Supporting Information (SI)

SI Method

Critical micelle concentration (CMC)

CMC was determined from DG and EMP@glycymicelles (with a EMP/DG mass ratio of 1:15) in water, artificial tears, and PBS using pyrene as the probe molecule.

A standard 6 μM pyrene solution in acetone was prepared, and then 100 μl of pyrene solution was added to 5-ml penicillin bottle and was kept in dark at room temperature to solvent evaporated completely. Then, gradient concentrations of DG or EMP@glycymicelles (with a EMP/DG mass ratio of 1:15) in water, PBS or artificial tear solution. 1.0 ml was added to each penicillin bottle, followed with 30 min ultrasonic in water bath. Then, these solutions were incubated for 12 h in a dark place. The fluorescence intensity of each solution sample was measured under the conditions that the excitation wavelength was 335 nm and the emission wavelength was 373 nm、384 nm. The determined concentration of DG in DG only or in EMP@glycymicelles (with a EMP/DG mass ratio of 1:15) was ranged from 0.1 mg/ml to 10 mg/ml. A plot of the fluorescence intensity as ordinate and the logarithm of concentration as abscissa, yielded two straight lines whose intersection was taken as the CMC.

SI Results and Figures

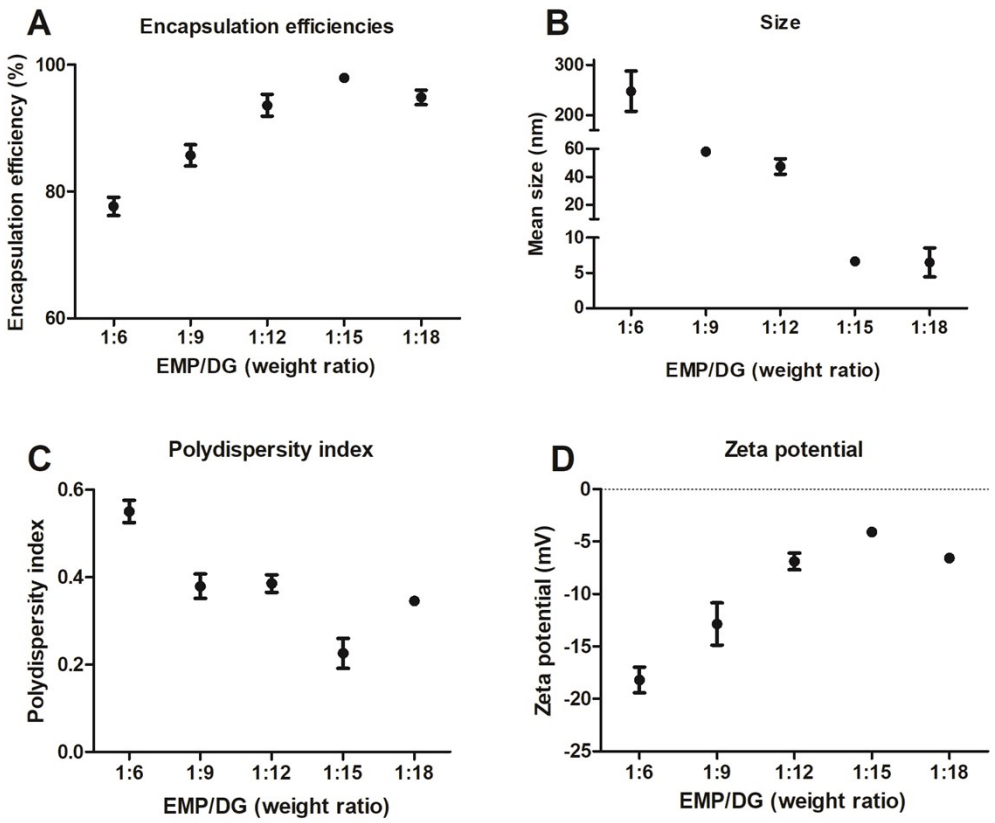


Figure S1 EMP@glycymicelles characterizations. Encapsulation efficiencies, particle size, polydispersity index, and zeta potential were tested as functions of different weight ratios of EMP to DG.

