

# Formulation conditions on the drug loading properties of polymeric micelles

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**Abstract:** Methyl poly(ethylene glycol) grafted poly (lactide-co-(glycolic acid)-alt-(glutamic acid) amphiphilic copolymers (PLG-g-mPEG) were fabricated polymeric micelles to load anticancer drug doxorubicin (DOX). Both blank and drug loaded micelles were spherical nanoparticles with the mean sizes around 50 and 100nm, respectively. The effects of formulation conditions including compositions, concentrations, temperature, feeding doses and solvents on the size and drug loading content were investigated, the storage of the drug loaded micelles was explored. The results showed that the short graft mPEG chain length was favorable for the loading of DOX. The increase of temperature was preferable for receive micelles with higher drug loading content and smaller size. The encapsulation of polymeric micelles could protect the bioactivity of DOX. In vitro drug release profiles illustrated that the drug release from polymeric micelles with long mPEG chains was much faster than from micelles with short mPEG chains. The release kinetics of drug from micelles fitted to the Ritger-Peppas equation well and the release process followed diffusion mechanism.

**Keywords:** Polymeric micelles, formulation, drug delivery, biodegradable.

## INTRODUCTION

Polymeric micelles have been attracted widespread attention to pharmacists as several polymeric micelles based nanomedicines have been in clinical trials and exhibited optimistic therapeutic effects. (Gong *et al.*, 2012, Hrkach *et al.*, 2012) Comparing to liposomes, polymeric micelles exhibited better stability during the transportation in blood vessels, which possessed the functions of long circulation and passive targeting tumors via EPR effect (Cao *et al.*, 2014, Yohan *et al.*, 2015). The micelles with core-shell architecture were self-assembled from the amphiphilic copolymers, and the lipophilic anticancer drug was encapsulated in hydrophobic cores, which reduced the extent of burst release in the delivery. (Cao *et al.*, 2013, Zhang *et al.*, 2014)

Biodegradable polymeric amphiphiles are important carriers to fabricate micelles, polymeric micelles self-assembled from PEG-polyester diblock or triblock copolymers were extensively reported in anticancer drug delivery, (Cao *et al.*, 2014, Zavanah *et al.*, 2006) besides block copolymers, amphiphilic graft copolymers were also reported for fabricating polymeric micelles. (Zhang *et al.*, 2010) Taking the advantages of plenty pendant functional groups on the backbones, many natural polymers including chitosan, dextran and pullulan were grafted with hydrophobic moieties to prepare polymeric amphiphiles with grafts (Choi *et al.*, 2008; Wang *et al.*, 2016). Although the critical micelle concentration (CMC) of natural polymer micelles was lower than that of block copolymer micelles, which revealed better stability (Chen

*et al.*, 2013, Guo *et al.*, 2010) the application of natural polymer micelles was limited due to the high molecular weight, strong hydrogen bonding and their enzyme-catalyzed degradation of natural polymers.

The polymerization of morpholine-2,5-dione derivative could receive a synthetic biodegradable polymer with pendant groups, poly (glycolic acid-alt-glutamic acid) was used to conjugate anticancer drug (Xie *et al.*, 2007). The self-assembly of the conjugates formed micelles and the drug was released through the hydrolysis of the ester linkages. Other polymeric micelles containing poly (glycolic acid-alt-glutamic acid) were also reported in drug delivery, they exhibited promising therapeutic efficiency both *in vitro* and *in vivo* (Feng *et al.*, 2001, Lu *et al.*, 2006)

To evaluate the formulation of drug loaded polymeric micelles, drug loading content and average size are two important parameters. It was reported that the preparation conditions including compositions, concentrations, temperature, feeding doses and solvents affected the properties of drug loaded liposomes, especially in the drug loading content and mean size (Aliabadi *et al.*, 2007; Cao *et al.*, 2010; Frank *et al.*, 2005). Although there were many papers reported the polymeric micelles based nanomedicine, most of the researches focused on the fabrication of polymeric micelle, *in vitro* and *in vivo* therapeutic efficacy of polymeric micelle nanomedicine, (Liang *et al.*, 2015) however, no systemic research on the formulation conditions to the properties of drug loaded micelles was reported.

In this paper, we reported the effects of formulation conditions on the properties, emphasized on drug loading

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efficiency and micelles' size. Methyl poly (ethylene glycol) graft poly (lactide-co-(glycolic acid)-alt-(glutamic acid) amphiphilic copolymers (mPEG-PLG) were used to fabricate polymeric micelles and load DOX. The effects of formulation conditions including compositions, concentrations, temperature, feeding doses and solvents on the micelles' size and DOX-loading content were explored. The release kinetics and release mechanism of DOX-loaded micelles were studied.

## MATERIALS AND METHODS

### Materials

Methyl poly (ethylene glycol) graft poly (lactide-co-(glycolic acid)-alt-(glutamic acid) amphiphilic copolymers were purchased from Clear Biomaterials Company, Wenzhou, China. The composition of morpholine-2,5-dione was 10% in the copolymers, the molecular weights (Mw) of grafted mPEG were 2000, 1100 and 500, respectively. Doxorubicin hydrochloride (DOX·HCl, Shanghai Yingxuan Chempharm Co. Ltd., China) was dissolved in water, NaOH solution (0.1M) was added in the DOX·HCl solution dropwise receive DOX. (Cao, Su *et al.*, 2014) All the solvents (Chengdu Kelong Chemical Co. Ltd.) were purified before used.

