TẠP CHÍ KHOA HỌC ĐẠI HỌC KHÁNH HÒA
tapchikhoahoc@ukh.edu.vn

SYNTHESIS, CHARACTERISTICS OF CURCUMIN-LOADED MICELLAR NANOSYSTEM

Phan Quoc Thong[1], Ly Ngoc Tai[1], Nguyen Minh Hiep[3], Ha Phuong Thu[2]

[1]*University of Khanh Hoa,*
[2]*Institute of Materials Science, Institute of Chemistry, Vietnam Academy of Science and Technology*
[3]*DaLat Nuclear research Institute*

Abstract: *Targeting delivery system use natural drugs for tumor cells is an appealing platform help to reduce the side effects and enhance the therapeutic effects of the drug. In this study, we synthesized curcumin (Cur) loaded (D, L Polylactic – Polyethylen glycol) micelle (Cur/PLA-PEG) with the ratio of PLA/PEG of 3/1 (w/w). The PLA-PEG copolymer was synthesized by ring opening polymerization method. After loading onto the micelle, solubility of Cur increased to 0.73 mg.ml^{-1}. The average size of prepared Cur/PLA-PEG micelles was 69 nm and the drug encapsulating efficiency was 91.3%. The results demonstrated that micelle (Cur/PLA-PEG) could serve as a potential nano carrier to improve solubility of the nanosystem.*

Keywords: *Curcumin (Cur), PLA-PEG copolymer, PLA-PEG/Cur, nanosystem.*

1. Introduction

Curcumin (Cur) or diferuloylmethane, bis(4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dion is a yellow polyphenol compound extracted from the rhizome of turmeric (*Curcumin longa*), a plant grown in tropical Southeast Asia [1-4]. Recently, a great deal of research has been reported that Cur has a wide range of pharmacologic activities such as anti-inflammation, anti-human immuno-deficiency virus, anti-microbial, anti-oxidant, anti-parasitic, anti-mutagenic and anti-cancer with low or no intrinsic toxicity. Among these pharmacologic activities, Cur has been paid most attention on its anti-cancer activity to prevent and inhibit the generation, metastasis of many of kinds of tumors, such as breast cancer, cervical cancer, colon carcinoma, stomach cancer, liver cancer, epithelial cell carcinoma, pancreatic cancer [5]. The clinical studies of Cur for cancer are still continuous. For example, the treatment of pancreatic by Cur is studied on phase II clinical [6], and studied on phase I clinical for breast cancer [7]. Despite of its excellent anti-cancer properties, low solubility in aqueous solution and rapid decomposition in physiological conditions of Cur make its clinic application become limited [8, 9]. Therefore, improving the stability, solubility and bioactivity of Cur is necessary.

It is pointed out that there are numerous ways to improve bioavailability of hydrophobic drugs, among which using polymeric micelle is one of the most attractive alternatives. The polymeric micelles have core-shell structure formed by amphiphilic block which can solubilize water poorly soluble drugs. The polymeric micelles have such more advantages than other delivery systems as: [10 - 13], (i) able to conjugate with targeting molecules via surface modification and achieve the possible targeting, (ii) capturing the hydrophobic drugs into hydrophobic inner core so that protecting drugs from adverse surrounding environments and improving the apparent dissolvability of drugs, (iii) reducing the nonspecific uptake by the reticuloendothelial system (RES).

2. Material and methods

2.1. Materials

Curcumin (Cur), lactic acid (LA), polyethylen glycol (PEG 2000), stannous octoate ($Sn(Oct)_2$), maleic anhydride, folic acid were purchased from Sigma (USA). Solvents (toluene, dichlomethan (DCM), methanol, ethanol, Phosphate Buffered Saline (PBS, pH 7.4)) were purchased from Merck (Darmstadt, Germany). All chemicals were used

without further purification. Distilled water was used for all experiments.

2.2. Methods

2.2.1. Synthesis of PLA-PEG block copolymer

PLA-PEG block copolymer was synthesized by ring-opening polymerization of lactide monomer in the present of polyethylene glycol (PEG) using stannous octoate as catalyst [Sabharanjak et al 2005] with the ratio of PLA/PEG (w/w) is 3:1. Polymerization reaction was performed at 130^{o}C under inert gas atmospherely. After 10 h reaction, solvent was evaporated at 110^{o}C. 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^{o}C for 48 h.

