Electronic Supplementary Information

In situ phase transition of microemulsions for parenteral injection yielding lyotropic liquid crystalline carriers of the antitumor drug bufalin

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Structure characterization

Small-angle X-ray scattering (SAXS) experiments: The blank and drug-loaded samples were characterized by SAXS measurements at 37 ^{o}C. The experiments were performed at the beamline BL19U2 of the National Center for Protein Science Shanghai (NCPSS) at Shanghai Synchrotron Radiation Facility (SSRF). The wavelength, λ, of X-ray radiation was set as 1.033 Å. The scattered X-ray intensities were measured using a Pilatus 1M detector (DECTRIS Ltd). The sample-to-detector distance was set so that the detected range of momentum transfer q [$q = 4\pi \sin\theta/\lambda$, where 2θ is the scattering angle] was between 0.01 and 0.17Å^{-1}. To reduce the radiation damage during the SAXS experiments, a flow cell made of a cylindrical quartz capillary with a diameter of 1.5 mm and a wall thickness of 10 μm was employed. The exposure time was set to 1-2 seconds. The X-ray beam was with a size of 0.40× 0.15 (H x V) mm^2 and was adjusted to pass through the center of the capillary. Ten 2D images were recorded for each sample and a buffer solution in order to obtain good signal-to-noise ratios. The 2D scattering images were converted into 1D SAXS curves through azimuthally averaging procedure after solid angle correction. The normalization by the intensity of the transmitted X-ray beam was done using the BioXTAS RAW software package.

Rheological experiments: Rheological measurements were performed on the AR 2000 EX rheometer (TA Instruments). Before every experiment, the sample was equilibrated at 37 ^{o}C and centrifuged for 20 min at 3000 rpm in order to ensure the absence of air bubbles. The cone/plate geometry was employed with a cone angle of 2^{o} and a plate diameter of 29 mm. After conditioning the sample at a constant temperature for about 10 min, the excess sample was carefully removed. Steady shear measurements were performed on samples B1, C1, B and C using a range of shear rate from 10^{-1} to 10^{3} s^{-1}. Controlled stress measurements were performed at 1.0 Hz. The applied shear stress was scanned in order to determine the linear viscoelastic region of the system. Then frequency sweep measurements were performed at a constant stress which was found to be in the linear viscoelastic domain. The frequency was varied from 10^{-1} to 10^{-2} rad/s.

Dynamic light scattering measurements (DLS): The DLS measurements were performed with a Delsat Nano C Particle Analyzer (Beckman Coulter, USA). The back-scattering angle was 165°. The mean hydrodynamic diameters of the particles and their size distributions were determined for every dispersed sample. All measurements were done in a triplicate.

Cryogenic transmission electron microscopy (Cryo-TEM): For cryo-transmission electron microscopy studies, a sample droplet of 2 μL was placed on a lacey carbon-filmed copper grid (Science Services, Muenchen), which was hydrophilized by air plasma glow discharge (Solarus 950, Gatan, Muenchen, Germany) for 30 s. Subsequently, most of the liquid was removed with blotting paper, leaving a thin film stretched over the lace holes. The specimens were instantly shock-frozen by rapid immersion into liquid ethane, cooled to approximately 90 K by liquid nitrogen in a temperature-controlled freezing unit (Zeiss Cryobox, Carl Zeiss Microscopy GmbH, Jena, Germany). The temperature was monitored and kept constant in the chamber during all sample preparation steps. After freezing the specimens, the remaining ethane was removed using blotting paper. The specimen was inserted into a cryo transfer holder (CT3500, Gatan, Muenchen, Germany) and transferred to a Zeiss/Leo EM922 Omega EFTEM (Zeiss Microscopy GmbH, Jena, Germany). Examinations were carried out at temperatures around 90 K. The TEM was operated at an acceleration voltage of 200 kV. Zero-loss filtered images ($\Delta E = 0$ eV) were taken under reduced dose conditions (100–1000 e nm^{-2}). All images were registered digitally by a bottom-mounted CCD camera system (Ultrascan 1000, Gatan, Muenchen，Germany), combined and processed with a digital imaging processing system (Digital Micrograph GMS 1.9, Gatan, Muenchen, Germany).

Table S1. Compositions of the investigated LC-ME systems (wt%).

