

# Supplementary Materials: Ionic Liquid Forms of the Antimalarial Lumefantrine in Combination with LFCS Type IIIB Lipid-Based Formulations Preferentially Increase Lipid Solubility, In Vitro Solubilization Behavior and In Vivo Exposure

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## **<sup>1</sup>H and <sup>13</sup>C NMR data, and HRMS data**

**Lumefantrine hydrochloride:** [C<sub>25</sub>H<sub>30</sub>I<sub>2</sub>NO<sup>+</sup>][Cl<sup>-</sup>] <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 10.17 (s, 1H), 8.20 (dd, J = 14.0, 3.9 Hz, 3H), 7.67 – 7.57 (m, 5H), 7.48 (dd, J = 8.4, 2.0 Hz, 1H), 7.37 (d, J = 2.0 Hz, 1H), 6.63 (d, J = 4.3 Hz, 1H), 5.95 – 5.85 (m, 1H), 3.44 – 3.37 (m, 1H), 3.29 – 3.27 (m, 1H), 3.27 – 3.10 (m, 3H), 1.77 – 1.58 (m, 4H), 1.41 – 1.26 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H), 0.91 (t, J = 6.8 Hz, 3H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 141.4, 139.1, 138.2, 135.0, 134.9, 134.4, 133.6, 133.5, 132.5, 131.6, 130.9, 130.5, 129.0, 128.4, 126.9, 125.8, 122.6, 121.1, 64.8, 56.9, 53.3, 52.7, 24.7, 24.5, 19.5, 19.4, 13.6, 13.5. *m/z* +ve mode – 528.2.

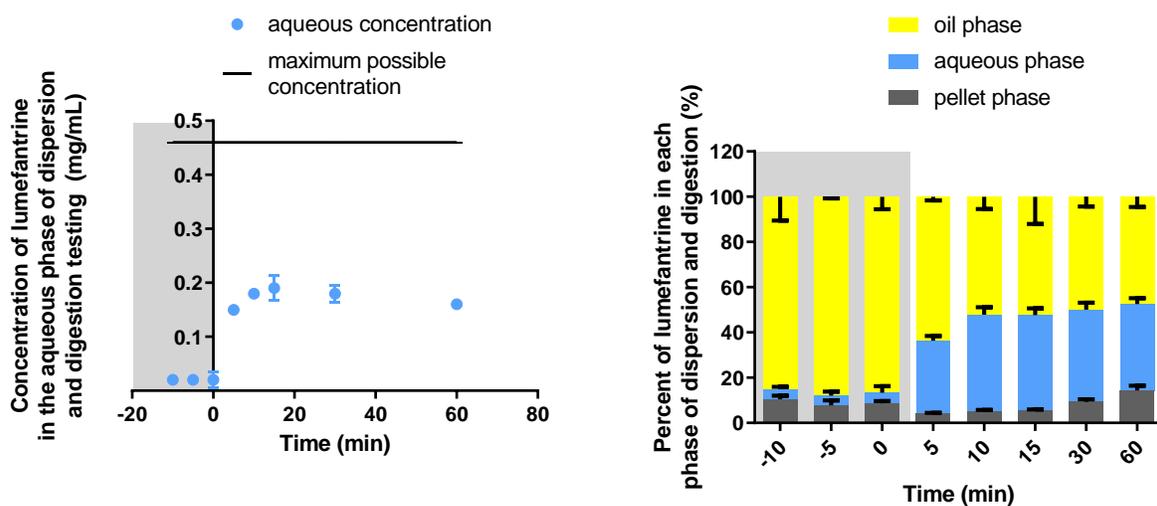
**Lumefantrine dodecyl sulfate:** [C<sub>25</sub>H<sub>30</sub>I<sub>2</sub>NO<sup>+</sup>][C<sub>12</sub>H<sub>25</sub>O<sub>4</sub>S<sup>-</sup>]. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 9.36 (s, 1H), 8.21 (m, 2H), 8.03 (d, J = 8.5 Hz, 1H), 7.71-7.54 (m, 5H), 7.52 (dd, J = 8.4, 1.8 Hz, 1H), 7.38 (d, J = 1.7 Hz, 1H), 6.60 (s, 1H), 5.75 (s, 1H), 3.66 (t, J = 6.7 Hz, 2H), 3.30-3.11 (m, 2H), 1.65 (s, 6H), 1.55-1.16 (m, 22H), 1.01-0.76 (m, 9H). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>) δ 141.4, 138.3, 135.1, 134.4, 133.6, 132.6, 131.6, 130.8, 130.7, 129.0, 128.3, 127.1, 125.3, 122.8, 121.2, 65.4, 56.5, 53.6, 51.9, 31.3, 29.1, 29.0, 28.8, 28.7, 25.5, 24.7, 22.1, 19.4, 13.9, 13.5. HRMS (*m/z*): [C<sub>30</sub>H<sub>33</sub>Cl<sub>3</sub>NO<sup>+</sup>][C<sub>12</sub>H<sub>25</sub>O<sub>4</sub>S<sup>-</sup>] requires [C<sub>30</sub>H<sub>33</sub>Cl<sub>3</sub>NO<sup>+</sup>] 528.1628 and [C<sub>12</sub>H<sub>25</sub>O<sub>4</sub>S<sup>-</sup>] 265.1474; found 528.1626 and 265.1464.

