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Research Article

Thermosensitive Copolymeric PLGA-PEG-PLGA Nanomicelles of Raloxifene: Synthesis, Formulation, and *In-vitro* Characterization

Pooja Dave*, Chetan Detroja

School of Pharmacy, RK University, Rajkot, Gujarat, India

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ABSTRACT

The objective of the present research study has been to synthesize (PLGA-PEG-PLGA), a thermolabile and biodegradable triblock copolymer. The polymer was subjected to differential evaluation using NMR, XRD, and FTIR techniques for characterization. Raloxifene, a potential therapeutic for breast cancer, exerts poor water solubility and a low fraction of bioavailability owing to its pharmacokinetic properties. The synthesized polymer was used to load raloxifene-encapsulating polymeric micelles, which were then subjected to several evaluations with the aim of improving solubility and bioavailability standards. Zeta potential for the developed formulation was approximately -0.73 mV, indicating a relatively neutral surface charge. It subsequently emerged that the sizes of the blank and raloxifene-loaded micelles were 40.18 and 42.18 nm, respectively. The drug encapsulating capacity and% drug loading capacity were determined to be 73.4 ± 0.34% and drug loading 6.04 ± 0.002% (w/w), respectively. *In-vitro* study illustrated a 94.37% sustained release drug profile for 120 hours. In this research study, polymeric raloxifene nanomicelles were successfully prepared to enhance solubility, bioavailability, and antitumor effect due to the smaller size of the micelles.

Introduction

In recent decades, breast cancer has been the leading cause of morbidity among women. In breast cancer, several therapies are available, such as radiotherapy, chemotherapy, immune therapy, targeted therapy, and hormonal therapy. Raloxifene has been found as an estrogen-receptor inhibitor, lowering the jeopardy of the invasive breast as well as endometrial cancer. However, it has a limited bioavailability in humans, nearly 2%, and poor bioavailability due to P-glycoprotein efflux and gut wall glucuronidation. Several methods like microemulsions and nanoparticles, were utilized to increase the water solubility and bioavailability of raloxifene. [1-6]

Polymeric micelles have become a promising carrier for poorly water-soluble drugs and for enhancing kinetic stability. Numerous studies have been conducted in the past decade, investigating various polymers and their potential applicability in biochemistry, biomedicine, and several other areas. Polymeric nanomicelles have a spherical shape, resulting from the self-assembly of such a hydrophobic micelle core surrounded by a hydrophilic palisade. Moreover, mixed nanomicelles draw much more attention over single polymeric micelles to enhance stability, low toxicity, high tumor selectivity, penetration, and high drug loading efficiency. Additionally, polymeric nanomicelles contain complex designs on size of particles and occurrence to prevent macrophage phagocytosis, hence dramatically increasing the drug's anticancer activity and minimizing its harmful and side effects. Therefore, it has been hypothesized that formulating thermosensitive biodegradable triblock copolymeric Nanomicelles may effectively enhance solubility and bioavailability.[7-9]

***Corresponding Author:** Ms. Pooja Dave

Address: School of Pharmacy, RK University, Rajkot, Gujarat, India

Email ✉**:** poojadave.pharma@gmail.com

Tel.: +91 9426492370

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A Thermosensitive, thermogelling and biodegradable - poly (D, L-lactide-co-glycolide)-b- poly(ethylene glycol)-b-poly(D,L-lactide-co-glycolide) [PLGA-PEG-PLGA] has been found through potential in drug delivery, biodegradability, biocompatibility, thermosensitivity in addition to its easy controlled release characters.[10-16] PEG has potential advantages to enhance the biocompatibility of micelles. This seems to be due to the hydrophilic nature of the biological environment and the apparent inverse relationship between lipophilicity and biocompatibility.[17-22] PLGA-PEG-PLGA has been widely applied to enhance drug solubility and bioavailability. It also attracts sol-gel, gel-sol behavior, and biomedical demands and is thus widely approached in targeted sustained-release drug delivery system.[23-30] Throughout this research, PLGA-PEG-PLGA was synthesized using a ring-opening mechanism, also simultaneously prepared raloxifene-loaded micelles. FTIR, NMR and XRD performed the polymer characterization. Raloxifene-loaded PLGA-PEG-PLGA nanomicelles were characterized by various parameters such as particle size, diameter, zeta potential, morphological characterization like TEM, SEM, %drug loading and %entrapment efficiency, *in-vitro* drug release study.[30-37]