### Critical Micelle Concentration (CMC)

The CMC of micelles was measured using pyrene as the fluorescence probe. Prescribed amount of pyrene was dissolved in distilled acetone, distilled water was added after acetone evaporated and the final concentration was  $6 \times 10^{-7}$  M. (Cao, Xiu *et al.*, 2012) The dilution of copolymer micelles with different concentrations was prepared from 1.0 to  $1.0 \times 10^{-8}$  mg/mL. Fluorescence spectra were recorded on Hitachi F-7000 fluorescence spectrometer with the excitation at 300nm, and the emission fluorescence at 338nm and 334nm was monitored. The CMC was estimated as the cross-point of extrapolating the intensity ratios  $I_{338}/I_{334}$  at low and high concentration regions.

### Preparation of drug-loaded micelles

Three methods of solvent evaporation, emulsion (O/W) or dialysis were involved to prepare drug-loaded micelles. The DOX-loaded micelles prepared by solvent method were used as an example. DOX was dissolved in the mixture solvents of tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO). After stirred a period of time, the DOX solution was slowly added into PLG-g-mPEG copolymer solutions (10mg/mL). The solution was stirred overnight to evaporate the organic solvent completely and was filtrated. After centrifugation, the inner phase was lyophilized to give DOX-loaded micelles. The whole procedure was carried out in dark. The preparation of drug loaded micelles by emulsion method was carried out as follow: the copolymer (10mg) in  $\text{CHCl}_3$  was dropwise added into distilled water. After stirred for 24h, the

micelle solution was filtrated.

The content of encapsulated DOX was confirmed by UV-vis spectra (Perkin-Elmer Ltd. Co., USA) using the maximum absorption wavelength of DOX at 485 nm. (Nan *et al.*, 2014) The standard curve was received from DOX-DMSO solutions with different DOX concentrations. Drug loading content (DLC) and Drug loading efficiency (DLE) were calculated according to the following equations:

$$DLC = \frac{\text{Weight of DOX in micelles}}{\text{Weight of drug loaded micelles}} \times 100\% \quad (1)$$

$$EE = \frac{\text{Weight of DOX in micelles}}{\text{Weight of DOX in feed}} \times 100\% \quad (2)$$

### Morphology of polymeric micelles

The morphology of micelles was observed by Transmission electron microscopy (TEM; JEM-100CX, JEOL, Japan). The samples were prepared by dropping freshly micelle solutions (0.1~0.5mg/mL) onto carbon-coated copper grids with Formvar film, the sample was dyed by a drop of 1% phosphotungstic acid solution. The samples were dried at room temperature.

The average hydrodynamic diameter and the size distribution of micelles were evaluated using dynamic light scattering (DLS) measurement (Zetasizer Nano ZS, Malvern Instrument Ltd. Co., Worcestershire, UK) with the concentration of 1mg/mL. The measurements were carried out at an angle of 173°C to the incident light at 25°C and each sample was recorded thrice to gain the mean diameter.

### Drug release profile

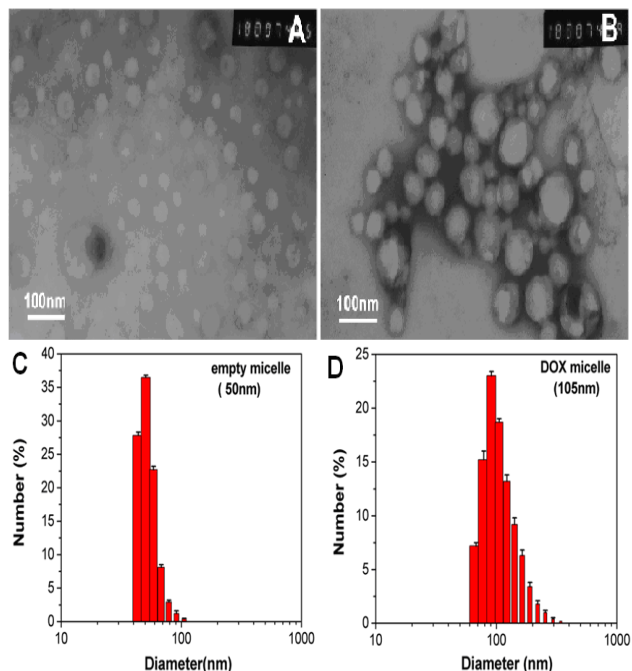
Lyophilized DOX-loaded micelles were dispersed in PBS with different pH (pH=7.4, 5.0; ionic strength=0.01M). 1 mL of solution in dialysis tubing (Spectra/Por, USA) with a molecular weight cut-off of 3kDa were immersed in vials containing 25mL of PBS (pH=7.4 or 5.0). The vials were put in a shaking bed with the shaking rate of 120 rpm at  $37 \pm 0.5^\circ\text{C}$ . The dialysate (1mL) was withdrawn for detection at time intervals and corresponding volume of fresh PBS was added to keep the volume. The amount of released DOX was detected by a fluorescence spectrometer with the excitation at 485nm and emission wavelength at 550nm. The releasing experiments were conducted in triplicate under sink condition and the results were expressed as mean value with standard deviation (SD).