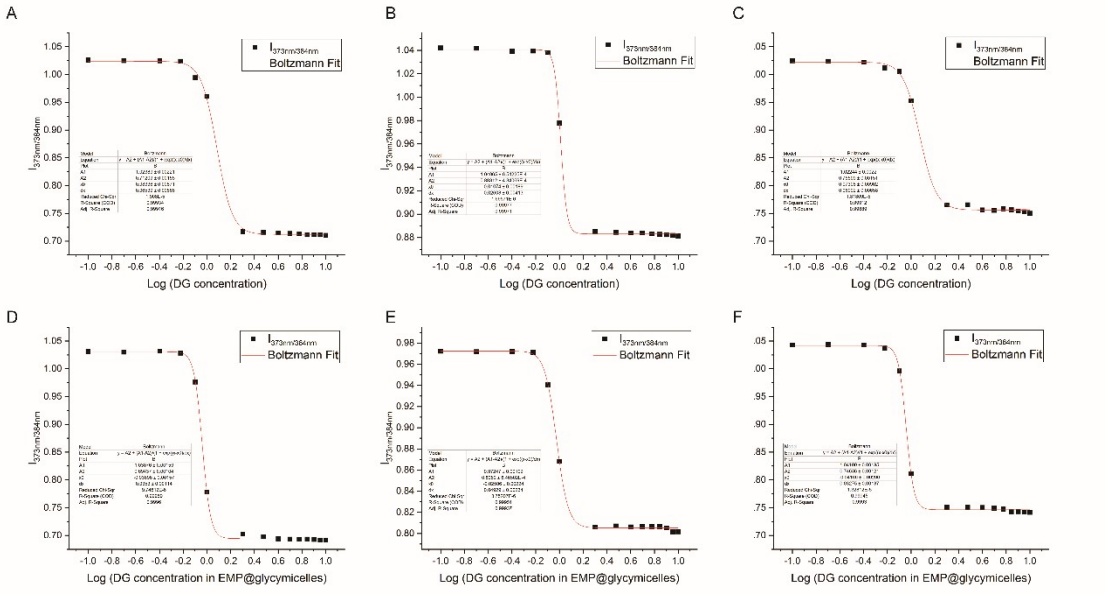
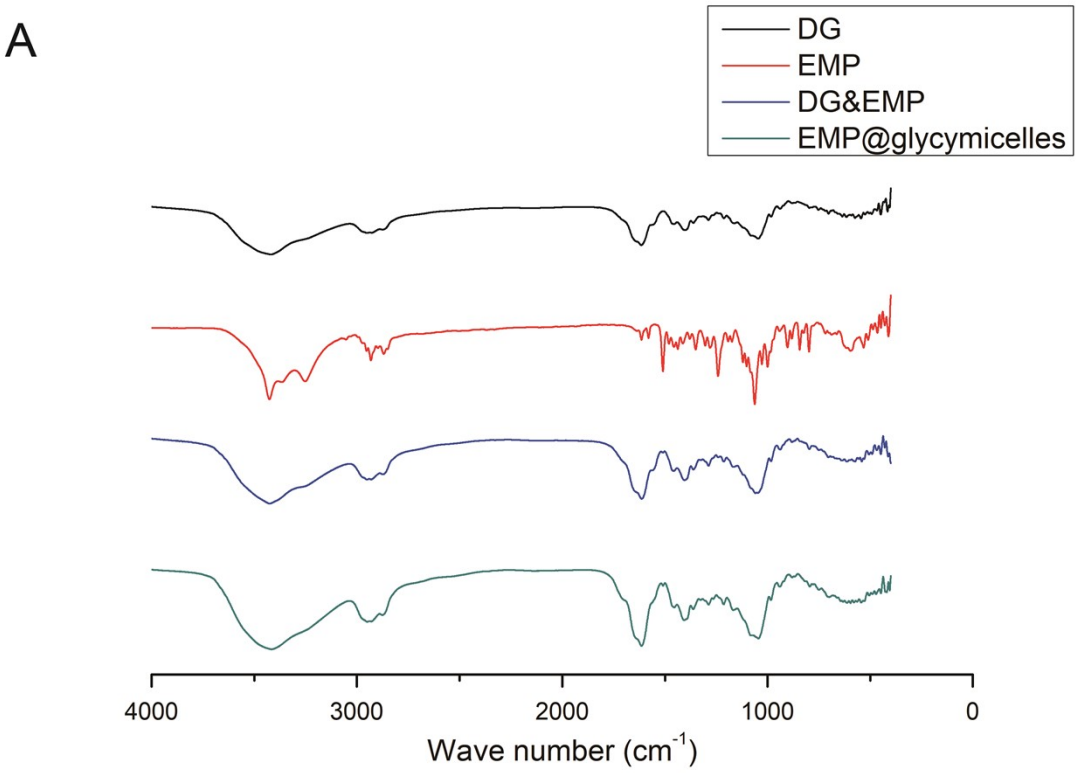


