Appendix

1 The thermodynamic study on micellization of F127-GA, F127-SS-PDLA and GA/F127-SS-PDLA

1.1 Methods

MicroCal ITC 200 isothermal titration calorimeter (ITC, Ge Asia Medical Equipment Co., Ltd) was used to obtain the heat change during the experiment. Normal saline was added to the weighed amount of F127-GA, F127-SS-PDLA and GA/F127-SS-PDLA. The mixture was sonicated for 5 min to form uniform solutions. The concentrations of copolymer were adjusted to approximately 4.4×10^{-1}, 1.1×10^{-2} and 2.0×10^{-2} mg/mL, respectively. After that, 40 μL of copolymer solution was added to the syringe, and 200 μL of normal saline was added to the sample cell. The titration temperature was set at 40℃. The stirring speed was set at 750 rpm with 2 μL per drop and 120 s one drop. The heat change was recorded, and the peak area was calculated by Origin 8.0 software to draw the corresponding calorimetric curve. CMC of the corresponding copolymer was the inflection point of the first-order derivative curve of calorimetric curve mentioned above. The Gibbs free energy (△G) was calculated according to the following formula[1]:

$$\triangle G = R \cdot T \cdot \ln(CMC')$$

where R and T represented the gas constant (R = 8.314 K/mol·K) and the absolute temperature of the experiment, respectively. In this equation, CMC' was the critical micellization concentration expressed in mole fraction units. In addition, the enthalpy difference between the initial droplet curve and the final droplet curve of the calorimeter curve is the enthalpy change value (△H). The formula provided below was utilized to calculate the corresponding entropy change value (△S):

$$\triangle G = \triangle H - T \cdot \triangle S$$

1.2 Results and discussions

The thermodynamic parameters of micellization of F127-GA, F127-SS-PDLA and GA/F127-SS-PDLA were displayed in Table 1. △G of F127-GA, F127-SS-PDLA and GA/F127-SS-PDLA were hereinafter referred to as $\triangle G_{FGA}$, $\triangle G_{FSSP}$ and $\triangle G_{MIX}$,

respectively. As shown in Table 1, △S of three polymer materials were positive, indicating that the micellization of these polymers was all spontaneous. If the two polymeric materials, F127-GA and F127-SS-PDLA, form micelles separately in solution, then $\triangle G_{MIX}$ should be equal to $\triangle G_{FSSP}$. Because the proportion of F127-GA in the system was very low, which could not reach its CMC at the experimental concentration, so there was no change in heat. The experimental results show that $\triangle G_{MIX}$ was between $\triangle G_{FSSP}$ and $\triangle G_{FGA}$, so it can be considered that F127-GA was involved in the formation of the mixed system. Based on our previous research results[2], it could be inferred that the reason for this change might be that the addition of FGA reduced the proportion of hydrophobic segments in the original system of F127-SS-PDLA, resulting in $\triangle G_{MIX}$ was greater than $\triangle G_{FSSP}$. Due to the lowest proportion of hydrophobic segments, the $\triangle G_{FGA}$ of F127-GA was the largest. In addition, by analyzing the CMC or enthalpy change, it also could draw the same conclusions. Therefore, it can be concluded the two polymers were truly co-assembled in solution, as opposed to merely self-sorting.

Table 1 The thermodynamic parameters of micellization of F127-GA, F127-SS-PDLA and GA/F127-SS-PDLA

Copolymers	△H (KJ/mol)	△S (KJ/mol·K)	△G (KJ/mol)	CMC (mg/mL)
F127-GA	84.425	0.411	-44.198	0.363×10^{-1}
F127-SS-PDLA	138.076	0.617	-54.967	0.940×10^{-3}
GA/F127-SS-PDLA	125.841	0.571	-52.996	0.187×10^{-2}

2 Gel permeation chromatography (GPC) and nuclear magnetic resonance (^{1}H-NMR) spectroscopy

2.1 Methods

Molecular weights of F127, F127-GA and F127-SS-PDLA were determined by Agilent 1260 infinity II GPC (Agilent Technologies, USA) equipped with a differential refractive index detector by employing two columns (PL MIXED-C). The mobile phase was N, N-dimethylformamide (DMF) and the flow rate was 1 mL/min. Polystyrene standards were used for calibration runs. Subsequently, the molecular

weight of samples was reported as the polystyrene equivalent molecular weight. In addition, the results of ^{1}H-NMR spectroscopy were further analyzed.