As a pharmaceutical aspect, numerous studies have been conducted on specifically stimuli-sensitive gel-forming polymers or those that sol forming hydrogels were attracting an abundance of anticipation as potential low-invasive injectable insertion methods.[38-41] At low temperatures, drugs or cells may easily be mixed alongside an aqueous copolymer solution before being injected into the body at a specified location in order to form a translucent matrix.[42] Raloxifene has poor water solubility and low bioavailability, which will hinder its application in breast cancer treatment. Thus, in this research work, main objective was to enhance raloxifene solubility, biodegradability, and biocompatibility within the body. Moreover, thermosensitive micelles also enhance stability of micelles as well as also improves drug loading capacity. Thermosensitive, biodegradable triblock copolymeric PLGA-PEG-PLGA nanomicelles were prepared by solvent evaporation technique. Due to biocompatibility, biodegradability and high kinetic stability, low toxicity with low cost, is the main reason to prefer formulation of polymeric micelles.

Materials and Methods

Materials

Dr. Reddy's Laboratory generously provided raloxifene (Hyderabad, India). The acquisition of PLGA from Nomisma Healthcare (Vadodara, India). Purchased from LOBA Chemie was N, N'-dicyclohexylcarbodiimide (DCC) of the synthesis grade (Mumbai, India). The remaining solvents all came from analytical grade.

Polymer Synthesis

In the ring-opening process, PLGA-PEG-PLGA was synthesized. PLGA-PEG-PLGA employed stannous octanate as a catalyst to complete ring-opening polymerization of lactide (5905 mg) and glycolide (595 mg) in the presence of PEG in attempt to develop biodegradable copolymer (3500 mg).After a 12 hour reaction at 150°C, the product was cleaned by being submerged in 80°C hot water three times. After deployment, the obtained product was lyophilized and kept at -20°C. In order to ascertain the structure and content of the produced PLGA-PEG-PLGA triblock copolymer, ^{1}H-NMR and FTIR spectroscopy were used. Copolymer was applied on potassium bromide tablets to get the FTIR spectrum.[43-45]

Characterization of Polymer

FTIR Analysis

The polymeric characterization has been carried out by FTIR analysis using NEXUS 470 FTIR spectroscopy (Nicolet, USA) for functional group analysis.

Proton nuclear magnetic resonance (^{1}H-NMR)

To determine the LA/GA ratio and investigate the copolymer's structure, ^{1}H-NMR spectra were extracted at 25°C using a 600 MHz Bruker spectrometer (AVANCE model, Germany). By integrating the signals from polymerization that impact one another, such as the maxima from CH but also CH_3 groups of LA in addition to those from CH_2 groups from ethylene glycol but also GA, the LA/GA ratio and Mn were calculated.

X-Ray Diffraction Chromatography

X-ray diffraction of drug-loaded micelles were obtained using a vertical goniometer (MS University, Baroda) with K-alpha radiation (1.54060 Å) within start position [°2Th.] 9.9972 to end position [°2Th.] 49.9892 at 40 mA and 45 kV.

Preparation and Characterization of Polymeric Nanomicelles

Preparation of Blank Micelles

Polymeric micelles were made by a solvent evaporation method. First, triblock copolymer (PLGA-PEG-PLGA 10 mg) was dissolved into the acetone. The mixture was then added into deionized water (10 mL) with the help of magnetic stirrers at 2-8°C until a blush tint color clear solution was formed.

Raloxifene Loaded Polymeric Micelles Preparation Method

Polymeric micelles were formulated by the solvent evaporation technique. In this method, polymer with raloxifene ratio were selected and dissolved in acetone: methanol (2:2) as a solvent afterwards put the solution for sonication by using probe sonicator for 5 minutes. The blended mixture was then added drop by drop into deionized water (10 mL) with the help of a syringe at

2000 rpm on a magnetic stirrer until the complete solvent was evaporated. After solvent evaporation, nanomicelles were undergone for filtration by using 0.45 µm pore-sized syringe filter paper to confiscate the undissolved drug.

Particle Size Measurement & Zeta Potential Measurement: Static and Dynamic Light Scattering

A dynamic laser-light scattering instrument measured the particle size and the zeta potential of raloxifene (Zeta sizer, Parul Institute of Pharmacy & Research, Parul University). Polymeric micelles size was determined using a static scattering angle of 90° at 25°C, and the data were estimated using volume distribution. Simultaneously, the zeta potential of nanomicelles was analyzed at 25°C.

