



Naloxone-initiated mechanochemical synthesis of poly(lactic acid)[†]

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A mechanochemical approach was utilized for the synthesis of naloxone covalently linked poly(lactic acid) and nanoparticles. This preparation was achieved using lactide as a monomer in anionic ring opening polymerization, naloxone as a drug initiator, and CHCl_3 to perform liquid-assisted grinding. This process resulted in the direct preparation of a naloxone nanoparticle with a drug loading of $\sim 8.3\%$ w/w and nanoparticles around 600 nm. These findings underscore the promise of mechanochemistry in developing drug delivery systems.

The fatal opioid epidemic that is currently affecting the United States,^{1–3} and other countries around the world, is fuelled by the widespread abuse of synthetic mu opioid receptor (MOR) agonists such as fentanyl and its derivatives.^{4–6} Due to continuously rising overdose rates, an overdose crisis has been declared by the United States Drug Enforcement Administration (DEA).^{7,8} Additionally, recent data from the Center for Disease Control and Prevention (CDC) indicated a dramatic increase in opioid deaths across the US to be nearly 79 000 opioid-related mortalities in 2021.⁸ The only antidote currently accessible for emergency intervention in cases of acute toxicity and respiratory depression resulting from an opioid overdose is the traditional MOR antagonist known as naloxone.^{9–12} Due to rapid metabolic clearance of naloxone (elimination half-life of ~ 30 min) repeated dosing or larger bolus doses are required to successfully reverse a synthetic opioid overdose.¹³ Methods to increase the bioavailability of naloxone can lead to dramatically improved overdose reversal outcomes.^{14–16} Utilizing the

biodegradability of poly(lactic acid) (PLA), our group has shown that covalently loaded naloxone PLA nanoparticle (cNLX-NP) technology can be used to create an effective, long-lasting MOR antagonist naloxone formulation against a high dose of morphine.⁹ In addition, cNLX-NPs have showed therapeutically relevant plasma concentrations that far outlast free naloxone and exhibited a 34-fold increase in the half-life when compared to free naloxone. This naloxone-poly(lactic acid) polymer was prepared under bulk polymerization conditions and produced well-defined NLX-PLA nanoparticles with a degree of polymerization (DP) of 64 and naloxone drug loading of 6.6% w/w.

Methods that enable the rapid scale up and production of the NLX-PLA NPs can dramatically simplify the complex multistep production process of covalently loaded drug particles. A synthetic approach that incorporates: (1) increased naloxone drug loadings in the PLA polymer and (2) one step polymerization and preparation of the covalently linked naloxone PLA nanoparticles can breakthrough current complex drug production barriers. Therefore, we have been seeking an alternate path that benefits from current developments in mechanochemistry methods. Using mechanochemistry, high molecular weight polymers of PLA have been synthesized by Kim and other workers *via* ring opening polymerization (ROP) of lactide using DBU as an organocatalyst and a primary alkyl alcohol as an initiator.^{17–20} Our research team has recently employed ball milling mechanochemistry to synthesize phenolic-PLA nanoparticles in a single step, utilizing thiourea/tertiary amine as an organocatalyst, K_2CO_3 as a base, CHCl_3 added to perform the liquid-assisted grinding (LAG) and phenols as initiators for phenolic-bearing polymers.²¹ Following a similar mechanochemical approach, we have successfully produced naloxone-PLA nanoparticles (NLX-PLA NPs) in one step, achieving a higher naloxone drug loading of 8.3% w/w compared to our prior study's 6.6% w/w under bulk polymerization conditions. The single-step mechanochemical synthesis and nanoparticle preparation of NLX-PLA NPs were accomplished using similar reaction conditions to those employed for phenolic-PLA polymers but without K_2CO_3 (Scheme 1).

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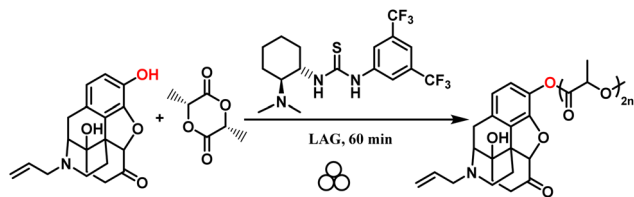
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Scheme 1 Mechanochemical ring-opening polymerization of L -lactide using naloxone as initiator.

