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RESEARCH PAPER

The Effect of Polymerization Method in Stereo-active Block Copolymers on the Stability of Polymeric Micelles and their Drug Release Profile

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ABSTRACT

Purpose To investigate the effect of polymerization method on the stability and drug release properties of polymeric micelles formed using stereo-active block copolymers.

Methods Diblock copolymers consisting of methoxy poly ethylene oxide (MePEO) and poly(lactide)s (PLA)s of different stereochemistry were synthesized by bulk or solution polymerization. Polymers and micelles were characterized for their chemical structure by ^{1}H NMR, optical rotation by polarimetry, critical micellar concentration by fluorescence spectroscopy, thermal properties by differential scanning calorimetry, morphology by transmission electron microscopy and size as well as kinetic stability by dynamic light scattering. Release of encapsulated nimodipine from polymeric micelles at different levels of loading was also investigated.

Results Solution polymerization yielded a higher degree of crystallinity for stereo-regular PLA blocks. Consequently, the related polymeric micelles were kinetically more stable than those prepared by bulk polymerization. At high drug loading levels, the release of nimodipine was more rapid from polymeric micelles with crystalline cores. At lower levels of drug loading, drug release was slower and independent of the stereochemistry of the core.

Conclusions The results underline the effect of polymerization method in defining core crystallinity in stereoregular block copolymer micelles. It also shows the impact of core crystallinity on enhancing micellar stability and drug release.

KEY WORDS drug release · kinetic stability · poly ethylene oxide · poly lactic acid · polymeric micelle

ABBREVIATIONS

CMC	Critical micellar concentration
DL	Drug loading
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
DSC	Differential scanning calorimetry
EE	Encapsulation efficiency
MD	Molecular dynamic
Mn	Number average molecular weight
NMR	Nuclear magnetic resonance spectroscopy
PDLA	Poly(D-lactide)
PD/LLA	Poly(D/L-lactide)
PDLLA	50–50 poly(D,L-lactide)
PEG	Poly ethylene glycol
PEO	Poly ethylene oxide
PI	Polydispersity index
PLA	Poly lactide
PLLA	Poly(L-lactide)
RAC	Racemic
ROP	Ring opening polymerization
SnO_2	Stannous octoate (tin(II) bis(2-ethylhexanoate))
SDS	Sodium dodecyl sulfate
TEM	Transmission electron microscopy
THF	Tetrahydrofuran

INTRODUCTION

Polymeric micelles have been the focus of much interest in research owing to a wide variety of potential applications in pharmaceutical and biomedical field (1–4). In pharmaceutical field, the interest is especially keen on their potential use for solubilization of poorly soluble drugs, sustained drug release

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following parenteral administration and/or drug targeting (5,6). For sustained and targeted drug delivery, development of biocompatible and biodegradable polymeric micelles with improved thermodynamic as well as kinetic stability, high capacity for drug solubilization and a slow or delayed rate of drug release are desirable properties.

Although polymeric micelles are superior to their low molecular weight surfactant counterparts in terms of thermodynamic and kinetic stability, their stability profile has yet to present the optimal characteristics (6). Often premature drug release occurs after systemic administration either because the polymeric micellar structure cannot retain their integrity upon introduction to infinite dilution in blood circulation, or because the drug cannot be kept within the micellar carrier (7). Retaining integrity is essential for prolonging the circulation time of polymeric micelles, attaining sustained drug release and/or preferential accumulation of polymeric micelles in the target (e.g., tumor or inflammation) sites. Holding the drug by the micellar carrier, on the other hand, will ensure similar fate and distribution for the encapsulated drug to that of the micellar carrier.

The aim of this study was to assess the effect of polymerization method (bulk *versus* solution polymerization) on the crystalline structure of the core-forming block in stereoactive block copolymers; and to evaluate the eventual influence of core crystallinity on micellar stability and drug release profile. Polymeric micelles with a poly(lactide) (PLA) segment are suitable candidates for such studies. Lactide has two chiral carbons in its structure and three different enantiomers; i.e., L-lactide, D-lactid and meso-lactide. Polymerization of either L-, D- or meso lactide under mild conditions is expected to lead to the production of poly(L-lactide) (PLLA), poly(D-lactide) (PDLA) or poly(D/L-lactide) (PD/LLA), respectively, which are mostly stereo-regular (i.e., either isotactic or syndiotactic) (Scheme 1). The stereo-regular PLAs can theoretically form semi-crystalline core structures in PEO-PLA micelles. On the other hand, polymerization of a mixture of L- and D-lactide can lead to the production of poly(D, L-lactide) (PDLLA) (Scheme 1) with less or no degree of stereo-regularity (i.e., atactic polymers), which are known to form majorly amorphous polymeric micellar cores (8).

Previous studies have shown an effect for the stereochemistry of PLA segment on the kinetic stability (9) and release behavior of assembled polymeric micelles (10). Enhanced resistance against sodium dodecyl sulfate (SDS) induced dissociation for PEO-PLLA micelles that had stereo-regular cores compared to PEO-PDLLA micelles with no stereo-regularity in their core has been reported (9). In addition, a lower partition coefficient for pyrene in stereo-regular and crystalline cores compared to those with no stereo-regularity in the core and amorphous structures has been observed (11). Other studies have indicated an effect for the degree of crystallinity of the PLA block on the rate of drug release from PLA-PEO-PLA micelles, where the release rates of sulindac and tetracaine was shown to be much faster from micelles with semi-crystalline PLA cores as compared to those with amorphous ones (10). In this manuscript, the effect of polymerization method on the crystallinity of the PLA block in methoxy poly(ethylene oxide)-poly(lactide)(PEO-PLA) block copolymers of different stereo-chemistry was investigated. The eventual effect of PLA crystallinity on the stability of polymeric micelles and their release profile for a model hydrophobic drug, i.e., nimodipine, at different loading levels was then evaluated. For this purpose, diblock copolymers consisting of methoxy PEO and PLAs of different stereochemistry (Scheme 1) were prepared by either bulk or solution polymerization using stannous octoate as catalyst. This led to the production of block copolymers with different degrees of crystallinity in the PLA block. Nimodipine, a second-generation dihydropyridine calcium antagonist with apparent selectivity for cerebral blood vessels (12) was selected as a model hydrophobic drug for incorporation in polymeric micelles. This choice was made based on the results of our previous study where molecular dynamics (MD) simulation have shown a preferential interaction of nimodipine with the core (rather than the shell) forming block in a series of micelle-forming block copolymers (13). The effect of the degree of PLA crystallinity on the kinetic stability of polymeric micelles and the release profile of nimodipine from the micellar carrier at various drug loading levels was then investigated.

MATERIALS AND METHODS

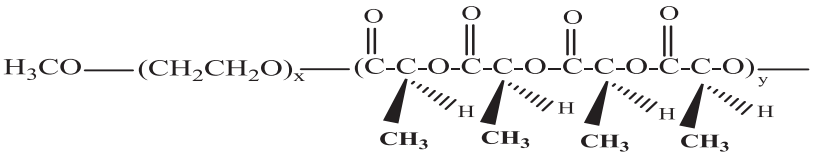
Materials

L-lactide (98%) and meso D/L-lactide (99%) were purchased from Alfa Aesar, Lancashire, UK. D-lactide (98%) was a generous gift from Purac, Schiedam, Netherlands. Methoxy-PEO 5000 (MePEO), sodium dodecyl sulfate (SDS) and tin(II) bis(2-ethylhexanoate) (stannous octoate) were obtained from Sigma, St Louis, MO, USA. Stannous octoate was dried and purified using anhydrous magnesium sulfate, dry toluene and vacuum distillation (14). All other chemicals and reagents used were of analytical grade.

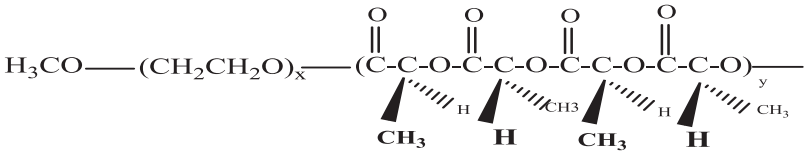
Synthesis of Diblock Copolymers in Bulk

L-lactide, D-lactide, meso D/L lactide and MePEO 5000 were dried in a vacuum oven at 65°C overnight. Diblock copolymers of MePEO (as the hydrophilic block) and poly(L-lactide) (PLLA), poly(D/L-lactide) (PD/LLA) and poly(D, L- lactide) were prepared using ring opening polymerization of either L-lactide, meso-lactide or a 50–50 racemic mixture of L-lactide and D-lactide, respectively. The prepared diblock copolymers are abbreviated as PEO-

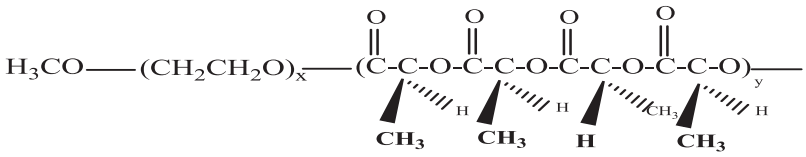
Scheme 1 The schematic model of synthesized diblock copolymers having isotactic PLLA, syndiotactic PD/LLA and atactic PDLLA 50–50 structures.