Morphology of Copolymer

The morphological studies were performed under transmission electron microscopy.

Drug Entrapment Efficiency

A UV–visible spectrophotometer set to 287 nm was used to determine the amount of drug in the micelles. The following formula was utilized to calculate the %drug loading of raloxifene content and %encapsulation efficiency (EE).[16,21]

$$EE\% = \frac{\text{Weight of Raloxifene in nanomicelles}}{\text{Initial weight of Raloxifene}} \times 100\%$$

% Drug Loading Efficiency

As a consequence of raloxifene is poorly water-soluble drug (11 ng/mL), thus, water content was omitted while comparing %loading capacity of nanomicelles. As a result, free raloxifene in water was not to be considered in the calculation of %DL and %EE.[46,47]

$$\text{Drug loading} = \frac{\text{Weight of Raloxifene in nanomicelles}}{\text{Weight of nanomicelles}} \times 100\%$$

In-vitro *Dissolution profile*

Drug-loaded micellar dispersion equivalent to 1-mg of drug was wrapped in a dialysis bag. The sealed bag was suspended in a 20 mL methanol: phosphate buffer (7.4 pH) solution. Micellar solution was stirred at 100 rpm at 37 ± 0.5°C. At specific time intervals, each 1-mL withdrew and substituted with an equivalent volume of fresh diffusion medium. Check absorbance in UV.

Stability Study

After 15 days after hydrolytic degradation of a block copolymer, size stability was performed to determine stability study. The stability of micellar dispersion will eventually be shown by the absence of alteration in size, hydrodynamic diameter, and PDI within 15 days after preparation. Samples will be visually inspected in order to prevent continuous DLS readings. Look for signs of instability, including agglomerations, changes in appearance, and coloration. DLS measured the sample in the case that such a change was found. After 15 days, if there had been no change, a sample was examined. After checking for empty micelles initially, raloxifene filled the micelles.

Raloxifene Polymeric Micelles Storage Stability

Over four weeks, raloxifene-loaded PLGA-PEG-PLGA nanomicelles with a 0.5% polymer content were stored at different temperatures such as 4 and 25, as well as 40°C. Once a week, drug loads and particle sizes have been examined through storage.

Results and Discussion

Characterization of Polymer

FTIR and NMR studies represent the structural identification of triblock copolymer and the compatibility study of polymer with drug. Nonionic block copolymer comprises both hydrophilic and hydrophobic segments of PEG and PLGA, respectively that are biodegradable and thermosensitive.

Figs 1-3, illustrate the infrared spectra containing Raloxifene, PLGA, PLGA-PEG-PLGA, as well as raloxifene containing PLGA-PEG-PLGA, sequentially. The peak at 3145.4 cm^{-1} is shown for the hydroxyl group. The peaks at 1466.76, 1642.35, and 1245.53 cm^{-1} are shown for aromatic rings, keto, and oxo group, respectively, similar to the site of PLGA. The PLGA-PEG-PLGA were obtainable in Fig. 3. The methyl group properties of the polymer were ascribed to the peaks at 1455 and 1352 cm^{-1}. The properties of the polymer's methylene group placed near the oxygen atom and the C-O-C group were observed to have maxima around 2877 and 1100 cm^{-1}. The presence of PEG linkage in the copolymer has thus been established. The terminal Hydroxyl group of LA or else GA structural portion in the polymer product and C-O linkage of ester in the polymer were ascribed to the peak at 3497 and 1189 cm^{-1}, respectively. As a result, it was possible to conclude that inducting LA and GA into PEG resulted in the formation of ester groups in the triblock copolymer. These findings validated the production of PLGA-PEG-PLGA.

Fig. 4 depicts a representative spectrum of the PLGA-PEG-PLGA copolymer, which is identical to the observed spectrum. The methylene protons within $-CH_2$ and terminal CH_2 groups of PEG were 3.64 and 4.28 ppm, respectively. In the lactic acid (LA) portions of PLGA, methylidyne (-CH) and methyl ($-CH_3$) protons were found at roughly 5.22 and 1.55 ppm, respectively. Methylene ($-CH_2$) in the glycolic acid (GA) portion of PLGA and acid amide (-CONH-) generated by the graft between PEG and PLGA were also detected in the peaks at about 4.8 and 1.26 ppm.