2.2.2. Preparation of curcumin loaded PLA-PEG polymeric micelle (Cur/PLA-PEG)

Curcumin loaded PLA-PEG micelle was prepared by emulsification/solvent evaporation method. In brief, PLA-PEG copolymer was dispersed in distilled water and magnetically stirring for 6 h to ensure complete homogeneity. Curcumin was dissolved in ethanol and was added with gentle stirring at room temperature. After 24 h stirring, ethanol was evaporated and the obtained mixture was centrifuged at 3000 rpm for 10 minutes to remove the excess curcumin.

2.2.3. Characterization methods

The microstructure and particle size of copolymer PLA-PEG, Cur/PLA-PEG were observed under the field emission scanning electron microscopy (FE-SEM, Hitachi S-4800). Curcumin loaded micelles were dissolved in methanol to determine the amount of curcumin loaded. The resultant solutions were then analyzed by an ultraviolet-visible spectrometer (UV-Vis Aligent 8453) at the wave length of 428 nm.

2.2.4. Curcumin entrapment efficiency (EE)

Curcumin EE of Cur/PLA-PEG-Fol was calculated by following formula:

$$EE\ (\%) = \frac{W_{total} - W_{unentrapped}}{W_{total}} \times 100$$

In which, W_{total} was the feeding curcumin, W_{excess} showed amount of excess curcumin in Cur/PLA-PEG-Fol In vitro Curcumin release

10 mg of Cur/PLA-PEG or Cur/PLA-PEG-Fol NPs was dispersed in 30 ml Phosphate Buffered Saline (PBS, pH 7.4). The dispersion was incubated in water bath at 37^{o}C. At desired time intervals, 2 ml sample was withdrawn and replaced with an equal volume of the fresh release medium. The curcumin concentration in each taken sample was determined by Ultraviolet-Visible spectroscopy.

3. Results and discussion

3.1. Synthesis and characterization of Cur/PLA-PEG

PLA-PEG block copolymer was synthesized by ring-opening polymerization [11]. After that, Cur was loaded on PLA-PEG polymeric micelle to form Cur/PLA-PEG. The characterization of PLA-PEG and Cur/PLA-PEG were demonstrated by ^{1}H NMR and IR spectra, which were reported in our previous study [14].

Comparing fluorescence spectra showed that the emission band was shifted from 540 nm of free Cur to 535 nm of Cur/PLA-PEG (fig 1a). Moreover, the absorption band of curcumin in blank solution was observed at 431 nm, while the absorption band of Cur/PLA-PEG shifted to 426 nm (fig 1b). The absorption band shifts of both fluorescence and UV-Vis spectra can be explained by the formation of hydrophobic interaction between Cur and hydrophobic segments PLA of PLA-PEG as well as conjugation between Fol and PLA-PEG micelles. In addition, the fluorescence intensity of encapsulated Cur solution was higher than that of free Cur solution. This probably came from the interaction of Cur with PLA-PEG copolymer.

According to the FT-IR spectra of PLA and PLA-PEG recorded (fig 2a), the CH stretching bands observed at 2924 cm^{-1} in PLA have shifted to 2978 cm^{-1} in PLA-PEG. The peak of –CO–O– groups of PLA appeared at 1554 cm^{-1} and changed to 1539 cm^{-1} in PLA-PEG.

Compared with that of pure Cur, the IR spectrum of Cur/PLA-PEG (fig 2a) shows band shifts from 1751 (C=O vibrations) to 1746 cm^{-1} and 1250 (aromatic C–O stretching vibration) to 1276 cm^{-1}. Especially, when comparing PLA-PEG and Cur/PLA-PEG, two shifts were observed from 2978 to 2985 cm^{-1} and from 1750 to 1746 cm^{-1} due to CH stretching and ester stretching, respectively. The IR data showed that Cur was encapsulated in the hydrophobic core of micelles of PLA-PEG.

