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Short communication

Microwave-enhanced reductive amination via Schiff's base formation for block copolymer synthesis

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ABSTRACT

Reductive amination via Schiff's base formation is a widely used reaction for laboratory and industrial applications ranging from protein immobilization to nanoparticle synthesis. One major limitation of this reaction is the slow kinetics and hence, it can take several days for the reaction to reach completion. Here we demonstrate that electromagnetic microwave can be used to accelerate the rate of reduction amination. To demonstrate proof of concept, we utilized the reductive amination between reducing end of dextran and primary amine from *N*-Boc-ethylenediamine as a model reaction. The reaction was conducted at room temperature to demonstrate that the enhancement was mainly due to electromagnetic effects of the microwave rather than thermal effects. We show that reductive amination reaction time was reduced from 72 h to 4 h using microwave irradiation. These results indicate non-thermal microwave effects to expedite reductive amination for synthesizing copolymers. The efficient conjugation of dextran using reductive amination provides an important tool for developing biocompatible copolymers using carbohydrates.

1. Introduction

Reductive amination is the reaction of aldehydes or ketones with a primary amine through the formation of Schiff's base and reduction of intermediate imine, which leads to the formation of a secondary amine (Borch, Bernstei, & Durst, 1971). Reductive amination has been explored in a variety of applications including small molecule synthesis (Borch et al., 1971), fluorescent labeling of biomolecules (Meunier & Wilkinson, 2002), immobilization of proteins on surfaces (Stults, Asta, & Lee, 1989), purification of proteins (Baues & Gray, 1977), building libraries of fluorescent molecules (Tesfu, Maurer, & Moeller, 2006), polymer synthesis (Ouchi, Uchida, Arimura, & Ohya, 2003), and ultrasmall superparamagnetic nanoparticle synthesis (Mornet, Portier, & Duguet, 2005). Reductive amination typically requires a reducing agent to convert intermediates such as an imine, to a stable compound. Sodium cyanoborohydride is a commonly used reducing agent due to its selectivity towards reduction of imines (Borch et al., 1971). Reductive amination with sodium cyanoborohydride is a very time consuming reaction and typically requires a few days to reach completion (Borch et al., 1971). Microwave irradiation has been explored previously for significant improvements in reaction times of reductive amination using sodium borohydride and clay (Varma & Dahiya, 1998), carbohydrates and glass surface (Yates et al., 2003) as well as *N*-substituted glycine derivatives (Santagada et al., 2005). Most of the previous experiments have been conducted at an elevated temperature, which makes it difficult to differentiate between the thermal and non-thermal effects of microwave irradiation (de la Hoz, Diaz-Ortiz, & Moreno, 2005). We here demonstrate the enhancement of reductive amination reaction by non-thermal effects of microwave at room temperature.

Dextran is a biocompatible polysaccharide with α-1,6 glycosidic linkages in the backbone and α-1,3 branches. An abundance of hydroxyl groups on the backbone are responsible for maintaining water solubility and biocompatibility of dextran. Each dextran polymer has one reducing end, which can be exploited for reactions without backbone modification. Reaction with the reducing end of dextran typically requires several days due to the low concentration of free aldehyde groups at equilibrium compared to the cyclic hemiacetal form (Schatz & Lecommandoux, 2010; Zhang, Dou, & Jin, 2010). Microwave can be used to increase the concentration of free aldehyde groups on the reducing end (Pagnotta, Pooley, Gurland, & Choi, 1993). Hence, it is postulated that microwave can allow efficient end to end conjugation using dextran.

2. Experimental

All chemical reagents were purchased from Sigma–Aldrich Canada unless otherwise noted. In a typical reaction, 6 g of dextran ($M_r \sim 6000$ Da) was added to 15 mL 0.05 M borate buffer (pH 8.2)

