Supporting Information

Self-microemulsifying Drug Delivery System of Curcumin with Enhanced Solubility and Bioavailability using a New Semi-synthetic Bicephalous Heterolipid: *in vitro* and *in vivo* Evaluation

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Materials

Oleic acid (technical grade, 90%) was purchased from Sigma, USA. 3-Amino-1-propanol and *tert*-butyl acrylate was obtained from Alfa-Aesar, USA. Thionyl chloride, *p*-dimethylaminopyridine (DMAP), toluene were purchased from S D Fine-Chem, India. All the solvents used were of analytical grade and procured from Merck. Merck precoated Silica-gel $60F_{254}$ plates were used for thin layer chromatography.

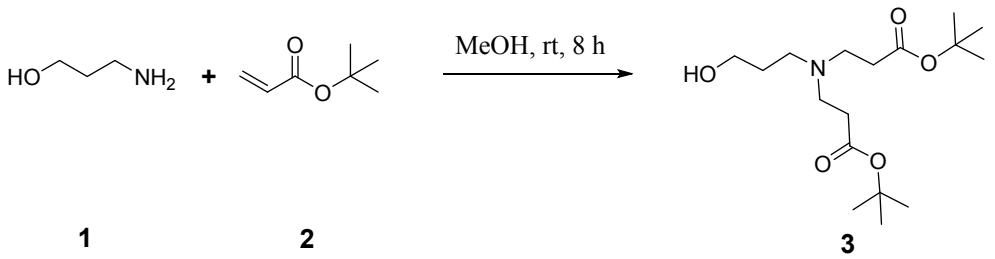
Instrumentation

FT-IR spectra were recorded using Shimadzu, FT-IR Spectrophotometer whereas ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker NMR spectrometer at 300 MHz and 75 MHz respectively. An electrospray ionization-mass spectrum (ESI-MS) was recorded on Bruker mass spectrometer.

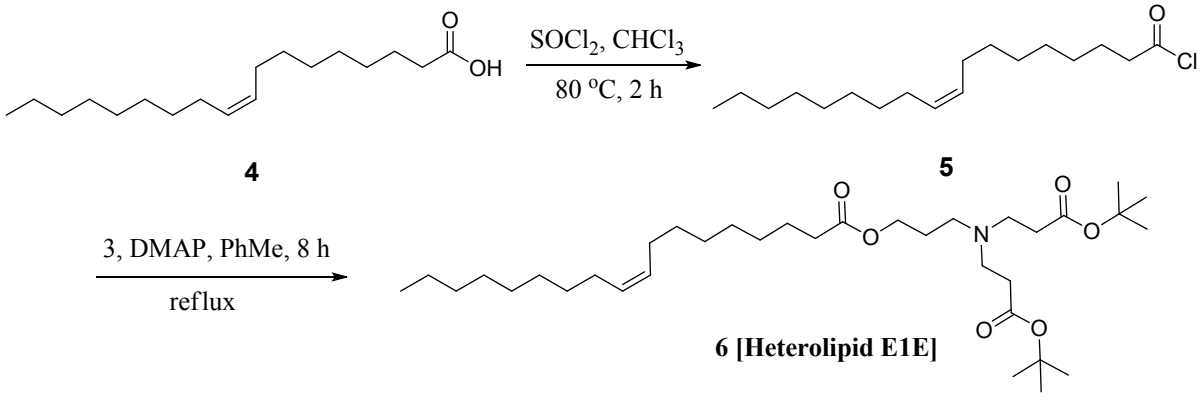
Synthesis and characterization of heterolipid E1E

