Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Fluorescence Imaging of Antibiotic Clofazimine Encapsulated Within Mesoporous Silica Particle Carriers: Relevance to Drug Delivery and the Effect on its Release Kinetics

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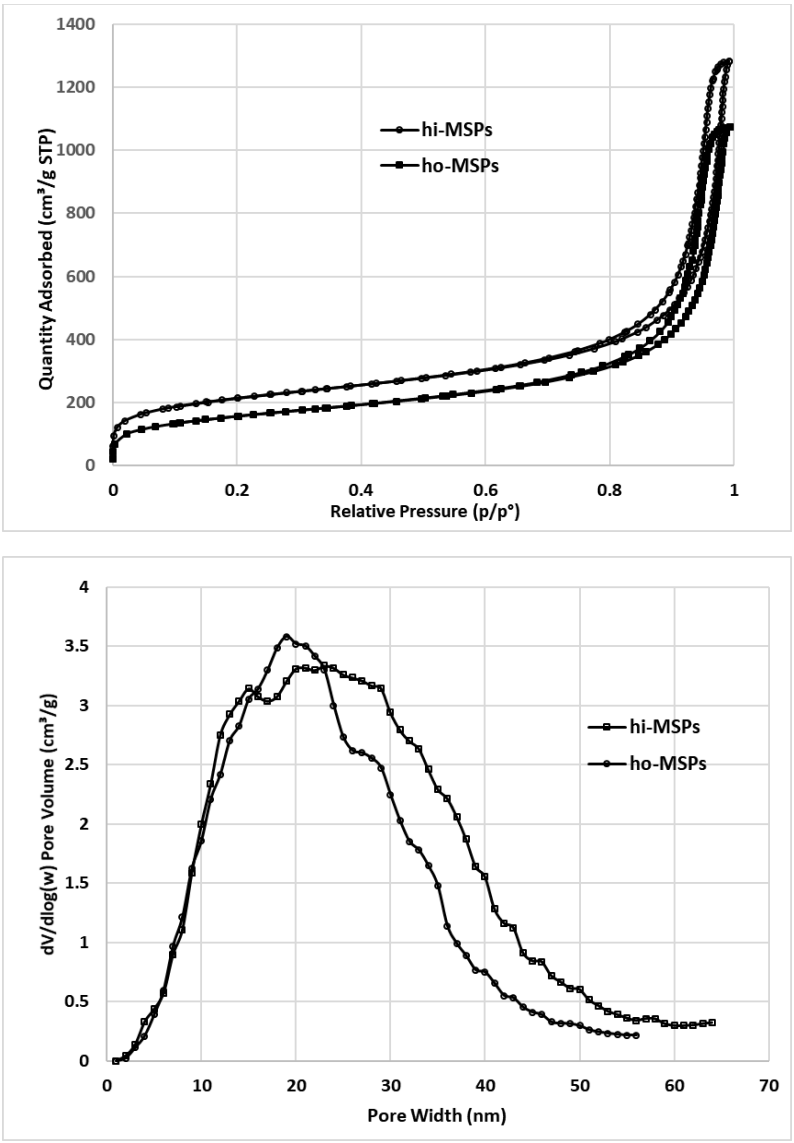

Figure S1. Nitrogen adsorption/desorption isotherm (top), and pore size distribution (bottom) obtained from BJH model using desorption branch of isotherm for hi-MSPs and ho-MSPs.