Figure S2 Critical micelle concentration of DG and EMP@glycymicelles (with a EMP/DG mass ratio of 1:15) in water, artificial tears, and PBS. (A) DG in water; (B) DG in artificial tears; (C) DG in PBS; (D) EMP@glycymicelles in water; (E) EMP@glycymicelles in artificial tears; (F) EMP@glycymicelles in PBS. n=3. EMP: empagliflozin; DG: dipotassium glycyrrhizinate.

CMC value of DG and EMP@glycymicelles (with a EMP/DG mass ratio of 1:15) in water, artificial tears, and PBS. DG exhibited critical micelle concentration (CMC) values of 1.21 ± 0.01, 1.02 ± 0.02, and 1.18 ± 0.02 mg/ml in water, artificial tears, and PBS, respectively. The CMC values of EMP@glycymicelles with a EMP/DG mass ratio of 1:15 were 0.91 ± 0.01, 0.94 ± 0.01, and 0.91 ± 0.02 mg/ml in water, artificial tears, and PBS, respectively.


A
DG
EMP
DG&EMP
EMP@glycymicelles
4000
3000
2000
1000
0
Wave number (cm-1)

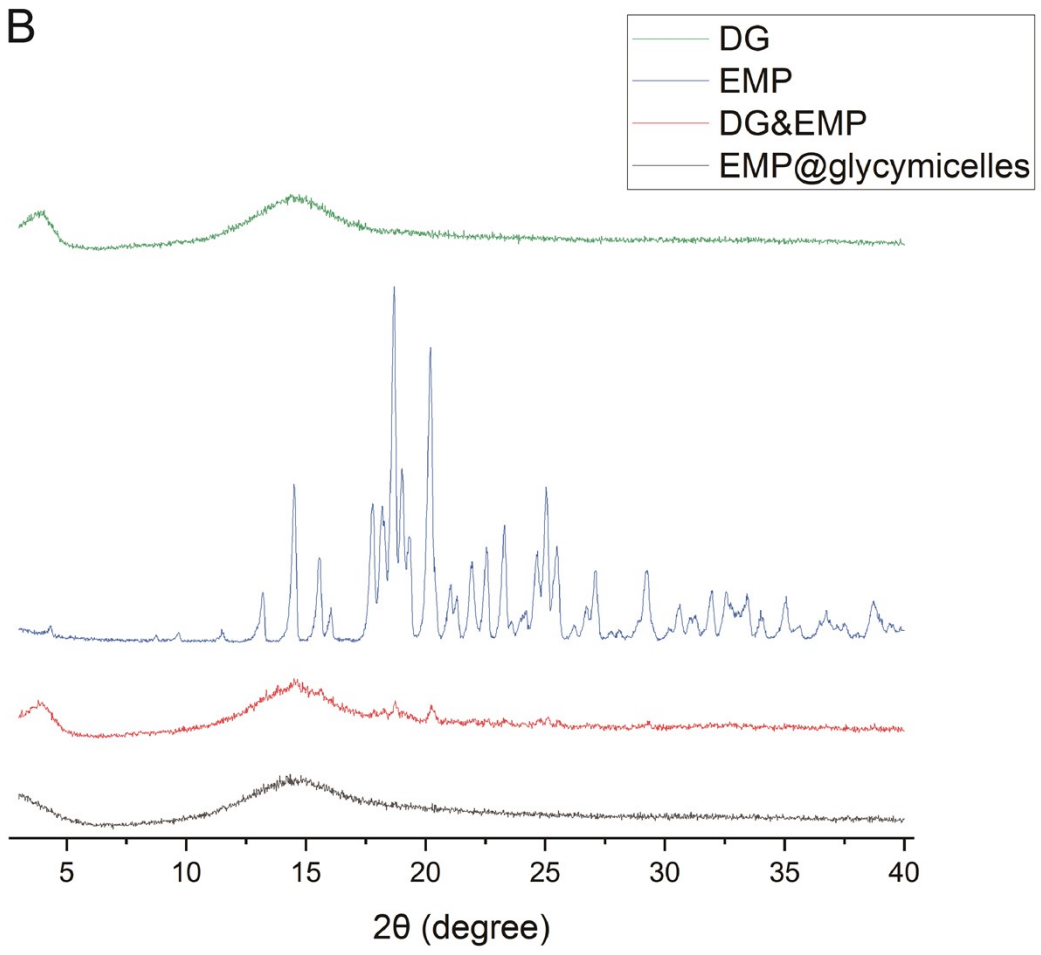

B
DG
EMP
DG&EMP
EMP@glycymicelles
5
10
15
20
25
30
35
40
2θ (degree)

Figure S3 Physicochemical properties of EMP@glycymicelles. (A) Fourier Transform InfraRed spectroscopy (FT-IR) with a Nicolet iS10 IR-spectrophotometer (ThermoFisher, Madison, WI, USA) and (B) XRD analysis with a x-ray diffractometer (D/max-2400; Rigaku, Tokyo, Japan) for dipotassium glycyrrhizinate (DG), empagliflozin (EMP), a physical mixture of DG and EMP, and EMP@glycymicelles freeze-dried powder were performed.