Isotactic PLLA



Syndiotactic PD/LLA



Atactic PDLLA 50-50

PLLA, PEO-PD/LLA and PEO-PDLLA 50–50 in the manuscript, respectively (Scheme 1). Briefly, MePEO (0.5 g, 0.1 mmol) was reacted with (L-lactide), (D/L-lactide) (0.5 g, 3.4 mmol) or a 50–50 ratio of L-lactide and D-lactide (total weight 0.5 g), in an ampule using 5 mg (0.5 w % of the weight of reactants) stannous octoate as catalyst. The ampule was then sealed and left at 160°C for 7 h. The obtained product was dissolved in dichloromethane, and precipitated by n-hexane, dried at room temperature in a vacuum oven overnight and washed with anhydrous ethyl ether. This was followed by drying in a vacuum oven at room temperature overnight.

Synthesis of Diblock Copolymers in Solution

To prepare diblock copolymers of PEO-PLLA, PEO-PD/LLA and PEO-PDLLA 50–50 in solution, MePEO (0.5 g, 0.1 mmol) was reacted with (L-lactide), (D/L-lactide) (0.5 g, 3.4 mmol) or a 50–50 ratio of L-lactide and D-lactide (total weight 0.5 g), respectively, in 10 ml of dry toluene. Purified stannous octoate (5 mg, 0.5 w % of the weight of reactants) was added to the solution. The reaction mixture was refluxed for 24 h. The synthesized polymers were precipitated in n-hexane, dried at room temperature in a vacuum oven overnight. The copolymers were washed using anhydrous ethyl ether and dried under vacuum at room temperature.

Self-Assembly of Prepared Block Copolymers

Preparation of colloidal dispersions from synthesized block copolymers was tried by co-solvent evaporation method adding organic solvent to water. Briefly, the diblock copolymer (20 mg) was dissolved in tetrahydrofuran (THF) (2.5 mL). After sonication for 10 min, this solution was added in a drop wise manner to 5 mL deionized water while stirring. The solution was stirred for 24 h under a fume hood to remove the organic solvent by evaporation.

Characterization of Block Copolymers and Their Self-Assembled Structures

^{1}H NMR Spectroscopy

^{1}H NMR was performed on a Bruker, ASEND^{TM} 600 MHz spectrometer. Polymers were dissolved in $CDCl_3$ or assembled in D_2O at a concentration range of 1–4 mg/ml. ^{1}H NMR spectra were generated using $CDCl_3$ as the solvent and dimethyl sulfone as the internal standard, respectively. All integrations were referenced to proton peaks for the internal standard at about $\delta = 3.0$ ppm.

Gel Permeation Chromatography (GPC)

The poly dispersity index of the copolymers were determined at room temperature by GPC using a Shimadzu LC-10 AD HPLC pump and M302 triple detector array (Viscotek Corp., Houston, TX) connected to a 10 μm Waters Strygel HT3 column. The mobile phase was THF with a flow rate of 1 ml/min. The injection volume of the sample was 20 μl at a polymer concentration of 1% (w/v). The molecular weights of the polymers were determined relative to polyethylene glycol standards using the universal calibration and OmniSEC 4.7 software (Malvern Instruments Ltd., Malvern, UK).

Determination of Thermal Behavior of Copolymers and Their Micelles

The melting and crystallization temperatures as well as corresponding enthalpies for block copolymers and polymeric micelles (empty and drug loaded freeze-dried ones) were identified by Differential Scanning Calorimetry (DSC), Q2000-1576, TA Instrument. The heating rate was 2°C/min under nitrogen atmosphere. Samples were weighed (3–5 mg) in Tzero aluminum pans. To determine reproducibility, melting temperatures and enthalpies were measured in three cycles. First cycle was from 8°C to 170°C, second cycle was from 170°C to 8°C and third cycle was from 8°C to 170°C.

Determination of Optical Activity of Block Copolymers

Specific rotation, [α], of various diblock copolymers was determined in dimethyl sulfoxide (DMSO) at a concentration of 1 g/dl at room temperature using Perkin-Elmer 241 polarimeter at a center wavelength of 589 nm.

Determination of Critical Micellar Concentration (CMC)

The CMC of diblock copolymers was determined using pyrene as the fluorescent probe. From a stock solution of 0.435 mM pyrene in acetone, a 1 μM solution was prepared by serial dilution. Aliquots of this solution (1.8 ml) were transferred to glass vials and the solvent was allowed to evaporate under vacuum while protected from light overnight. A series of polymer solution (0.060 to 125 μg/ml) in deionized water were added to the vials. The final pyrene concentration in each vial was 0.6 μM. The solutions were heated in water bath for 1 h at 65°C and then cooled overnight. Nitrogen gas was used for deoxygenation of solutions prior to measurements. Excitation fluorescence spectra of pyrene was recorded between 300 and 360 nm, keeping the emission wavelength and excitation slit at 390 and 5 nm, respectively. The ratio of fluorescence intensity of pyrene excitation peak at 337 nm over the one at 334 nm was plotted *vs.* logarithm of the polymer concentration. The intersection of the two linear graphs in the sigmoidal curve, i.e., the onset of a rise in the excitation fluorescence intensity ratio of pyrene, was defined as the CMC value.

Determination of the Size of Self-Assembled Structures

The hydrodynamic diameter of assembled structures with or without drug at a polymer concentration of 4 mg/ml was measured using dynamic light scattering (DLS) ZETA-Sizer Nano (Malvern Instruments Ltd., Malvern, UK). The analysis was performed at a scattering angle of 173° at 25°C at least three times.

Determination of the Morphology of Self-Assembled Structures

Transmission electron microscopy (TEM) (FEI Morgagni ™ 268, North America NanoPort, Oregon, USA) was used to observe the morphology of self-assembled structures. For TEM measurements, the polymer concentration in micellar solutions was 1 mg/ml. Samples were placed onto copper grid, dried at room temperature, and examined using TEM by negative staining with 4% uranyl acetate.

Assessing the Kinetic Stability of Nanostructures

The micellar solutions (1 mg/ml) were mixed with sodium dodecyl sulfate (SDS) (20 mg/ml) at a 2:1 v/v ratio. Scattered light intensity and polydispersity index (PI) were measured at different incubation time intervals using ZETA-Sizer Nano (Malvern Instruments Ltd., Malvern, UK) at scattering angle of 173° at 25°C.

Encapsulation of Nimodipine in Polymeric Micelles

Drug-loaded polymeric micelles were prepared using the same procedure as described for the self-assembly conditions, except nimodipine (1 or 2 mg) was dissolved along with the block copolymers in THF. After the evaporation of THF, the micelle solution was centrifuged to remove the precipitated drug. To measure the concentration of loaded drug, UV spectroscopy was used. First, micellar solution was diluted with equal volume of methanol. Nimodipine concentration was measured by UV spectroscopy at 357 nm against a calibration curve plotted at 5–120 μg/ml nimodipine concentration in methanol:water 50:50 solution (13). The level of encapsulated nimodipine was calculated by multiplying nimodipine concentration by sample volume while taking the dilution factors into consideration.

The encapsulation efficiency (EE) and drug loading (DL) percentages were calculated from the following equations.

$$EE(\%) = \frac{\textit{the amount of encapsulated nimodipine}}{\textit{the total feeding amount of nimodipine}} \times 100$$

$$DL(\%) = \frac{\textit{the amount of encapsulated nimodipine}}{\textit{the total amount of polymer}} \times 100$$

For block copolymers prepared in bulk, encapsulation of nimodipine was also tried by adding water (5 ml) to a solution of nimodipine (1 or 2 mg) and polymer (20 mg) in THF (2.5 ml).

Assessing the In Vitro Drug Release

A dialysis bag (Spectraphor, Mw cutoff 3500 g/mol) containing 5 ml of the drug-loaded micelle solution was placed in 500 ml deionized water at 37°C in a Julabo SW 22 shaking water bath (Seelbach, Germany). At predetermined time intervals, 200 μl of the dialysis bag content was collected and diluted with methanol. The 500 ml deionized water in the recipient media was taken out and replaced by fresh water at the same time interval. The concentration of nimodipine remained in the dialysis bag at each time point was determined using UV spectroscopy (Beckman Coulter DU 730) at 357 nm as described before. The remaining drug concentration was subtracted from the initial concentration of the drug and used to plot the cumulative drug release (%) *versus* time.