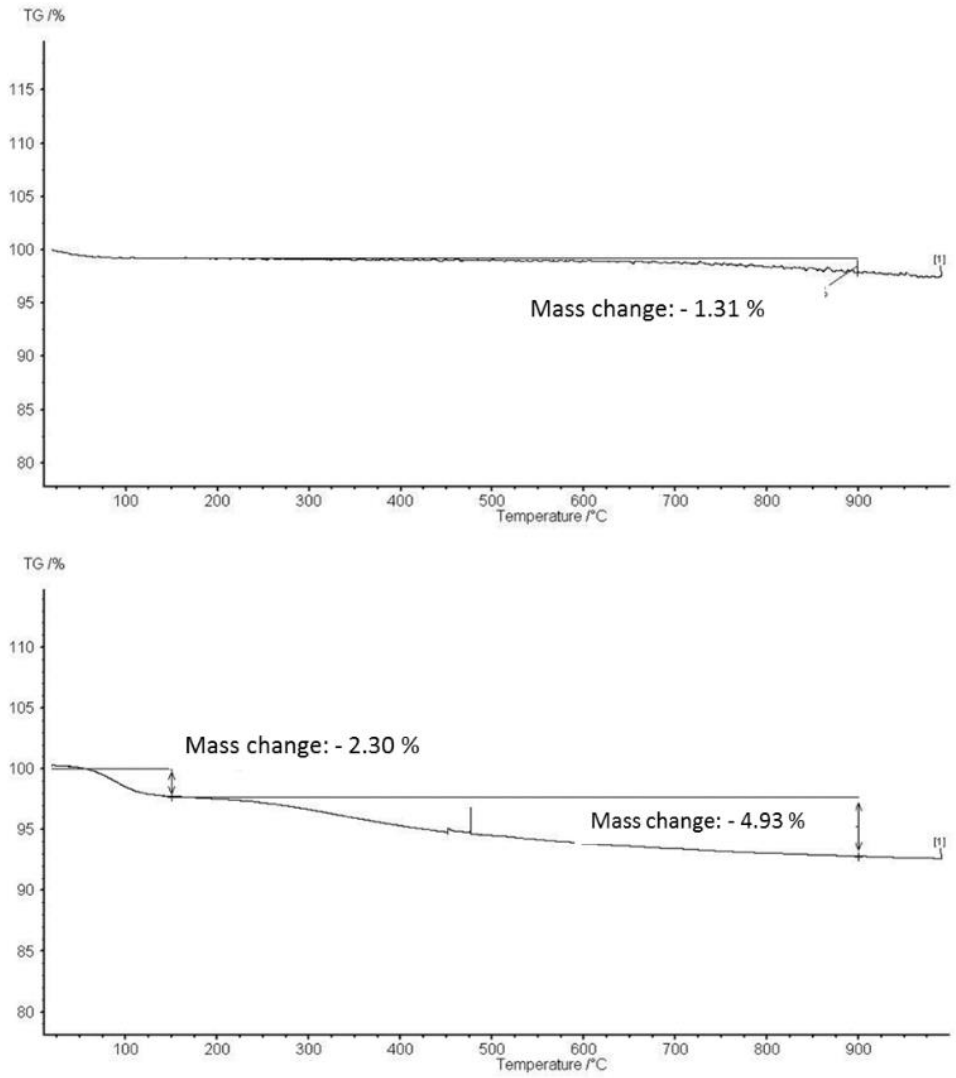

Figure S2. Thermogravimetric analysis (TGA) profile for ho-MSP (top) and hi-MSP (bottom). Decomposition temperatures of silanol groups is within the range of 150-900°C. Mass changes from 20°C to 150 °C are related to presence of water and solvent.

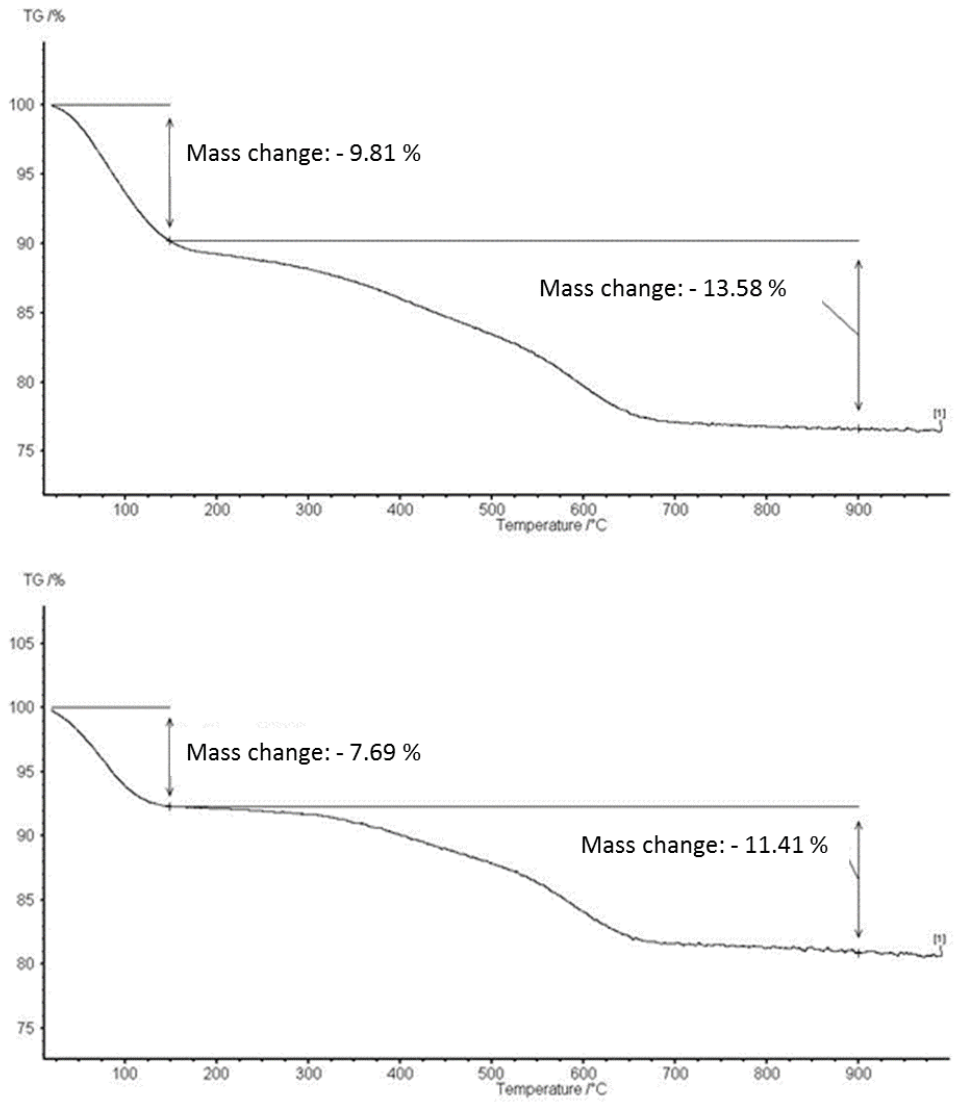

Figure S3. Thermogravimetric analysis (TGA) profile for hi-MSP-CLZ$_h$ (top) and ho-MSP-CLZ$_h$ (bottom). Decomposition temperatures of CLZ is within the range of 150-900°C. Mass changes from 20°C to 150 °C are related to presence of water and solvent.

