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Impact of PLA/PEG ratios on Curcumin solubility and encapsulation efficiency, size and release behavior of Curcumin loaded poly(lactide)-poly(ethylenglycol) polymeric micelles

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Abstract

Curcumin, a natural compound isolated from rhizomes of the herb Curcuma longa, is suggested as a potential therapeutic agent thanks to its multiple biological and pharmaceutical activities including anti-inflammatory, anti-oxidant, wound healing, anti-microbial and anti-cancer activities. Particularly, Curcumin has demonstrated efficacy as an anticancer agent for various kinds of cancer. However, its low aqueous solubility and bioavailability hamper its clinical application. Therefore, many drug delivery systems have been developed to overcome these limitations. In this study, by using polymeric micelles composed by poly (lactide)-poly (ethylenglycol) (PLA-PEG) copolymers, the aqueous solubility of Curcumin was increased to 0.73 mg.mL-1 compared to 0.11 10-4 mg.mL-1 of pure Curcumin. In addition, we found that the ratio of PLA/PEG has large impact on Curcumin solubility, Curcumin encapsulation efficiency, size and Curcumin release behavior of polymeric micelles. The increase in Curcumin solubility, Curcumin encapsulation efficacy and particle size but decrease in Curcumin release rate were observed when increasing the PLA/PEG ratio.

Keywords: Chemotherapy, Curcumin, Poly(lactide)-polyethylene glycol (PLA-PEG),copolymer, polymeric micelle.

Introduction

Chemotherapy is the most common method applied in cancer treatment. It can be used alone or in combination with other cancer treatment methods in order to improve the therapeutic efficacy. However, drugs used in chemotherapy still remain many problems relating to severe adverse effects for patients. The reason is that anti-cancer drugs can not be able to recognize the difference between cancer cell and normal cell leading to destroying both of them. Therefore, developing new drugs or drug formulations that are safe for patients but still induce high anti-cancer activity is very necessary.

Curcumin, a natural compound isolated from rhizomes of the herb Curcuma longa, has received considerable attentions because of its multiple biological and pharmaceutical activities including anti-inflammatory, anti- oxidant, wound healing, anti-microbial and anti-cancer activities [1]. The anti-cancer activity has been extensively studied. It was suggested Curcumin as a potential agent for both prevention and treatment of various cancers such as gastrointestinal, melanoma, genito-urinary, breast, lung, hematological, head and neck, neurological and sarcoma [2-4]. Interestingly, Curcumin has been demonstrated having no toxic effects on normal cells at the concentration as high as 50 µM while still exhibiting good anti-cancer activity [5]. Recent clinical study also indicated that oral administration of Curcumin was well tolerated at doses of 12g/day [6].

Despite of the potential biological and pharmaceutical properties of Curcumin, its clinical applications are still limited because of its poor water solubility and low bioavailability. It was reported that the solubility of Curcumin in aqueous buffer is only 0.11 10^{-4} mg.mL^{-1} [7] and its oral bioavailability is 1% [8]. In order to increase the water solubility and bioavailability of Curcumin, many drug delivery systems have been studied such as liposome, polymeric micelle, polymeric nanoparticle, conjugate, peptide/protein carrier [9]. Among them, polymeric micelles have attracted great deal of attention thanks to their small size, highly structural stability, high drug loading capacity and good bioavailability [10, 11]. Polymeric micelles have a core-shell structure with the hydrophobic core serving as microenvironment for incorporating hydrophobic drugs and the hydrophilic shell stabilizing the micelles and protecting hydrophobic drugs from physiological environment of the body [12]. Core/shell ratio of polymeric micelle was demonstrated having large impact on hydrophobic drug loading capacity, stability, pharmaceutical and biological activities of polymeric systems [13, 14].

In this study, we aimed to study the impact of PLA/PEG ratios on Curcumin solubility, Curcumin encapsulation efficacy, size and also the Curcumin release behavior of Curcumin loaded polymeric micelles composed by PLA-PEG copolymers.