Statistical Analysis

Statistical analysis was performed either using unpaired Student's *t*-test or one way ANOVA with Tukey post-test analysis. The significance level (α) was set at 0.05. For nonlinear regression analysis, Graphpad prism was used (version 5.00, Graphpad Software Inc., La Jolla, CA, USA). All experiments were conducted in triplicate unless mentioned otherwise in the text, Tables or Graphs.

RESULTS

Characterization of Synthesized Block Copolymers

^{1}H NMR Spectroscopy

The ^{1}H NMR spectra of synthesized PEO-PLLA, PEO-PD/LLA and PEO-PDLLA 50–50 diblock copolymers prepared by bulk polymerization are shown in Fig. 1a–c. Similar spectra were obtained for polymers prepared by solution polymerization (Fig. S1a–c). Signals at 3.7, 3.4, 5.2 and 1.5 ppm in ^{1}H NMR spectra correspond to protons in CH_2 (b), CH_3 (a), CH (c) and CH_3 (d), respectively. The molecular weight of PLLA, PD/LLA and PDLLA 50–50 segments was determined from ^{1}H NMR spectra by examining the peak intensity ratio of methine proton of the PLA segment ($COCH(C\underline{H}_3)O$: δ= 5.2 ppm) and the methylene protons of MePEO ($OC\underline{H}_2C\underline{H}_2$: δ=3.7 ppm). The theoretical and calculated molecular weights for block copolymers were in good agreement with each other for both methods of polymer synthesis (Table I).

Optical Activity of Copolymers

PEO-PLLA copolymer yielded high optical purity irrespective of the method of polymerization. This was revealed by a high specific rotation in the block copolymer (−53, 38% of pure PLLA) which was reflective of the corresponding w % of PLA block (42%) in the block copolymer structure under study (Table I), whereas PEO-PD/LLA and PEO-PDLLA 50–50 diblock copolymers were racemized in both methods of polymerization. Optical purity of the PLLA block was significantly higher in the PEO-PLLA copolymer synthesized by solution polymerization compared to PEO-PLLA of bulk polymerization (Student's *t* test, $P< 0.05$). Racemization of lactide can occur at high temperatures used in bulk polymerization (8,15) leading to lower optical activity of PLLA.

Thermal Behavior of Copolymers

Thermal behavior of block copolymers polymerized in bulk and solution were investigated using DSC. To determine reproducibility, melting temperatures and enthalpies were measured at a heating rate of 2°C/min in three cycles. The thermograms of the second and third cycles are shown in Fig. 2a and b for clarity of presentation. Homopolymer of PEO showed a melting peak at 63.53°C. Crystallization of the PEO occurred after slow cooling of the homopolymers from the melt at 44.78°C. The enthalpies of melting and crystallization for the PEO homopolymer were 216 and 189 J/g, respectively. The melting and crystallization temperatures and enthalpies for PEO in the block copolymers were reduced irrespective of the stereochemistry of the PLA block or the method of PLA copolymerization (Table II). This indicates a lower degree of PEO crystallinity in the copolymer, possibly due to the interference of the PLA block with the crystallization of MePEO (19). The interference of core forming block in crystallization of PEO seem to be higher for PLLA than PD/LLA or PDLLA 50–50 (ΔH of crystallization and melting of PEO is lower in PEO-PLLA compared to PEO-PD/LLA and PEO-PDLLA 50–50, irrespective of polymerization method, Table II). This may indicate a better interaction between crystalline segments of core forming block (which is higher in PLLA compared to PD/LLA and PDLLA 50–50) and PEO chains leading to interference in the crystalline PEO structure.

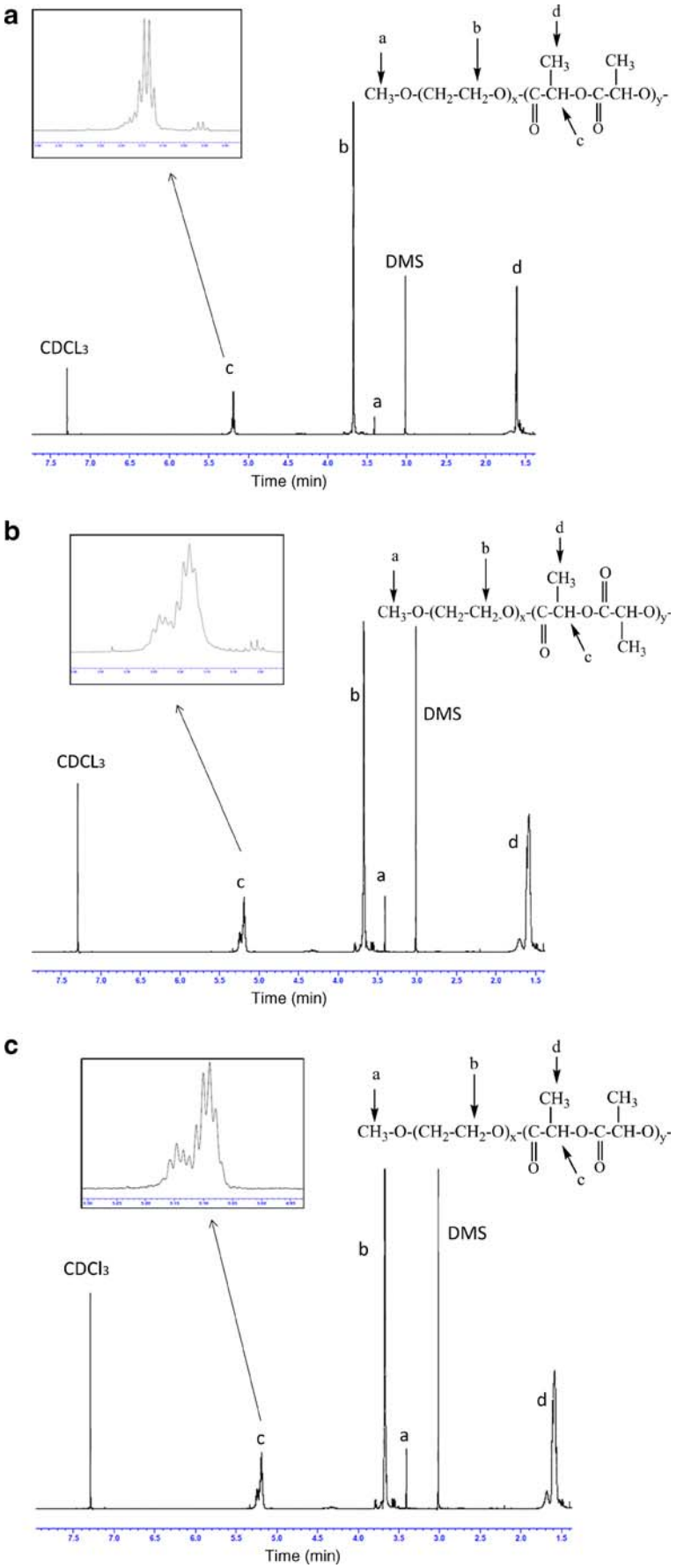


Fig. 1 ^{1}H NMR spectra and peak assignments for (**a**) PEO-PLLA; (**b**) PEO-PD/LLA; and (**c**) PEO-PDLLA 50–50 block copolymers in $CDCl_3$ with dimethyl sulfone (DMS) as the internal standard.

Table I Characteristics of Synthesized Block Copolymers

Polymer	Polymerization method	Theoretical M.wt. ($g.mol^{-1}$)	M_n ($g.mol^{-1}$) [a]	$M_w/M_n \pm SD$ [b]	PLA (w %) [c]	$[\alpha] \pm SD$ [d]	$[\alpha]/140$ (%) [e]
PEO-PLLA	Bulk	9968	8582	1.46 ± 0.06	42	−53 ± 0.08[f]	38 ± 0.06
PEO-PD/LLA			8629	1.24 ± 0.05	42	0.64 ± 0.05	0.5 ± 0.01
PEO-PDLLA 50-50			8629	1.30 ± 0.11	42	−4 ± 0.01	2.0 ± 0.01
PEO-PLLA	Solution		9224	1.52 ± 0.10	46	−59 ± 0.04[f]	42 ± 0.02
PEO-PD/LLA			7845	1.30 ± 0.13	36	0.97 ± 0.03	0.7 ± 0.01
PEO-PDLLA 50-50			7868	1.34 ± 0.09	36	−2 ± 0.02	1.4 ± 0.01

[a] Number- average molecular weight measured by ^{1}H NMR

[b] Measured by GPC, relative to polyethylene glycol standard

[c] $\frac{\text{The number average molecular weight of PLA}}{\text{The number average weight of polymer as determined by HNMR}} \times 100$

[d] Specific optical rotation of diblock copolymer measured in DMSO

[e] $\frac{\text{Specific optical rotation of diblockpolymer}}{\text{Optical rotations of higly pure polylactide}} \times 100$. The reported optical rotations of enantiomerically highly pure PLLA and PDLA typically lie between |140| and |156| (8,16–18)

[f] Means optical purity of the PLLA block was significantly higher in the PEO-PLLA copolymer synthesized by solution polymerization compared to PEO-PLLA of bulk polymerization (*t*-test, $P < 0.05$)

Crystallization of the PLA segment was not detected for PEO-PD/LLA or PEO-PDLLA 50–50 block copolymers irrespective of the method of polymerization (Fig. 2). Only PEO-PLLA showed crystallization and melting peaks for the PLA segment. The PLLA related crystallization and melting peaks were observed at 75.1 and 126.9°C, respectively, for PEO-PLLA copolymers prepared by bulk polymerization. For polymers prepared by solution polymerization, crystallization and melting peaks were observed at 107.5 and 148.0°C, respectively. The degree of crystallinity in the hydrophobic block is significantly higher for PEO-PLLA block copolymers synthesized through solution polymerization with ΔH crystallization of 16.7 *versus* 9.5 J/g for solution and bulk polymerized PLLAs, respectively (Student's *t* test, $P < 0.05$) (Table II).