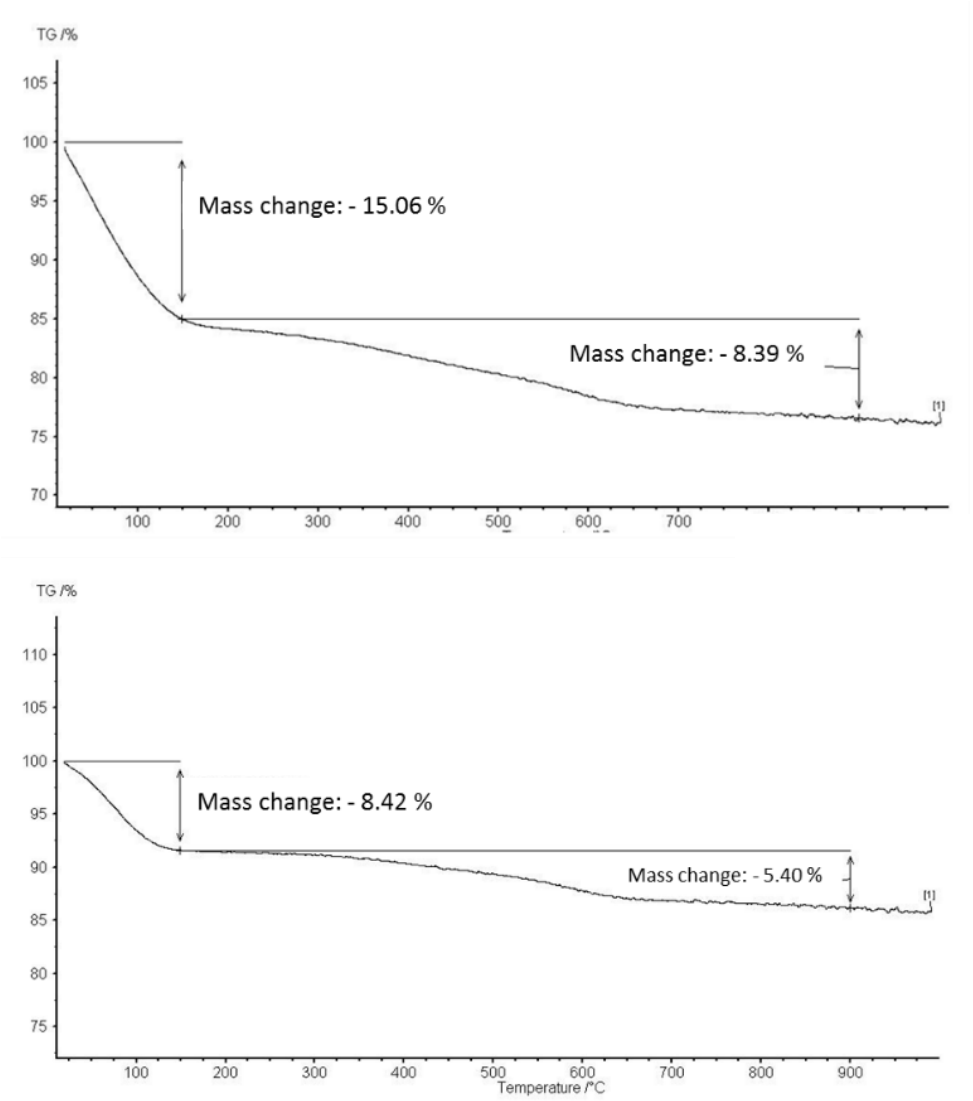

Figure S4. Thermogravimetric analysis (TGA) profile for hi-MSP-CLZ$_i$ (top) and ho-MSP-CLZ$_i$ (bottom). Decomposition temperatures of CLZ is within the range of 150-900°C. Mass changes from 20°C to 150 °C are related to presence of water and solvent.