FT-IR spectroscopy was used to investigate possible chemical reactions or H-bonding formation in binary systems of EMP with DG. The FT-IR spectra of EMP, DG, the DG&EMP physical mixture, and the EMP@glycymicelles are shown in Figure S3A. The spectra of EMP consisted of characteristic absorption peaks at 3425.83 cm^{-1} (O-H stretching vibration), 3251.69 cm^{-1} (stretching of the aromatic groups C-H), 1614.17 cm^{-1} (stretching of the aromatic rings C=C),1102.14 cm^{-1} (C-O stretching vibration). For DG, absorption bands appeared at 3419.43 cm^{-1} (O–H stretching vibration of Phenols), 2949.67 cm^{-1} (C–H stretching vibration), 1615.38 cm^{-1} and 1403.00 cm^{-1} (stretching vibration of C=C) and 1045.75 cm^{-1} (stretching vibration of C–O–C). Comparison of the IR spectra with EMP, DG, and DG&EMP, EMP@glycymicelles revealed no new absorption peaks, indicating the absence of any chemical reactions during the sample preparation procedures.

XRD patterns for EMP, DG, the DG&EMP physical mixture, and the EMP@glycymicelles were shown in Figure S3B. The powder X-ray diffraction pattern of EMP revealed several high intensity peaks at different diffraction angles (2θ) of 14.50°, 18.68°, 20.20°, 23.30° and 25.04°, suggesting that EMP existed in a crystalline nature; however, the diffraction patterns of DG corresponded to the amorphous state. The patterns for the physical mixture of DG and EMP showed some typical bands of EMP, such as 14.50°, 18.74°, 20.22°, 23.26° and 25.12°. In the EMP@glycymicelles, the diffraction pattern was similar to that of the amorphous structure of DG, and as the spectra did not reveal any characteristic peaks, indicating the amorphous state of EMP in the glycymicelles.