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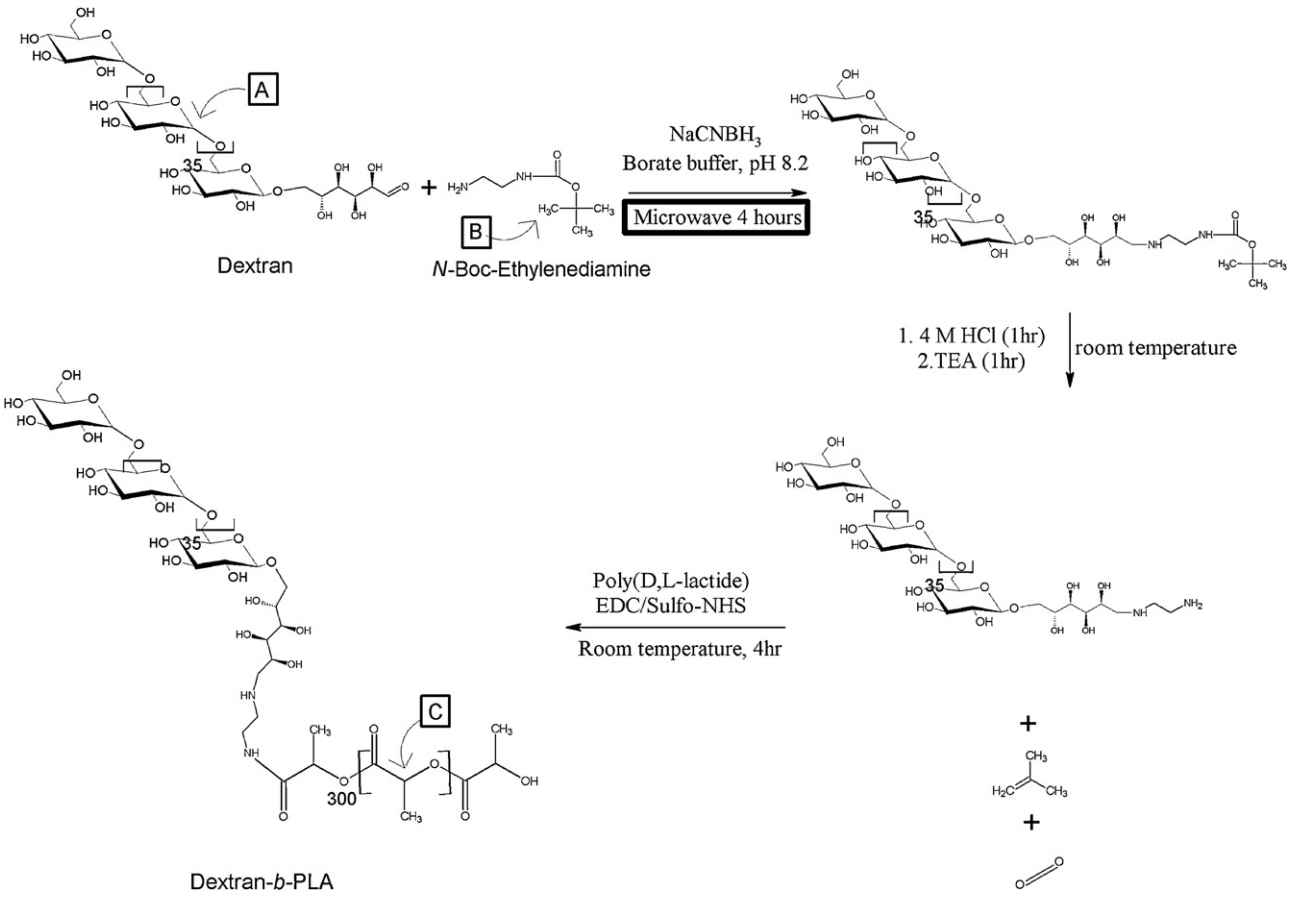

Scheme 1. Reaction scheme for the synthesis of dextran-*b*-PLA copolymer enhanced by microwave. A–C indicate protons which are identified in NMR spectra in Figs. 1 and 2.

along with 4 g of *N*-Boc-ethylenediamine (CNH Technologies, Massachusetts, USA) and 1 g sodium cyanoborohydride. The reductive amination proceeded at room temperature in dark for either 4 h or 72 h. When a Discover Microwave System (CEM, Canada) was used, the reaction temperature was set to 20 °C and microwave power was set to a maximum of 100 W. Reductive amination was conducted in a sealed 35 mL cylindrical glass reaction vessel. To maintain the temperature of reagents at 20 °C throughout the reaction, the reaction vessel was cooled down using pressurized air and microwave irradiation power was modulated by a feedback loop built into the microwave system. ^{1}H NMR samples of dextran and dextran conjugated with *N*-Boc-ethylenediamine (dextran-Boc) were prepared in D_2O at 30 mg/mL after washing with methanol. NMR spectroscopy was conducted in a Bruker 300 MHz instrument.