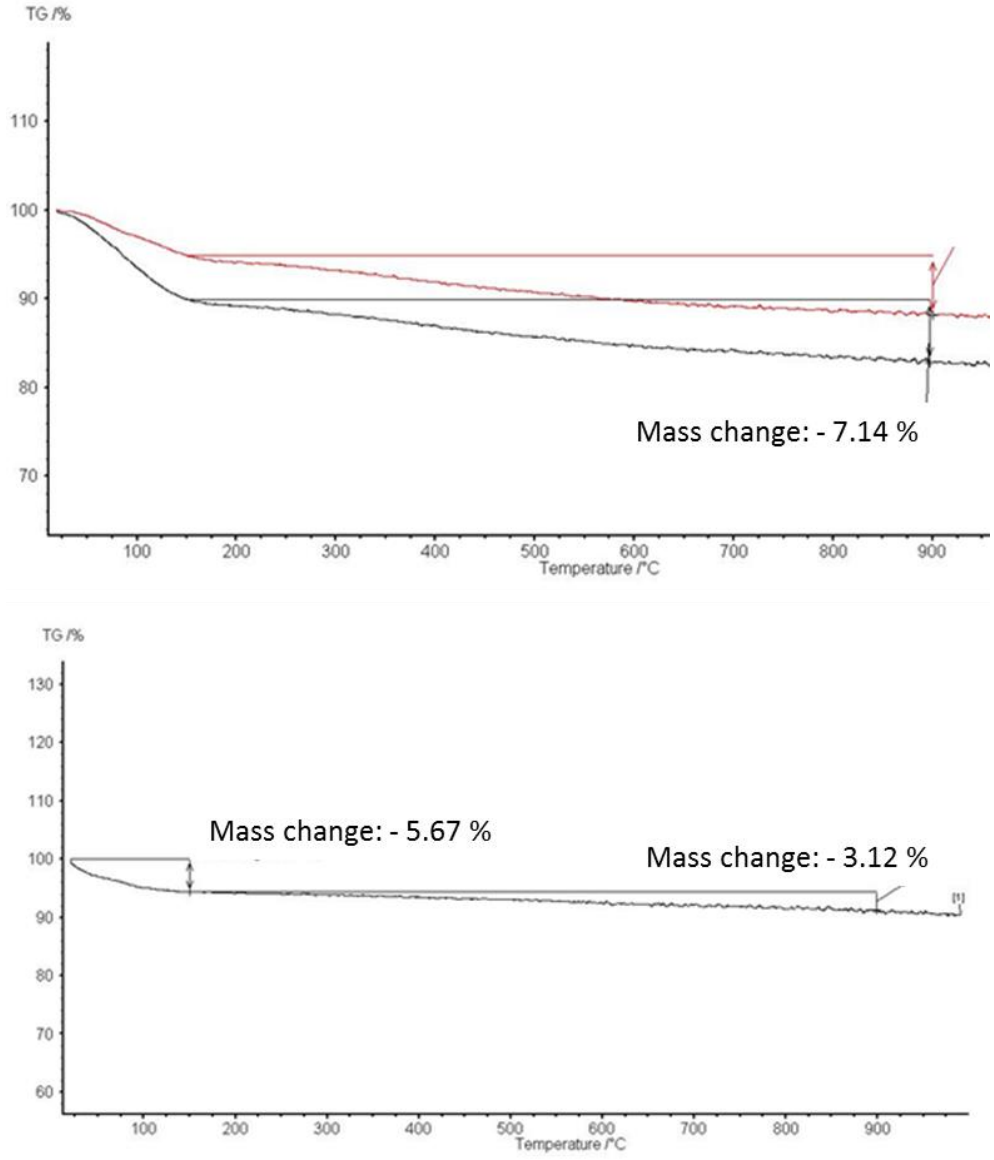

Figure S5. Thermogravimetric analysis (TGA) profile for hi-MSP-CLZ$_l$ (top – black line) and ho-MSP-CLZ$_l$ (bottom). Decomposition temperatures of CLZ is within the range of 150-900°C. Mass changes from 20°C to 150 °C are related to presence of water and solvent,

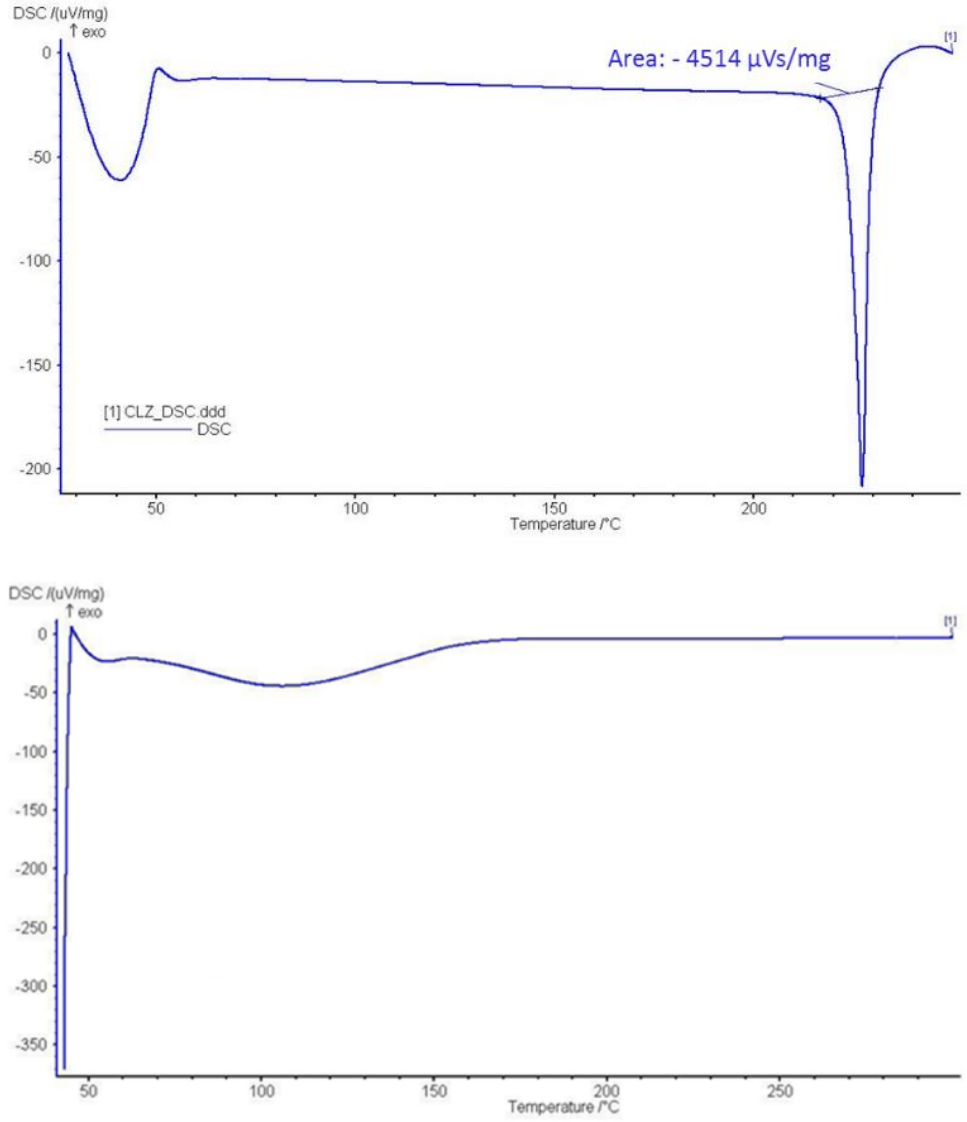

Figure S6. Typical differential scanning calorimetry (DSC) profile for (top) CLZ as free drug showing its crystallinity (melting point peak around 230°C) and (bottom) all MSP-CLZ particles synthesized in this study showing the amorphous state of the encapsulated CLZ (suppression of the melting point).