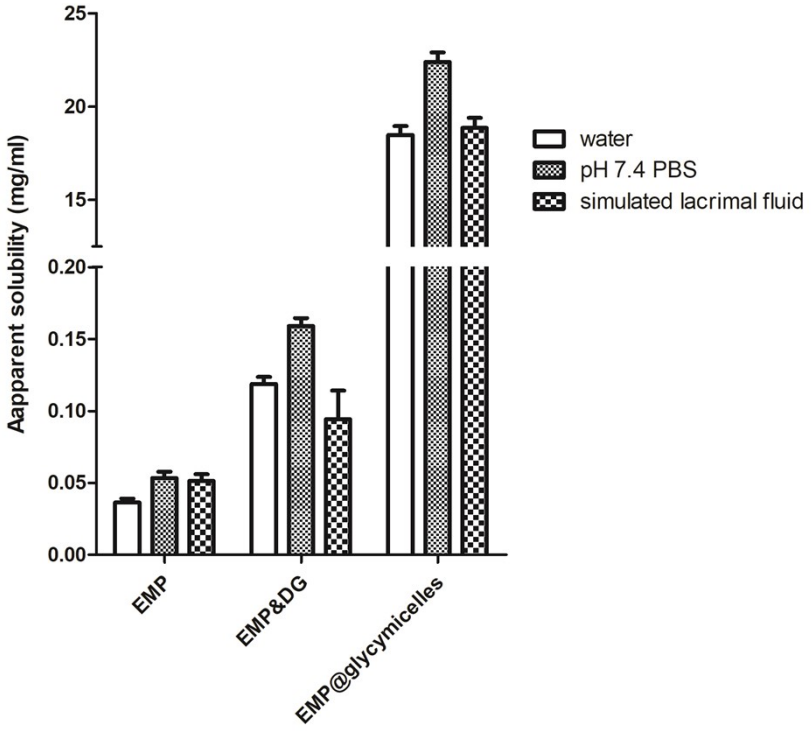


Figure S4 Apparent solubility profiles of EMP and EMP@glycymicelles.

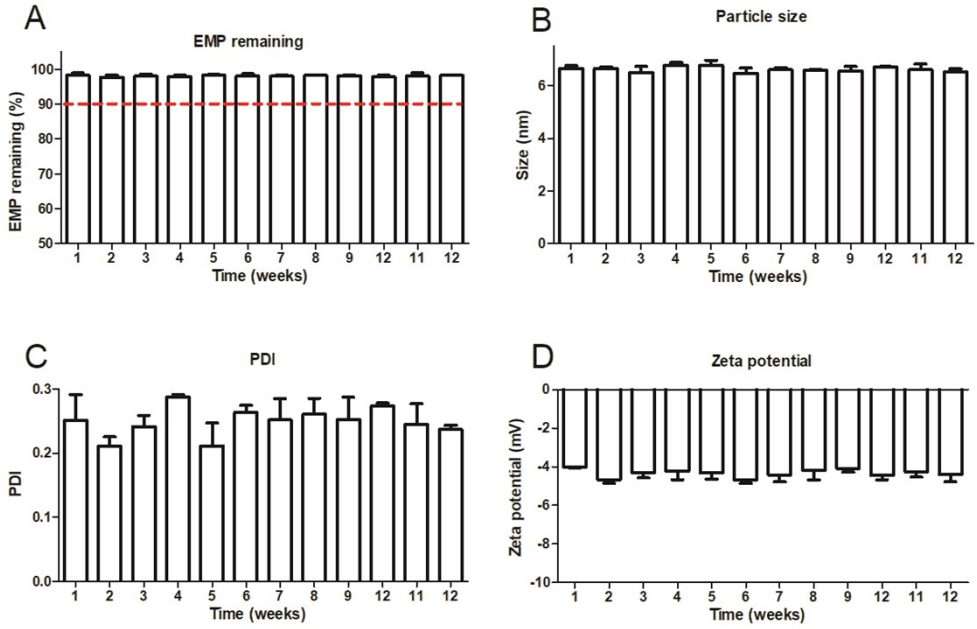


Figure S5 EMP@glycymicelles (EMP/DG weight ratio 1:15) characterizations changing

profiles during short-term storage at 25 °C. (A) EMP remaining in glycymicelles, (B) size, (C) polydispersity index (PDI), and (D) zeta potential were tested during storage. (n=3).