Characterization of Self-Assembled Structures

The characteristics of the self-assembled structures prepared from block copolymers of this study are reported in Table III. Irrespective of the method used for the polymerization of block copolymers, the Z average diameter of their self-assembled structures was below 50 nm and with a relatively narrow polydispersity in micellar size distribution. All block copolymers showed a

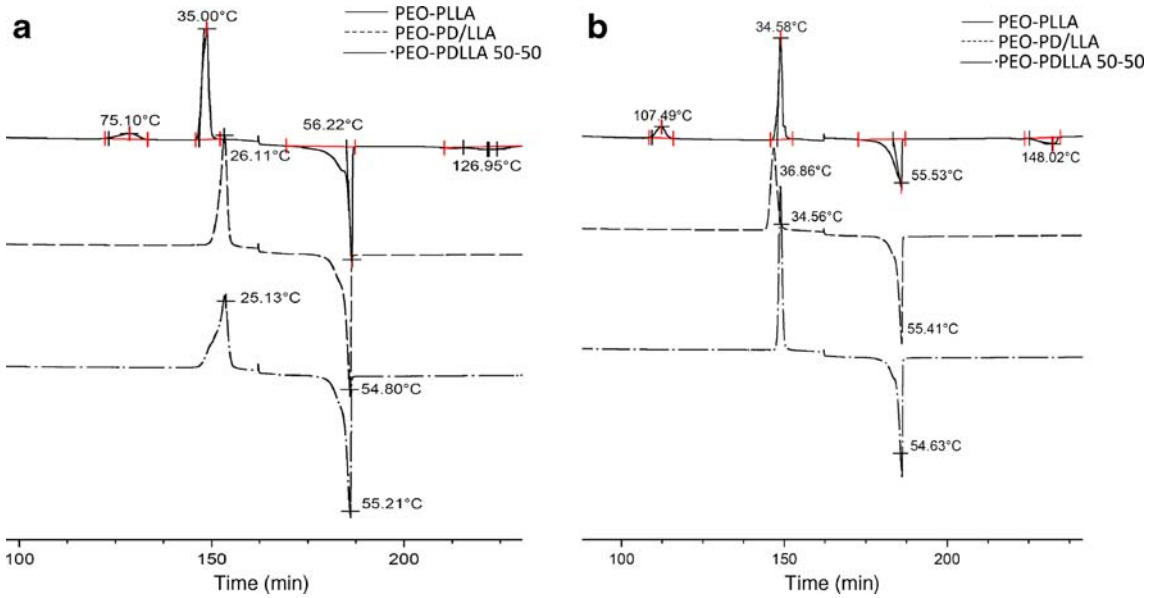


Fig. 2 DSC thermograms of diblock copolymers polymerized in (**a**) bulk and (**b**) solution. To determine reproducibility, melting temperatures and enthalpies were measured at a heating rate of 2°C/min in three cycles. The thermograms of the second and third cycles are shown.

Table II Thermal Properties of the Diblock Copolymers Polymerized in Bulk and Solution

Polymerization Method		$T_{m,PEO} \pm SD$ (°C) [a]	$\Delta H_{m,PEO} \pm SD$ (J/g)	$T_{cr,PEO} \pm SD$ (°C) [b]	$\Delta H_{cr,\ PEO} \pm SD$ (J/g)	$T_{m,PLA} \pm SD$ (°C)	$\Delta H_{m,\ PLA} \pm SD$ (J/g)	$T_{cr,PLA} \pm SD$ (°C)	$\Delta H_{cr,\ PLA} \pm SD$ (J/g)
PEO		63.53 ± 0.15	216.0 ± 0.89	44.78 ± 0.56	189.1 ± 0.10	–	–	–	–
PEO-PLLA		56.22 ± 0.13	70.0 ± 1.21	35.00 ± 0.91	66.9 ± 0.97	126.9 ± 1.35	8.3 ± 0.73[c]	75.1 ± 0.31	9.5 ± 0.34[d]
PEO-PD/LLA	Bulk	55.02 ± 1.15	80.5 ± 0.79	26.13 ± 0.75	77.9 ± 1.69	–	–	–	–
PEO-PDLLA 50-50		54.91 ± 0.78	81.6 ± 1.78	25.44 ± 0.56	79.3 ± 1.01	–	–	–	–
PEO-PLLA		55.53 ± 0.43	68.5 ± 3.39	34.58 ± 0.89	67.6 ± 0.45	148.0 ± 1.64	16.4 ± 0.86[c]	107.5 ± 0.42	16.7 ± 0.87[d]
PEO-PD/LLA	Solution	55.37 ± 1.20	85.7 ± 1.07	39.87 ± 0.64	84.1 ± 1.38	–	–	–	–
PEO-PDLLA 50-50		55.25 ± 0.96	92.1 ± 1.89	35.30 ± 3.29	90.8 ± 1.66	–	–	–	–

[a] Melting point (T_m)

[b] Crystallization temperature (T_{cr})

[c] Means $\Delta H_{m,\ PLA}$ of PEO-PLLA copolymer synthesized by solution polymerization was significantly higher compared to PEO-PLLA of bulk polymerization (*t*-test, $P < 0.05$)

[d] Means $\Delta H_{cr,\ PLA}$ of PEO-PLLA copolymer synthesized by solution polymerization was significantly higher compared to PEO-PLLA of bulk polymerization (*t*-test, $P < 0.05$)

CMC in μg/ml range. Among different block copolymers prepared by bulk polymerization, PEO-PDLLA 50–50 showed a significantly higher CMC (One way ANOVA, $P < 0.05$). This result is in line with previous findings in which stereo-regularity in the hydrophobic block led to decrease in CMC. This observation was attributed to the facilitation of micelle formation by polymeric cores capable of forming semi-crystalline structure (20). In line with this explanation, polymers prepared in solution have shown significantly lower CMCs compared to those prepared in bulk (Student's *t* test, $P < 0.05$). However, no significant difference in CMC of polymers prepared by solution polymerization was observed irrespective of the stereochemistry of the core forming. This could be due to the low sensitivity of the method of CMC determination used in our study.

The morphology of empty polymeric micelles from polymers synthesized by bulk polymerization was spherical and uniform in most cases except for the PEO-PDLLA 50–50 where a tendency for micellar aggregation was apparent (Fig. 3a). Moreover, for polymeric micelles from PEO-PLLA polymers synthesized by solution polymerization in addition to spherical particles, rod and worm-like micelles were also observed (Fig. 3b). Worm micelles from PEO-PLLA polymers prepared in solution were observed in different sections of TEM sample (Fig. S3).

This phenomenon is in line with previous reports on the deviation of crystalline micellar core structure from spherical morphologies, where the micelles assembled with crystalline core took a cylindrical shape whereas those formed by non-crystalline structures adopted a classical spherical shape. It has been assumed that the crystallization of the core-forming blocks is the driving force governing the morphology of micelles in solution and when the core-forming block is a crystalline chain, the energy of crystallization is so large that this block must pack in a folded structure to keep its stability, leading to formation of non-spherical structures (11,21).