**Lumefantrine docosate:** [C<sub>30</sub>H<sub>33</sub>Cl<sub>3</sub>NO<sup>+</sup>][C<sub>20</sub>H<sub>37</sub>O<sub>7</sub>S<sup>-</sup>]. Melting point: 52-60 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 9.28 (s, 1H), 8.35-8.11 (m, 3H), 8.01 (d, J = 8.5 Hz, 1H), 7.71-7.55 (m, 5H), 7.52 (dd, J = 8.4, 1.8 Hz, 1H), 7.38 (d, J = 1.7 Hz, 1H), 6.61 (d, J = 3.7 Hz, 1H), 5.73 (s, 1H), 3.99-3.77 (m, 4H), 3.61 (dd, J = 11.5, 3.6 Hz, 1H), 3.30-3.10 (m, 4H), 2.99-2.71 (m, 2H), 1.65 (d, J = 6.8 Hz, 4H), 1.49 (s, 2H), 1.42-1.15 (m, 20H), 1.03-0.73 (m, 18H). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>) δ 171.0, 168.3, 141.4, 138.3, 135.1, 134.4, 133.6, 133.5, 132.6, 131.6, 130.8, 130.7, 129.0, 128.3, 127.1, 125.3, 122.8, 121.2, 66.1(2C), 66.0, 61.4,

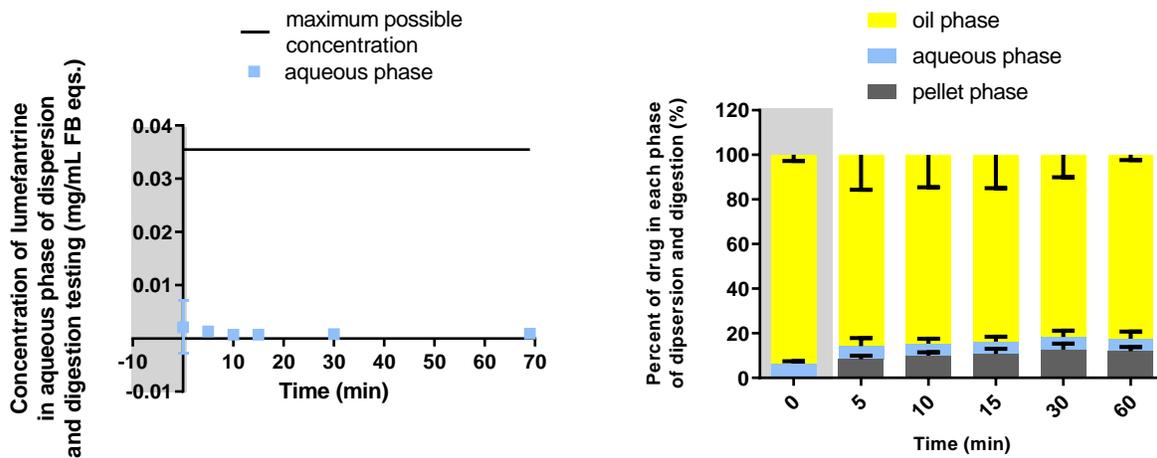
56.5, 53.6, 51.9, 38.1, 34.1, 29.7, 29.6, 28.3, 23.2, 23.0, 22.4 (2C), 19.4, 13.9 (3C), 13.5, 10.8(3C), 10.7.

HRMS ( $m/z$ ):  $[C_{30}H_{33}Cl_3NO^+][C_{20}H_{37}O_7S^-]$  requires  $[C_{30}H_{33}Cl_3NO^+]$  528.1628 and  $[C_{20}H_{37}O_7S^-]$  421.226; found 528.1632 and 421.2246.

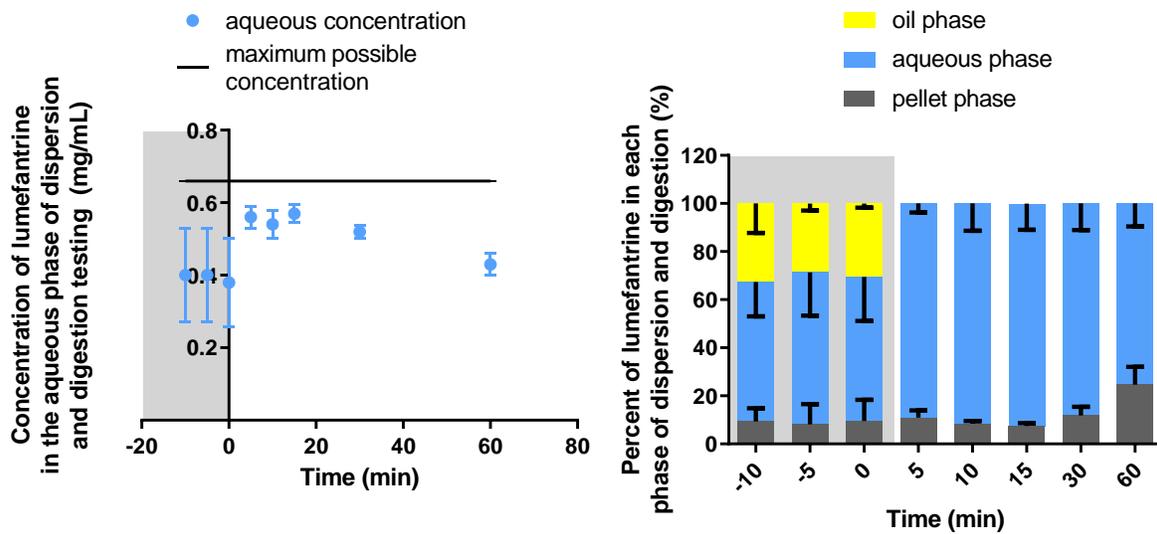
### Drug Distribution and Aqueous Phase Concentrations for Lumefantrine Free Base and Lumefantrine Docusate in Type I-IV Formulations