Synthesis of heterolipid E1E was performed as per earlier reported method (Scheme S1 and S2).*1*In brief, first generation poly (propyl ether imine) dendron **3** was synthesized by a Michael addition reaction between 3-amino-1-propanol and *tert*-butyl acrylate. For this purpose a solution of *tert*-butyl acrylate **2** (19.22 g, 150 mmol) in MeOH (500 ml) was added drop wise to a solution of 3-amino-1-propanol **1** (3.75 g, 50 mmol) in MeOH (1000 ml) at room temperature. The reaction mixture was stirred at room temperature for 8 h,allowed to stand overnight and evaporated in vacuo to obtain pure compound **3**(16.23 g, 98%) as a colorless liquid. In the next step, oleoyl chloride(3.01 g, 10 mmol) synthesized by reaction with thionyl chloride at 80 ^{o}C using $CHCl_3$ as a solvent was added at room temperature to the mixture of compound **3**(3.31 g, 10 mmol) and DMAP (1.22 g, 10 mmol) in toluene (50 ml) which was previously refluxed for 3 h in an assembly consisting of a two necked flask fitted with Dean-Stark and reflux condenser.This reaction mixture was refluxed for 8 h, solvent was

removed under reduced pressure and the residue obtained was purified by column chromatography (SiO_2, 60–120 mesh) using hexane/EtOAc, 9:1 as eluent to afford heterolipid E1E **6** as colorless to slightly yellow liquid (5.59 g, 91%).. FT-IR (neat) *v*: 1730, 1460, 1365, 1249, 1155, 2854, 2926 cm^{-1}.^{1}HNMR ($CDCl_3$) *δ*: 0.83 (t, 3H, $C_{16}H_{33}C\mathbf{H_3}$), 1.23 **(**m, 20H,$CH_3(C\mathbf{H_2})_6CH{=}CH(C\mathbf{H_2})_4CH_2CH_2CO$), 1.42 (s, 18H, 2 × $C(C\mathbf{H_3})_3$,1.64(q, 2H, $C_{15}H_{29}CH_2C\mathbf{H_2}$-CO), 1.73 (m, 2H, -$OCH_2CH_2C\mathbf{H_2}$N-),2.19 **(**m, 4H, -$C\mathbf{H_2}CH{=}CHC\mathbf{H_2}$-**)**, 2.34 (m, 6H**,** -$C\mathbf{H_2}$CO-), 2.47 **(**t,2H**,** -$OC\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}$N-), 2.71 (m, 4H**,** -$NC\mathbf{H_2}C\mathbf{H_2}$CO- **)**, 4.03 (t, 2H,-$OC\mathbf{H_2}C\mathbf{H_2}C\mathbf{H_2}$N- **)**, 5.32 (m**, 2H,** -C**H**=C**H**- **)**.^{13}C NMR ($CDCl_3$) *δ*:14.09, 22.66, 24.97, 26.57, 27.16, 27.20, 28.08, 29.11, 29.15, 29.30, 29.50, 29.65, 29.75, 31.92, 33.89, 34.33, 49.34, 50.12, 62.41, 76.61, 77.03, 77.46, 80.32, 129.73, 129.97, 171.90, 173.82. ESI-MS m/z: 596 [M+].



Scheme 1. Synthesis of compound **3** with primary alcohol as a focal functionality.