The Boc group from the dextran-Boc conjugate was deprotected for 1 h using 4 M hydrochloric acid. This was followed by deprotonation of the NH_3^+ groups on the reducing terminus of dextran using triethylamine to raise the pH to 9 for 1 h. After washing with methanol, the amine terminated dextran (dextran-NH_2) samples were assessed using ^{1}H NMR to confirm the absence of Boc peak. Subsequently, the dextran-NH_2 (~3 g) was added to 40 mL DMSO in the presence of 5 g acid-terminated poly(D,L-lactide) (PLA, M_w ~ 20 kDa Lakeshore Biomaterials, Alabama, USA) and conjugated with the aid of 120 mg *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC) and 300 mg *N*-hydroxysulfosuccinimide (Sulfo-NHS, CNH Technologies, Massachusetts, USA) at room temperature for 4 h. The product was washed with methanol and then purified using acetone to remove unreacted dextran. Upon addition of acetone, a cloudy suspension was formed, which was centrifuged at 4000 rpm for 10 min. The supernatant from this suspension was extracted carefully and purged with air followed by drying *in vacuo*. After purification, ^{1}H NMR samples were prepared as follows: pure dextran sample was prepared in D_2O at 30 mg/mL, pure PLA samples were prepared in $CDCl_3$ at 5 mg/mL, whereas the block copolymer of dextran and PLA (dextran-*b*-PLA) samples were prepared in DMSO-d6 at 30 mg/mL.

Nanoparticles were formed by dissolving dextran-*b*-PLA in DMSO at 10 mg/mL and adding 1 mL of the solution in a dropwise manner to 10 mL of Millipore filtered water under magnetic stirring. The particle sizes were measured by 90Plus Particle Size Analyzer (Brookhaven, λ = 659 nm at 90°) at room temperature. The volume averaged multimode size distributions (MSD) were reported.

3. Results and discussion

Reductive amination reaction was conducted between the reducing end of dextran and reactive amine terminal of *N*-Boc-ethylenediamine in the presence of a reducing agent sodium cyanoborohydride. Reductive amination was carried out for either 4 h or 72 h under ambient conditions. To characterize the effect of microwave, reductive amination was carried out in a microwave system at room temperature for 4 h. The reductive amination of dextran was characterized by ^{1}H Nuclear Magnetic Resonance (NMR) analysis. The ^{1}H NMR spectra showed that the proton on carbon 1 of dextran (Scheme 1) repeating units appeared at 4.86 ppm (Cheetham, Fialabeer, & Walker, 1990) (Fig. 1, peak A). The integration of peak A was compared to the integration of the Boc peak (Scheme 1) at 1.3 ppm (Geall, Taylor, Earll, Eaton, & Blagbrough,