Kinetic Stability of Polymeric Micelles

Micelle kinetic stability was studied by DLS in the presence of SDS, which acted as a destabilizing agent. SDS is consisting of

Table III Characteristics of Assembled Structures from Block Copolymers Under Study

Polymer	Polymerization method	Diameter ± SD[a] (nm)	PI	CMC ± SD (μg/ml)
PEO-PLLA	Bulk	44.9 ± 1.3	0.252 ± 0.061	0.625 ± 0.014[b]
PEO-PD/LLA		28.5 ± 1.0	0.184 ± 0.162	0.606 ± 0.050[b]
PEO-PDLLA 50-50		24.2 ± 3.7	0.129 ± 0.038	0.952 ± 0.014[bc]
PEO-PLLA	Solution	43.9 ± 4.5	0.269 ± 0.078	0.352 ± 0.020
PEO-PD/LLA		39.13 ± 6.2	0.226 ± 0.010	0.335 ± 0.043
PEO-PDLLA 50-50		41.2 ± 2.5	0.293 ± 0.078	0.359 ± 0.024

[a] Z average measured by DLS

[b] Means polymers prepared in bulk have shown significantly higher CMCs compared to those prepared in solution (*t*-test, $P < 0.05$)

[c] Means PEO-PDLLA 50–50 copolymer showed a significantly higher CMC among other polymers prepared in bulk polymerization (one way ANOVA with Tukey post-test, $P < 0.05$)

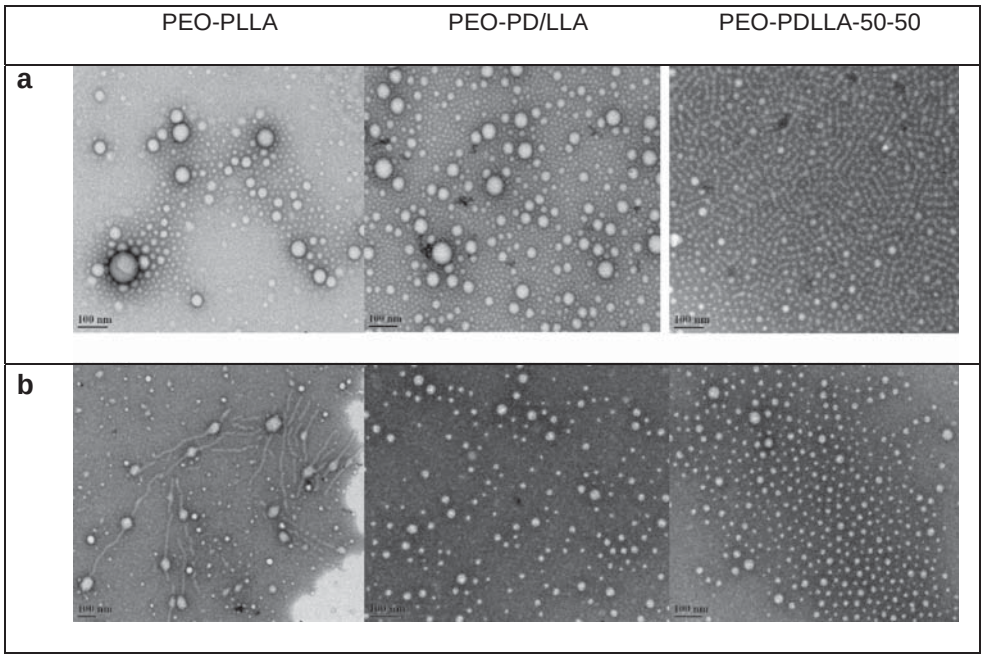


Fig. 3 TEM images of polymeric micelles from block copolymers synthesized in (**a**) bulk and (**b**) solution.

a 12-carbon tail attached to a sulfate group bearing a negative charge, giving the material the amphiphilic properties. When incubating micelles in the presence of SDS, its straight hydrophobic tail can adsorb onto the hydrophobic segment of the micelles and destabilize them due to electrostatic repulsion between negatively charged head of SDS (22). Figure 4 shows the time dependence in (a) scattered light intensity and (b) polydispersity index (PI) of micelles. SDS-treated PEO-PD/LLA or PEO-PDLLA 50–50 micelles prepared in bulk exhibited a drastic decrease in scattered light intensity and an increase in PI within 2 h. The same trend but at a reduced extent was observed with PEO-PD/LLA or PEO-PDLLA 50–50 micelles where the polymers were prepared using solution polymerization. Micellar structures of PEO-PLLA, particularly those prepared by solution polymerization, showed an enhanced kinetic stability in comparison with other structures when exposed to SDS. In general, these results indicated that the polymeric micelles prepared from copolymers synthesized in solution were more stable compared with their counterparts prepared in bulk. Among different structures under

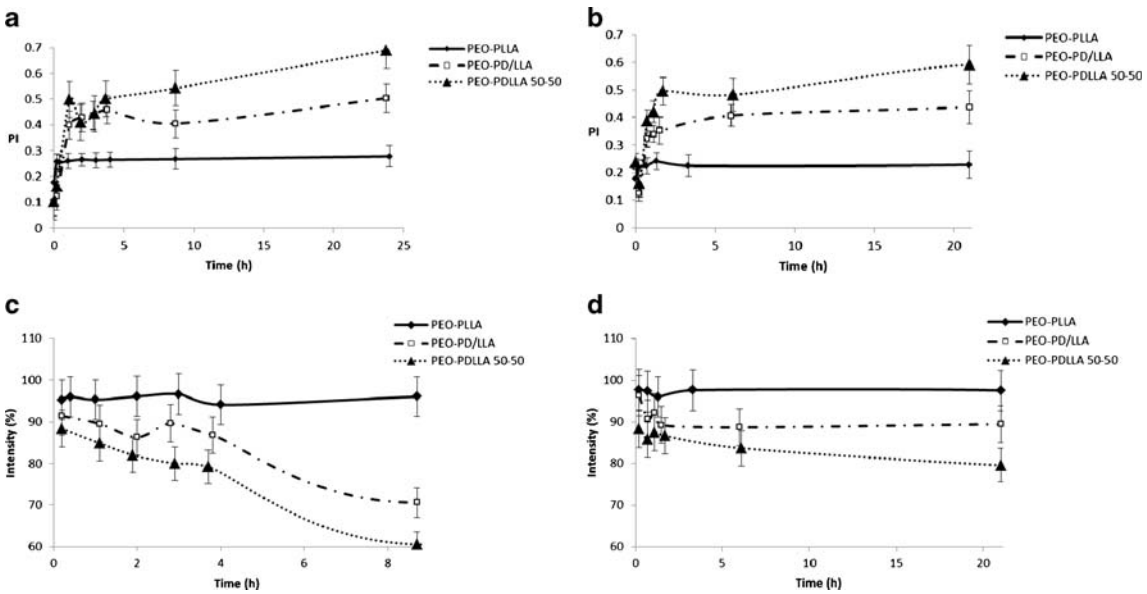


Fig. 4 The time dependent changes in PI and scattered light intensity for micelles from polymers prepared by either (**a**, **c**) bulk or (**b**, **d**) solution polymerization. The data was gathered after addition of SDS solution to micelles at different incubation time points.

study, PEO-PLLA micelles prepared by solution polymerization were the most stable ones.

Thermal Behavior of Freeze-Dried Polymeric Micelles

The thermal behavior of freeze-dried polymeric micelles was investigated using DSC. Similar to what was observed for the block copolymers, freeze-dried micelles of PEO-PLLA synthesized in bulk and solution methods were the only structures that exhibited PLA crystallinity in their thermograms. The PLLA related crystallization and melting points for freeze-dried polymeric micelles of bulk polymerized block copolymers were observed at 79.57 and 127.72°C, respectively (Fig. 5a, Table IV). For polymeric micelles of block copolymers prepared in solution, these temperatures were 105.83 and 148.28°C, respectively. The degree of crystallinity in the micellar core is significantly higher for those block copolymers synthesized through solution polymerization with ΔH crystallization of 16.45 *versus* 8.50 J/g for solution *versus* bulk prepared PLLAs, respectively (Student's *t* test, $P< 0.05$). This data is in good agreement with the thermal behavior of block copolymers in solid form (Fig. 2).

Characterization of Nimodipine Loaded Polymeric Micelles

Encapsulation of nimodipine in micelles of block copolymers prepared by bulk polymerization was examined using either the addition of water to polymer/drug THF solution or adding drug/polymer THF solution to water. Characteristics of nimodipine loaded polymeric nanocarriers using addition of water to THF solution are summarized in Table V. Overall, the encapsulation efficiency and loading level of nimodipine in polymeric micelles using addition of water to THF were relatively low.

Characteristics of nimodipine loaded polymeric micelles using addition of THF to water are summarized in Table VI. Compared to the previous method of encapsulation (addition of water to THF), yielded micelles were with higher encapsulation efficiency. This observation is in line with our previous data (23). Using addition of THF to water, drug loading was increased for polymers prepared by bulk polymerization when a higher concentration of nimodipine in polymer (10 w/w %) was used in the encapsulation process. The opposite trend was observed for polymers prepared by the solution polymerization; where encapsulation efficiency and drug loading both decreased when a higher amount of drug was used. At high drug loading levels, a comparison of micellar size showed a significantly lower size only for PEO-PLLA micelles prepared from solution *versus* bulk polymers (Student's *t* test, $P< 0.05$). The polymerization method did not appear to significantly affect the average diameter of loaded micelles prepared from PEO-PD/LLA and PEO-PDLLA 50–50 (Student's *t* test, $P> 0.05$).

