Supporting Information

Rapid Recovery of Clofazimine-loaded Nanoparticles with Extended Shelf Life as Anti-Cryptosporidium Therapy

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Information of AFFINSOL™ HPMCAS

Table S1. Physical properties of Dow AFFINISOL™ HPMCAS from https://www.dow.com/en-us/pharma/products/affinisol

	AFFINISOL™ HPMCAS		
	716	912	126
Hydroxypropyl	5.0-9.0%	5.0-9.0%	6.0-10.0%
Methoxyl	20.0-24.0%	21.0-25.0%	22.0-26.0%
Viscosity*	2.4-3.6 cP	2.4-3.6 cP	2.4-3.6 cP
Free Acids	<1.0%	<1.0%	<1.0%
Acetate Substitution	5.0-9.0%	7.0-11.0%	10.0-14.0%
Succinate Substitution	14.0-18.0%	10.0-14.0%	4.0-8.0%
Acetic Acid	0.5%	0.5%	0.5%

Calculation of effective Reynolds number for MIVM

The definition of effective Reynolds number is

$$Re = \sum_{i=1}^{4} \frac{U_i D_i}{\nu_i} \quad , \qquad (1)$$

where ρ_i is the density, U_i is the velocity, ν_i is the kinematic viscosity of stream i, and D_i is the characteristic dimension [1]. The current MIVM set-up has a cross-section area of each inlet as 1.1×1.5 mm,^{2} and the diameter of the mixing geometry as D_i=6 mm. Regarding the HPMCAS and lecithin formulations, the total flow rate is 160 mL/and 120 mL/min, respectively, with volumetric flow rate of organic:water=1:3. Tables S2 and S3 list the flow velocities and

kinematic viscosities of each stream for HPMCAS and lecithin NPs. Therefore, the effective Reynolds numbers are Re=8.8×10^3 and 1.2×10^4 for HPMCAS and lecithin, respectively.

Table S2. Velocity and kinematic viscosity of each stream for the formulation of HPMCAS NPs

	Organic stream 1	Aqueous stream 2	Aqueous stream 3	Aqueous stream 4
U_i (m/s)	0.16	0.48	0.48	0.48
ν_i (m^2/s)	0.38×10^{-6}	0.89×10^{-6}	0.89×10^{-6}	0.89×10^{-6}

Table S3. Velocity and kinematic viscosity of each stream for the formulation of lecithin NPs

	Organic stream 1	Aqueous stream 2	Aqueous stream 3	Aqueous stream 4
U_i (m/s)	0.12	0.36	0.36	0.36
ν_i (m^2/s)	0.54×10^{-6}	0.89×10^{-6}	0.89×10^{-6}	0.89×10^{-6}

Calculation for *Pe* at the spray-drying process for HPMCAS and lecithin nanoparticles

In the spray-drying process, the Péclet number is defined as [2]

$$Pe = \frac{d^2}{4D\tau_{\mathrm{dry}}} \quad , \qquad (2)$$

where d is the radius of the droplet in the spray dryer, D is the diffusion coefficient of the solute or NPs, and τ_{dry} is the time required for a droplet to dry. We estimate τ_{dry} with the aspirator setting for a gas flow rate of 35 m^3/h. Since the drying chamber has a cross-section diameter of 0.16 m and a length of 0.5 m, the flow velocity is 0.5 m/s and hence $\tau_{\mathrm{dry}}\approx O(1)$ s in the SD conditions for both formulations. Additionally, D is calculated using Stokes-Einstein equation as

$$D = \frac{k_B T}{6\pi\mu R_{\mathrm{H}}} \quad , \qquad (3)$$

where k_B is the Boltzmann's constant, T is the absolute temperature (≈360 K), μ is the viscosity of the solvent (≈8.9×10^{-4} Pa·s,)and R_H is the hydrodynamic radius of the solute (≈10 nm for HPMC E3 [3], ≈1 nm for mannitol) or NPs (≈150 and 432 nm for HPMCAS and lecithin NPs, respectively).