## RESULTS

### Size and morphology of micelles

The size distribution of polymeric micelles was evaluated by DLS and the morphology was characterized by SEM (Wen and Meng, 2014). The nanomicelles of average size

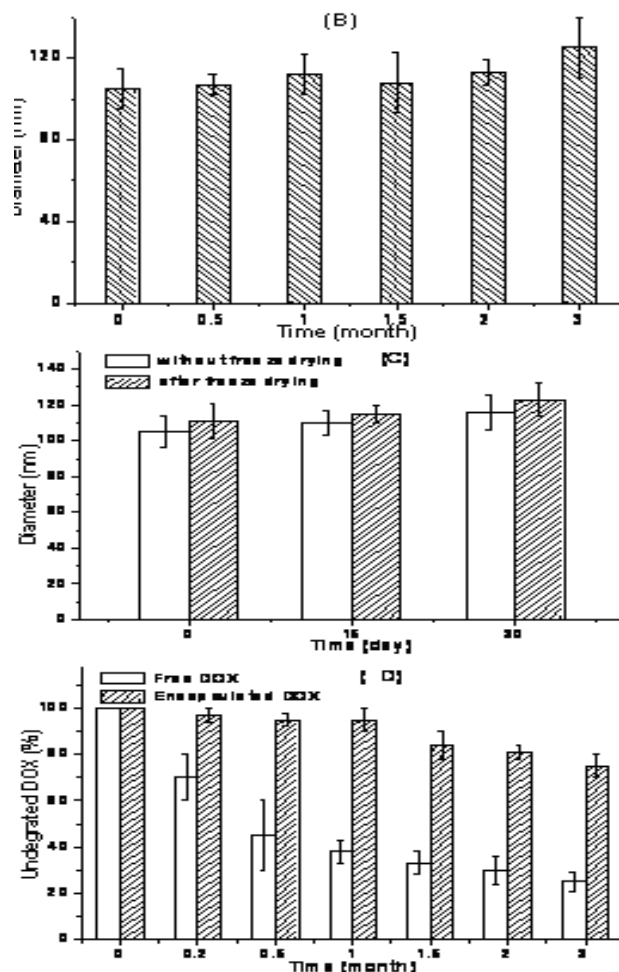
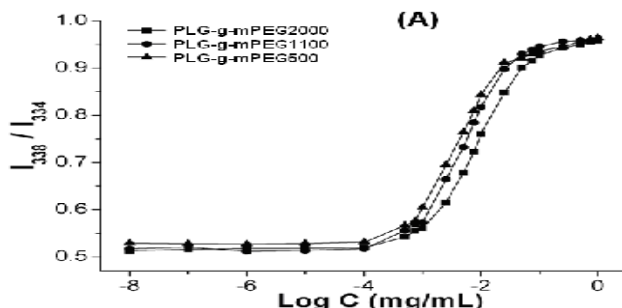
of 50nm with monodisperse were obtained by self-assembly of copolymer (fig. 1). After DOX was trapped, the size of micelles increased, indicating the successful encapsulation. Well dispersed and spherical morphology of PLG-g-mPEG2000 micelles were appeared in TEM images. The mean diameter of the blank micelle observed in TEM was around 50nm and that of drug-loaded micelle was around 100nm, which was in consistent with the DLS results.



**Fig. 1:** TEM images of empty (A) and DOX-loaded (B) PLG-g-mPEG2000 micelles, the size distribution of empty (C) and DOX-loaded (D) PLG-g-mPEG2000 micelles determined by DLS.

#### Stability of micelles

The critical micelle concentration (CMC) was an important parameter to characterize the stability of polymeric micelles. As shown in fig. 2A, the CMCs of those copolymer micelles were 1.04, 0.55 and 0.13 $\mu$ g/mL when the molecular weight of mPEG decreased from 2000 to 500.

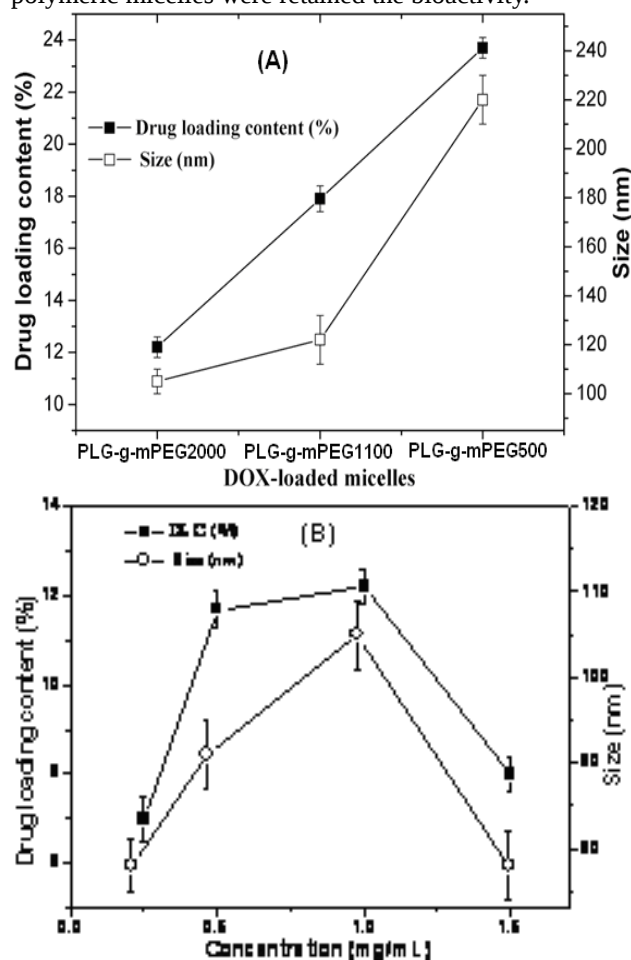


**Fig. 2:** Stability of drug loaded micelles, A: critical micelle concentration of polymeric micelles; B: stability in storage, stored at 4°C and pH7.4 for 3 months; C: stability with freeze-drying process with the storage for 30 days at 25°C; D: fraction of un-degraded drug for pure DOX (10%) and encapsulated DOX (eq.10%) stored at 4 °C and pH 7.4, determined by UV (485 nm) at 25°C.