Materials and Methods

Materials

Curcumin (Cur), lactic acide (LA), polyethylen glycol (PEG 2000), stannuos octoate ($Sn(Oct)_2$) were purchased from Sigma (USA). Solvents (toluen, dichlomethan, methanol, ethanol, phosphate buffered saline PBS (pH 7.4)) were purchased from Merck (Germany). All chemicals were used without further purification. Distilled water was used for all experiments.

Synthesis of copolymers

PLA-PEG copolymers were synthesized by ring-opening polymerzation of lactic monomer (LA) and PEG using stannuous octoate as catalyst [15]. The ratios of LA/PEG (w/w) were changed in the range of 3:1; 2:1; 1:1; 1:2; 1:3. Polymerization reaction was performed at 145^0C under magnetical stirring. After 10h reaction, solvent was evaporated at 110^0C. Obtained copolymer was dissolved in DCM and then purified by precipitating in cool methanol. Purification process was repeated three times and the copolymer was dried under vacuum at 45^0C for 48 h.

Preparation of PLA-PEG polymeric micelles (PLA-PEG NPs)

PLA-PEG NPs were prepared by dissolving 60 mg copolymer in 40 ml distilled water and magnetically stirring for 6h. After that, the mixture was centrifuged at 5000 rpm for 10 minute to remove undispersed copolymer. The obtained solution was lyophilized to collect dry PLA-PEG NPs and then stored at 4^0C for further uses.

Preparation of Curcumin loaded PLA-PEG polymeric micelles (Cur/PLA-PEG NPs)

Five kinds of copolymer with the different ratios of PLA/PEG (3:1; 2:1; 1:1; 1:2; 1:3 (w/w)) were used to prepare Cur/PLA-PEG NPs by emulsification/solvent evaporation method. In brief, 150 mg copolymer was dispersed in 100 ml distilled water (solution A) and magnetically stirring for 6h to ensure complete homogeneity. 80 mg Curcumin was dissolved in 100 ml ethanol (solution B). The solution B was added dropwise to the solution A under magnetically stirring at room temperature. After 48 h stirring, the solvent (ethanol) was evaporated and the obtained mixture was centrifuged at 5000 rpm for 10 minutes in order to remove unencapsulated Curcumin. A half of obtained transparent solution was lyophilized to obtain dry Cur/PLA-PEG NPs. Solution and dry samples were stored at 4^0C for further use.

Curcumin encapsulation efficiency

Curcumin encapsulation efficiency (EE) was calculated by following formula:

$$\text{EE}\ (\%) = \frac{\text{Total amount of Cur} - \text{unencapsulated amount of Cur}}{\text{Total amount of Cur}} \times 100$$

Curcumin release behavior

5 mg dry Cur/PLA-PEG NPs was redispersed in 20 ml phosphate buffered saline (PBS, pH 7.4). The dispersion was incubated in water bath at 37^0C. After each period of time, 3 ml sample was taken and 3 ml distilled water was added. The taken sample was centrifuged at 5000 rpm for 10 minutes to remove released Curcumin. Concentration of Curcumin in obtained solution was measured by Ultraviolet-Visible spectroscopy. Curcumin release was calculated based on the following formula:

$$\text{Cur release}\ (\%) = \frac{C_0 - C_t}{C_0} \times 100$$

Where, C_0: initial concentration of Curcumin
C_t: Concentration of Curcumin at time t

Characterization methods

Surface morphology and particle size of nanoparticles were investigated by by Field Emission Scanning Electron Microscopy (FE-SEM, Hitachi S-4800). Curcumin concentration was determined by Ultraviolet-Visible Spectrometer (UV-Vis Aligent 8453).