Polymeric micelles prepared by adding THF to water and containing similar drug/polymer level of ~1.5% (w/w) were used for further studies. The thermal behavior of freeze-dried nimodipine loaded polymeric micelles was investigated and

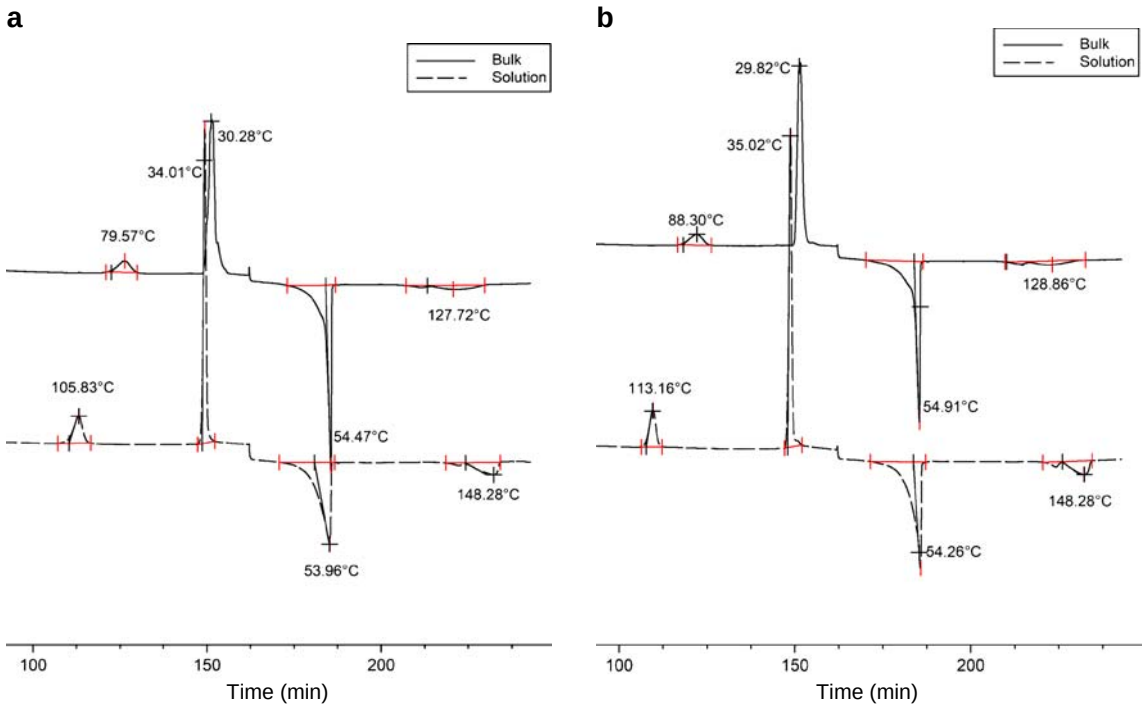


Fig. 5 DSC thermograms of freeze-dried (**a**) empty and (**b**) nimodipine loaded micelles of PEO-PLLA. To determine reproducibility, melting temperatures and enthalpies were measured at a heating rate of 2°C/min in three cycles. The thermograms of the second and third cycles are shown.

Table IV Thermal Properties of the Freeze-dried Empty and Nimodipine Loaded PEO-PLLA Micelles as Determined by DSC

Method of Synth.	Freeze-dried micelles	$T_{m,PEG}$ ± SD °C [a]	$\Delta H_{m,PEG}$ ± SD (J/g)	$T_{cr,PEG}$ ± SD °C [b]	$\Delta H_{cr,PEG}$ ± SD (J/g)	$T_{m,PLA}$ ± SD °C	$\Delta H_{m,PLA}$ ± SD (J/g)	$T_{cr,PLA}$ ± SD °C	$\Delta H_{cr,PLA}$ ± SD (J/g)
Bulk	Empty	54.47 ± 0.65	55.76 ± 1.59	30.28 ± 1.93	54.53 ± 1.56	127.72 ± 0.22	8.18 ± 0.89[b]	79.57 ± 0.35	8.50 ± 1.05[c]
Solution		53.96 ± 2.44	60.40 ± 0.98	34.01 ± 1.03	59.97 ± 0.94	148.28 ± 1.14	16.30 ± 1.01[b]	105.83 ± 0.58	16.45 ± 0.99[c]
Bulk	Loaded	54.91 ± 0.13	60.67 ± 1.41	29.82 ± 0.85	58.47 ± 1.09	128.86 ± 0.66	9.15 ± 1.12[b]	88.30 ± 1.50	9.13 ± 0.12[c]
Solution		54.26 ± 0.19	59.71 ± 1.02	35.02 ± 0.01	59.54 ± 0.98	148.28 ± 1.56	15.31 ± 1.53[b]	113.16 ± 1.94	15.04 ± 1.31[c]

[a] Melting point (T_m)

[b] Crystallization temperature (T_{cr})

[b] Means $\Delta H_{m,PLA}$ of freeze-dried empty and nimodipine loaded PEO-PLLA micelles synthesized by solution polymerization was significantly higher compared to their counterparts of bulk polymerization (*t*-test, $P < 0.05$)

[c] Means $\Delta H_{cr,PLA}$ of freeze-dried empty and nimodipine loaded PEO-PLLA micelles synthesized by solution polymerization was significantly higher compared to their counterparts of bulk polymerization (*t*-test, $P < 0.05$)

compared to that of empty micelles (Fig. 5b, Table IV). Similar to empty micelles, only PEO-PLLA prepared by bulk and solution methods showed crystallization and melting points for the PLA block. In line with what was observed for empty micelles the degree of crystallinity appeared to be higher for micelles in which the block copolymer was synthesized through solution polymerization. The PLLA melting temperature and enthalpy of melting appeared to be similar for nimodipine loaded *versus* empty micelles. The PLLA crystallization temperatures and enthalpies; however, slightly increased for loaded *versus* empty micelles irrespective of the used method of polymerization. This indicates nimodipine loading has facilitated the crystallization of the core-forming block. No extra peak was observed for nimodipine.

The effect of nimodipine loading on kinetic stability of polymeric micelles from different block copolymers under study was then investigated. Similar to empty micelles, nimodipine loaded micellar structures of PEO-PLLA showed an enhanced kinetic stability in comparison with other structures (Fig. 6). PEO-PDLLA 50–50 loaded with nimodipine showed the lowest resistance toward dissociation by SDS in comparison to other formulations. In line with previous observation for empty micelles, the drug loaded polymeric micelles prepared from copolymers polymerized in solution were more stable than their counterparts prepared in bulk. This was characterized by a smaller decrease in scattered light intensity and a smaller increase in PI index as a function of incubation time with SDS. Interestingly, nimodipine loading enhanced micellar stability irrespective of the method of polymerization (Fig. 6 for loaded micelles *versus* Fig. 4 for empty micelles).

We have then assessed the effect of different factors on the release profile of nimodipine from polymeric micelles. Figure 7 shows the release profile of nimodipine from polymeric micelles of block copolymers prepared by bulk method using addition of water to THF. Under these conditions, low levels of nimodipine loading were achieved (1–3.5%). For these micelles, similar (~65%) nimodpine release within 24 hs of study irrespective of the stereo-chemistry of the block copolymer was seen.

Figure 8 shows the effect of stereochemistry of the block copolymer on the release profile of nimodipine from polymeric micelles prepared by adding THF to water at two different drug loaded levels. For polymeric micelles with high drug loading ~9% (w/w), maximum accumulative drug release was observed for PEO-PLLA micelles. The lowest rate of drug release was observed for PEO-PDLLA 50–50 micelles (Fig. 8a). In the case of polymeric micelles with low drug loading ~1.5% (w/w), however, no difference was observed between polymeric micelles with various stereo-chemistry in their core structure (Fig. 8b).