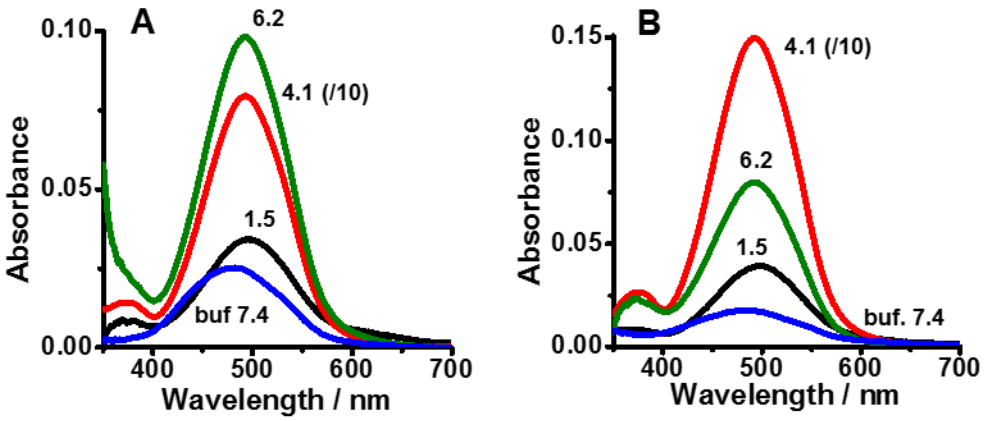

Figure S7. Steady-state absorption spectra of CLZ released after 30 minutes of stirring, separated by centrifugation from 5 mg of **(A)** ho-MSP-CLZ_h and **(B)** hi-MSP-CLZ_h in 3 mL of water at indicated pH and in buffer at pH 7.4, where the intensity at pH 4.1 has been divided by 10.

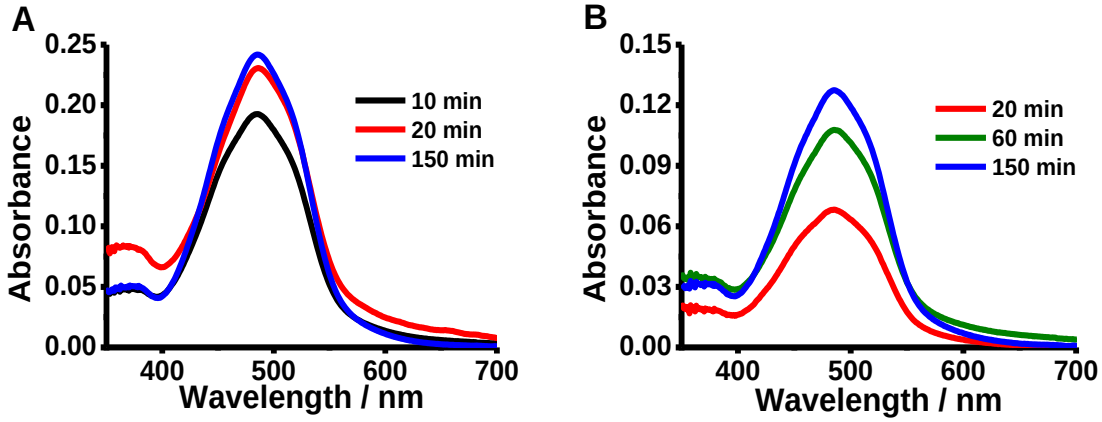

Figure S8. Steady-state absorption spectra of CLZ **(A)** released at different time from 10 mg of hi-CLZ-MSPs (CLZ 9.9% w/w) and **(B)** from the free drug (1 mg), in 50 ml of water at pH 4.1

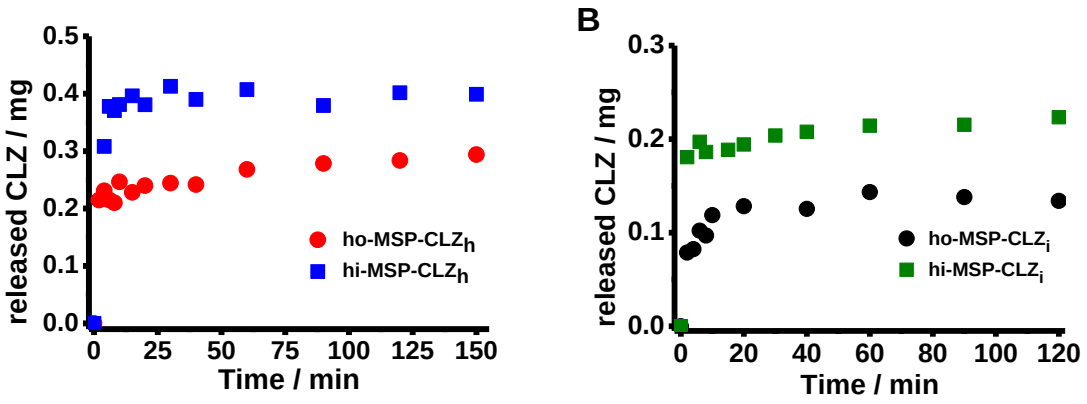

Figure S9. Amount of CLZ (mg) released from (**A**) ho- and hi-MSPs-CLZ_h, and (**B**) ho- and hi-MSPs-CLZ_i, calculated from the absorbance intensity collected at indicated times at 493 nm.