CHCl_3 was used to perform LAG in these mechanochemical ring-opening polymerization (ROP) reactions since the initial neat reaction resulted in no reaction similar to published results.²¹

Initially, the naloxone-initiated mechanochemical synthesis of covalently linked PLA was conducted according to our previous work in a vibratory ball mill (FormTech Scientific, FTS 1000).²¹ However, the percent conversion (conv. %) and initiator efficiency (IE%) were relatively low at 13% and 12%, respectively. Previous reports have highlighted the temperature as a crucial factor for promoting ball-milled reactions.^{22–24} In the case of this mechanochemical ROP reaction in the vibratory mill, an average milling jar temperature of 29 °C was measured using an Etekcity infrared thermometer (Table 1). Our group has demonstrated that the ball-milled reaction in the FlackTek speedmixer resulted in an increase in the average milling jar temperature compared to a vibratory ball mill.²⁵ Therefore, we conducted the mechanochemical naloxone-initiated ROP reaction in a FlackTek speedmixer under the same reaction protocol (Table 1). The ROP reaction in the speed mixer with a milling frequency of 1800 RMP and milling time of 60 min exhibited an average milling jar temperature of 41 °C. An increase in the milling jar temperature resulted in a slight improvement of the reaction conversion (21%) and initiator efficiency (25%). As a result, we have chosen the speed mixer for further optimization.

To evaluate the influence of equivalence of CHCl_3 and milling frequency in the speedmixer on the initiation of ROP of L -lactide using naloxone was conducted and shown in Table 2. We speculate that incorporating small quantities of CHCl_3 could facilitate the solvation of the polymer chain end, thus enabling efficient chain-growth.²⁶ As anticipated, the monomer conversion increased upon addition of CHCl_3 . With the addition of low CHCl_3 loading (10 μL), lactide conversion and initiator efficiency increased from 0% to 23%, and 0% to 13%,

Table 2 Impact of using CHCl_3 to perform LAG and milling frequency using the speedmixer on naloxone-initiated ROP of L -lactide^a

Entry	Milling frequency (rpm)	CHCl_3 (μL)	Conv. ^b (%)	IE ^b (%)
1	2100	10	23	13
2	2100	20	49	27
3	2100	30	55	25
4	2250	20	57	22
5	2500	20	20	19

^a Polymerization conditions: L -lactide (44 mg, 0.310 mmol, 10 equiv.), naloxone (10 mg, 0.031 mmol, 1 equiv.) and thiourea catalyst (6.4 mg, 0.015 mmol, 5 mol%), in a 5 mL stainless-steel jar with 5 mm ($\times 5$) stainless-steel balls for 60 minutes. ^b Determined *via* crude ^1H NMR spectra.

respectively (Table 2, entry 1). With the addition of 20 μL of CHCl_3 , the lactide conversion and initiator efficiency increased to 49%, and 27% respectively (Table 2, entry 2). However, for the high CHCl_3 loadings (30 μL), the conversion (55%) and IE% (25%) were statistically similar to the 20 μL CHCl_3 loading results (Table 2, entry 3). As a result, we chose entry 2 reaction conditions with 20 μL of CHCl_3 for further optimization of the naloxone-initiated mechanochemical ROP of lactide.

To investigate the effect of milling frequency, we conducted naloxone-initiated mechanochemical ROP of lactide at four different milling frequencies, 1800, 2100, 2250 and 2500 rpms. At 1800 rpm, as indicated in Table 1, the lactide conversion and initiator efficiency was 41% and 21%, respectively. There was a slight increase in lactide conversion (49%) and initiator efficiency (27%), respectively at 2100 rpm. At 2250 rpm, there was a slight increase in lactide conversion (57%) but there was a decrease in initiator efficiency (22%). At higher milling frequency (2500 rpm), we noticed that the colour of the crude product had changed from off white to brown during the milling process and we observed impurities in the crude ^1H NMR (Fig. S7†). This also resulted in low conversion% (20%) and IE% (19%) (Table 2, entry 4). Consequently, we postulated that the optimal frequency for subsequent optimizations would be 2100 rpm.