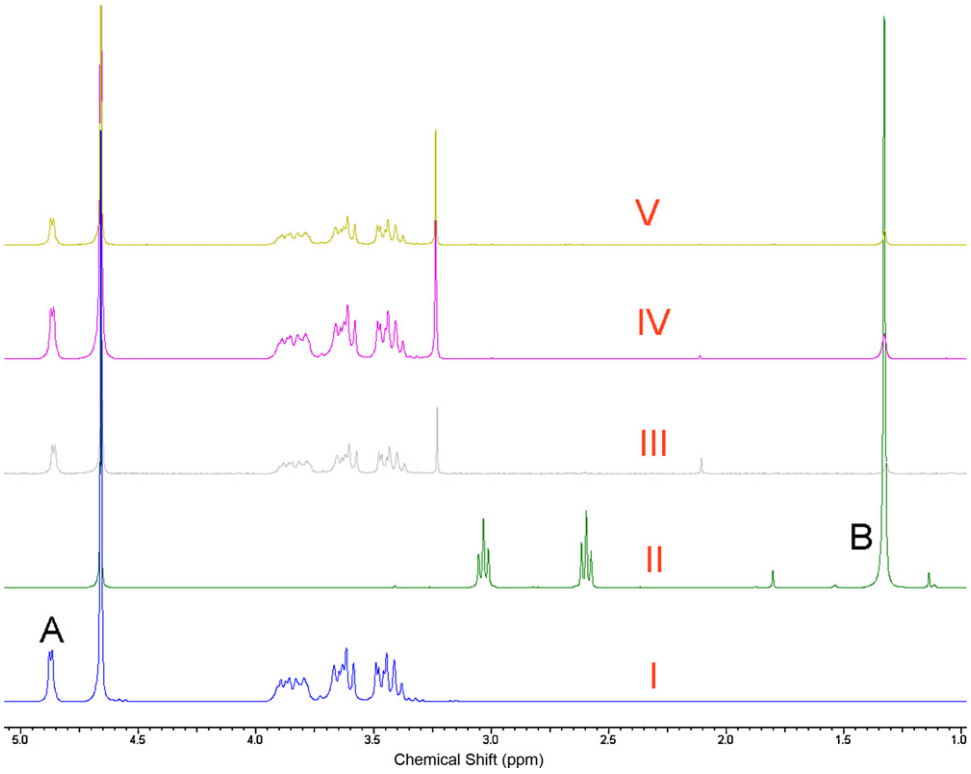

Fig. 1. ^{1}H NMR spectra in D_2O for reductive amination reaction between dextran and *N*-Boc-ethylenediamine. Peak A resembles C1 proton peak on dextran repeating units and peak B resembles Boc peak on *N*-Boc-ethylenediamine. Labels are I: pure dextran sample; II: pure *N*-Boc-ethylenediamine; III–V: dextran conjugated to *N*-Boc-ethylenediamine with reaction conditions of non-microwave 4 h, non-microwave 72 h and microwave 4 h respectively. Peak assignments are outlined in Table 3.