Scheme 2. Synthesis of heterolipid E1E

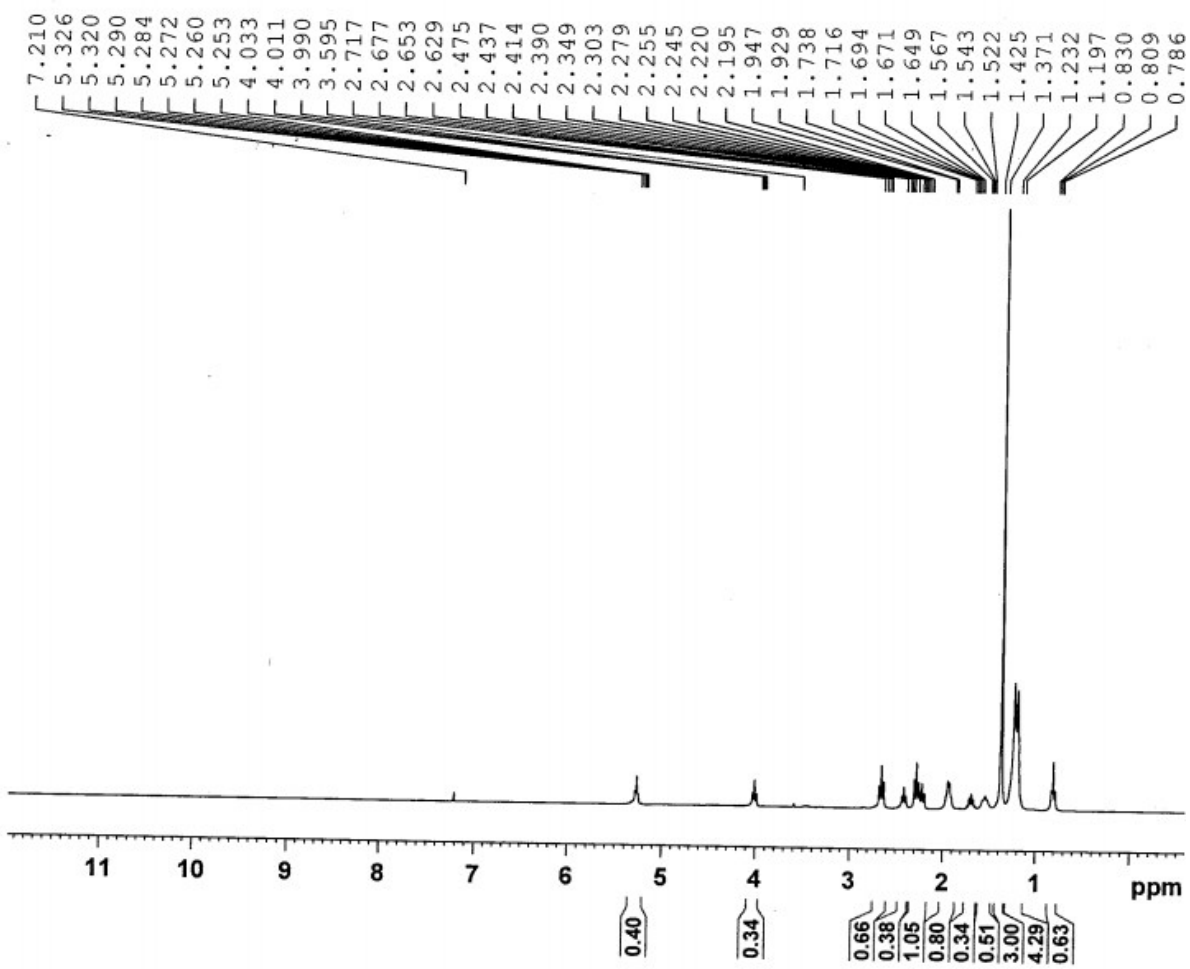


Fig. S1. ^{1}H NMR of heterolipid E1E.