Moreover, the impact of catalyst loading on naloxone-initiated ROP of L -lactide was investigated. Thiourea/tertiary amine catalyst (C-T) is known to play two roles, as it contains both thiourea and tertiary amine functional groups, which are required for monomer and nucleophile activation, respectively.^{26,27} Thus, we investigated the impact of catalyst loading (5.0%, 7.5%, and 10%) on naloxone-initiated ROP reactions as

Table 1 Impact of vibratory mill and speed mixer grinding on naloxone-initiated ROP of L -lactide^a

Method	Milling frequency (rpm)	Milling time (min)	Temp (°C)	Conv. ^b (%)	IE ^b (%)
Ball mill	1800	60	29	13	12
Speedmixer	1800	60	41	21	25

^a Polymerization conditions: L -lactide (44 mg, 0.310 mmol, 10 equiv.), naloxone (10 mg, 0.031 mmol, 1 equiv.) and thiourea catalyst (6.4 mg, 0.015 mmol, 5 mol%), CHCl_3 (20 μL), in a 5 mL stainless-steel jar with 5 mm ($\times 5$) stainless-steel balls. ^b Determined *via* crude ^1H NMR spectra analysis.



Table 3 Impact of catalyst loadings on naloxone-initiated ROP of L-lactide^a

Entry	Cat. (mol%)	Conv. ^b (%)	IE ^b (%)	DP ^c	M _n ^d (kDa)	D ^d	NLX loadings (1H NMR)	NLX loadings (UV-vis)	NLX loadings (LC-MS)	Yield ^e (%)
1	5	49	27	52	5.0	1.16	8.03	8.31	8.25	43
2	7.5	65	27	58	5.5	1.19	7.24	7.40	ND	47
3	10	74	33	63	6.2	1.23	6.73	6.43	ND	59

^a Polymerization conditions: L-lactide (44 mg, 0.310 mmol, 10 equiv.), naloxone (10 mg, 0.031 mmol, 1 equiv.) and CHCl₃ (20 μL), in a 5 mL stainless-steel jar with 5 mm (×5) stainless-steel balls for 60 minutes. ^b Determined *via* crude ¹H NMR spectra. ^c Determined use pure ¹H NMR analysis. ^d Determined *via* waters GPC with THF as eluent. ^e Isolated yield for a pure product. ND: not determined.

shows in Table 3. NLX-PLA_{5.0}, NLX-PLA_{7.5}, and NLX-PLA₁₀ were used as abbreviations for the 5.0%, 7.5%, and 10% of the catalyst loading amounts, respectively. The corresponding drug loading of naloxone was determined using ¹H NMR, UV-vis, and LC-MS as described in the ESI (S13–S17†). Table 3 displays that increasing the catalyst loading led to an increase in the conversion and degree of polymerization (DP). However, the drug loadings decreased with increasing catalyst loadings. The increase in lactide conversion and degree of polymerization (DP), consequently leading to a decrease in drug concentration (loading), is likely attributable to the heightened activation of the lactide carbonyl group. Even though 10 mol% (NLX-PLA₁₀) has the highest IE%, conversion%, M_n, and yield, we chose 5.0 mol% (NLX-PLA_{5.0}) as the highest drug loading (~8.3% w/w) and the lowest molecular weight (5.0 kDa) for further therapeutic studies.^{7,9} The findings indicated that the drug loading for NLX-PLA_{5.0} determined *via* LC-MS closely matched our initial assessments from ¹H NMR and UV-vis spectroscopy, demonstrating good agreement (Table 3, entry 1). The polymer structure for NLX-PLA polymers was confirmed by ¹H NMR analysis and was in a good agreement with the previous study by Averick and coworkers.⁹

The initiator efficiency (IE) of ROP reactions was based on the degree of incorporation of the initiators (naloxone) into the polymer chain that was calculated *via* a crude ¹H NMR spectrum. DP was estimated from the ratio of integrals of the vinylic proton from end group naloxone to the methine proton from lactic acid polymer chain that was calculated *via* an ¹H NMR spectrum of a pure product. The calculations of IE%, conversion%, and DP values were described in the ESI (S7).†

In previous research, nanoparticles of various materials have been successfully produced using ball milling mechanochemistry.^{28,29} Additionally, as mentioned earlier, our group successfully synthesized phenolic-PLA_{5.0} polymer nanoparticles in a previous study.²¹ In this current study, mechanochemical synthesis was employed to create NLX-PLA_{5.0} polymer nanoparticles in a single step (Fig. 1). However, the results from this study showed that the average particle size distribution for the NLX-PLA_{5.0} polymer was 591 nm, which is larger than the particle size of the phenolic-PLA_{5.0} polymer (140 nm) observed in our prior investigation.