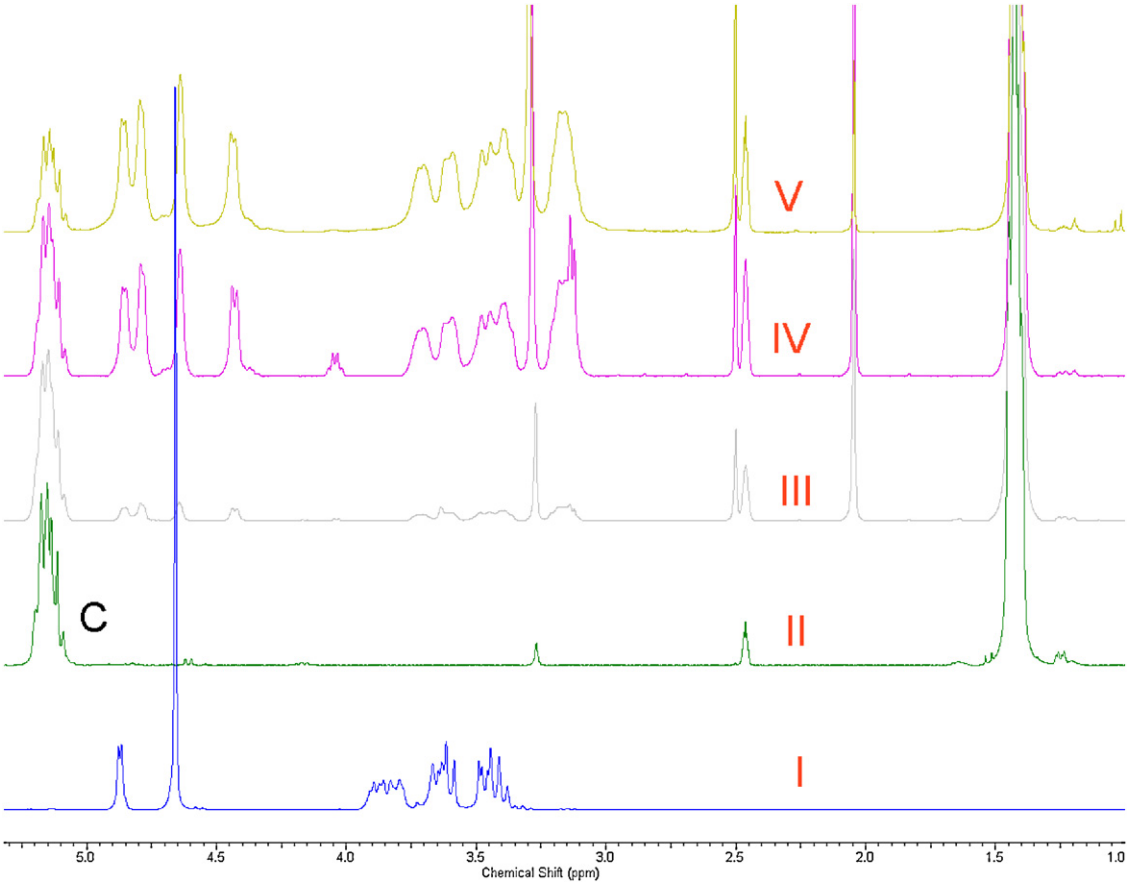

Fig. 2. ^{1}H NMR spectra for conjugated dextran-*b*-PLA. Peak C resembles CH proton peak on PLA repeating units. Labels are I: pure dextran sample in D_2O, II: pure PLA sample in $CDCl_3$; III–V: dextran-*b*-PLA with initial reaction conditions of non-microwave 4 h, non-microwave 72 h and microwave 4 h respectively in DMSO-d6. Peak assignments are outlined in Table 3.

Table 1
Integrals from ^{1}H NMR of dextran conjugated to *N*-Boc-ethylenediamine.

Peak label	Peak position (ppm)	Relative integrals under the peaks for different reactions		
		Non-microwave 4 h	Non-microwave 72 h	Microwave 4 h
Boc peak (peak B)	[1.28 . . . 1.38]	0.243	0.384	0.326
Dextran C1 peak (peak A)	[4.80 . . . 4.94]	1.00	1.00	1.00
% Boc conjugated		63%	100%	85%

Table 2
Integrals from ^{1}H NMR of dextran-*b*-PLA block copolymer.

Peak label	Peak position (ppm)	Relative integrals under the peaks for dextran-*b*-PLA		
		Non-microwave 4 h	Non-microwave 72 h	Microwave 4 h
Dextran C1 peak (peak A)	[4.82 . . . 4.92]	0.0468	0.335	0.864
PLA CH peak (peak C)	[5.00 . . . 5.27]	1.00	1.00	1.00
% PLA conjugated		5%	39%	100%

2000) (Fig. 1, peak B) from *N*-Boc-ethylenediamine. Peak A (Fig. 1) is used as reference for the integrals in each spectrum and % Boc conjugated is determined by comparing each integral to the maximum integral from peak B (Fig. 1) among the three reaction conditions. These results shown in Table 1 indicate that dextran-Boc synthesized using microwave has a significantly higher Boc conjugation efficiency compared to the non-microwave reaction for 4 h. The conjugation efficiency of 4-h microwave reaction is also comparable to the 72-h non-microwave reaction. When in solution, glucose residues are known to undergo an equilibration process between closed unreactive ring structures, which proceeds via the open chain reactive intermediate (Pagnotta et al., 1993; Yates et al., 2003), a process known as mutarotation (Pagnotta et al., 1993). It is postulated that the increased reaction rate from microwave irradiation is due to increased rate of mutarotation of the reducing end glucose unit. This transition allows access to the reducing end of dextran molecule and since imine formation is the rate limiting step (Borch et al., 1971) in reductive amination, the conjugation process is significantly enhanced.

The dextran-Boc conjugate can be utilized to form an end to end block copolymer with the biodegradable PLA. Such an amphiphilic block copolymer can be used for self-assembly of nanoparticles. These nanoparticles have the potential of encapsulating hydrophobic drugs in the PLA core, while being biocompatible and water soluble due to the dextran shell (Verma, Liu, Chen, Meerasa, & Gu, 2011). Consequently, the dextran-Boc conjugate is deprotected. The resulting amine dextran-NH_2 is conjugated to acid terminated PLA to form the linear block copolymer, dextran-*b*-PLA. The block copolymer is characterized using ^{1}H NMR in DMSO-d6 to determine the relative amounts of PLA conjugated to dextran processed with and without microwave exposure. In the ^{1}H NMR spectra, the multiplet at 5.2 ppm belongs to the CH on the lactic acid (Scheme 1) repeating unit of PLA (Espartero, Rashkov, Li, Manolova, & Vert, 1996) (Fig. 2, peak C) and is compared to peak A from dextran repeating units. Using peak C as reference, integrals were calculated and normalized by the maximum integral values for the dextran peaks. This data is presented in Table 2 and demonstrates that microwave irradiated dextran provides a 20-fold increase in conjugation efficiency of PLA when compared to non-microwave irradiated dextran with the same reductive amination reaction time of 4 h. The PLA conjugation efficiency of microwave irradiated dextran-*b*-PLA was 2.5 times higher compared to 72 h reductive amination reaction sample. Thus, microwave provides an extremely useful route for polymer conjugation by reducing the reaction time by about 18-fold. Table 3 summarizes the peak assignments from Figs. 1 and 2 based on previous literature (Cheetham et al., 1990; Espartero et al., 1996; Geall et al., 2000; Zhang et al., 2010).