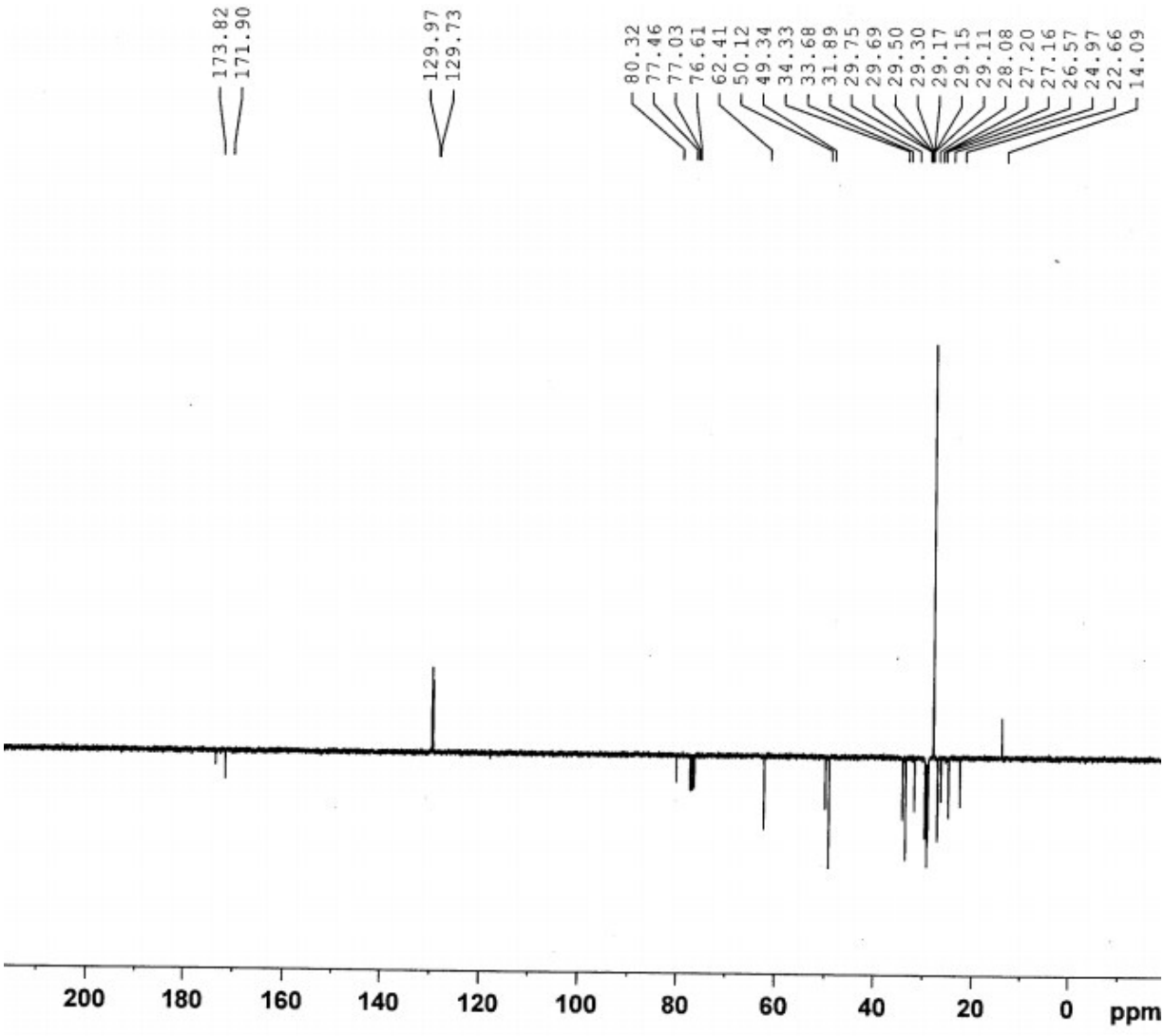


Fig S2. ^{13}C NMR of heterolipid E1E.