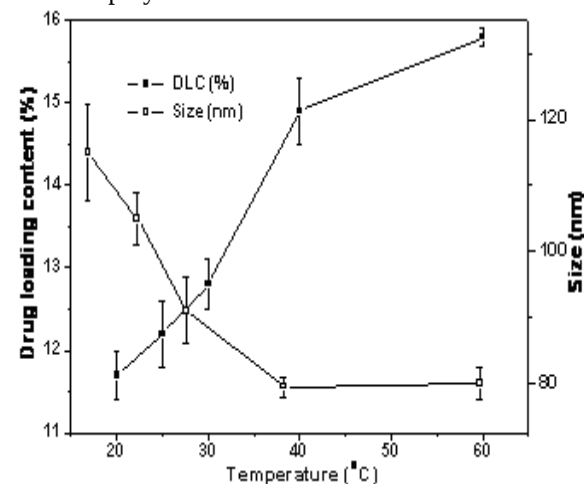
The stability of those micelles was further studied by storing at 4°C and freeze drying. Little size change was observed even the storage time was up to 3 months (fig. 2B), it meant those micelles possessed excellent stability suitable for clinical application. After the drug loaded micelles were freeze-dried, the sizes of the re-dispersed samples were comparable to the original micelles when the storage time was 1 month as shown in fig. 2C. It suggested that the polymeric micelles were not destroyed during the freeze-drying.

The activity of DOX in polymeric micelle determined their further therapeutic efficiency. The activity of DOX in polymeric micelles in storage was investigated. The results in fig. 2D showed that the activity of free DOX decreased rapidly, which was probably attributed to the automatically oxidation and hydrolysis rate (Beijnen *et al.*,

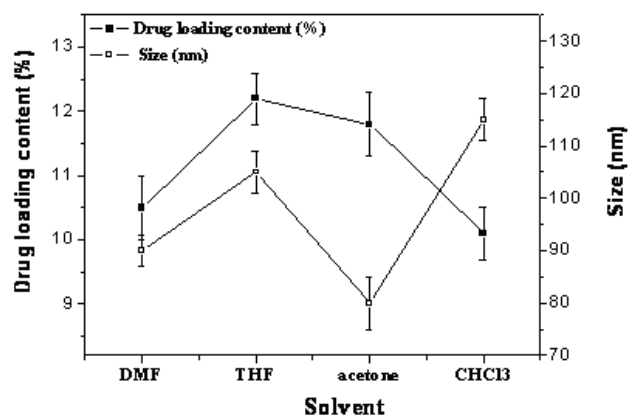
1986, Janssen *et al.*, 1985) However, the DOX encapsulated in polymeric micelles kept its bioactivity even the time was extended to 3 months, 80% DOX in polymeric micelles were retained the bioactivity.



**Fig. 3:** The size and DLC of (A) DOX-loaded PLG-g-mPEG micelles with different molecular weight of mPEG and (B) DOX-loaded PLG-g-mPEG2000 micelles in different polymer concentrations.



**Fig. 4:** The size and DLC of DOX-loaded PLG-g-mPEG2000 micelles prepared in different temperatures.



**Fig. 5:** Effects of solvent on the size and DLC of DOX-loaded micelles.

#### Formulation conditions on size and DLC

To further understand the effect of polymer composition on the size and drug loading, PLG-g-PEG with different compositions were used for fabricating drug loaded micelles. As shown in fig. 3, an increase of mPEG molecular weight from 500 to 2000 led to the reduction of DOX loading content and mean diameter.

Besides compositions, the concentration played a significant role in determining DOX loading and micelle size. As shown in fig. 3B, both DOX loading content and mean diameter of polymeric micelles increased with the increase of polymer concentration when the concentration was in the range of 0.02~1mg/mL, and the two indexes decreased with further increase of polymer concentration to 1.5mg/mL, 1mg/mL was the critical polymer concentration for the highest drug loading content.

#### Temperature effect on size and DLC

The temperature effect on the size and DLC of micelles was presented in fig. 4. A higher drug loading content was obtained when the preparation was carried out at higher temperatures. Interestingly, a reduction of size was observed with the increase of preparation temperature. The radius of micelle core increased with increasing temperature. 40 is the optimal temperature to load drug as the micelles possessed the highest drug loading content and the smallest size.

#### The ratio of organic solvent and water

To achieve an optimum polymeric micelle carrier for the delivery of DOX, the ratio of organic solvent to water in the formulation was explored. Different organic solvents including DMF, acetone, THF and CHCl<sub>3</sub> were used as organic solvents to dissolve the polymer, the solutions were added in aqueous phase with 1:20 (V: V) to receive micelles. The selection of organic solvent was based on their miscibility with water, dissolution of DOX and copolymer. The PLG-g-mPEG2000 and DOX in three different solvents (DMF, THF, acetone) showed the lower

average diameter and higher loading content comparing to those in  $\text{CHCl}_3$  (fig. 5). DOX exhibited better miscibility with PLG segment when DMF or THF or acetone was used as organic solvent, in the process of solvent evaporation, more DOX was retained in PLG cores. THF was the best organic solvent to prepare drug loaded micelles. The effect of THF to water volume fraction on the DOX loading content in polymeric micelles and the diameter of micelles was studied as shown in table 1, little raise in micelle size and a reduction in the encapsulated DOX when was observed when the volume fraction of THF to water increased.