2.2 Results and discussions

Molecular weight distribution and polydispersity index (PDI) of F127, F127-GA and F127-SS-PDLA were displayed in Table 2 and Fig. 1. As shown in Table 2, there was a certain deviation between the molecular weight of F127 and its theoretical value. This may be due to GPC measuring the relative molecular weight of copolymers, which is highly dependent on standard samples. Therefore, there are some deviations in the test results. The yields of F127-GA and F127-SS-PDLA obtained from GPC were 93.51% and 90.32%, respectively. The ^{1}H-NMR spectra marked the integral curve of F127-GA and F127-SS-PDLA were shown in Fig. 2. By analyzing the integration ratio of the peak l at 3.99 ppm (CH_2 in F127-GA), peak r at 5.18 ppm (CH in PDLA) and peak c at 3.39 ppm (CH in F127) in the ^{1}H-NMR spectra, the yields of F127-GA and F127-SS-PDLA could be calculated to be 98.25% and 97.06%, respectively. In conclusion, the yields of F127-GA and F127-SS-PDLA measured by different methods were over 90%, indicating successful synthesis of both polymer materials.

Table 2 Molecular weight of copolymers analyzed by GPC

Copolymers	$\overline{Mn}$	$\overline{Mw}$	PDI[a]	Yield (%)
F127	15713	18596	1.18	-
F127-GA	16592	19603	1.18	93.51
F127-SS-PDLA	24582	29321	1.19	90.32

[a] polydispersity index (PDI) = $\overline{Mw}$ / $\overline{Mn}$

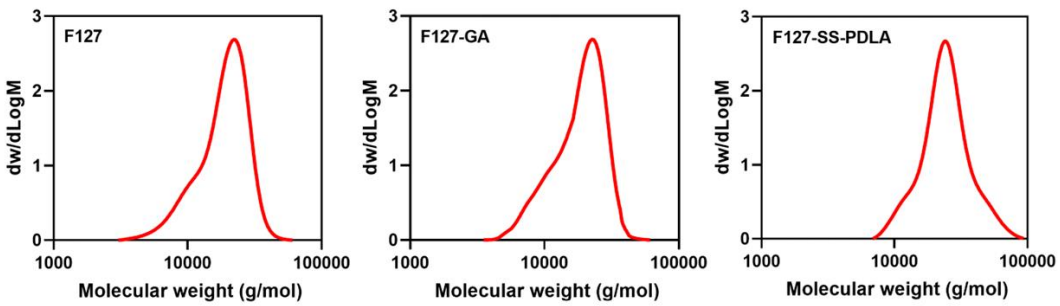
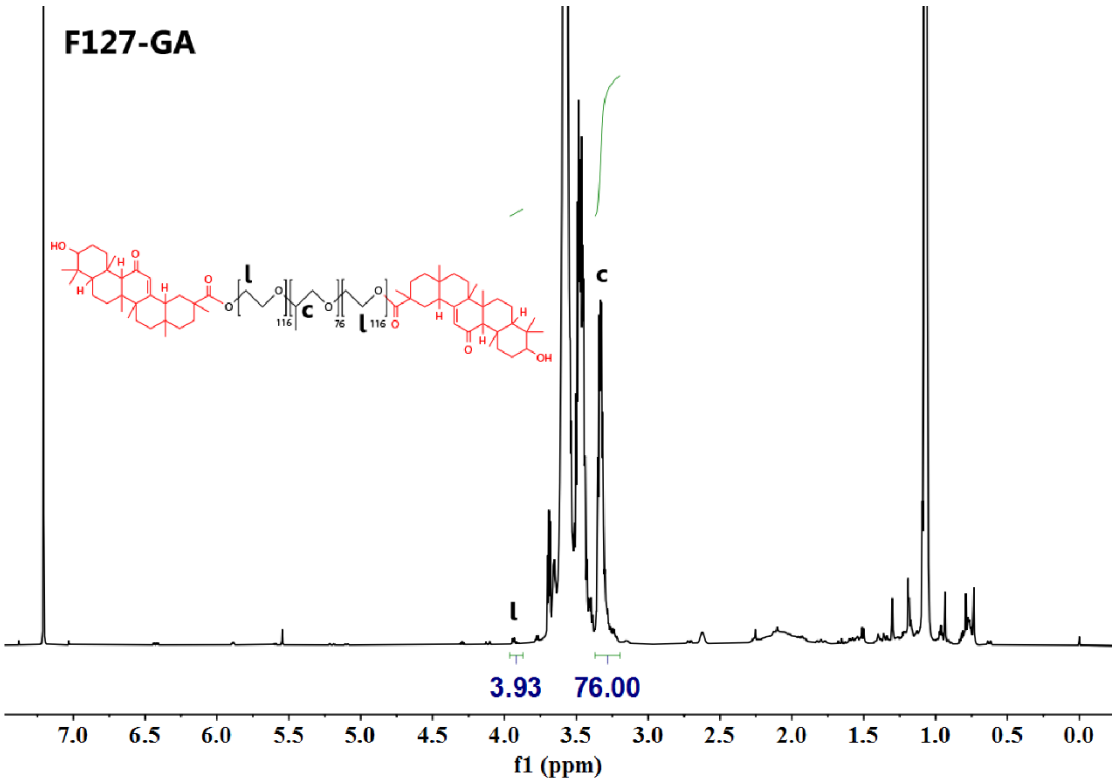