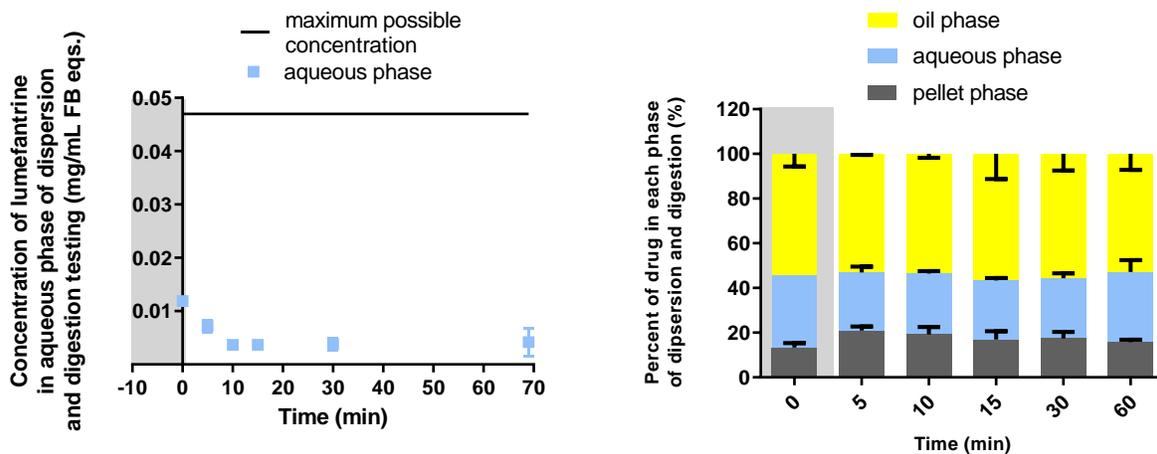
**Figure S1.** *In vitro* dispersion and digestion of lumefantrine free base in Type I-MCF. The concentration of lumefantrine free base in the aqueous phase of the medium-chain digest as a function of time (left), and the proportion of lumefantrine free base in the pellet, aqueous, and oil phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



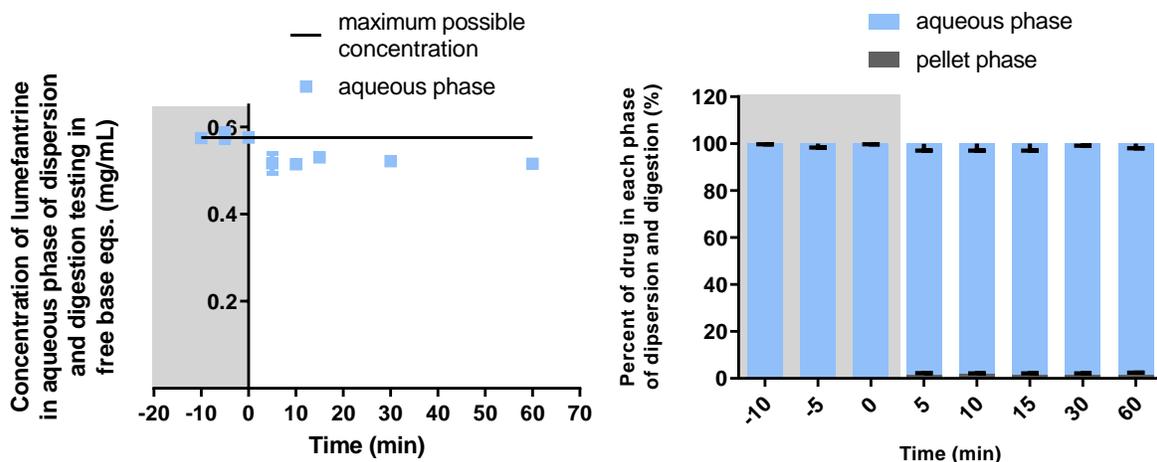
**Figure S2.** *In vitro* dispersion and digestion of lumefantrine free base in Type I-LCF. The concentration of lumefantrine free base in the aqueous phase of the long-chain digest as a function of time (left), and the proportion of lumefantrine free base in the pellet, aqueous, and oil phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



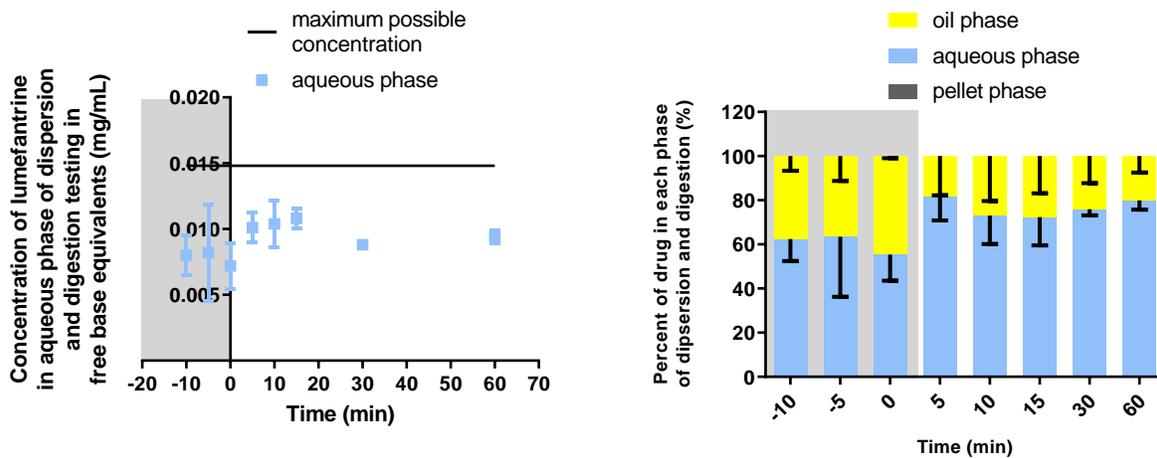
**Figure S3.** *In vitro* dispersion and digestion of lumefantrine free base in Type II-MCF. The concentration of lumefantrine free base in the aqueous phase of the medium-chain digest as a function of time (left), and the proportion of lumefantrine free base in the pellet, aqueous, and oil phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