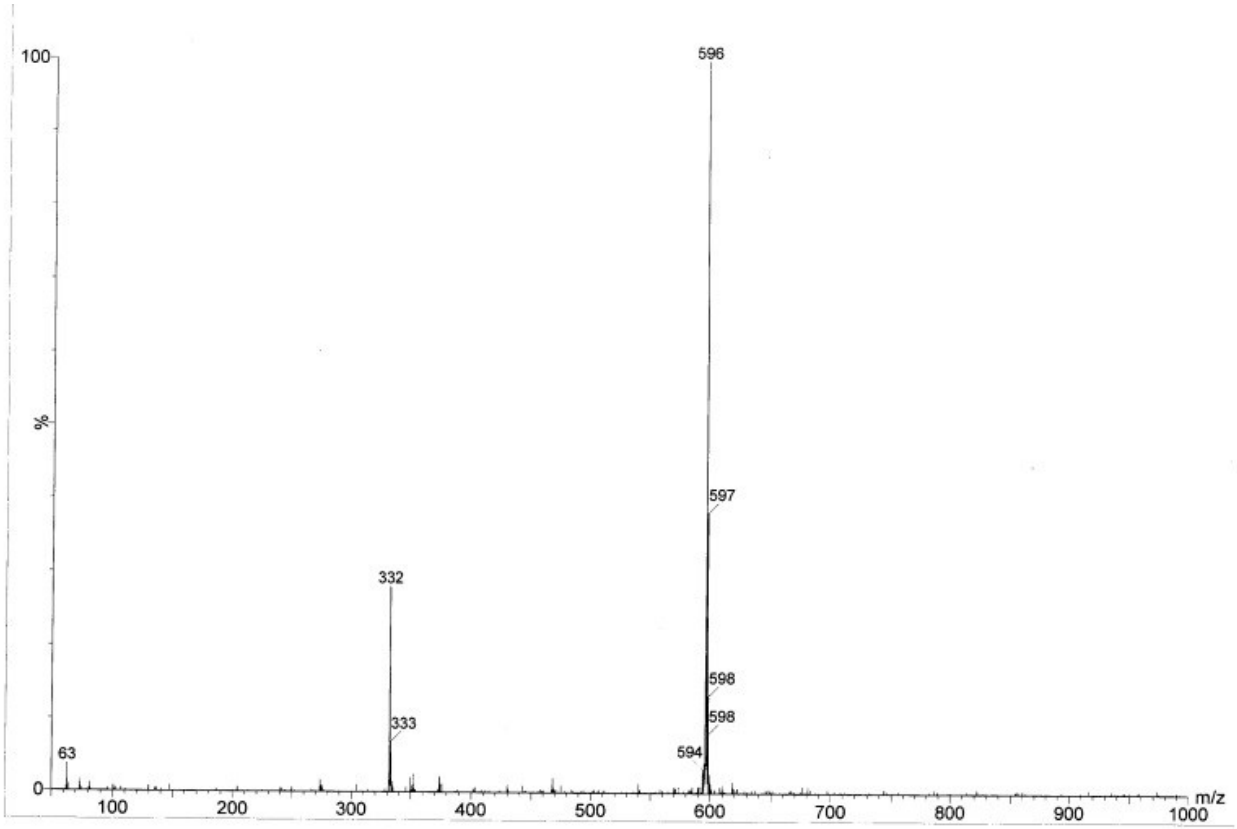


Fig S3. Mass spectrum of heterolipid E1E.

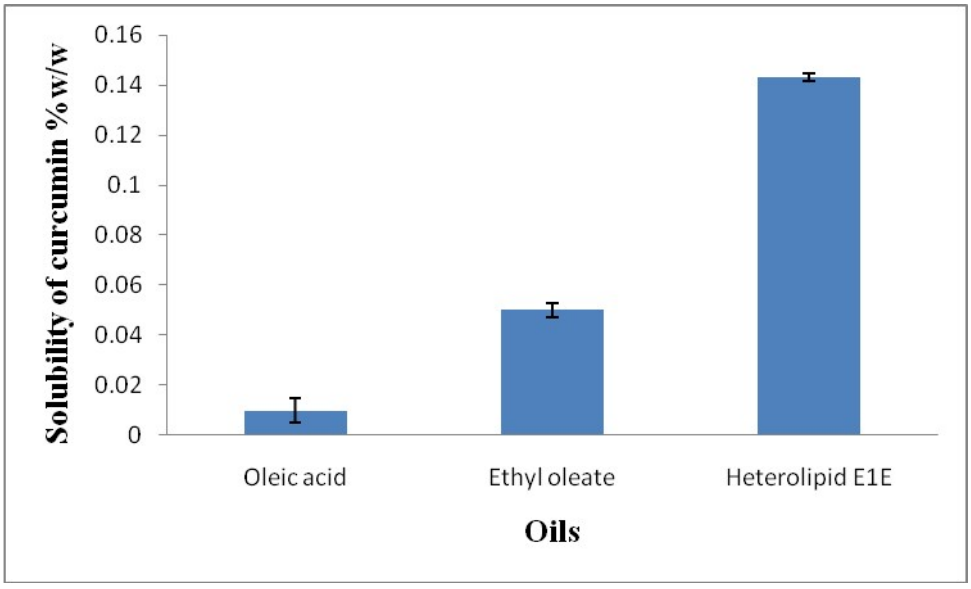


Fig. S4. Curcumin solubility in oleic acid, ethyl oleate and heterolipid E1E, data expressed as mean ±S.D. (n=3)