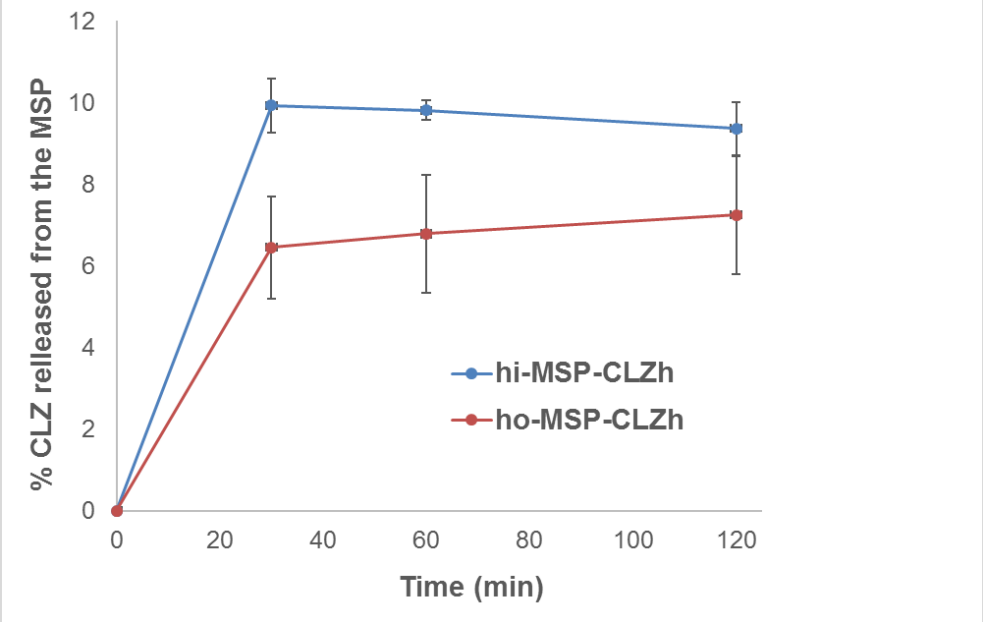

Figure S10. Drug release profile of CLZ from hi-MSP-CLZ_h and ho-MSP-CLZ_h in SIF (phosphate buffer pH 6.8 with 0.1% w/w SDS).

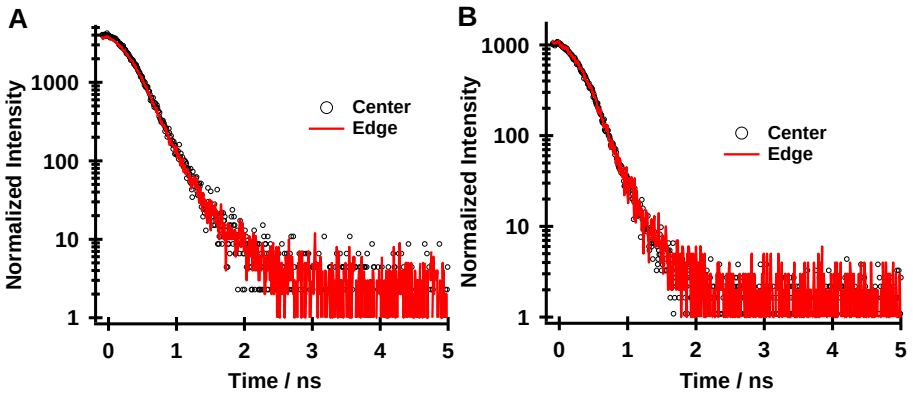

Figure S11. Emission decays collected at the center (open circles) and the edge (solid lines) of the equator cross-sections for **A)** ho-MSP-CLZ$_h$ and **B)** hi-MSP-CLZ$_h$ particles prior to washing with acetone, in air.

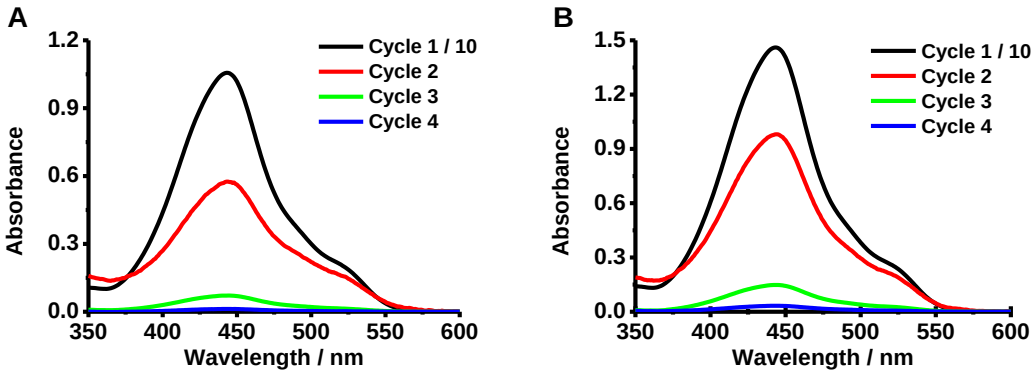

Figure S12. UV-visible absorption spectra of the supernatant following the indicated washing cycles with acetone for (**A**) ho-MSP-CLZ$_h$ and (**B**) hi-MSP-CLZ$_h$ silica microparticles, collected using a 1 mm cell. Note that the spectrum after the first washing cycle is divided by 100.

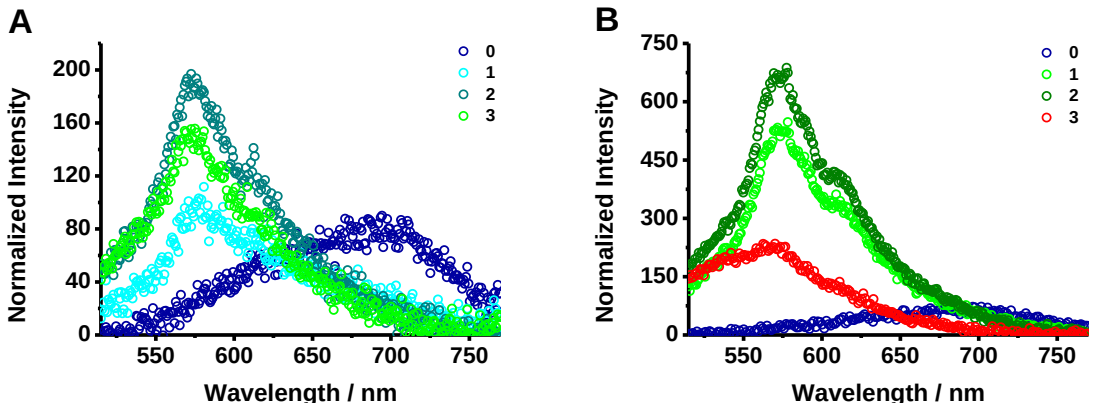

Figure S13. Raw single particle emission spectra (exciting at 470 nm) of (**A**) ho-MSP-CLZ$_h$ and (**D**) hi-MSP-CLZ$_h$ collected without washing (0), and after one (1), two (2) and three (3) washing cycles with acetone.