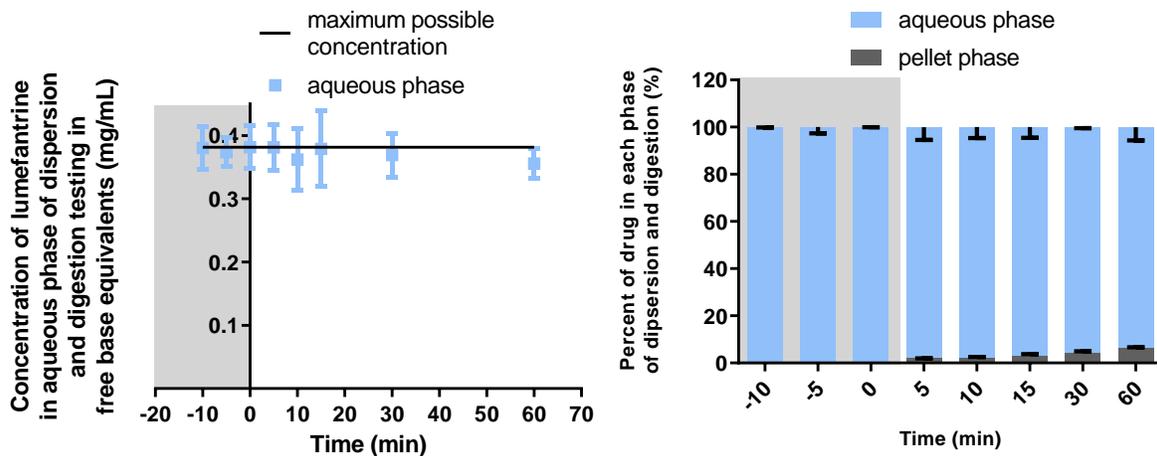
**Figure S4.** *In vitro* dispersion and digestion of lumefantrine free base in Type II-LCF. The concentration of lumefantrine free base in the aqueous phase of the long-chain digest as a function of time (left), and the proportion of lumefantrine free base in the pellet, aqueous, and oil phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



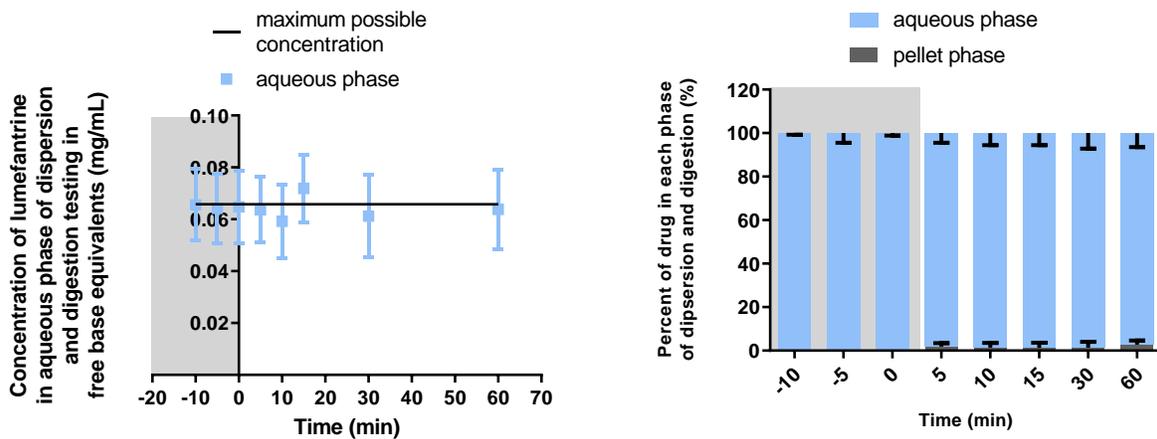
**Figure S5.** *In vitro* dispersion and digestion of lumefantrine free base in Type IIIA-MCF. The concentration of lumefantrine free base in the aqueous phase of the medium-chain digest as a function of time (left), and the proportion of lumefantrine free base in the pellet and aqueous phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



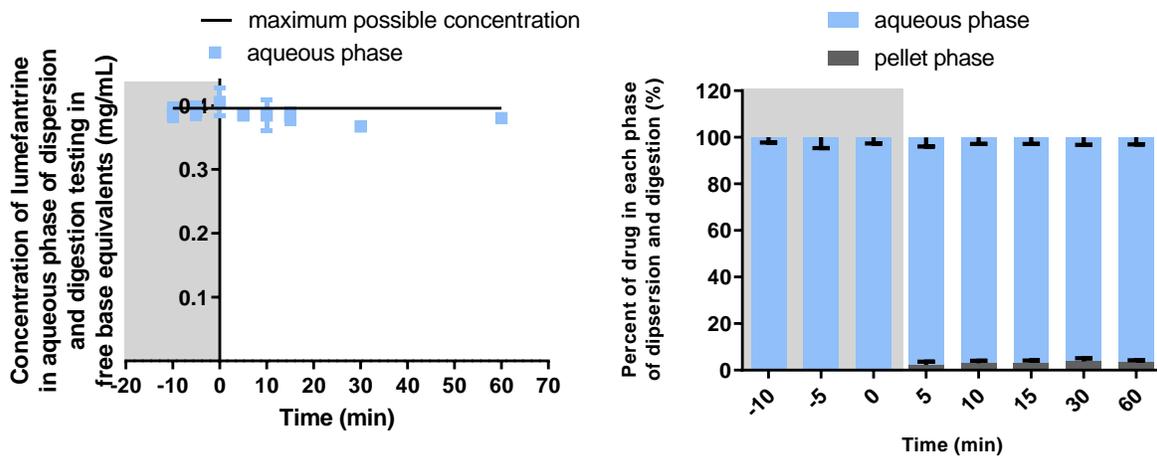
**Figure S6.** *In vitro* dispersion and digestion of lumefantrine free base in Type IIIA-LCF. The concentration of lumefantrine free base in the aqueous phase of the long-chain digest as a function of time (left), and the proportion of lumefantrine free base in the pellet, aqueous, and oil phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