Fig. 1 Molecular weight distribution of copolymers



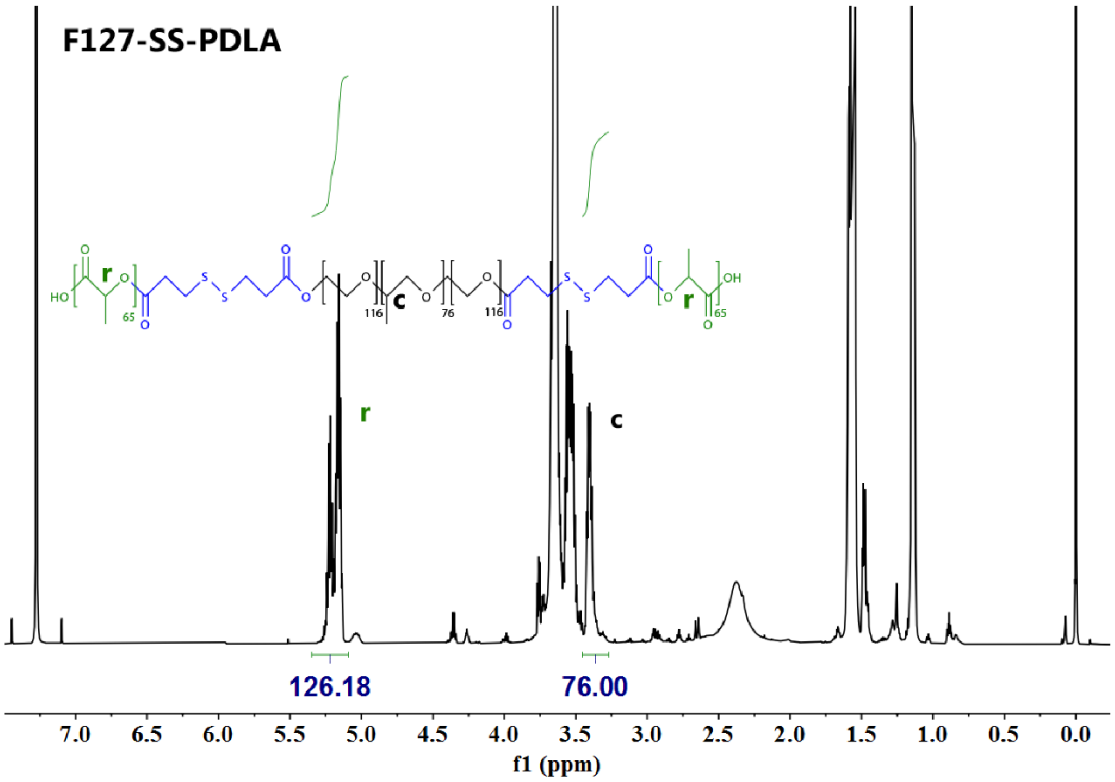


Fig. 2 The ^{1}H-NMR spectra of F127-GA and F127-SS-PDLA (marked the integral curve)

3 Release mechanism

3.1 Methods

Drug release mechanism after cleavage of disulfide bond was analyzed through drug release assay and DLS of F127, PDLA and CTD mixtures. The cumulative release curve and particle size distribution of F127, PDLA and CTD mixtures were determined according to the experimental methods described in items 2.9 and 2.10 in the manuscript.