Assuming that one final dry particle results from the drying of one droplet and there is no coalescence of the droplets during spray drying, we can estimate d by mass conservation as follows:

$$\rho_l d^3 = \rho_s d_{90}^3 \quad , \qquad (4)$$

where ρ_l is the density of the NP dispersion (≈1×10^3 kg/m^3), ρ_s is the density of the dry powder (estimated by the density of clofazimine, ≈1.3×10^3 kg/m^3) and $d_{90\%}$ is the diameter at which 90% of the total particle volume is comprised of particles of diameter $d_{90\%}$ (≈9.1 and 8.4 μm for spray-dried HPMCAS and lecithin NPs, respectively). Therefore, using equations (2-4), $Pe\approx O(1\text{-}10)$ for HPMC E3 and mannitol, while $Pe\approx O(10^{2\text{-}3})$ for HPMCAS and lecithin NPs.

Clofazimine calibration curves in THF, FaSSGF, FaSSIF and FeSSIF

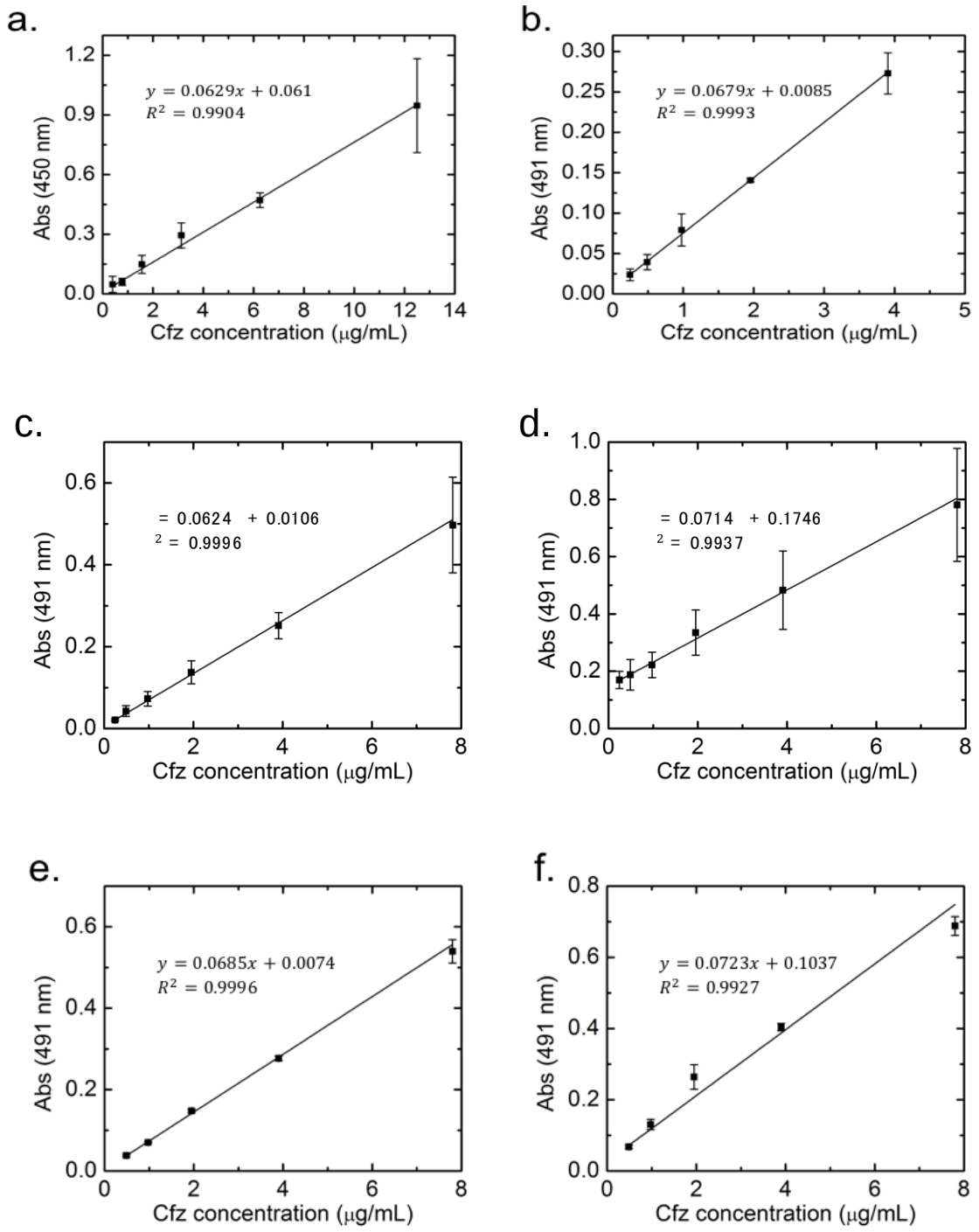

Figure S1. Clofazimine calibration curves in (a) THF, (b) FaSSGF, (c) FaSSIF, (d) FeSSIF, (e) FaSSGF:FaSSIF=1:9, and (f) FaSSGF:FeSSIF=1:9