Results and Discussion

Morphology and size of PLA-PEG NPs and Cur/PLA-PEG NPs

FE-SEM method was used to investigate morphology and size of PLA-PEG NPs and Cur/PLA-PEG NPs (fig.1). The results showed that both PLA-PEG NPs and Cur/PLA-PEG NPs have spherical shape. There were changes in size of polymeric micelles composed by different PLA-PEG copolymer. Size of PLA-PEG NPs increased from 49 to 58 nm when ratio of PLA/PEG (w/w) increased from 1:3 to 3:1. Interestingly, size of PLA-PEG NPs almost had no changes when the ratio of PLA/PEG increased from 1:3 to 1:1 and remarkably increased when this ratio increased from 1:1 to 3:1 (Table 1). This result led us to assume that higher PLA component induces the larger nanoparticle. This result is in good agreement with other results studying the role of hydrophobic segment of copolymers on size of polymeric micelles [13]. High PEG component, meanwhile, seems not to impact to the size of nanoparticle. However, PEG component was demonstrated playing very important role for improving blood circulation time and avoiding recognition of reticuloendothelial system of drug delivery systems [16]. Similar to PLA-PEG NPs, size of nanoparticles containing drug Cur/PLA-PEG NPs also increased from 60 nm to 69 nm when the ratio of PLA/PEG increased from 1:3 to 3:1 (w/w)

(Table 1). This size range is most suitable for drug delivery system applied in cancer treatment [17].

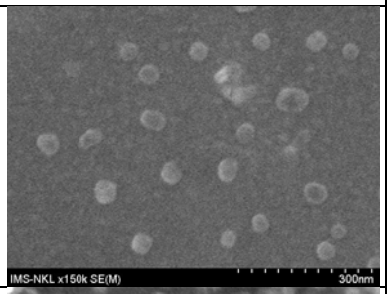
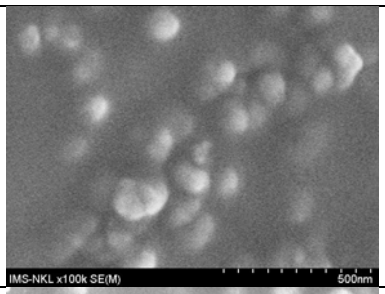
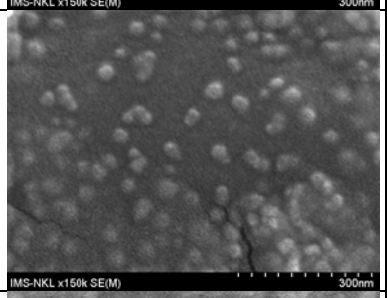
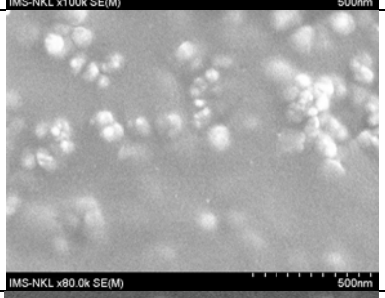
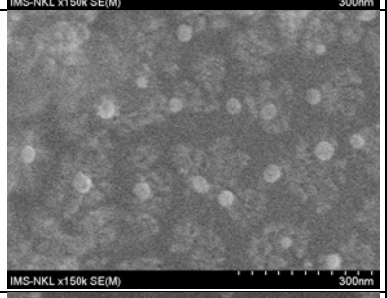
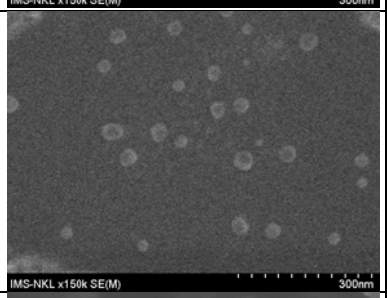
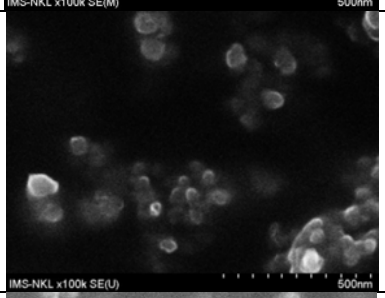
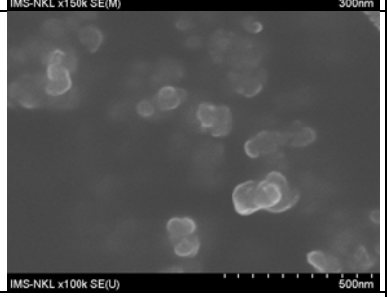
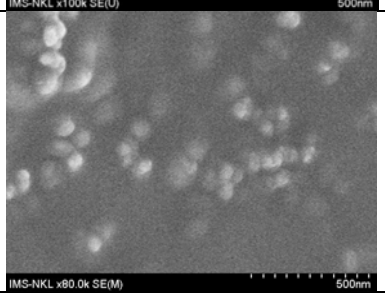
PLA/PEG(w/w)	PLA-PEG NPs	Cur/PLA-PEG NPs
3:1		
2:1		
1:1		
1:2		
1:3		
	(A)	(B)