X-Ray Diffraction Chromatography

XRD was especially used to characterized drug-polymer interaction and which results to changes in the drug's

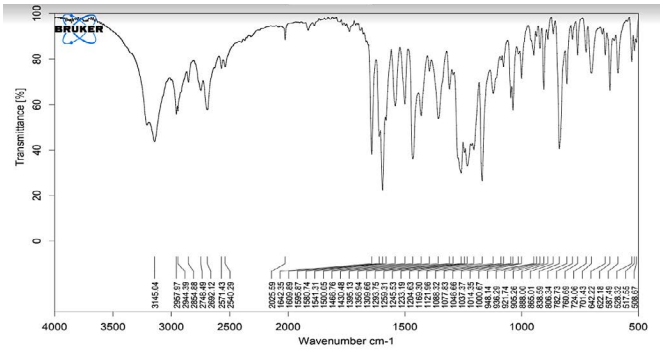

Fig. 1: FTIR of Raloxifene

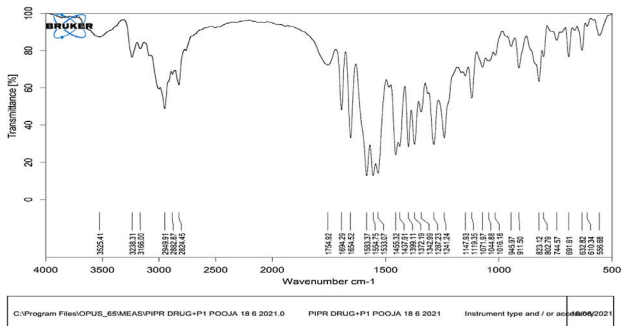

Fig. 2: FTIR of PLGA

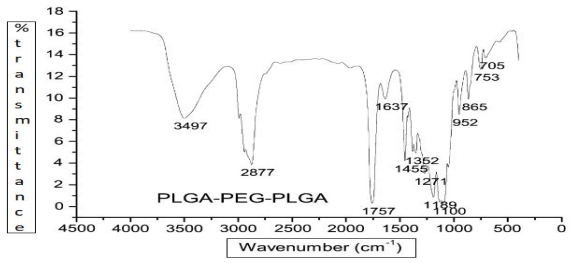

Fig. 3: FTIR of triblock copolymer

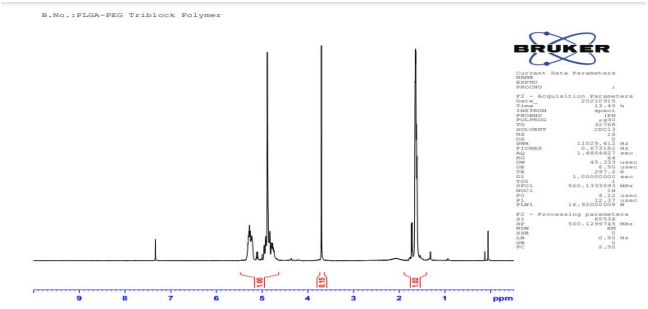

Fig. 4: ^{1}H-NMR of PLGA-PEG-PLGA

molecular mobility, crystallization inhibitory effect of drug-polymer molecules and polymer's amorphous dispersion. In the present study, an XRD pattern of raloxifene-loaded PLGA-PEG-PLGA micelles was shown in Fig. 5. In which a broad peak was observed which confirm that it was an amorphous state. XRD studies represent that PLGA-PEG-PLGA was amorphous in nature. Raloxifene is hydrophobic and poorly water soluble in nature thus by PLGA-PEG-PLGA - raloxifene loaded micelles enhances

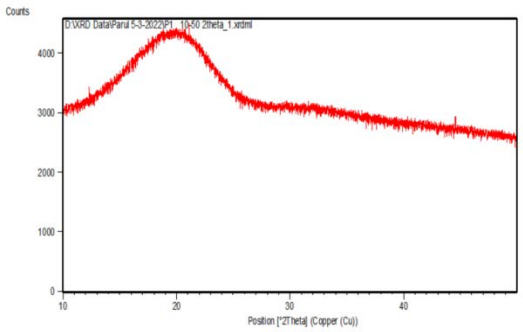

Fig. 5: XRD of drug loaded PLGA-PEG-PLGA micelles

solubility as well as enhances drug loading capacity by using solvent evaporation method. Raloxifene drug molecules were distributed well in triblock copolymer and intimately contacted with the PLGA segment of the polymer after the evaporation.