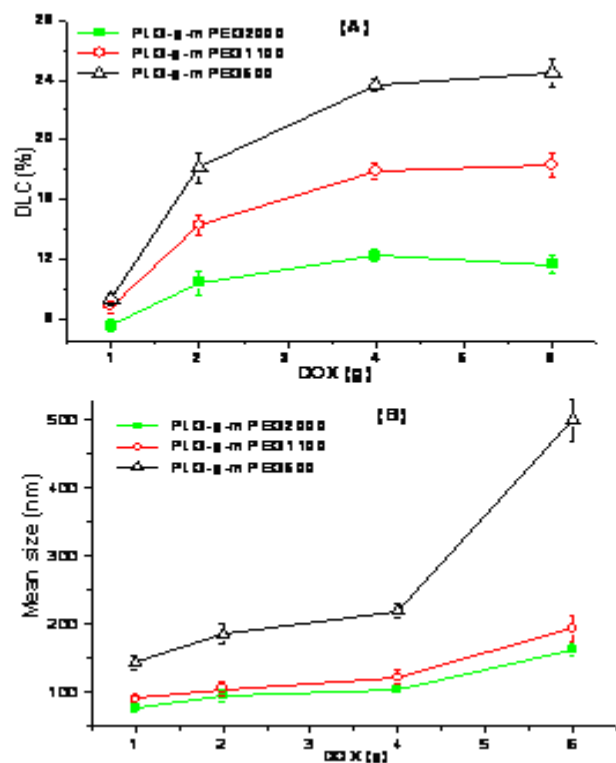


Fig. 6: Effects of drug/polymer feeding doses on drug loading content (A) and size (B), the amount of polymer was 10g.

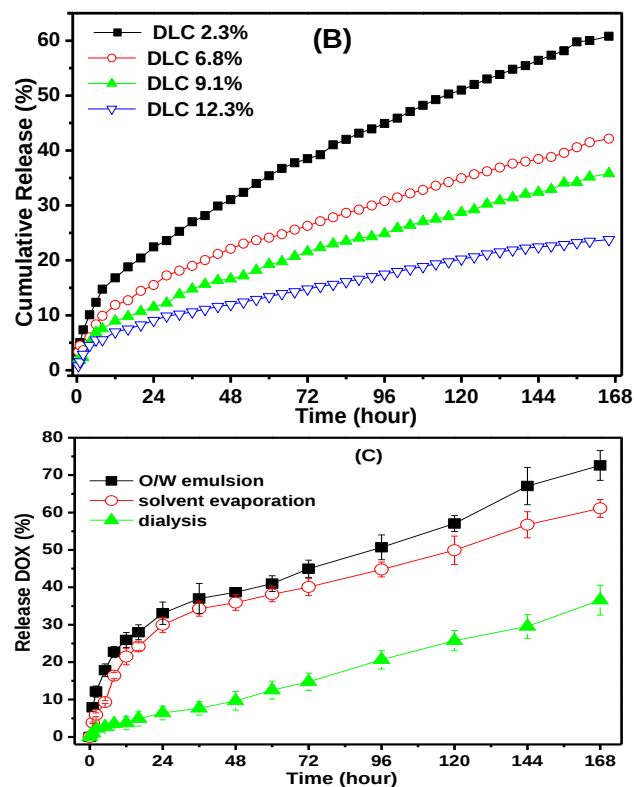
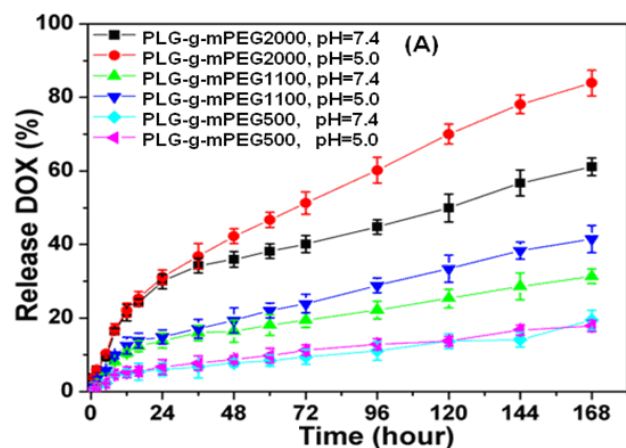


Fig. 7: *In vitro* release profiles of DOX loaded PLG-g-mPEG micelles, A: in the media with different pH values; B: with different drug loading content in PBS with pH=7.4; C: micelles prepared by different methods.

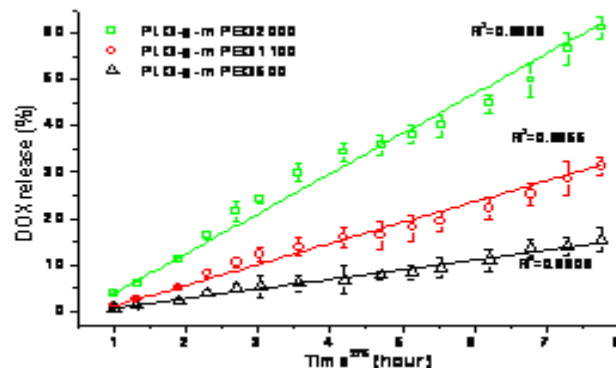


Fig. 8: Kinetics model fitted for the *in vitro* drug release profiles (pH=7.4, DLC=10%).

The mass ratio of drug to copolymer was 8mg: 20mg, the copolymer concentration was 1mg/mL.

#### The effect of feeding dose on size and DLC

The DOX loading content and micelle size increased with the increase of drug content in fig. 6. The drug loading content presented a biphasic manner with a rapid increase when the drug content was less than 4g and then a sustained increase. Within all the three groups, PLG-g-mPEG500 micelles exhibited the highest DLC and the largest micelle size. The highest DLC observed in PLG-g-mPEG500 micelles was because that the PLG-g-

**Table 1:** Effects of the ratio (organic solvent: water) on the properties of DOX-loaded micelles.

| THF/H <sub>2</sub> O (mL /mL) | Drug-loaded content (%) | Encapsulation Efficiency (%) | Size (nm) |
|-------------------------------|-------------------------|------------------------------|-----------|
| 0.5/20                        | 12.8±0.3                | 33.1±2.0                     | 78±5      |
| 0.8/20                        | 12.2±0.3                | 32.6±1.0                     | 91±4      |
| 1/20                          | 12.2±0.4                | 32.3±1.3                     | 105±4     |
| 2/20                          | 11.7±0.3                | 30.5±1.1                     | 114±10    |
| 5/20                          | 11.5±0.5                | 28.7±0.7                     | 120±5     |

The mass ratio of drug to copolymer was 8mg: 20mg, the copolymer concentration was 1mg/mL.

mPEG500 micelles possessed the largest core, which could entrap more drugs in micelles.