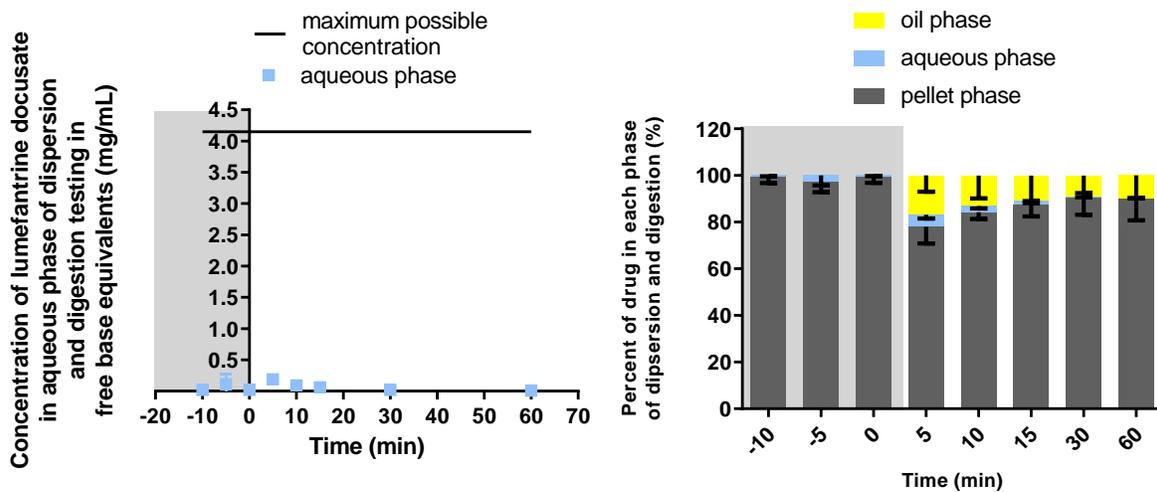
**Figure S7.** *In vitro* dispersion and digestion of lumefantrine free base in Type IIIB-MCF. The concentration of lumefantrine free base in the aqueous phase of the medium-chain digest as a function of time (left), and the proportion of lumefantrine free base in the pellet and aqueous phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



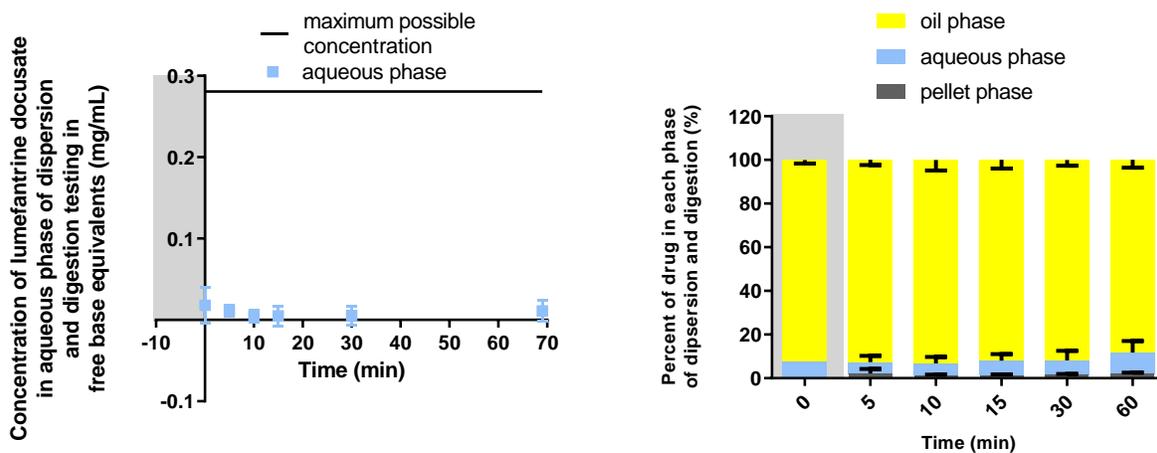
**Figure S8.** *In vitro* dispersion and digestion of lumefantrine free base in Type IIIB-LCF. The concentration of lumefantrine free base in the aqueous phase of the long-chain digest as a function of time (left), and the proportion of lumefantrine free base in the pellet and aqueous phases as a function of time (right). Data are n = 3, mean  $\pm$  SD.



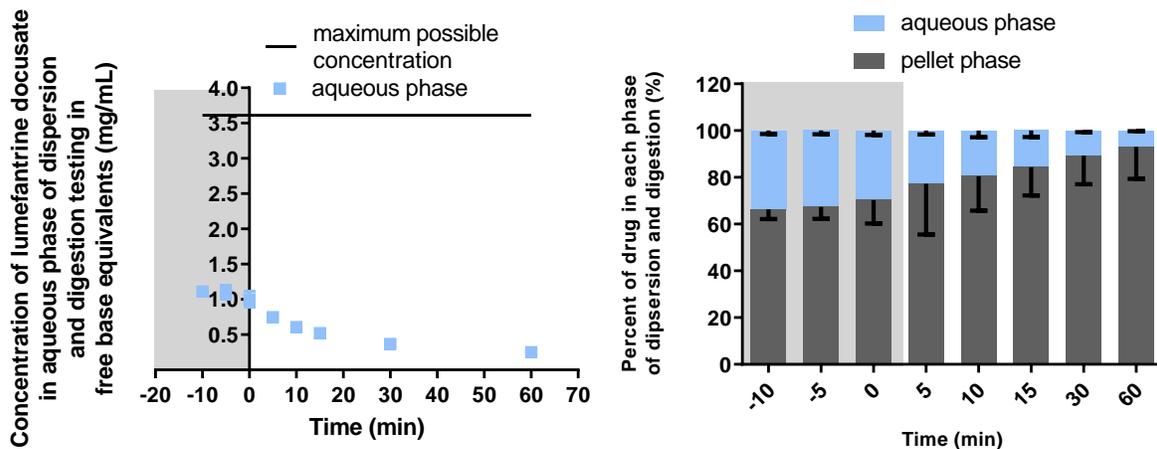
**Figure S9.** *In vitro* dispersion and digestion of lumefantrine free base in Type IV. The concentration of lumefantrine free base in the aqueous phase of the digest as a function of time (left), and the proportion of lumefantrine free base in the pellet and aqueous phases as a function of time (right). Data are n = 3, mean  $\pm$  SD.