3.2 Results and discussions

As illustrated in Fig. 3A, the cumulative release curves of the mixture were basically consistent with those of free CTD whatever the concentrations of GSH, indicating that free polymers had no influence on the release of CTD. Combined with the cumulative release curve of micelles, it could be seen that F127-SS-PDLA/CTD and GA/F127-SS-PDLA/CTD micelles were gradually decomposed to release drugs in the reducing environment. By comparing the particle size distribution of micelles with that of mixtures under the reducing environment, it could also be inferred that micelles were gradually dissociated and there were free F127 and PDLA fragments as well as some incompletely cleaved micelles in the final system (Fig. 3B).

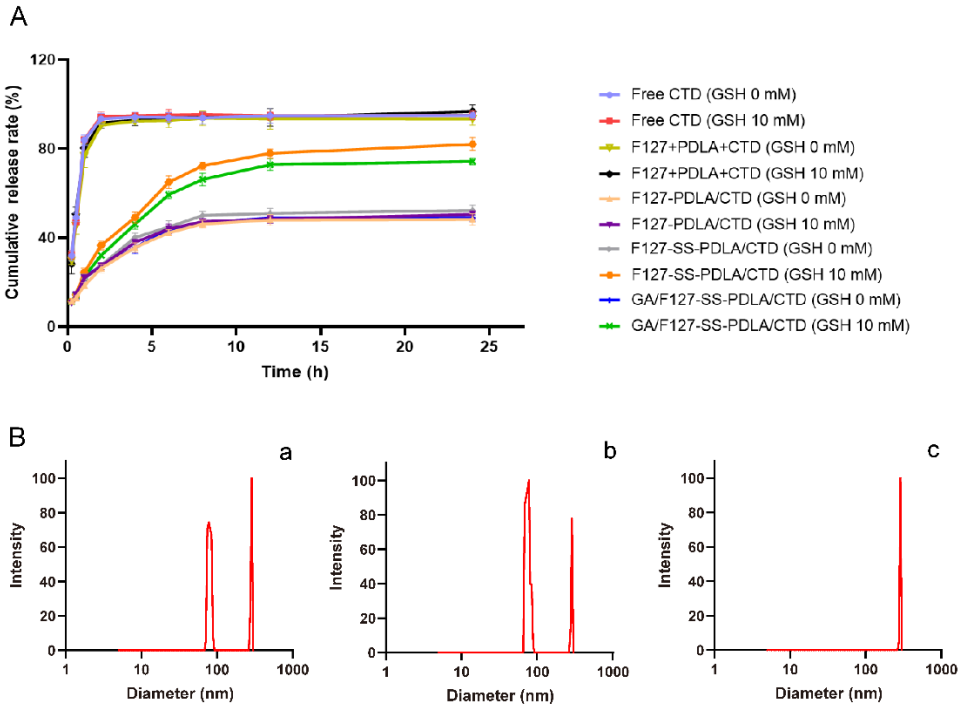


Fig. 3 (A) Cumulative release profiles of free CTD, CTD-loaded micelles as well as the mixtures of F127, PDLA and CTD. (B) Size distributions of F127-SS-PDLA/CTD (a), GA/F127-SS-PDLA/CTD (b) as well as the mixtures of F127, PDLA and CTD (c) under reductive environment for 24 h. Data were presented as mean ± SD; n = 3.

References

[1] Bouchemal K., Agnely F., Koffi A., et al. A concise analysis of the effect of temperature and propanediol-1, 2 on Pluronic F127 micellization using isothermal titration microcalorimetry[J]. *J Colloid Interface Sci*. 2009;338(1):169-176.

[2] Song J. Z. Study on glycyrrhetinic acid modified and pH-sensitive curcumin mixed micelle for anti-hepatoma cell[D]. Beijing: China Academy of Chinese Medical Science,2020.