Formulation	Water	Oil (MCT)	HS 15	Span 80	Ethanol
B	20.0	15.0	43.3	14.5	7.2
B1	30.0	13.1	37.9	12.7	6.3
B2	35.0	12.2	35.2	11.8	5.8
B3	40.0	11.2	32.5	10.9	5.4
B4	45.0	10.3	29.7	10.0	5.0
B5	90.0	1.9	5.4	1.8	0.9
C	24.0	6.0	46.7	15.5	7.8
C1	35.0	5.1	39.9	13.3	6.7
C2	40.0	4.7	36.9	12.2	6.2
C3	45.0	4.3	33.8	11.2	5.7
C4	50.0	4.0	30.7	10.2	5.1
C5	90.0	0.8	6.1	2.0	1.0

Table S2. Entrapment efficiency (%) and drug loading (%) of BUF in B-LC-ME-BUF and C-LC-ME-BUF carriers and their evolution during 90 days of storage. The results were presented as mean values (n=3).

Sample code	EE（%）			DL（%）		
	Day 1	Day 30	Day 90	Day 1	Day 30	Day 90
B-LC-ME-BUF	92.20±1.0	96.50±0.2	96.03±1.0	3.30±1.0	3.46±0.2	3.44±1.0
C-LC-ME-BUF	91.30±1.0	92.25±0.7	95.72±1.4	7.71±1.0	7.78±0.7	8.07±1.4

Table S3. IC_{50} values determined by MTT assays with A549 cells (n=3).

Formulation	IC_{50} (ng/mL)
Free BUF drug	10.61
B-LC-ME-BUF	9.236
C-LC-ME-BUF	7.523
B-LC-ME	＞5000
C-LC-ME	＞5000

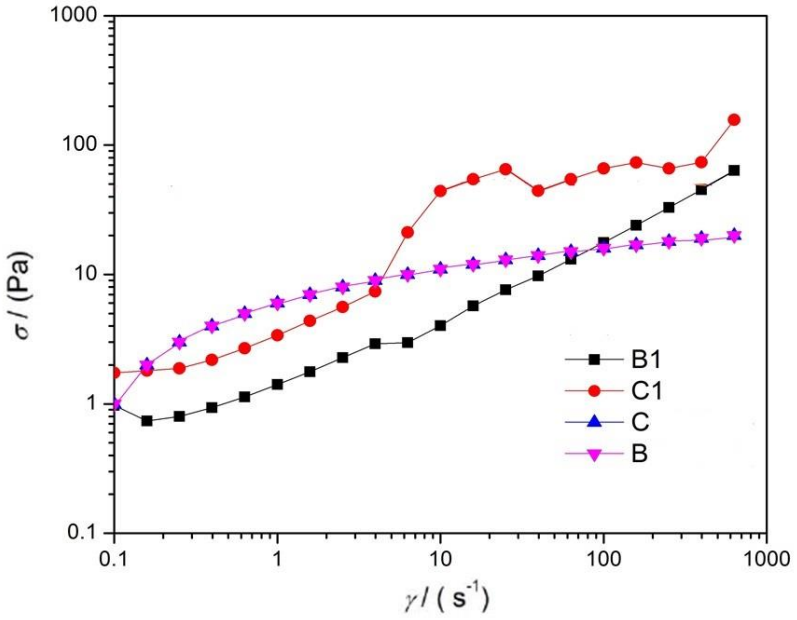

Fig. S1 The shear stress curves versus shear rate.

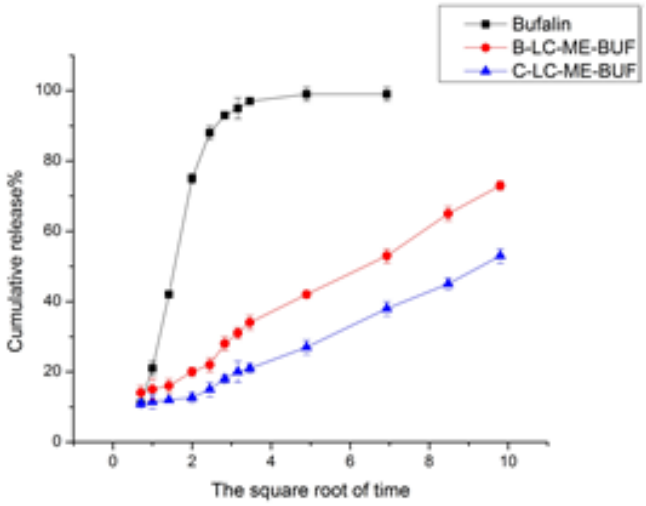

Fig. S2 The bufalin release curves versus the square root of time.