinitiator to monomer) molar ratio to produce PLA blocks with various lengths. According to the previous studies, ROP of lactide in the presence of DBU can proceed *via* two routes of initiation depending on the availability and amount of the alcohol group(s) of an initiator.[15] Molar excess of DBU relative to the alcohol group(s) provides initiation both by directly activating the monomer and by deprotonating the OH-group(s). To the best of our knowledge, there are only a few studies of the kinetics of lactide polymerization in the presence of poly(ethylene glycol) and DBU. Typically, the molar ratio of macroinitiator to catalyst ([OH]/[DBU]) is less than 2.1, while the reaction time varies from 15 min to 4 h.[13,15–18] As far as we know, it is still unclear if DBU is a biocompatible catalyst; therefore, it is favorable to minimize its content. In order to carry out a controlled synthesis and to minimize side reactions, a lower DBU concentration ([OH]/[DBU] = 2.5) was used for the synthesis of diblock (PEG_{113}-PLA_n) and triblock copolymers (PLA_n-PEG_{104}-PLA_n).

Earlier it was demonstrated that DBU-catalyzed ROP of lactide stopped at incomplete conversion of the monomer.[15–17] Base DBU is an amidine derivative with weak nucleophilicity, and so acidic impurities from reagents and solvents can deactivate the free catalyst during the reaction. Since a number of such acidic impurities are present in reagents, firstly we performed test reactions using reagents and solvents prepared by two different purification methods (methods A and B, for details, see Online Supplementary Materials). Synthesis of diblock copolymers with a short polylactide block (degree polymerization DP of PLA $\leqslant$ 35) was successful with the reagents prepared by both purification methods. High conversion values (>90%) were achieved in less than 15 min. The advantage of method B was clear for synthesis of polymers with a longer polylactide block (DP of PLA $\geqslant$ 70). Reactions which were performed with reagents prepared by method A resulted in a low degree of conversion not exceeding 60%. At the same time, repeated purification of the reagents and solvents according to method B provided both high lactide conversion and controlled DP of PLA, as well as significantly reduced the reaction time (see Online Supplementary Materials, Table S1). For the synthesis of triblock copolymers, the purification of reagents and solvents was carried out only by method B. For each block copolymer PEG_{113}-PLA_n and PLA_n-PEG_{104}-PLA_n, the optimal reaction time was determined considering the conversion and molecular weight distribution. It is important to note that at various time points the samples were characterized by monomodal molecular weight distribution curves with a constant width indicating minimal side reactions (Figure S1).[15] Finally, di- and triblock copolymers with molecular weights from 6.0 to 12.5 kDa and different stereoregularities were synthesized. The degree of polymerization of the polylactide block was varied from 14 to 104 monomeric units (Table 1). All samples exhibited a desired correlation between the targeted and experimental values of DP of PLA, monomodal molecular weight distribution and polydispersity index of less than 1.15.

The structure of the synthesized block copolymers was studied by ^{1}H NMR (Figure 1). The spectra contained signals for C*H* and CH_3 protons of the polylactide repeating units and CH_2 protons in the PEG block. The small signals at 4.22–4.32 ppm and 4.34 ppm correspond to the C*H* group of the terminal PLA unit and to the CH_2 methylene spacer group of the PEG block, which directly confirm the successful polymerization of the monomer on the hydroxy groups of the macroinitiator and confirm the formation of the block copolymer structure. The relative integral intensity of signals for terminal methine C*H* of the PLA block (δ = 4.33–4.40 ppm) and the spacer methylene CH_2 of the PEG block correlate in all cases as 1 : 2, which confirms the absence of possible lactide polymerization initiated by hydroxyl-containing impurities or free DBU molecules by the nucleophilic mechanism.

The final number-average degree of polymerization of polylactide was calculated from the ratio of the relative intensities of the peaks from the methine protons (C*H*) and/or methyl (CH_3) protons of the main polylactide chain to the CH_2 protons of the poly(ethylene glycol) block. The obtained values correlated well with the targeted ones.

Thus, it was found that at molar ratios of [LA]/[DBU] $\leqslant$ 150 and [OH]/[DBU] = 2.5, the polymerization of L- and D,L-lactide proceeded successfully at the hydroxy groups of mono- and bifunctional poly(ethylene glycol) with the formation of di- and triblock copolymers, while the reaction time depended on the structure of the polymer and ranged from 10 min to 2 h. In addition, regardless of the stereoregularity of the monomer and

Table 1 Characterstics of block copolymers synthesized at [OH]/[DBU] = 2.5, *T* = 25 °C, CH_2Cl_2.

Sample (targeted)	[LA]/[OH]	[LA]/[DBU]	*t*/min	M_n/kDa[a]	PDI[a]	DP PLA[b]	M_n/kDa[b]
PEG_{113}-P(D,L)LA_{14}	7	17	10	6.7	1.10	13	6.0
PEG_{113}-P(D,L)LA_{36}	18	45	15	8.6	1.06	33	7.4
PEG_{113}-P(D,L)LA_{70}	35	86	30	11.4	1.15	65	9.7
PEG_{113}-P(L)LA_{120}	105	150	120	12.9	1.15	106	12.6
P(L)LA_{15}-PEG_{104}-P(L)LA_{15}	15	18	3	8.0	1.07	14	6.6
P(L)LA_{30}-PEG_{104}-P(L)LA_{30}	30	37	4	10.3	1.07	24	8.0
P(L)LA_{70}-PEG_{104}-P(L)LA_{70}	70	86	5	15.2	1.15	68	14.3

[a] GPC measurement (polystyrene standards), PDI is the polydispersity index. [b] Calculated based on NMR spectroscopy; DP is the degree of polymerization of the block and M_n is the molecular weight of the block copolymer.