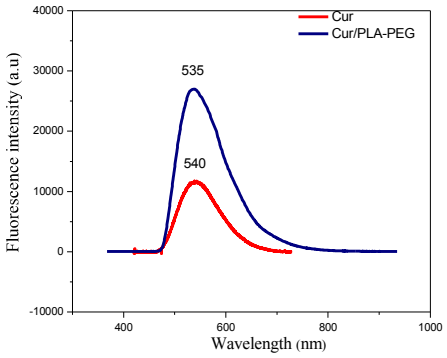


Fig 1a. Fluorescence spectra of Cur and Cur/PLA-PEG

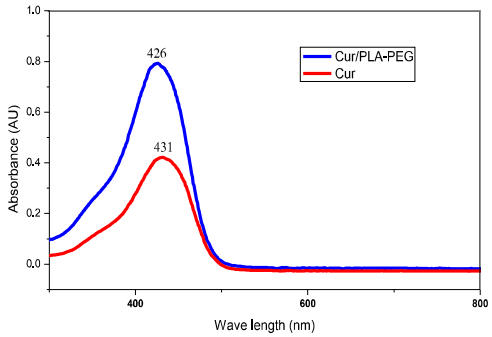


Fig 1b. UV-Vis spectra of Cur and Cur/PLA-PEG

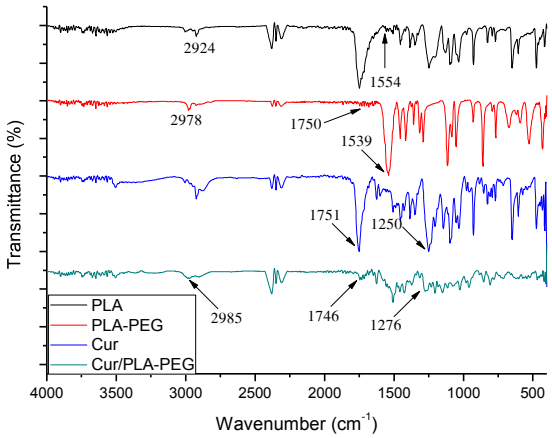


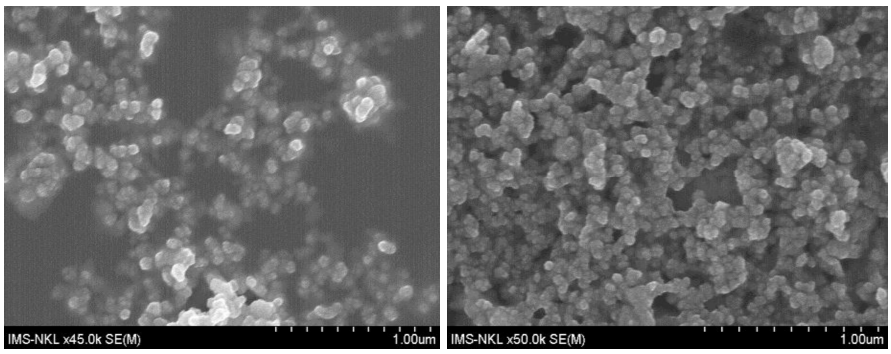
Fig 2a. FT-IR spectra of PLA, PLA-PEG, Cur and Cur/PLA-PEG

3.2. Size distribution and morphology

The size distribution and morphology of PLA-PEG with and without Cur were measured by FE-SEM. The results showed that PLA-PEG, Cur/PLA-PEG nanoparticles are all spherical and monodisperse (fig 3). The average size of Cur/PLA-PEG NPs (69.2 ± 1.9 nm) containing drug Cur is larger than that of PLA-PEG NPs without drug Cur (58.2 ± 1.8 nm), meanwhile the average size of Cur/PLA-PEG-Fol NPs is about 90 nm ± 2.3 nm (table 1). This can be explained by the core-shell structure of polymeric micelles and hydrophobic interactions of PLA core and hydrophobic drug (Cur) (which causes Cur to be trapped in the inner core of polymeric micelles) as well as the effect of targeted factor (folate) on the size of nanoparticles [13]. With the size range smaller than 100 nm, the polymeric micelles can escape from mononuclear phagocytic system (MPS) and prolong their time in the blood. Tumor capillaries have pore with size ranging from 200 nm to 1.2 µm [15] and the polymeric micelles prepared in this study can easily extravasate into the tumor tissue.

Table 1: Characteristic of PLA-PEG and Cur/PLA-PEG NPs

Copolymers PLA:PEG 3:1	Size of NPs (nm)	Cur solubility $mg.mL^{-1}$	EE%
PLA-PEG	58.2 ± 1.8		
Cur/PLA-PEG	69.2 ± 1.9	0.73	91.3%



(A) (B)

Fig 3. FE-SEM of PLA-PEG NPs (A), Cur/PLA-PEG NPs (B)

3.3. Drug encapsulation efficiency

The Cur loaded PLA-PEG samples were determined by UV-Vis absorbance at 428 nm, this wavelength is not interfered by the presence of copolymers. The average EE of the optimized Cur/PLA-PEG NPs formulation were 91.3% (tab 1). The Cur solubility of NPs in aqueous solution was improved to be about 0.73 mg/ml (tab 1), which was about as 7 x 10^2 times as the solubility of pure Cur in water (11 μg/ml) [16]. Therefore, the results indicated that the obtained micelles system significantly enhance the solubility of Cur in aqueous solution.