### Drug release profiles

The drug release profiles were conducted in different pH medium at 37°C the release profiles of micelles with different mPEG block length were shown in fig. 7a. Since the initial burst release was not observed in all examined samples, it confirmed that there was no residual drug on the surface of micelles. Moreover, The DOX loaded in the inner core of micelles showed significant sustained release characteristic of less than 60%, 31% and 19% drug released from PLG-g-mPEG2000, PLG-g-mPEG1100 and PLG-g-mPEG500 micelles even the time was elongated to 168h. The slow drug release behavior indicated that most of the drug remained in the status of being packaged in pH7.4.

Fig. 7b showed the drug release from PLG-g-mPEG polymeric micelles with different drug loading contents. The high drug loading content would reduce the release rate due to the stronger hydrophobic interaction, which slowed the diffusion of DOX into medium. The impact of preparation method on the release of DOX from PLG-g-mPEG micelles was presented in fig. 7c. Burst release was not observed in the micelles prepared by dialysis.

### Drug release kinetics

As we known, the drug release from polymeric micelles was a rather complicated process. The release rate might be mainly determined by the diffusion of the drug from the cores. In order to confirm the drug release mechanism, the drug release data obtained from the in vitro study was analyzed using the Ritger-Peppas (Haag, 2004) drug release equation given below:

$$\frac{M_t}{M_\infty} = kt^n \quad (3)$$

Where  $M_t/M_\infty$  is the fraction of drug release at any time  $t$ ,  $k$  is the release rate constant and  $n$  is the diffusion exponent indicative of drug release. For diffusion-erosion controlled drug release system, a value of  $n < 0.43$  indicates fickian;  $0.43 < n < 0.85$  for non-fickian or anomalous release; and  $n > 0.85$  indicates erosion. (Cao, Lu *et al.*, 2013, Haag, 2004) The fitting results of drug release profiles were given in fig. 8 and the release of DOX from micelles followed Ritger-Peppas equation very well, with satisfactory coefficients of 0.9896, 0.9855, 0.9808 for PLG-g-mPEG2000, PLG-g-mPEG1100, PLG-

g-mPEG500, respectively. Moreover, the drug release rate could be obtained by the slope of those equations as shown in fig. 8. It was observed that the slope of those equations corresponded to PLG-g-mPEG2000, PLG-g-mPEG1100, PLG-g-mPEG500 polymeric micelles decreased with decreasing the molecular weight of PEG, which were in good accordance with experimental results as shown in fig. 7. The value of  $n$  was all lower than 0.43, thus the release mechanism was the drug diffusion control (Cao *et al.*, 2013).

## DISCUSSION

In order to explore the effects of formulations including compositions, concentrations, the ratio of oil to water, the type of organic solvent, feeding dose and temperature on the DLC, DLE and size of DOX-loaded micelles, three amphiphiles of (PLG-g-mPEG2000, PLG-g-mPEG1100 and PLG-g-mPEG500) were used for drug loading.

The size results revealed that the DOX-loaded micelles were spherical particles with a uniform diameter. The TEM sizes were a little smaller than the mean sizes of DLS due to the evaporation of water (Liang *et al.*, 2015).

To investigate the stability of micelles, CMC and stability in storage were studied. The low CMC of the three kinds of micelles implied that the micelles were thermally stable against diluting in the blood vessel circulation. Moreover, as previous reported (Allen *et al.*, 1999, Cao *et al.*, 2012) the CMC value decreased with decreasing the mass ratio of hydrophilic block to hydrophobic block. The micelles solution stored at 4°C were very stable up to 3 months and its frozen powder stored at 25°C could be stable for 1 months. During the storage period, the activity of DOX has a slight decrease. The encapsulation of micelles prevented DOX from degradation and the drug released from polymeric micelles was much slow at low temperature (4°C), thus, the high bioactivity of DOX was protected. The above results demonstrated that the formulations would be preferable for cancer chemotherapy PLG-g-mPEG with different compositions was used for fabricating drug loaded micelles. It was found the increase of mPEG molecular led to the reduction of DOX loading content and mean diameter, which meted with the results observed by Papadimitriou (Papadimitriou and Bikiaris, 2009). The decrease of micelle size was attributed to the formation of compact

micelles because of the increase of hydrophobic interaction between polymer and drug. The increase of drug loading content could be ascribed to more hydrophobic aggregations in the micelles, which led to large volume of core in micelles and resulted in more drug trapped in micelles (Lee *et al.*, 2003). As for the polymer concentration, Some studies suggested that in higher polymer concentration, the viscosity increased and resulted in more serious aggregation to load more hydrophobic drug and enlarge the particle size, however, mPEG segment in copolymer would be difficult to stretch in water as more molecular chains were tangled with the further increase of concentration. This entanglement would reduce the drug loading content and decrease of micelle size.

Temperature is also an important factor affecting the drug loading and particle morphology. It was found that the higher temperature reduced the particle size because of enhancing the interaction of hydrophilic segment and solvent to prompt the solvent "crowd out" from the transition layer as the thickness of transition layer became slim (Bittner *et al.*, 1998) Moreover, the reduced thickness of shell was larger than that of increased core of micelle size, thus, the drug loaded micelles became more compact, showing smaller size.