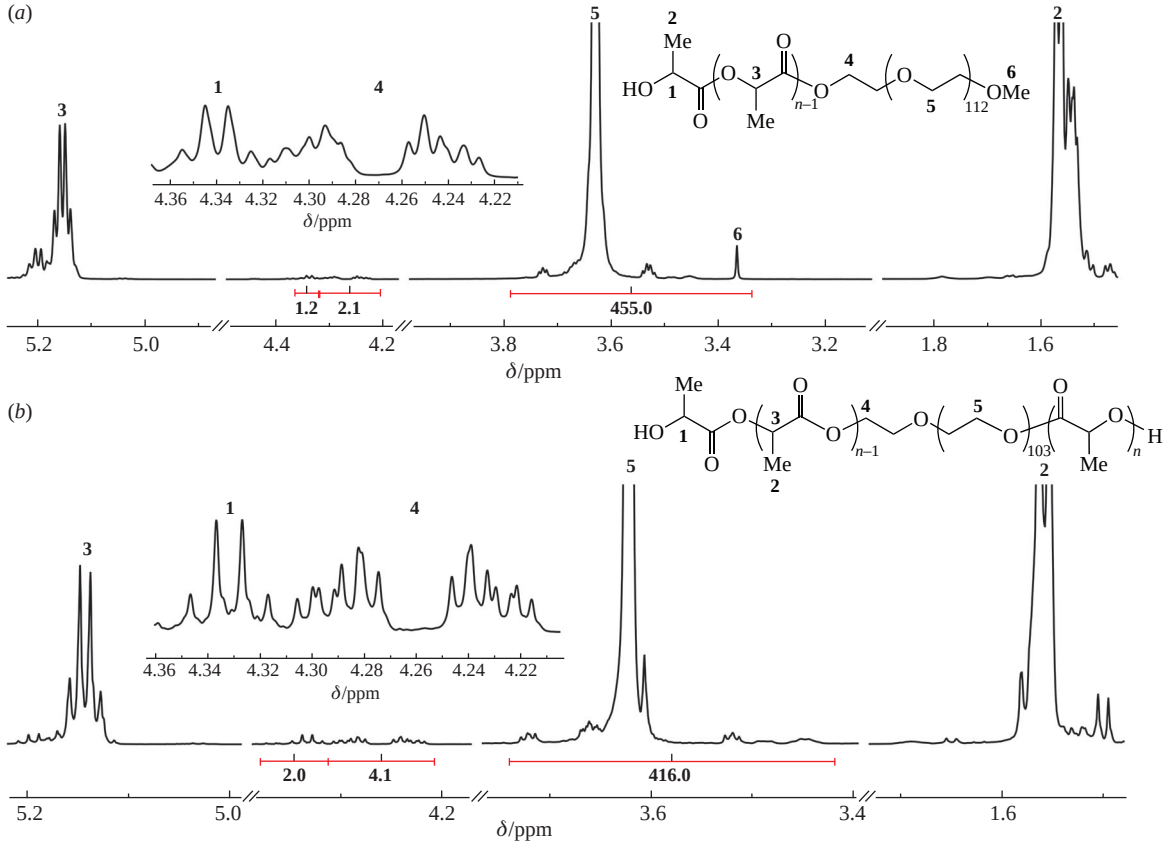


Figure 1 ^{1}H NMR spectra (600 MHz, $CDCl_3$) of block copolymers: (*a*) PEG_{113}-P(D,L)LA_{70} and (*b*) P(L)LA_{30}-PEG_{104}-P(L)LA_{30}.

the functionality of the macroinitiator, the polydispersity index of the block copolymers in the presence of DBU varied from 1.07 to 1.15. Fast solution polymerization at room temperature is a technological advantage of a metal-free catalyst compared to tin octoate. The absence of transesterification and cyclization side reactions, the controlled production of block copolymers of a given structure and composition, and the absence of heavy metal impurities determine the effectiveness of the DBU catalyst in the synthesis of polymers for various biomedical applications.[20]

To assess the biocompatibility of the synthesized block copolymers, nanoparticles were prepared according to the procedure described in our previous work.[3] An average hydrodynamic diameter of the particles determined by dynamic light scattering was 20–25 nm. A representative transmission electron microscopy image of the nanoparticles is shown in Figure S2. It was established that the particles were spherical and characterized by a narrow size distribution.

Cytotoxicity of nanoparticles PEG_{113}-P(D,L)LA_n was studied on WI38 normal embryonic human lung fibroblasts using the MTT assay according to the standard method.[19] It was found that PEG_{113}-P(D,L)LA_n nanoparticles showed no toxicity in the concentration range of 0.001–1.000 mg ml^{-1} (Figure S3). The absence of toxicity *in vitro* suggests the biocompatibility of diblock copolymers with a P(D,L)LA-block synthesized with a DBU organocatalyst under the described above conditions.

The synthesis, characterization of polymers, preparation and studies of nanoparticles were carried out with the financial support of the Russian Science Foundation (grant no. 18-73-10079-P). The preparation and analysis of reagents were supported by the Ministry of Science and Higher Education of Russia (topic no. FFSM-2022-0003), using the equipment of the Center for Collective Use 'Polymer Research Center' of ISPM RAS.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.04.033.

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Received: 21st December 2022; Com. 22/7071