ho-MSP-CLZ$_h$						
Before rinsing (a)	**t_1 (ns)**	**a_1 (kcnts)**	**t_2 (ns)**	**a_2 (kcnts)**	**t_3 (ns)**	**a_3 (kcnts)**
LP Center	0.03	17.47 (%90)	0.2	1.86 (%10)	-	-
LP Edge	0.03	27.42 (89%)	0.2	3.38 (11%)	-	-
rinsing cycle 1 (b)	**t_1 (ns)**	**a_1 (kcnts)**	**t_2 (ns)**	**a_2 (kcnts)**	**t_3 (ns)**	**a_3 (kcnts)**
LP Center	0.01	92.77 (92%)	0.2	7.28 (7%)	0.8	0.7 (1%)
LP Edge	0.01	86.83 (91%)	0.2	8.02 (8%)	0.8	0.82 (1%)
rinsing cycle 2 (c)	**t_1 (ns)**	**a_1 (kcnts)**	**t_2 (ns)**	**a_2 (kcnts)**	**t_3 (ns)**	**a_3 (kcnts)**
LP Center	-	-	0.5	3.89 (83%)	1.4	0.78 (17%)
LP Edge	-	-	0.4	4.55 (84%)	1.4	0.84 (16%)
rinsing cycle 3 (d)	**t_1 (ns)**	**a_1 (kcnts)**	**t_2 (ns)**	**a_2 (kcnts)**	**t_3 (ns)**	**a_3 (kcnts)**
LP Center	0.01	156 (97%)	0.7	3.99 (2%)	2.0	1.1 (1%)
LP Edge	-	-	0.7	2.82 (80%)	2.0	0.72 (20%)

Table 1S. Averaged fluorescence lifetime decays over 10 particles for ho-MSP-CLZ$_h$ (hydrophobic) without washing (**a**), and after one (**b**), two (**c**) and three (**d**) washing cycles with acetone at the center and the edge of the equator (15 μm) cross-section, at low and high power conditions. The error in lifetimes values is about 10-15%.

hi-MSP-CLZ$_h$						
Before rinsing (a)	**t_1 (ns)**	**a_1 (kcnts)**	**t_2 (ns)**	**a_2 (kcnts)**	**t_3 (ns)**	**a_3 (kcnts)**
LP Center	0.06	7.77 (86%)	0.27	1.2 (14%)	-	-
LP Edge	0.06	4.45 (87%)	0.27	0.7 (13%)	-	-
rinsing cycle 1 (b)	**t_1 (ns)**	**a_1 (kcnts)**	**t_2 (ns)**	**a_2 (kcnts)**	**t_3 (ns)**	**a_3 (kcnts)**
LP Center	-	-	0.6	4.41 (81%)	1.7	1.01 (19%)
LP Edge	-	-	0.6	5.34 (81%)	1.7	1.23 (19%)
rinsing cycle 2 (c)	**t_1 (ns)**	**a_1 (kcnts)**	**t_2 (ns)**	**a_2 (kcnts)**	**t_3 (ns)**	**a_3 (kcnts)**
LP Center	-	-	1.0	6.66 (81%)	2.7	1.56 (19%)
LP Edge	-	-	0.9	7.28 (80%)	2.6	1.77 (20%)
rinsing cycle 3 (d)	**t_1 (ns)**	**a_1 (kcnts)**	**t_2 (ns)**	**a_2 (kcnts)**	**t_3 (ns)**	**a_3 (kcnts)**
LP Center	-	-	1.30	1.08 (76%)	4.2	0.35 (24%)
LP Edge	-	-	1.4	1.01 (76%)	4.2	0.32 (24%)

Table 2S. Averaged fluorescence lifetime decays over 10 particles for hi-MSP-CLZ$_h$ (hydrophilic) without washing (**a**) and after one (**b**), two (**c**) and three (**d**) washing cycles with acetone at the center and the edge of the equator (15 μm) cross-section, at low and high power conditions. The error in lifetimes values is about 10-15%.

Calculation of CLZ % released from the MSP:

Calibration curve of CLZ in Methyl Acetate for ε at 493nm.

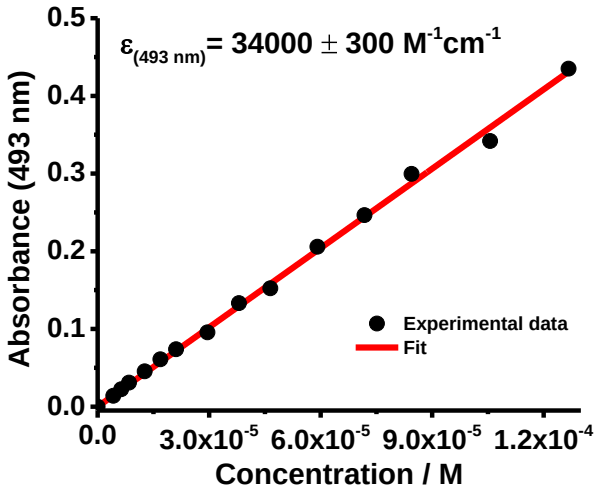

Figure S12. Calibration curve of Clofazimine in Methyl Acetate (MA) for ε at 493 nm.