In term of the ratio of organic solvent and water, the phenomenon might be explained by a longer aggregation time of micellization because of lower polymer concentration. This hypothesis theory was also proposed by Vangeyte (Vangeyte *et al.*, 2004) and Johnson (Johnson and Prud'homme, 2003) who recognized that a higher polymer concentration in organic phase could decrease the aggregation time of micellization and lead to the formation of more compact nanoparticles. Moreover, the decrease of DOX loading content was due to less chance of drug molecules contacting with hydrophobic segments.

The trend of effect of feeding dose on size and DLC was also reported in other studies (Cao *et al.*, 2013). The lowest drug loading content in PLG-g-mPEG2000 was derived from that free water could quickly penetrate into the core of micelles to accelerate drug precipitation. It was a competition process, the drug could entrapped into the core of micelles efficiently when in low input drug content, and higher input drug content decreased the drug loading due to the aggregation and precipitation of drugs (Gao *et al.*, 2008)

The drug release results could be concluded that the releasing feature of this anticancer drug delivery vehicle in neutral environment was a merit to prevent the release during the transportation in blood, the release rate of drug was proportional to the molecular weight of PEG segments. In medium with weak acidity, the drug release

from micelles was accelerated due to protonation of DOX with the transition from hydrophobic to hydrophilic, which would improve the diffusion of drug to enhance the therapeutic efficiency (Lai *et al.*, 2012).

## CONCLUSION

In this study, three amphiphilic copolymers of PLG-g-mPEG2000, PLG-g-mPEG1100 and PLG-g-mPEG500 were self-assemble into micelles to load anticancer drug. The drug loaded micelles possessed good stability exhibiting as low critical micelle concentration, little size change after stored at low temperature and freeze drying. The composition of copolymer played a key role in determining their drug loading content and size. The formulation conditions such as compositions, concentrations, organic solvent/water ratios, feeding doses and temperatures affected the drug loading capacity and mean size. The *in vitro* drug release rates were depended on the composition of copolymer, drug loading content and the preparation method. The drug release followed the Rigter-Peppas equation well and was corresponded to the drug diffusion control.

## REFERENCES

- Aliabadi HM, Elhasi S, Mahmud A, Gulamhusein R, Mahdipoor P and Lavasanifar A (2007). Encapsulation of hydrophobic drugs in polymeric micelles through co-solvent evaporation: The effect of solvent composition on micellar properties and drug loading. *Int. J. Pharm.*, **329**(1): 158-165.
- Allen C, Maysinger D and Eisenberg A (1999). Nano-engineering block copolymer aggregates for drug delivery. *Colloids Surf. B Biointerfaces*, **16**(1): 3-27.
- Beijnen J, Van der Houwen O and Underberg W (1986). Aspects of the degradation kinetics of doxorubicin in aqueous solution. *Int. J. Pharm.*, **32**(2): 123-131.
- Bittner B, Morlock M, Koll H, Winter G and Kissel T (1998). Recombinant human erythropoietin (rhEPO) loaded poly (lactide-co-glycolide) microspheres: influence of the encapsulation technique and polymer purity on microsphere characteristics. *Eur. J. Pharm. Biopharm.*, **45**(3): 295-305.
- Cao J, Chen Y, Chen N and Luo X (2010). The preparation of phosphorylcholine-containing poly (Llactide) nanoparticles with solvent evaporation method. *e-Polymers*, **10**(1): 813-821.
- Cao J, Lu A, Li C, Cai M, Chen Y, Li S and Luo X (2013). Effect of architecture on the micellar properties of poly ( $\epsilon$ -caprolactone) containing sulfobetaines. *Colloids Surf. B Biointerfaces*, **112**: 35-41.
- Cao J, Su T, Zhang L, Liu R, Wang G, He B and Gu Z (2014). Polymeric micelles with citraconic amide as pH-sensitive bond in backbone for anticancer drug delivery. *Int. J. Pharm.*, **471**(1): 28-36.
- Cao J, Xie X, Lu A, He B, Chen Y, Gu Z and Luo X