4. Conclusions

In this study, we prepared a polymeric micellar formulation of Cur. Cur was loaded to hydrophobic core of the block copolymer by a hydrophobic linkage to produce Cur/PLA-PEG. This linkage would support Cur to enter the core of the block copolymer therefore increase the solubility of Cur in water. This formulation can provide active targeting and controlled release of Cur under the physiological environmental conditions of cancer cells. The nano copolymer drug delivery targeting systems have a unique potential for delivery of Cur because of the ability to prolong circulation time in the blood due to its chemical surface, high tumor accumulation due to the appropriate size. Therefore, the micellar NPs can be expected to strongly increase the effectiveness of Cur in nanosystem.

REFERENCES

1. Shishodia S, S.G., Aggarwal BB, *Curcumin getting back to the roots.* Ann N Y Acad Sci, 2005. 1056: p. 206-217.
2. M.A. El-Moselhy, A.T., S.S. Sharkawi, S.F.I. El-Sisi, A.F. Ahmed, Food Chem.Toxicol. 49, 2011. 1129.
3. R.M. Srivastava, S.S., S.K. Dubey, K. Misra, A. Khar, Int. Immunopharmacol.11, 2011. 331.
4. K. Hossain, H.M., M. Kato, T. Miyata, R. Inagi, J. Du, K. Takeda, Y. Kawamoto,H. Suzuki, K. Kurokawa, I. Nakashima, Free Radic, Free Radic. Biol. Med. 31, 2001. 20.
5. A. Banerji, J.C., A. Mitra, A. Chatterjee, Cancer Lett 211, 2004. 235.
6. T.H. Kim, H.H.J., Y.S. Youn, C.W. Park, K.K. Tak, S. Lee, H. Kim, S. Jon, X. Chen, K.C. Lee, Int.J. Pharm 403, 2011. 285.
7. N. Dhillon, B.B.A., R.A. Newman, R.A. Wolff, A.B. Kunnumakkara, J.L.Abbruzzese, C.S. Ng, V. Badmaev, R. Kurzrock, Clin. Cancer Res. 14 2008. 4491.
8. M. Bayet-Robert, F.K., M. Leheurteur, F. Gachon, E. Planchat, C.Abrial, M.A. Mouret-Reynier, X. Durando, C. Barthomeuf, P. Chollet, Cancer Biol.Ther. 9, 2010. 8.
9. X. Wu, J.X., X. Huang, C. Wen, *Drug Dev.* Ind. Pharm 37, 2011. 15.
10. Jurenka, J.S., Altern. Med. Rev. 14, 2009. 141.
11. S. Chen, X.Z.Z., S.X. Cheng, R.X. Zhuo, Z.W, Biomacromolecules 9, 2008. 2578.
12. Y. Xiao, H.H., A. Javadi, W. Engle, W. Xu, Y. Yang, Y. Zhang, T.E. Barnhart, W.Cai, S. Gong, Biomaterials, 2012. 3071: p. 33.
13. K.M. Huh, S.C.L., Y.W. Cho, J. Lee, J.H. Jeong, K. Park, J. Control, Release, 2005. 59: p. 101. L. Liu, C.L., X. Li, Z. Yuan, Y. An, B. He, J. Appl. Polym. Sci. 80, 2001. 1976.
14. Ha.P.T, D.T.Q., Mai.T.T.T, Tran.T.H.H, Nguyen.H.N, Nguyen.X.P, Tran.T.M.N, Phan.Q.T, Phan.T.H.T, Vuong.T.K.O and Le.M.H, Chem. Lett, 2013. 42: p. 255257.
15. S.K. Hobbs, W.L.M., F. Yuan, W.G. Roberts, L. Griffith, V.P. Torchilin, R.K.Jain, Proc. Natl. Acad. Sci. U. S. A. 95, 1998. 4607.
16. Z. Song, R.F., M. Sun, C. Guo, Y. Gao, L. Li, G. Zhai, J. Colloid Interf. Sci. 354, 2011. 116.