Figure.1. FE-SEM images of PLA-PEG NPs (A) and Cur/PLA-PEG NPs (B)

Table 1. Characteristics of Cur/PLA-PEG NPs

Ratios of PLA/PEG (w/w)	Size of nanoparticles (nm)		Cur Solubility ($mg.mL^{-1}$)	EE (%)
	PLA-PEG NPs	Cur/PLA-PEG NPs		
1:3	49.5 ± 1.6	60.6 ± 1.4	0.39	48.8
1:2	49.7 ± 1.6	60.7 ± 1.4	0.40	50
1:1	50.2 ± 1.6	60.5 ± 1.5	0.43	53.8
2:1	55.2 ± 1.7	65.7 ± 1.7	0.63	78.8
3:1	58.2 ± 1.8	69.2 ± 1.9	0.73	91.3

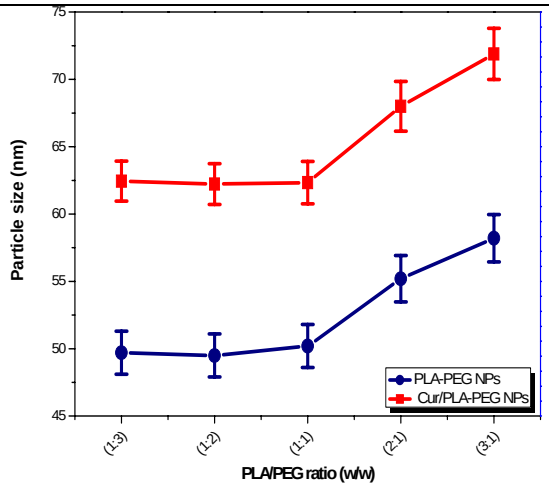


Figure.2. Impact of PLA/PEG ratios on particle size of PLA-PEG NPs and Cur/PLA-PEG NPs

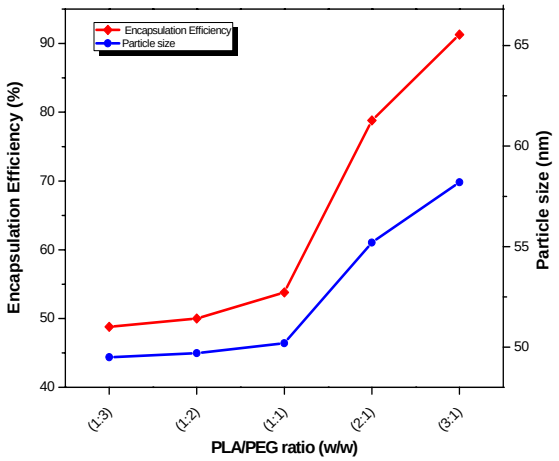


Figure.3. Changes of particle mean size and Curcumin encapsulation efficiency of Cur/PLA-TPGS when increasing PLA/PEG ratio