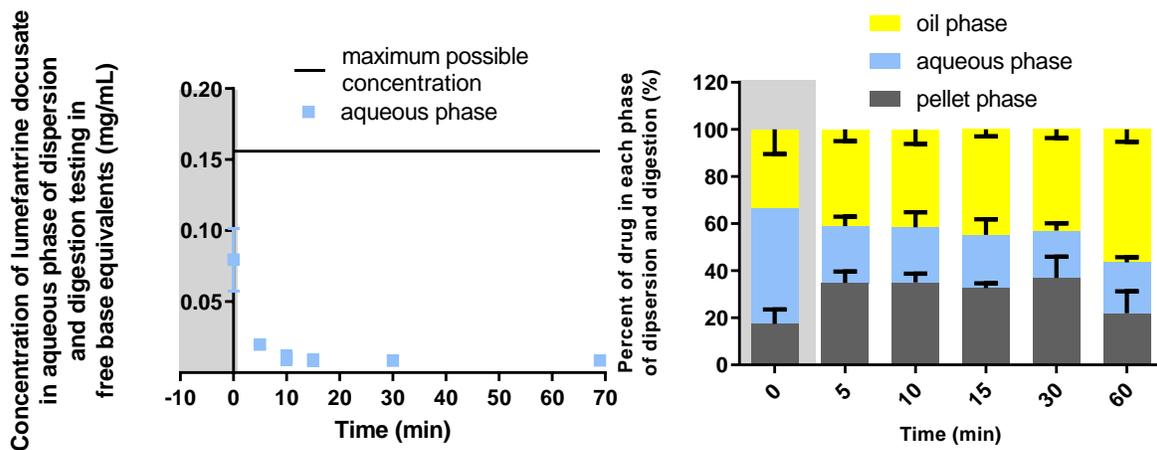
**Figure S10.** *In vitro* dispersion and digestion of lumefantrine docusate in Type I-MCF. The concentration of lumefantrine docusate (in free base equivalents) in the aqueous phase of the medium-chain digest as a function of time (left), and the proportion of lumefantrine docusate in the pellet, aqueous, and oil phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



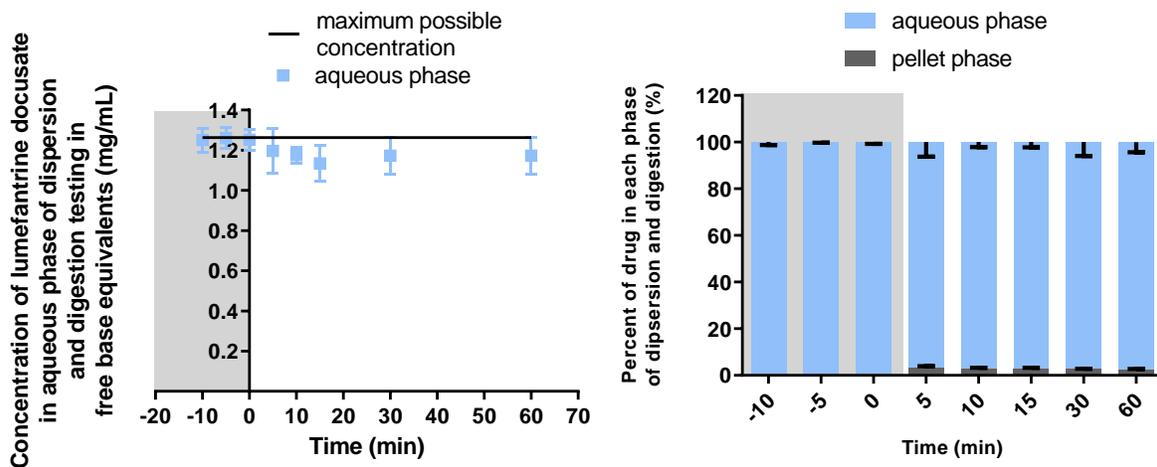
**Figure S11.** *In vitro* dispersion and digestion of lumefantrine docusate in Type I-LCF. The concentration of lumefantrine docusate (in free base equivalents) in the aqueous phase of the long-chain digest as a function of time (left), and the proportion of lumefantrine docusate in the pellet, aqueous, and oil phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



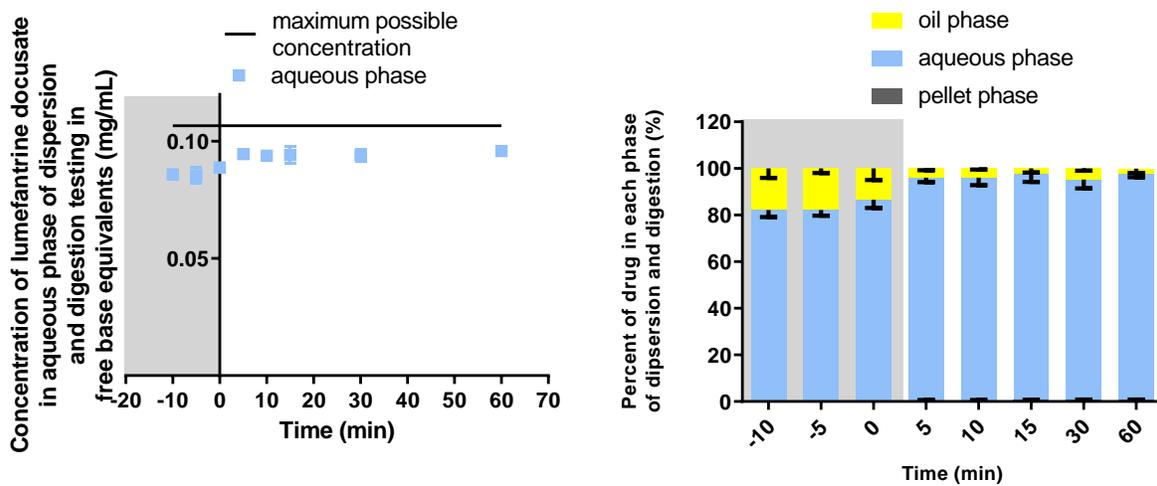
**Figure S12.** *In vitro* dispersion and digestion of lumefantrine docusate in Type II-MCF. The concentration of lumefantrine docusate (in free base equivalents) in the aqueous phase of the medium-chain digest as a function of time (left), and the proportion of lumefantrine docusate in the pellet and aqueous phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