- (2014). Cellular internalization of doxorubicin loaded star-shaped micelles with hydrophilic zwitterionic sulfobetaine segments. *Biomaterials*, **35**(15): 4517-4524.
- Cao J, Xiu KM, Zhu K, Chen YW and Luo XL (2012). Copolymer nanoparticles composed of sulfobetaine and poly ( $\epsilon$ -caprolactone) as novel anticancer drug carriers. *J. Biomed. Mater. Res. A*, **100**(8): 2079-2087.
- Cao J, Zhai S, Li C, He B, Lai Y, Chen Y, Luo X and Gu Z (2013). Novel pH-sensitive micelles generated by star-shape copolymers containing zwitterionic sulfobetaine for efficient cellular internalization. *J. Biomed. Nanotechnol.*, **9**(11): 1847-1861.
- Chen W, Zou Y, Jia J, Meng F, Cheng R, Deng C, Feijen J and Zhong Z (2013). Functional poly ( $\epsilon$ -caprolactone) s via copolymerization of  $\epsilon$ -caprolactone and pyridyl disulfide-containing cyclic carbonate: Controlled synthesis and facile access to reduction-sensitive biodegradable graft copolymer micelles. *Macromolecules*, **46**(3): 699-707.
- Choi KC, Bang JY, Kim PI, Kim C and Song CE (2008). Amphotericin B-incorporated polymeric micelles composed of poly (d, l-lactide-co-glycolide)/dextran graft copolymer. *Int. J. Pharm.*, **355**(1): 224-230.
- Feng Y, Klee D, Keul H and Hocker H (2001). Synthesis and characterization of new block copolymers with poly (ethylene oxide) and poly [3 (S)-sec-butylmorpholine-2, 5-dione] sequences. *Macromol. Biosci.*, **1**(1): 30-39.
- Frank A, Rath SK and Venkatraman SS (2005). Controlled release from bioerodible polymers: Effect of drug type and polymer composition. *J. Control. Release*, **102**(2): 333-344.
- Gao J, Ming J, He B, Fan Y, Gu Z and Zhang X (2008). Preparation and characterization of novel polymeric micelles for 9-nitro-20 (S)-camptothecin delivery. *Eur. J. Pharm. Sci.*, **34**(2): 85-93.
- Gong J, Chen M, Zheng Y, Wang S and Wang Y (2012). Polymeric micelles drug delivery system in oncology. *J. Control. Release*, **159**(3): 312-323.
- Guo S, Qiao Y, W. W, He H, Deng L, Xing J, Xu J, Liang XJ and Dong A (2010). Poly ( $\epsilon$ -caprolactone)-graft-poly (2-(N, N-dimethylamino) ethylmethacrylate) nanoparticles: pH dependent thermo-sensitive multifunctional carriers for gene and drug delivery. *J. Mater. Chem.*, **20**(33): 6935-6941.
- Haag R (2004). Supramolecular drug-delivery systems based on polymeric core-shell architectures. *Angewandte Chemie International Edition*, **43**(3): 278-282.
- Hrkach J, Von Hoff D, Ali MM, Andrianova E, Auer J, Campbell T, De Witt D, Figa M, Figueiredo M and Horhota A (2012). Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile. *Science Translational Medicine*, **4**(128): 128ra139-128ra139.
- Janssen M, Crommelin D, Storm G and Hulshoff A (1985). Doxorubicin decomposition on storage. Effect of pH, type of buffer and liposome encapsulation. *Int. J. Pharm.*, **23**(1): 1-11.
- Johnson BK and Prud'homme RK (2003). Mechanism for rapid self-assembly of block copolymer nanoparticles. *Phys. Rev. Lett.*, **91**(11): 118302.
- Lai Y, Long Y, Lei Y, Deng X, He B, Sheng M, Li M and Gu Z (2012). A novel micelle of coumarin derivative monoend-functionalized PEG for anti-tumor drug delivery: *in vitro* and *in vivo* study. *J. Drug Target*, **20**(3): 246-254.
- Lee SC, Kim C, Kwon IC, Chung H and Jeong SY (2003). Polymeric micelles of poly (2-ethyl-2-oxazoline)-block-poly ( $\epsilon$ -caprolactone) copolymer as a carrier for paclitaxel. *J. Control. Release*, **89**(3): 437-446.
- Liang Y, Deng X, Zhang L, Peng X, Gao W, Cao J, Gu Z and He B (2015). Terminal modification of polymeric micelles with  $\pi$ -conjugated moieties for efficient anticancer drug delivery. *Biomaterials*, **71**: 1-10.
- Lu C, Chen X, Xie Z, Lu T, Wang X, Ma J and Jing X (2006). Biodegradable amphiphilic triblock copolymer bearing pendant glucose residues: Preparation and specific interaction with concanavalin A molecules. *Biomacromolecules*, **7**(6): 1806-1810.
- Nan J, Chen Y, Li R, Wang J, Liu M, Wang C and Chu F (2014). Polymeric Hydrogel Nanocapsules: A Thermo and pH Dual-responsive Carrier for Sustained Drug Release. *Micro. Nano. Lett.*, **6**(3): 200-208.
- Papadimitriou S and Bikiaris D (2009). Novel self-assembled core-shell nanoparticles based on crystalline amorphous moieties of aliphatic copolyesters for efficient controlled drug release. *J. Control. Release*, **138**(2): 177-184.
- Vangeyte P, Gautier S and Jerome R (2004). About the methods of preparation of poly (ethylene oxide)-b-poly ( $\epsilon$ -caprolactone) nanoparticles in water: Analysis by dynamic light scattering. *Colloids Surf. A Physicochem. Eng. Asp.*, **242**(1): 203-211.
- Wang Y, Wang J, Wang T, Xu Y, Shi L, Wu Y, Li L and Guo X (2016). Pod-Like supramicelles with multicompart ment hydrophobic cores prepared by self-assembly of modified chitosan. *Nanomicro Lett.*, **8**(2): 151-156.
- Wen Y and Meng WS (2014). Recent *in vivo* evidences of particle-based delivery of small-interfering RNA (siRNA) into solid tumors. *J. Pharm. Innov.*, **9**(2): 158-173.
- Xie Z, Guan H, Chen X, Lu C, Chen L, Hu X, Shi Q and Jing X (2007). A novel polymer-paclitaxel conjugate based on amphiphilic triblock copolymer. *J. Control. Release*, **117**(2): 210-216.
- Yohan D, Cruje C, Lu X and Chithrani D (2015). Elucidating the uptake and distribution of nanoparticles in solid tumors via a multilayered cell culture model. *Micro. Nano Lett.*, **7**(2): 127-137.
- Zawaneh PN, Doody AM, Zelikin AN and Putnam D (2006). Diblock copolymers based on dihydroxyace

- tone and ethylene glycol: Synthesis, characterization, and nanoparticle formulation. *Biomacromolecules*, **7**(11): 3245-3251.
- Zhang B, Hu N, Wang Y, Wang Z, Wang Y, Kong ES and Zhang Y (2010). Poly (glycidyl methacrylates)-grafted zinc oxide nanowire by surface-initiated atom transfer radical polymerization. *Micro. Nano Lett.*, **2**(4): 285-289.
- Zhang L, Su T, He B and Gu Z (2014). Self-assembly polyrotaxanes nanoparticles as carriers for anticancer drug methotrexate delivery. *Micro. Nano Lett.*, **6**(2): 108-115.