Curcumin solubility and Encapsulation efficiency

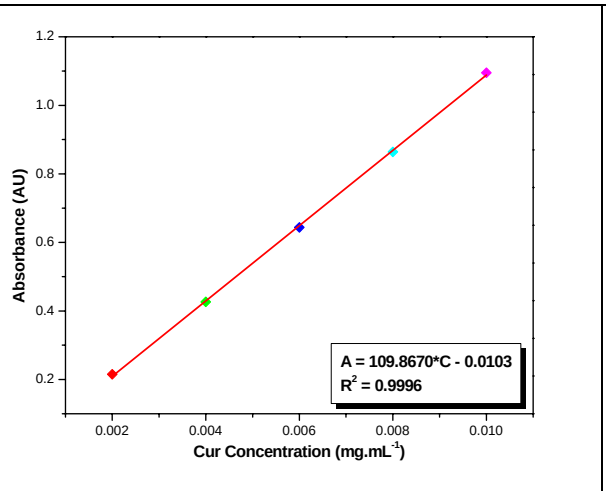


Figure.4 Calibration curve of Curcumin dissolved in ethanol

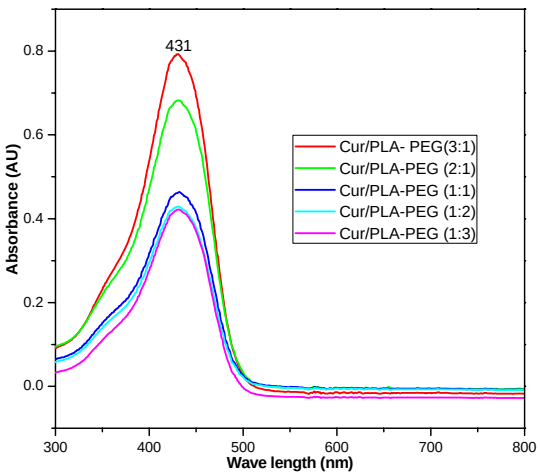


Figure. 5. UV spectra of Cur/PLA-PEG NPs dissolved in ethanol

Curcumin solubility was measured by UV spectrometer. 0.1 ml of each solution sample was added to 9.9 ml ethanol and then measured in the wavelength range of 300-800 nm. The Curcumin solubility was calculated from the absorbance of Curcumin at 431 nm based on the equation obtained from the calibration curve of Curcumin in ethanol (fig.4). This wavelength is not interfered by the presence of copolymers. The Curcumin solubility in aqueous solution was greatly increased with the increased component of PLA, achieving highest value (0.73 $mg.mL^{-1}$) at the ratio of PLA/PEG being 3:1. Meanwhile, there was no obvious increase of Curcumin solubility when the ratio of PLA/PEG varied from 1:3 to 1:1 (around 0.4 $mg.ml^{-1}$). For this result, we suggested that the PLA component significantly influences on the Curcumin solubility. Higher PLA component results to better Curcumin solubility. The increase in Curcumin solubility may be attributed to the increased hydrophobic interactions between drug and hydrophobic core of polymeric micelles [18].

Curucumin encapsulation efficiency also increased remarkably when increasing the PLA/PEG ratio. It was around 50% when PLA/PEG ratio was from 1:3 to 1:1 and increased strongly reaching 91.3% when the PLA/PEG ratio was 3:1.