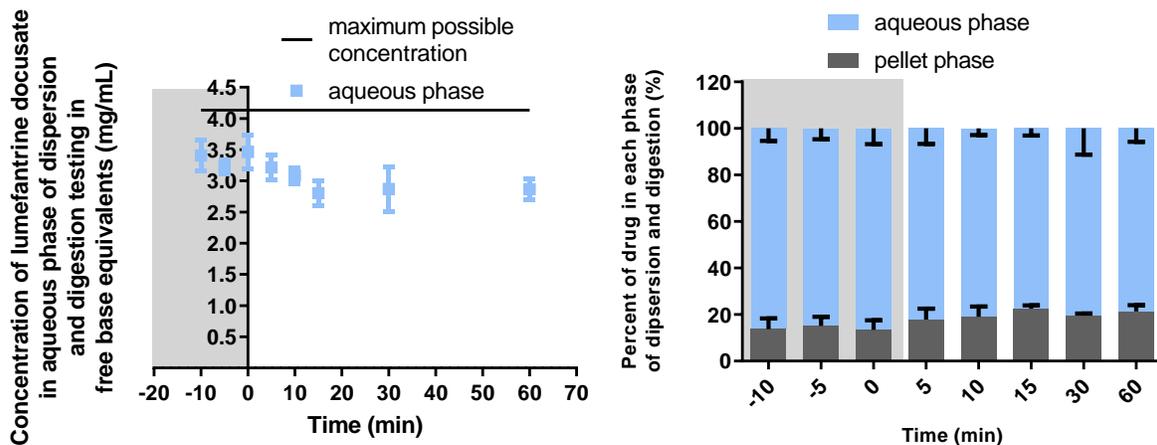
**Figure S13.** *In vitro* dispersion and digestion of lumefantrine docusate in Type II-LCF. The concentration of lumefantrine docusate (in free base equivalents) in the aqueous phase of the long-chain digest as a function of time (left), and the proportion of lumefantrine docusate in the pellet, aqueous, and oil phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



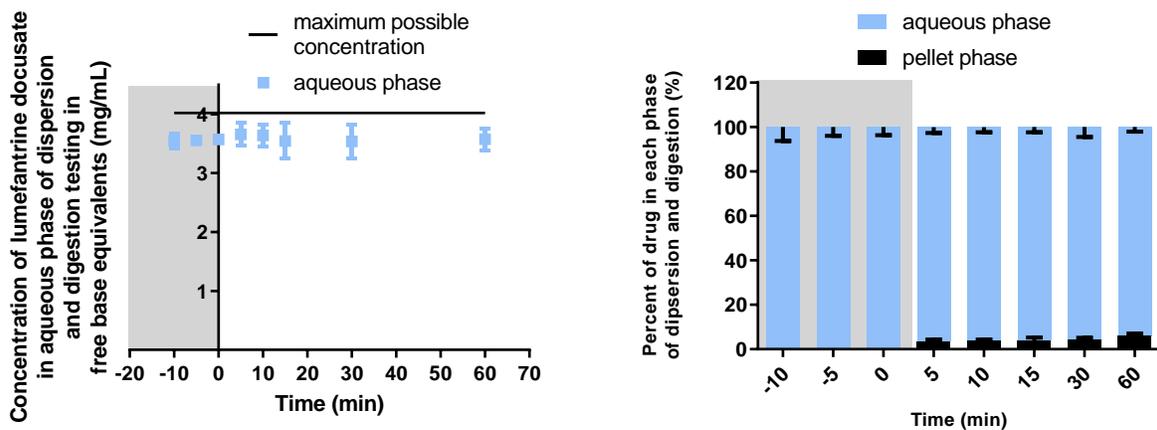
**Figure S14.** *In vitro* dispersion and digestion of lumefantrine docusate in Type IIIA-MCF. The concentration of lumefantrine docusate (in free base equivalents) in the aqueous phase of the medium-chain digest as a function of time (left), and the proportion of lumefantrine docusate in the pellet and aqueous phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



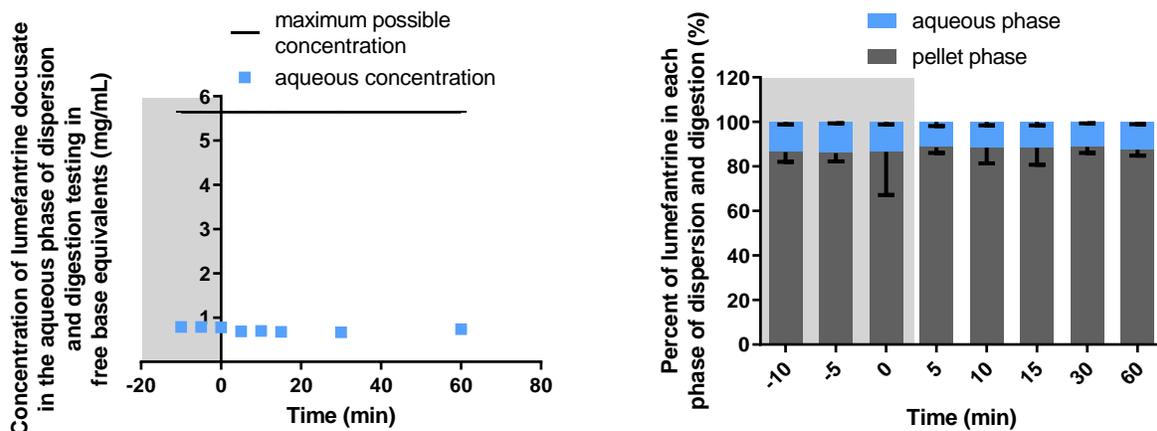
**Figure S15.** *In vitro* dispersion and digestion of lumefantrine docusate in Type IIIA-LCF. The concentration of lumefantrine docusate (in free base equivalents) in the aqueous phase of the long-chain digest as a function of time (left), and the proportion of lumefantrine docusate in the pellet, aqueous, and oil phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



**Figure S16.** *In vitro* dispersion and digestion of lumefantrine docusate in Type IIIB-MCF. The concentration of lumefantrine docusate (in free base equivalents) in the aqueous phase of the medium-chain digest as a function of time (left), and the proportion of lumefantrine docusate in the pellet and aqueous phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.