Physicochemical Characterization of Copolymer drug-loaded Micelles

Particle Size, PDI, Zeta Potential and Morphological Characterization

The role of size is a very important parameter in the formulation of micelles, representing the diffusion characteristics across the mucous layer or cellular uptake of epithelial cells. Size and surface charge are important characterization in micelles as they regulate biodistribution, stability, diffusivity, and pharmacokinetic measurements. Drug-loaded polymeric micelles had a zeta potential of roughly -0.73 mV, implying a practically neutral surface charge. Blank micelles and raloxifene-loaded micelles had similar average sizes of 40.18 and 42.18 nm (Figs. 6 and 7), respectively, with a tight distribution pattern which can certainly accumulate in highly permeable tumors by passive diffusion. The micelles' PDI value was found to be 0.028.

The TEM investigation of drug-loaded micelles revealed a spherical form with a smooth surface, as illustrated in Fig. 8.

%Entrapment Efficiency (EE) and %drug Loading (DL)

In drug loading and entrapment efficiency was measured by interacting with the hydrophobic group of PLGA-PEG-PLGA micelles, larger ratio of PLGA-PEG-PLGA nanomicelles generated through raloxifene more readily loaded into the core of micelles. %DL and %EE were found to be 73.4 ± 0.34% and drug loading 6.04 ± 0.002% (w/w) respectively. Significant EE & DL were measured due to the synergistic action of the unique micellar encapsulation.

In-vitro Dissolution profile

The *in-vitro* data showed in Fig. 9 that drug released sustained from the hydrotropic polymer micelles. Furthermore, as polymeric micelles contained PLGA-PEG-

PLGA, it would help micelles retain in systemic circulation for a prolonged time and sustained release effect for longer period of time. It will enhance the retention time of micelles in blood and gives controlled release drug delivery (Fig. 10).

Storage Stability of Drug-Loaded Micelles

After 15 days, it was demonstrated that particle sizes and size distributions did not correspond to similar approach in the storage stability of micelles under multiple storage conditions. There were no considerable changes in PS and ZP. Surprisingly, PDI is slightly increased with time (PS: 74 nm),the diameters and PDI of liquid formulations hardly changed, meaning that micelles are stable, homogeneous with nanosized (1–80 nm) (Fig. 11).