Curcumin release behavior

Fig. 6. shows the Curcumin release from Cur/PLA-PEG NPs. For all cases, the Curcumin release from nanoparticles displayed a biphasic release profile. The initial burst associated with the fast release of drug molecules located in the shell or at the core-shell interface of polymeric micelles took place in the first 12h. In the second phase, Curcumin was progressively released corresponding to the diffusion of Curcumin from the inner core into the outer aqueous environment. This release kinetic has been well-documented for most polymeric micelles [19-21]. However, Curcumin release rate was different for each kind of Cur/PLA-PEG NPs. It was fastest when curcumin was encapsulated in polymeric micelles composed by PLA-PEG at the ratio of PLA/PEG being 1:3. It was slower at higher PLA/PEG ratios. These results could be explained by two reasons. Firstly, higher PLA component could lead to stronger hydrophobic interaction between Curcumin and the hydrophobic core [22]. Secondly, at lower loading, Curcumin maybe present as a dispersed state in the core segment whereas a crystallization of drug in the PLA core occurs at higher loading [23]. The crystalized drug would be more slowly dissolved and diffused into the outer aqueous environment [24].

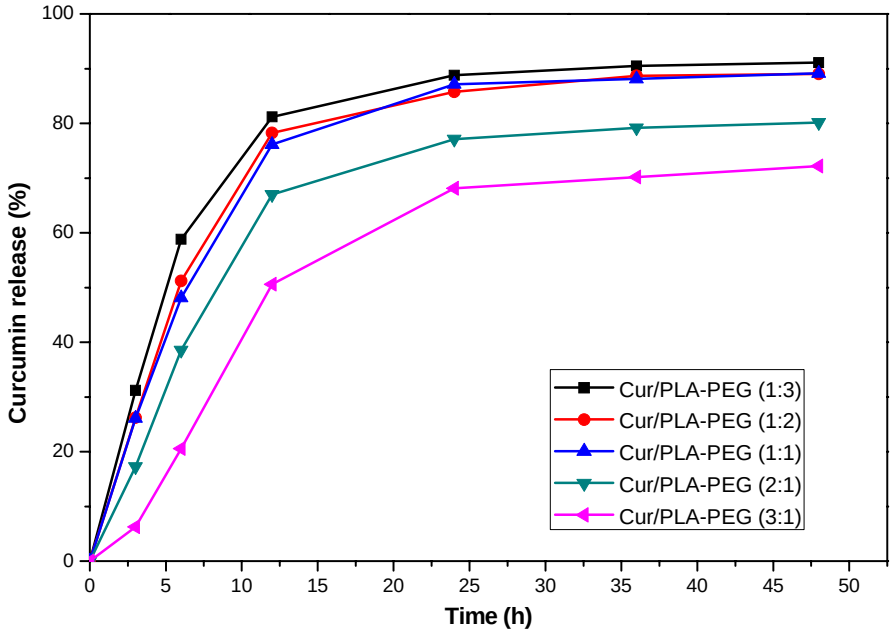


Figure.6. Curcumin release profiles of Cur/PLA-PEG NPs

Conclusion

Curcumin is a potential therapeutic agent owning a lot of bioactivities including anti-inflammatory, anti-oxidant, wound healing, anti-microbial and anti-cancer activities. However, its low aqueous solubility and bioavailability are major obstacles for its clinical applications. In this study, polymeric micelles composed by PLA-PEG copolymers were applied in order to improve Curcumin limitations. Systematic investigation showed that PLA/PEG ratio of PLA-PEG copolymer has large impact on Curcumin solubility, encapsulation efficiency, size and Curcumin release behavior of Cur/PLA-PEG NPs. Higher PLA/PEG ratio results in better Curcumin solubility, encapsulation efficiency and larger particle

size. Reversely, Curcumin release rate decreased when PLA/PEG increased. Noticeably, these changes were clearly happened when the PLA/PEG ratio increase from 1:1 to 3:1. However, as discussed, PEG component plays very important role determining efficiency of drug delivery system after administration. Therefore, optimum PLA/PEG ratio is only achieved by further intensive studies on cellular uptake and bioavailability of Cur/PLA-PEG NPs.

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