Figure 9 shows the effect of polymerization method on the release profile of nimodipine from block copolymer micelles with low level of drug loading (1.5%). The results showed that

Table V Characteristics of Nimodipine Loaded Micelles Prepared Adding Water to Organic Solvent ($n = 1$)

Polymerization method	Polymer	Drug added (w/w %)	Diameter [a] (nm)	PI	EE%	DL%
Bulk	PEO-PLLA	10	49	0.439	35.61	3.56
	PEO-PD/LLA		36	0.394	18.23	1.82
	PEO-PDLLA 50-50		51	0.479	19.22	1.92

[a] Z average measured by DLS

Table VI Characteristics of Nimodipine Loaded Micelles Prepared by Adding Organic Solvent to Water

Polymerization method	Polymer	Drug added (w/w %)	n	Diameter ± SD[a] (nm)	PI ± SD	EE%	DL%
Bulk	PEO-PLLA	5	1	72.2	0.160	22.4	1.12
		10	3	62.1 ± 4.7	0.184 ± 0.031	96.8	9.67
	PEO-PD/LLA	5	1	50.3	0.228	21.3	1.06
		10	3	45.2 ± 4.5	0.236 ± 0.011	93.5	9.35
	PEO-PDLA 50-50	5	1	51.4	0.251	22.9	1.14
		10	3	36.2 ± 1.1	0.226 ± 0.053	89.6	8.96
Solution	PEO-PLLA	5	1	49.2	0.232	70.2	3.51
		10	3	41.3 ± 6.0	0.182 ± 0.074	12.4	1.24
	PEO-PD/LLA	5	1	40.4	0.057	101	5.05
		10	3	44.4 ± 7.5	0.222 ± 0.133	11.7	1.17
	PEO-PDLA 50-50	5	1	39.3	0.097	51.2	2.55
		10	3	40.1 ± 9.4	0.200 ± 0.016	11.8	1.18

[a] Z average measured by DLS

method of polymerization did not affect the overall pattern of cumulative drug release at the early time points. Significant differences were only seen at later time points (> 8 h) for PEO-PLLA and (> 2 h) for PEO-PD/LLA micelles, where the cumulative drug release appeared to be significantly higher in micelles prepared from polymers synthesized by solution polymerization compared to those prepared in bulk ($P< 0.05$, student's t test). In PEO-PDLLA 50–50 there was not a significant difference in the cumulative drug release from micelles prepared from polymers synthesized by solution polymerization compared to those prepared in bulk ($P< 0.05$, student's t test).

DISCUSSION

For the goal of depot and/or targeted drug delivery, development of stable polymeric micelles that can solubilize high levels of therapeutics and, at the same time, release it in a sustained or even delayed manner is of interest. Achieving this goal has been a challenging task, however. Previous studies have presented a case for the effect of micellar core crystallinity on the stability of micellar structure and their drug release profile. For instance, enhancing core crystallinity has been shown to improve the stability of micellar structure (9), while restricting the partitioning of pyrene into the micellar core, leading to lower loaded pyrene levels (11). The increase in core crystallinity also led to a rapid release of sulindac and tetracaine from polymeric micelles (10). Here we studied the effect of polymerization method on the crystallinity of core-forming block in PEO-PLA block copolymers and its eventual influence on the properties of resulted polymeric micelles. To minimize the detrimental effect of high temperature on stereoregularity and gain more control over stereochemistry of polymer backbone, method of polymerization was changed from bulk to solution in this study.

Among different structures under study, PEO-PLLA micelles prepared by solution polymerization were the most stable ones, owing to their greater stereoregularity and subsequent higher crystallinity. Higher degree of crystalinity in the core seems to be the main reason behind higher kinetic stability and resistance to micellar dissociation to single chains

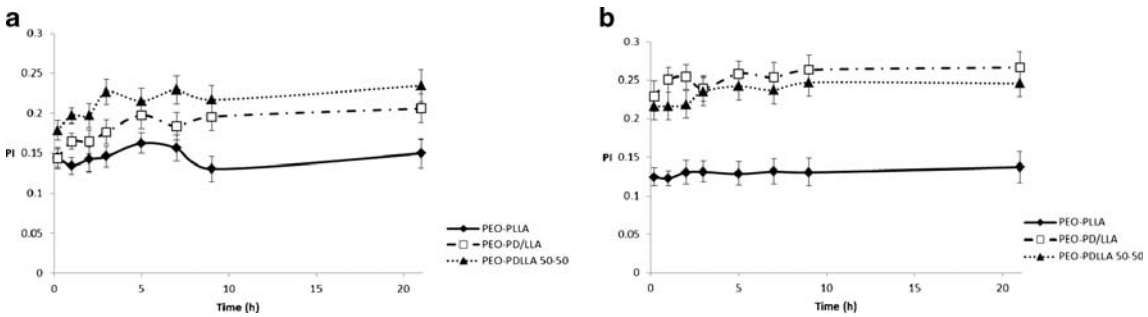


Fig. 6 The time dependent changes in PI for nimodipine loaded micelles prepared from polymers by either (**a**) bulk or (**b**) solution polymerization. The data was gathered after addition of SDS solution to micelles at different incubation time points.

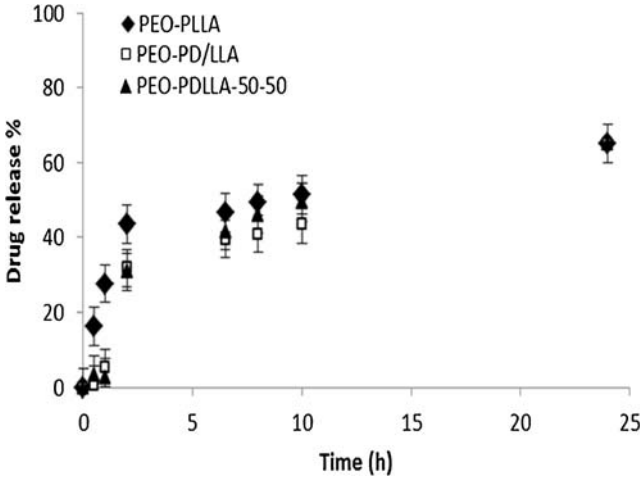


Fig. 7 The effect of the stereochemistry of the block copolymer structure on the release profile of nimodipine from micelles prepared through addition of water to THF solution. The block copolymers were prepared by bulk polymerization.

when incubating with SDS (15). The detrimental effect of temperature on stereoregularity can be avoided by changing the method of polymerization from bulk to solution. Since solution polymerization employs lower temperatures during reaction, the stereoregularity of produced polymers can be preserved better. In the present study, we did not observe a significant difference in the polydispersity index of polymers synthesized by bulk *versus* solution polymerization. However, the use of solution polymerization led to the production of more stereoregular PLLA.

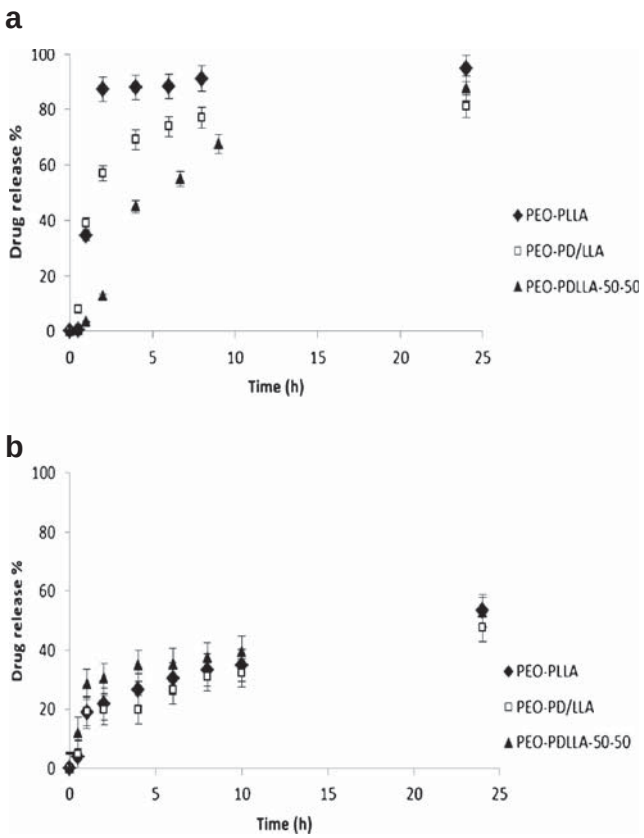


Fig. 8 The effect of the stereochemistry of block copolymer structure on the release profile of nimodipine from micelles prepared by adding THF to water with (**a**) high drug loading ~9% (w/w); and (**b**) low drug loading ~1.5% (w/w). The block copolymers were prepared by bulk polymerization.

High temperatures used for polymerization in the bulk method, lead to racemization and subsequently lower the stereoregularity, therefore the polymer chains cannot stack on top of each other in an orderly melting. This results in a lower melting and crystallization. Application of solution polymerization instead of bulk polymerization was shown to favor the crystallization of the PLA segment in PEO-PLLA block copolymers (made from polymerization of L-lactide) (Fig. 2, Table II) and micelles (Fig. 5, Table IV). However, no change in the crystallinity of PEO-PD/LLA and PEO-PDLLA 50–50 block copolymers (Fig. 2, Table II) and micelles (Fig. 5, Table IV) was detected when the method of polymerization was switched to solution from bulk. Because the tin-catalyzed ROP of lactide does not differentiate between the monomer enantiomers, it will likely produce random atactic PLA with racemic-lactide (24). The same scenario might have happened to meso-lactide resulting in the non-selective addition of monomers to polymer chain and production of an atactic polymer with a random distribution of stereocenters along the polymer backbone (that is amorphous) instead of syndiotactic one with alternating arrangement of stereocenters in the polymer. Despite the recent development of complexes for the ROP of lactide, relatively few well-defined metal catalysts are capable of achieving high stereochemical control in the ROP of meso- or rac-lactide (25).