The variation observed likely stemmed from differences in the instruments utilized and the nature of the resulting products. More investigations are needed to determine the reasons for the variations of the average particle size between the two

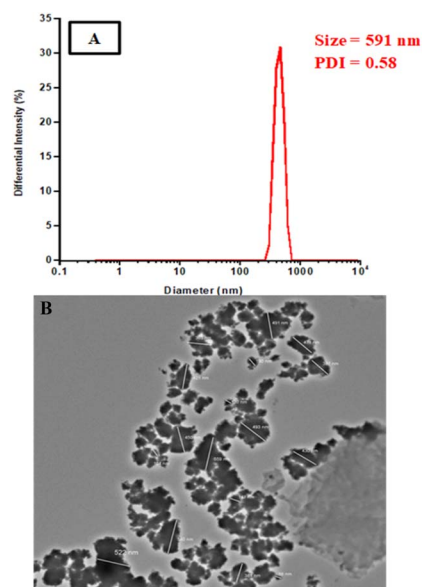


Fig. 1 Characterization of a pure NLX-PLA_{5.0} NPs *via* (A) dynamic light scattering size distribution analysis and (B) transmission electron microscopy. The size value is the average of four measurements *via* DLS.

different PLA-polymers. Characterization of NLX-PLA_{5.0} NPs was done *via* transmission electron microscopy (TEM) and dynamic light scattering (DLS). Fig. 1 displayed the average particle size at 591 nm with a broad unimodal size distribution (PDI = 0.58). In general, the average particle size for the NLX-PLA_{5.0} nanoparticles was ~530 nm from the TEM measurements which was in close with the hydrodynamic size gained from DLS (591 nm).^{30,31} NLX-PLA_{5.0} NPs exhibited a strong, negative ζ potential value (−43 mV) which is consistent with the presence of lactic acid chain ends and verifies their stability in nature.^{7,9} Further investigations will focus on optimizing methods to achieve smaller nanoparticle sizes and narrower size distributions.

Conclusion

This communication emphasizes a mechanochemical approach and optimization strategy for synthesizing naloxone-poly(lactic acid) (PLA) employing both a vibratory ball mill and speed mixer. The mechanochemical synthesis and preparation of naloxone-PLA was achieved using lactide as a monomer,



naloxone as a drug initiator, thiourea as catalyst, and CHCl_3 to perform liquid-assisted grinding (LAG). Additionally, different moles % of catalysts were used (5.0, 7.5, and 10 mol%) to investigate the variation of catalyst loading on IE%, Conv.%, Mn and drug loading%. The results displayed that the reaction performance was improved in the speed mixer when compared to the ball mill due to the increase in average milling jar temperature. Additionally, the result revealed that NLX-PLA_{5,0} with 5.0 mol% loading yielded the highest drug loading of naloxone of 8.3% w/w with the lowest molecular weight (5.0 kDa) compared to other polymers (NLX-PLA_{7,5} and NLX-PLA₁₀). The DLS and TEM were used to determine the hydrodynamic particle size of the NLX-PLA_{5,0} polymer. The results revealed that the average particle size was 591 nm with a broad unimodal size distribution (PDI = 0.58). The corresponding drug loading of naloxone was determined and confirmed using ^1H NMR, UV-vis, and LC-MS techniques. Utilizing this approach, we have successfully synthesized naloxone-PLA nanoparticles with a higher naloxone drug loading of (8.3% w/w) compared to our previous study (6.6% w/w) in the bulk polymerization conditions. Thus, the results point to the potential of mechanochemistry for a drug delivery system. Utilizing this approach, future work will focus on the optimization of naloxone-initiated mechanochemical ROP of lactide to prepare naloxone-PLA nanoparticles that vary in diameters, has narrow dispersities and has increased drug loadings to be used as opioid reversal agents in *in vivo* and *in vitro* studies.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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