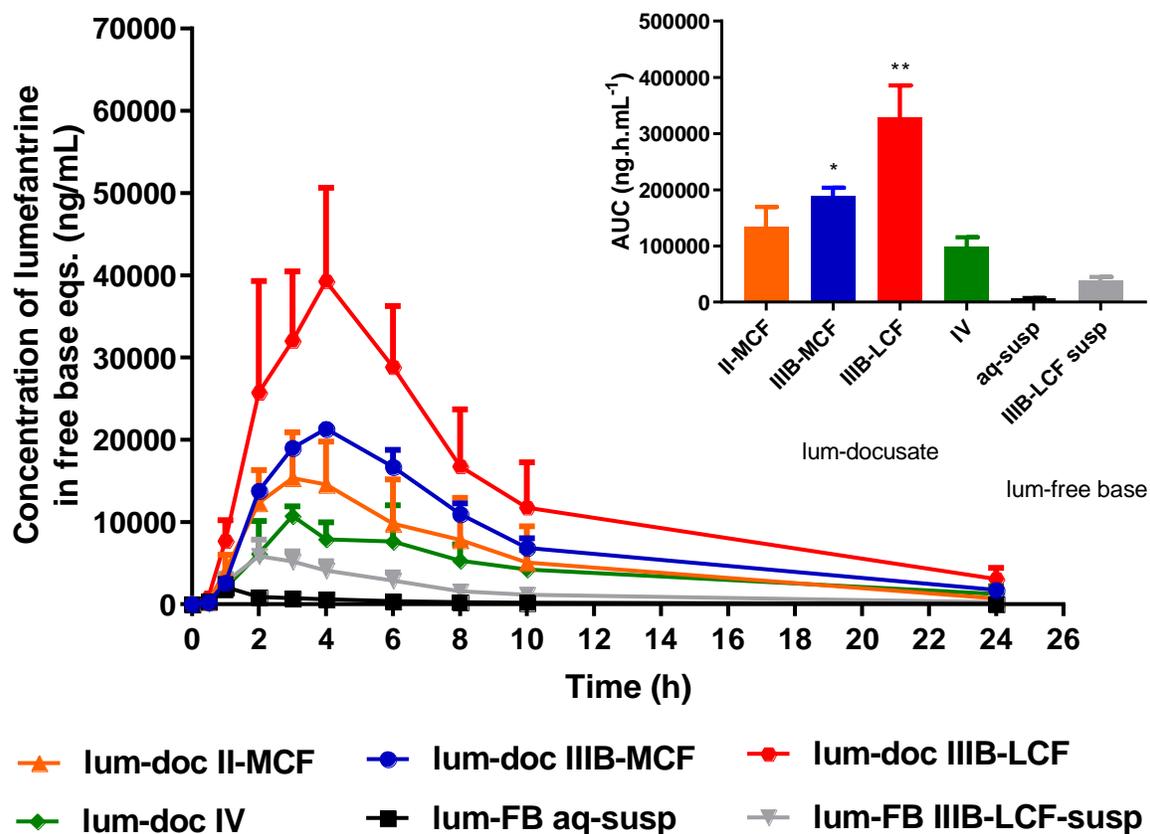


**Figure S17.** *In vitro* dispersion and digestion of lumefantrine docusate in Type IIIB-LCF. The concentration of lumefantrine docusate (in free base equivalents) in the aqueous phase of the long-chain digest as a function of time (left), and the proportion of lumefantrine docusate in the pellet and aqueous phases as a function of time (right). Data are  $n = 3$ , mean  $\pm$  SD.



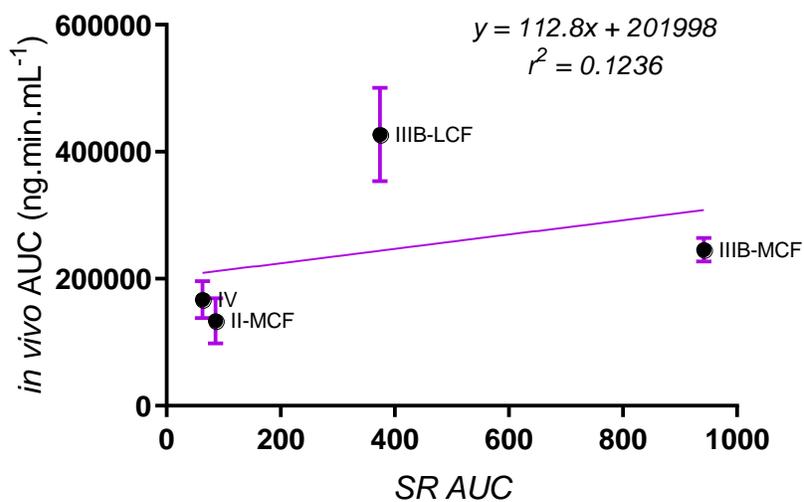
**Figure S18.** *In vitro* dispersion and digestion of lumefantrine docusate in Type IV. The concentration of lumefantrine docusate (in free base equivalents) in the aqueous phase of the digest as a function of time (left), and the proportion of lumefantrine docusate in the pellet and aqueous phases as a function of time (right). Data are n = 3, mean ± SD.

## Dose Normalised (all doses 50 mg/kg) Pharmacokinetic Data



**Figure S19.** Dose normalized lumefantrine plasma concentration versus time data after oral administration of lumefantrine docusate in Type II-MCF, IIIB-MCF, IIIB-LCF, and IV LBF, as well as lumefantrine free base as an aqueous suspension, and a lipid suspension (in the Type IIIB-LCF). All data have been dose normalized to 50 mg/kg (reflecting the lowest dose administered (Type II-MCF)). Data represented as mean (n = 4) ± SEM. Insert: Total lumefantrine exposure over 24 h. Data represented as mean (n = 4) ± SEM. \*Exposure was statistically higher (p < 0.05) than both suspension formulations. \*\*Exposure was statistically higher than all other formulations.

### *In vivo* AUC vs. Supersaturation Ratio Correlation Plot



**Figure S20.** Apparent supersaturation ratio/*in vivo* correlation plot, displaying the AUC of the apparent supersaturation ratio across the *in vitro* experiment, and the AUC for *in vivo* exposure. Data are expressed as mean  $\pm$  SEM for *in vivo* AUC (n = 4).