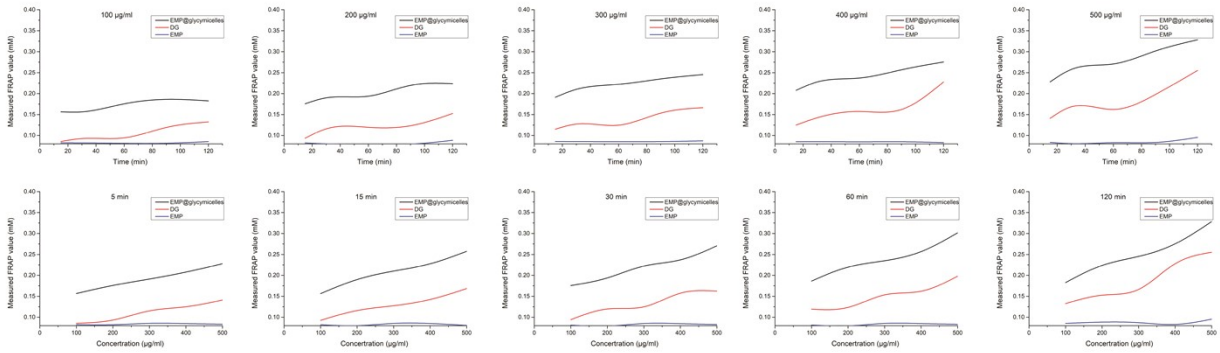


Figure S6 Measured antioxidant characterizations. Measured FRAP values of the bare EMP, DG and EMP@glycymicelles with (A) different concentrations as functions of time and (B) different incubation times as functions of concentration.

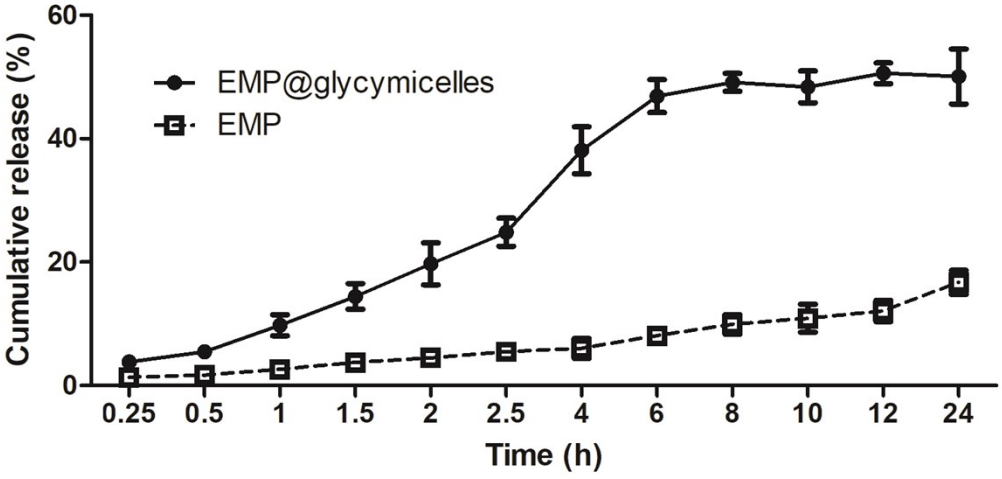


Figure S7 Characterizations of in vitro release. In vitro release profiles of EMP from EMP@glycymicelles with dialysis method.