In line with enhanced crystallinity of the core in PEO-PLLA block copolymers prepared by solution polymerization, PEO-PLLA micelles showed a tendency for the formation of cylindrical and worm like micelles (instead of spherical ones) (Fig. 3b) and demonstrated improved kinetic stability (Fig. 4b, d). A slight increase in the kinetic stability of spherical micelles formed from PEO-PD/LLA and PEO-PDLLA 50–50 was also noted (Fig. 4).

In the next step, we investigated the effect of core crystallinity, on the stability and release profile of drug loaded polymeric micelles. Nimodipine was used as the model drug for these studies because of its proven preferential interaction with the polyester core structure (13). Similar to empty micelles (Fig. 4), drug loaded micelles with semi-crystalline cores were shown to be more stable than those with amorphous ones (Fig. 6). The application of solution polymerization also enhanced the kinetic stability of prepared micelles (Fig. 6b). In general, the presence of drug in the micellar core was shown to enhance the stability of polymeric micelles, irrespective of the core crystallinity (Fig. 6). An interaction between the drug and the core-forming block is possibly responsible for enhanced kinetic stability of drug loaded polymeric micelles (26).

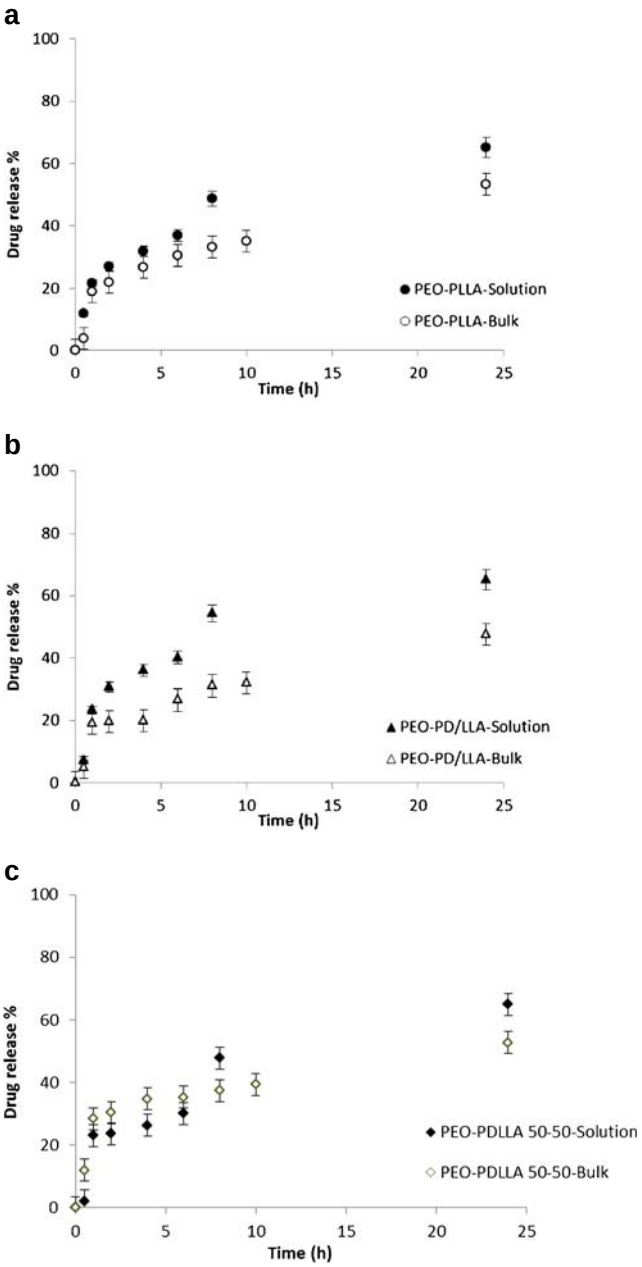


Fig. 9 Assessing the effect of polymerization method on the release profile of nimodipine from (**a**) PEO-PLLA (**b**) PEO-PD/LLA and (**c**) PEO-PDLLA 50–50 micelles.

We then investigated the effect of core crystallinity on the release of nimodipine from PEO-PLA polymeric micelles. Since drug release from polymeric micelles is predominantly governed by diffusion of drug through amorphous regions of the micellar structure, an increase in the crystalline structure of the core was expected to increase the diffusion path and as a result, reduce the rate of drug release from the micellar carrier. This is provided to the localization of the drug in the core of polymeric micelles. Our observations, however, were in contrary to this expectation. When addition of organic solvent to water was used as the method of micellization for polymers prepared by bulk method, at high drug loaded levels ~9% (w/w), the PEO-PLLA micelles showed more rapid drug release compared to PEO-PD/LLA and PEO-PDLLA 50–50 micelles (Fig. 8a). When drug loading levels were low 1.5% (w/w) drug release from polymeric micelles was similar irrespective of the core crystalinity and structure (Fig. 8b). A higher release of nimodipine from micelles was observed when PEO-PLLA and PEO-PD/LLA polymers were prepared by solution polymerization compared to those prepared by bulk polymerization (Fig. 9a, b).

The observed difference between the release of nimodipine from polymeric micelles of different core crsytallinity may in fact reflect the influence of core crystallinity on the localization of drug within the micellar structure rather than the diffusion path of drug from the micellar core. In fact when data from all sample studied here is considered, a good correlation between drug loading level and the time required to release 50% of drug ($R^2 = 0.8896$) is observed. Rapid formation of micellar structure from PEO-PLLA block copolymers that have crystalline PLLA blocks may prevent the uniform dispersion of the drug in the micellar core. In this case, the drug may be squeezed out of the core and eventually accumulate in the surrounding regions of core (core/shell interface or the shell) and released with a rapid rate from micelles particularly at high drug loading levels. In contrast, PEO-PD/LLA and PEO-PDLLA 50–50 copolymers that have less crystalline core forming structure may allow better drug loading within the micellar core (10). Formation of polymeric micelles from block copolymers prepared in solution may also be more rapid, owing to higher stereoregularity, compared to polymers prepared in bulk. In comparison, micelles of diblock copolymers

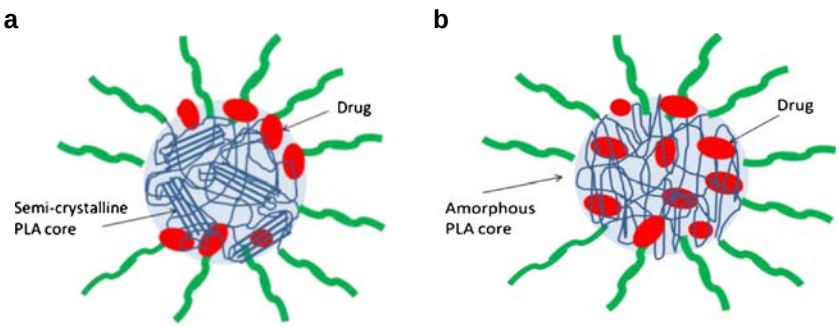


Fig. 10 Depiction of drugs dispersion in the (**a**) semi-crystalline PLA core and (**b**) amorphous PLA core.

prepared by bulk polymerization can provide a higher chance for drug loading in the micellar core, leading to slower rate of drug release (Fig. 10).

With this in mind we have investigated whether a change in the method of micelle preparation by addition of water to polymer/drug solution in THF can slow down the kinetics of micelle formation, leading to better drug encapsulation within micellar core and a slower release profile for nimodipine from PEO-PLLA micelles that have crystalline core structure. Our results did not show any benefit for this approach in terms of drug release (Fig. 7). Instead, the encapsulation efficiency of nimodipine was drastically reduced.

CONCLUSIONS

The application of solution polymerization led to better preservation of stereoregularity and crystallinity of the PLA block in PEO-PLLA block copolymers compared to polymers made from bulk polymerization. Consequently, polymeric micelles prepared from polymers synthesized by solution method showed enhanced kinetic stability. Loading of a core-compatible drug, i.e., nimodipine, in PEO-PLA polymeric micelles enhanced the stability of prepared polymeric micelles. Nimodipine loading in polymeric micelles prepared from block copolymers synthesized by solution polymerization was reduced and its release was enhanced compared to those prepared by bulk polymerization. The latter observation may be a reflection of the enhanced kinetics of micelle formation leading to drug loading in the micellar shell or core/shell interface for the more stereoregular PEO-PLAs prepared through solution polymerization.

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