Residual moisture for spray-dried HPMCAS and lecithin NPs in the long-term storage

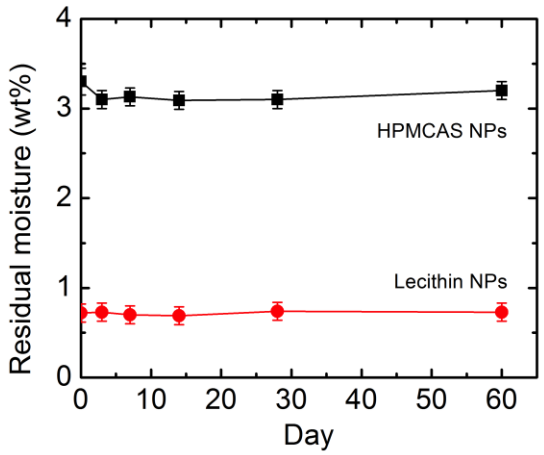

Figure S2. Residual moisture for spray-dried HPMCAS and Lecithin NPs

stability study

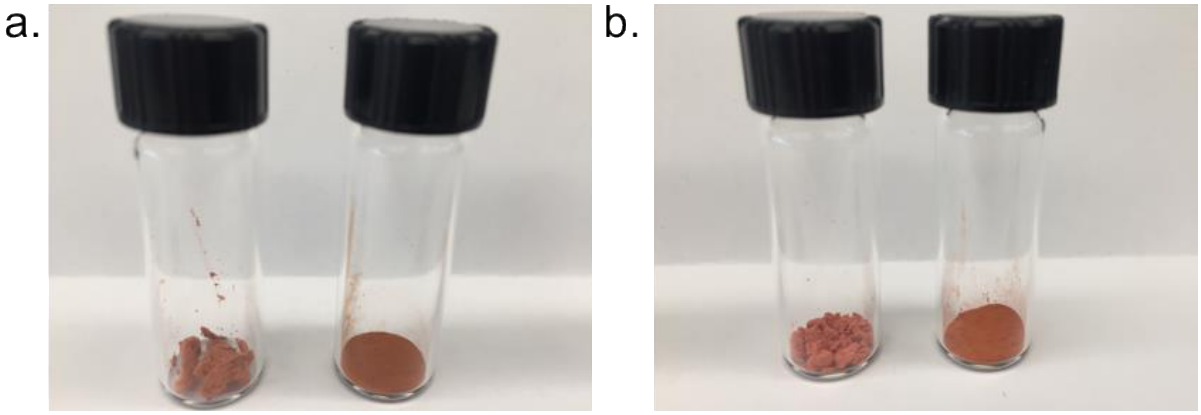

Figure S3. Appearance of lyophilized and spray-dried (a) HPMCAS and (b) lecithin nanoparticles

Appearance of lyophilized and spray-dried nanoparticles

Compressed mini-tablet test

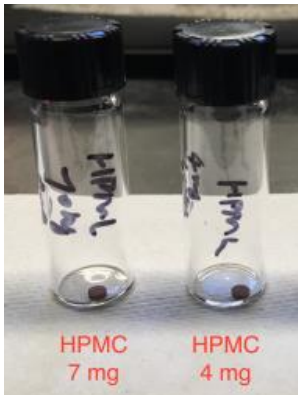

Figure S4. Appearance of the mini-tablet for spray-dried HPMCAS NPs

References:

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3. Huang, W.J., T. Mandal, and R.G. Larson, Computational Modeling of Hydroxypropyl-Methylcellulose Acetate Succinate (Hpmcas) and Phenytoin Interactions: A Systematic Coarse-Graining Approach. Mol Pharmaceut, 2017. **14**: 733-745.