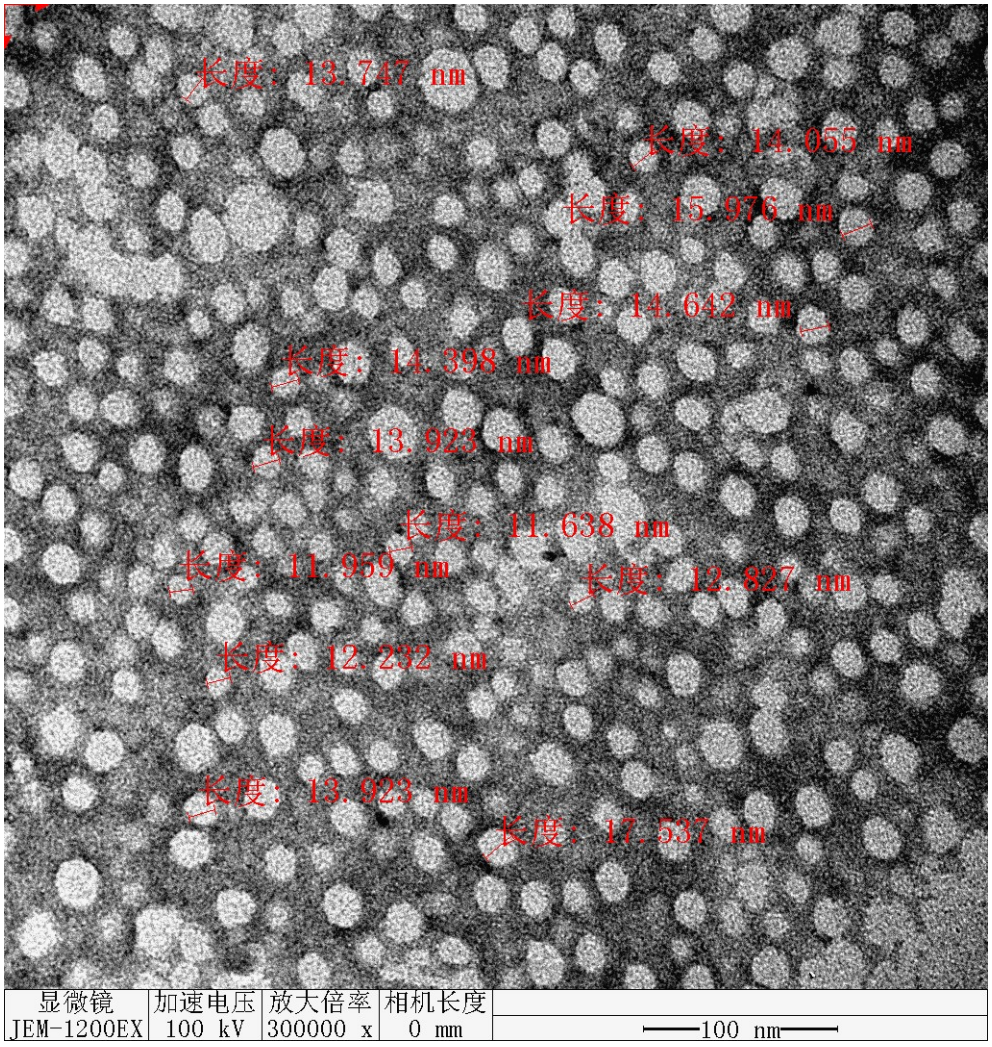


Figure S8 TEM observation (×30 k magnification, bar = 100 nm) glycymicelles only (without loading EMP). The TEM images revealed that glycymicelles only were uniform and without aggregation. The size observed was about 13.67 ± 1.82 nm, and this size was consistent with our previous observation[1]. The size observed to glycymicelles only was some bigger than the EMP@glycymicelles. This might be explained that EMP had an amphiphilic structure, and EMP could potentially self-assemble and interpenetrate glycymicelles with DG. So, EMP@glycymicelles assembled tightly in aqueous solution with stronger intermolecular interactions, such as hydrogen bonds and van der Waals forces, and presented as a smaller size.

Table S1 Results of fitting models

	EMP@glycymicelles		EMP	
	Fitted equation	*r*	Fitted equation	*r*
Zero order	Q=2.1051t+17.519	0.7641	Q=0.6592t+2.971	0.9592
First order	Ln(100-Q)=-0.0312t+4.3992	0.7831	Ln(100-Q)=-0.0073t+4.5758	0.9662
Higuchi	$Q=13.073t^{1/2}+2.6183$	0.8992	$Q=3.6192\ t^{1/2}-0.6969$	**0.9980**
Korsmeyer-Peppas	LgQ=0.6505Lgt+1.0396	**0.9622**	LgQ=0.5847Lgt+0.4497	0.9961
Hixson-Crowell	$(100-Q)^{1/3}=-0.0422t+4.3403$	0.7770	$(100-Q)^{1/3}=-0.0109t+4.5959$	0.9640

The drug release data were applied to various release kinetic models including zero order model, first order model, Higuchi model, Korsmeyer-Peppas model, and Hixson-Crowell model. As showed in Table S1, results indicated that the bare EMP followed Higuchi kinetic model (r =0.9980), suggesting a diffusion in vitro release mechanism of bare EMP under sink conditions. EMP@glycymicelles followed Korsmeyer-Peppas model (r =0.9622), suggesting a multiple in vitro release mechanisms involved of EMP releasing from glycymicelles; release exponent (n) of the Korsmeyer-Peppas model was calculated to determine the main release mechanism, and (n = 0.6505, falling into the $0.45 < n < 0.89$ to non-Fickian transport) suggested the non-fickian transport mechanism through glycymicelles. The release pattern of the EMP obtained was found similar to the reported results of micelle formulation for ocular drug delivery[2].

Reference

1. H. Yang, Q. Cao, Z. Yuan, X. Wu and M. Li, *Nanomedicine (Lond)*, 2021, **16**, 2431-2448.
2. N. Mehra, M. Aqil and Y. Sultana, *Eur J Pharm Sci*, 2021, **159**, 105735.