Table 3
Peak assignment for ^{1}H NMR spectra presented in Figs. 1 and 2.

Figure	Peak position (ppm)	Peak assignment
Fig. 1	[1.28 . . . 1.38]	CH_3 Boc peak
	[2.55 . . . 2.65]	CH_2 peak from –NH–CH_2–CH_2–NH_2
	[2.95 . . . 3.10]	CH_2 peak from –NH–CH_2–CH_2–NH_2
	[3.10 . . . 4.00]	Glycosidic H from dextran (C2–C6)
	[4.80 . . . 4.94]	Anomeric H from dextran (C1)
Fig. 2	[1.30 . . . 1.55]	CH_3 peak from PLA
	[5.00 . . . 5.27]	CH peak from PLA

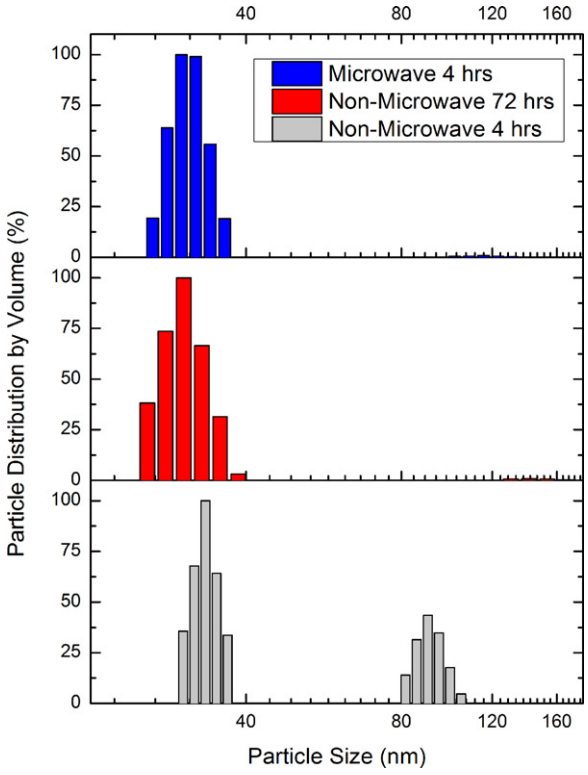

Fig. 3. Particle size distribution of nanoparticles formed by nanoprecipitation of dextran-*b*-PLA copolymer using dextran from various reaction conditions. Gray columns indicate non-microwave conditions for 4 h, red columns indicate non-microwave conditions for 72 h and blue columns indicate microwave exposure for 4 h. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The amphiphilic block copolymer dextran-*b*-PLA was assessed for self-assembly of nanoparticles by using nanoprecipitation. The resulting nanoparticles were characterized using dynamic light scattering. The nanoparticles obtained using microwave exposure or 72 h of reaction time were both comparable in size being 29.3 ± 2.0 nm and 29.0 ± 2.0 nm respectively and were half the size (60.3 ± 11.4 nm) of that obtained from 4 h reaction without microwave exposure. The size distributions of these formulations are shown in Fig. 3 and demonstrate that the 4 h reaction sample without microwave has a considerable population with bigger particle sizes which are absent in the other two reaction conditions. It is postulated that the bigger particle size population around 90 nm is a result of nanoparticles formed from unmodified PLA since particle sizes of about 100 nm have been observed in literature for PLA nanoparticles (Musumeci et al., 2006). The nanoparticle size and distribution exemplifies that microwave irradiation provides considerable savings in time of reaction while creating products with same properties. Small particle size is important for the application of these nanoparticles in cancer drug delivery to allow accumulation by enhanced permeation and retention effect (Cho, Wang, Nie, Chen, & Shin, 2008; Verma et al., 2011).

4. Conclusion

In this communication, we have highlighted a method that utilizes the non-thermal effects of microwave irradiation for enhancing reductive amination reaction in polymer conjugation. This method has allowed us to synthesize the desired nanoparticles in a fraction of the time. This advancement provides promising opportunities for the scale-up and combinatorial synthesis of products involving reductive amination reactions.

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