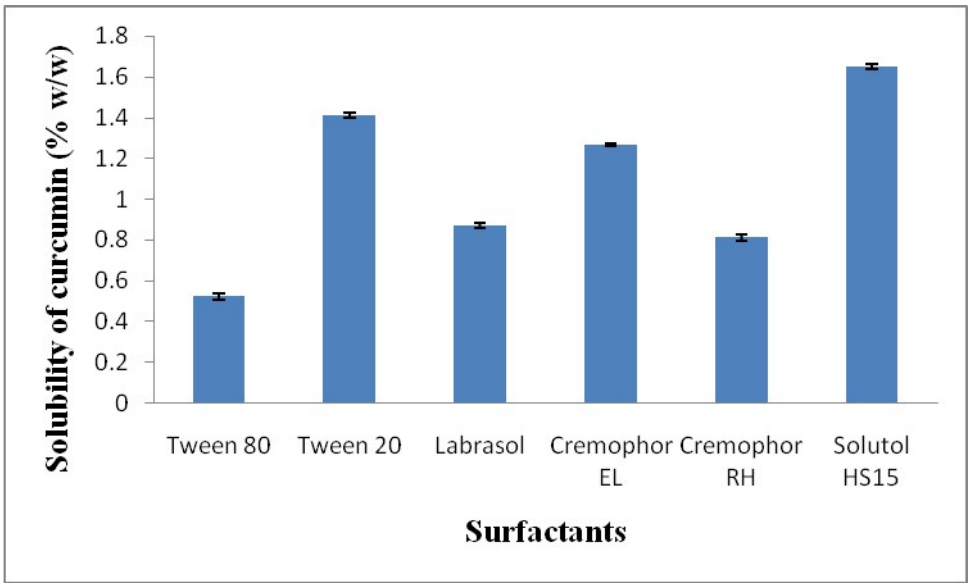


Fig. S5. Solubility of Curcumin in different surfactants, data expressed as mean ±S.D. (n=3).

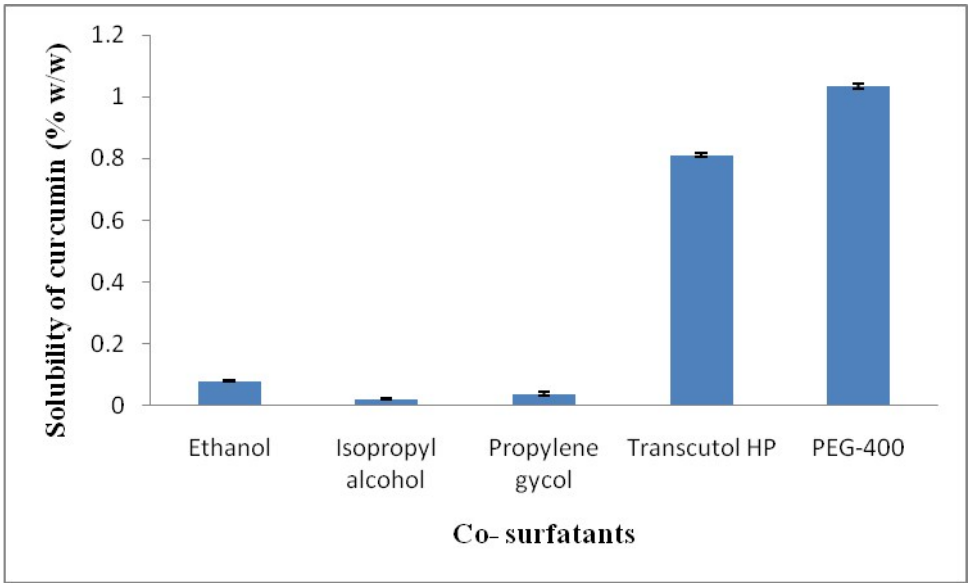


Fig. S6. Solubility of Curcumin in different co-surfactants, data expressed as mean ±S.D. (n=3).