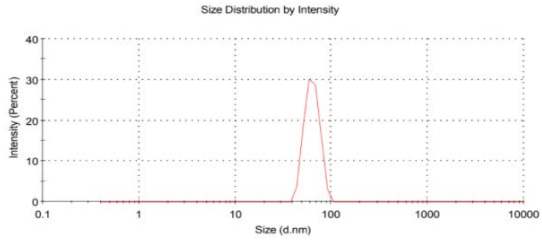

Fig. 6: Particle size of Blank micelles

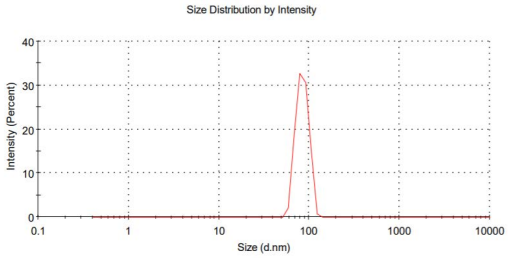

Fig. 7: Particle size of raloxifene-loaded micelles

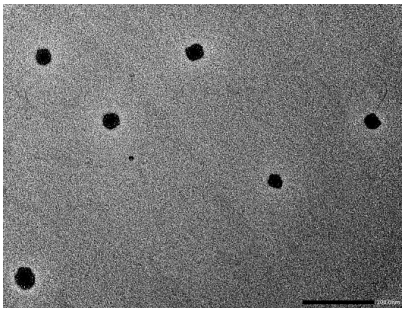

Fig. 8: TEM of raloxifene-loaded micelles

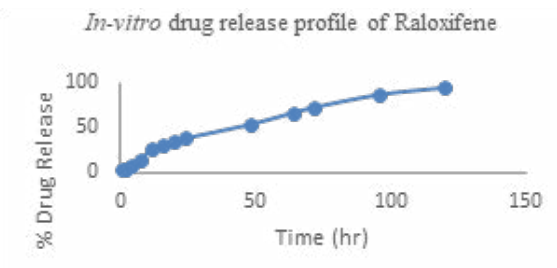

Fig. 9: *In-vitro* Drug release profile of raloxifene-loaded micelles

Storage Stability of Drug-loaded Micelles

The stability of nanomicelles and drug encapsulation was investigated by storing of raloxifene nanomicelles at various temperatures. %Drug loading, sizes of micelles, and particle size distributions were examined every week. The drug loads decreased with storage time, regardless of the storage circumstances. The magnitude of the reduction was inversely proportional to the storage temperature (from high to low): 40°C>25°C>4°C. The % drug loading of nanomicelles was abridged by 3% after 4 weeks of storage at 4°C, but it was lowered by significantly more (5–10%) at 25 and 40°C. It was remarkable that particle sizes but also size distributions deviated from this pattern. Surprisingly, the diameters and PDI of liquid formulations remained practically unaltered throughout time.

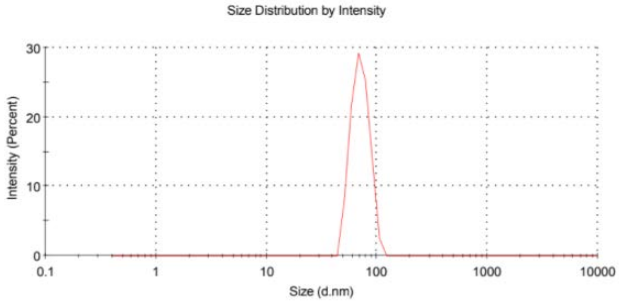

Fig. 10: Particle size of raloxifene-loaded micelles

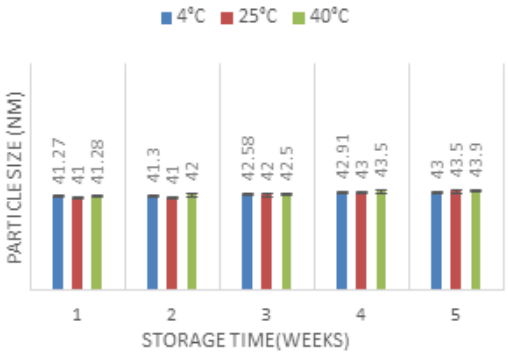

Fig. 11: Raloxifene-loaded PLGA-PEG-PLGA micelles under different storage conditions. Particle size (bars) obtained from DLS upon storage

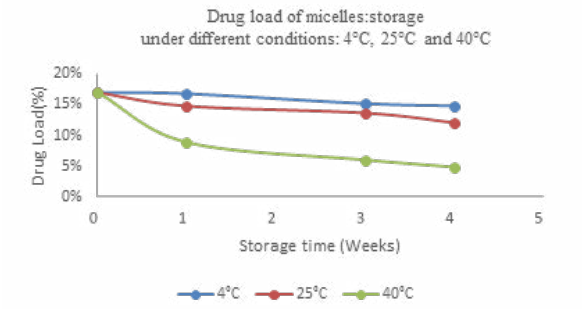

Fig. 12: Raloxifene-loaded PLGA-PEG-PLGA micelles determined by HPLC-UV

Conclusion

The ring-opening reaction synthesized biodegradable triblock copolymer PLGA-PEG-PLGA. Nanomicelles were prepared using biodegradable triblock copolymer using solvent evaporation and successfully encapsulated hydrophobic drugs (Raloxifene). PLGA-PEG-PLGA micelles have less size (40–100 nm) due to the smaller size it gives a better absorption and good dynamic stability thus it enhances drug solubility as well as it improves stability of raloxifene-loaded micelles. These findings suggested that PLGA-PEG-PLGA nanomicelles are highly suitable drug carrier for water-insoluble drugs, potentially improving solubility, biodegradability, and bioavailability (Fig. 12). Furthermore, from *in-vitro* dissolution studies putting in nutshell, PLGA-PEG-PLGA would help micelles give sustained release profile for longer and could be controlled by temperature to achieve real-time and accurate site drug delivery prolong circulation time. As a result, PLGA-PEG-PLGA nanomicelles have enormous promise as tumor-targeted therapeutic carriers.

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