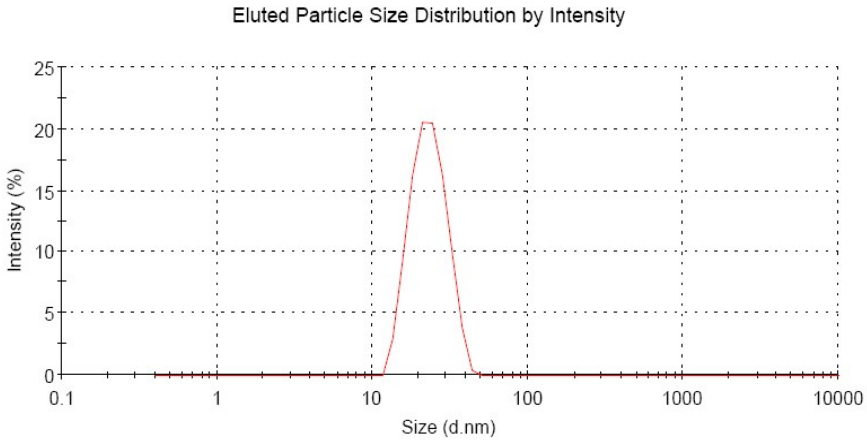


Fig. S7. Globule size distribution curve of Curc-E1E_SMEDDS5e.

HPLC analysis of plasma samples

Spiking technique was used to perform calibration curves of Curcumin. Briefly, plasma samples were previously collected using heparinized tubes and stored in deep freezer until required. A standard stock solution of Curcumin was prepared in methanol at a concentration of 0.1 mg/mL. The Curcumin stock standard was diluted with methanol to working solutions at concentrations of 1, 5, 10, 20, 40, 50 and 100µg/mL. Each plasma matrix (100 µl) was spiked with 100 µl of previously prepared stock solutions containing varying amounts of Curcumin. In resulting plasma samples acetonitrile was added to make up to 1 mL and then centrifuged at 12,000 rpm 20°C for 15 min. Each supernatant composed of final concentrations of Curcumin standards (0.1, 0.5, 1, 2, 4, 5 and 10 µg/ mL), was injected directly into the HPLC column three separate times (n= 3), and analysed by the HPLC method reported under solubility studies in the main article. A good linearity was achieved with a correlation coefficient of > 0.999 over the concentration range of 0.1–10 µg/mL.

Antimicrobial activity

Strains used

The control strains used in this study were *E. Coli* ATCC 25922 and *S. aureus* ATCC 29213

Inoculums Preparation and Inoculation

Bacterial isolates were removed from storage, streaked on to Mueller-Hinton (MH) Agar plates and incubated for 24 h at 35°C. A working bacterial suspension was prepared by suspending 3–5 isolated colonies in 3 mL of Mueller-Hinton broth(MHB). The turbidity of this suspension was carefully adjusted photometrically to equal that of a 0.5 McFarland standard. Within 15 minutes of preparation, inoculum suspension was adjusted withMHB so as each well contains approximately 5×10^5 CFU/mL.

The minimum inhibitory concentrations (MICs) determination by broth micro-dilution Technique[4,5]

Stock solution

Stock solutions of test samples were prepared in sterile water for injection. The stock solutions were further diluted with MHB so as to get two fold of required concentration in the well.

NCCLS broth micro-dilution and MTT assay

The broth micro-dilution tests were performed by using sterile, disposable, multiwell micro-dilution plates (96 U-shaped wells). Each well received 100 µL of MHB followed by 100µL of 2x test sample concentrations dispensed into the wells of Rows 1 to 12 of the micro-dilution with a multichannel pipette. Row 1 contained the highest (12500 µg/mL) Test sample concentration, and Row 12 contained the lowest drug concentration (6.08 µg/mL). Each well was mixed well by repeated pipetting. Serial dilutionswere done by transferring 100 µL of solution from Row 1 to Row 2 mix well transfer 100 µL from this to next well ROW 3and continue till row 12. From Row 12 remove 100 µL and discard. To each well 100 µL of the corresponding 2x diluted inoculum suspension was added, incubated at 35 °C for 24 h and observed for the presence or absence of visible growth. After visual observation, 10 µL of (5 mg/mL) 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) dye solution was added to each well and incubated further for 6 h. Content from each well was withdrawn

and centrifuge at 15,000 rpm for 5 min. The supernatant was discarded and 100 μL of DMSO was added to the residue and re-plated on another 96 well plate for optical density (OD) measurement at 540 nm. Data were obtained from triplicate wells.

References

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