We calculated the ε at 493 nm for CLZ in methyl acetate (MA) preparing solution with same volume but different concentration of CLZ and built the calibration curve. We repeated the experiment 3 times in order to obtain reproducibility of the measurements.

$$\varepsilon\ (493\text{nm}) = 34000\ M^{-1}cm^{-1}\ (\pm\ 300\ M^{-1}cm^{-1},\ R = 0.99765)$$

The procedure to measure the amount of CLZ released involved the preparation of a solution with 10 mg of ho-MSP-CLZ_h or hi-MSP-CLZ_h, added to 50 mL of water at pH 4.1. The solutions were put under magnetic stirring during the experiment. At increasing time delay 1 mL aliquots of solution were collected, put in a falcon and centrifuged for 10 minutes at 4200 RPM. After centrifugation the supernatant (0.8 mL) was separated from the particles present in the aliquot and moved to a vial. 2 mL of methyl acetate were added to vials, which were closed and agitated for 2 minutes in order to extract the released CLZ from the water to the methyl acetate. After complete separation of the two solvent phases the methyl acetate was took and put in a 1 cm quartz cell to measure the absorption spectra. From the obtained absorbance we built the absorbance/stirring time graphic, showing the release behavior. Using the absorbance values collected at each time delay we calculated the amount of CLZ

released. Here we report the calculation for the released at initial delay (2min) and at the end of the experiment (150 min), for both particles).

The hi-MSP-CLZ_h in 10 mg of particles present 0.865 mg of CLZ encapsulated, which correspond to 8.65% w/w loaded, in the 50 mL solution. After 2 minutes of stirring the observed absorbance in 2 mL of methyl acetate was A= 0.17709 that gives a concentration of C= $5.2*10^{-6}$ M.

$C = A / (\varepsilon d) = 0.17709 / (34000\ M^{-1}cm^{-1} \times 1\ cm) = 5.2*10^{-6}$ M.

From this, we were able to calculate the total amount of CLZ moles present in the methyl acetate (MA) solution.

In 2 mL of methyl acetate: $mole = C \times V = 5.2*10^{-6} \times 0.002 = 1.04*10^{-8}$ mole.

Assuming a 100% efficiency of extraction from water $1.04*10^{-8}$ mol of CLZ have been transferred from the initial water volume of 0.8 mL, the same amount of moles is found in MA and in the 0.8 mL aliquot of water.

Thus, in 1 mL the total amount of moles is $1.3*10^{-8}$ mole:

$$mole_2 = (mole_1/V_1) \times V_2 = (1.04*10^{-8}\ mole / 0.8\ mL) \times 1\ mL = 1.3*10^{-8}\ mole.$$

Having the molecular weight of CLZ (MW = 473.4 $g.mol^{-1}$) we calculated the amount expressed in weight of released CLZ in 1 mL, which corresponds to $6.15*10^{-6}$ g.

Assuming a homogenous dispersion of the particles in the 50 mL, the 0.865 mg of encapsulated CLZ in 50 mL can be approximated to $1.73*10^{-5}$ g of encapsulated CLZ in 1 mL. Then, it is possible to calculate the percentage of released CLZ in this 1 mL aliquot:

$$(6.15*10^{-6}\ g / 1.73*10^{-5}\ g) \times 100 = 36\%.$$

After 2 minutes of stirring 35.6% of CLZ is released from hi-MSP-CLZ_h.

Using this procedure we calculated the amount of released CLZ after each time delay, and repeated it for the ho-MSP-CLZ_h solutions. The steps of the calculation for 2 and 150 min are summed up as follows:

- **hi-MSP-CLZ_h** (10 ± 0.1 mg)

8.65% CLZ w/w correspond to 0.865 mg in 50 mL of water pH 4.1.

in 1 mL corresponds to: (0.865/50) = 0.0173 mg = $1.73*10^{-5}$ g.

At 2 minutes of stirring:

$A_{(493\ nm)}$ = 0.17709, ε (493nm) = 34000 $M^{-1}cm^{-1}$.

Concentration of CLZ molecules is C= $5.2*10^{-6}$ M (in 2 mL of MA).

Moles of CLZ in 2mL MA: ($5.2*10^{-6}$ M × 0.002 L) = $1.04*10^{-8}$ mole.

2mL of MA have same moles as 800 µl H_2O.

Moles in 1 mL of H_20: ($1.04*10^{-8}$ mol / 0.8 mL) × 1 mL = $1.3*10^{-8}$ mol

The CLZ released mass is: m= ($1.3*10^{-8}$ mole × 473.4 $g.mol^{-1}$) = $6.15*10^{-6}$ g.

% released: ($6.15*10^{-6}$ g / $1.73*10^{-5}$ g) × 100 = 36%.

At 150 minutes of stirring:

$A_{(493\ nm)}$ = 0.22918, and ε (493nm) = 34000 $M^{-1}cm^{-1}$.

Concentration of CLZ is C= $6.7*10^{-6}$ M (in 2 mL of MA).

Moles of CLZ in 2mL of MA: ($6.7*10^{-6}$ M × 0.002 L) = $1.34*10^{-8}$ mole.

2mL of MA have same moles as 800 µl H_2O.

Moles in 1 mL of H_20: ($1.34*10^{-8}$ mole / 0.8 mL) × 1 mL = $1.685*10^{-8}$ mole.

The amount of CLZ released is:

m= mol × MW = ($1.685*10^{-8}$ mole × 473.4 $g.mol^{-1}$) = $7.98*10^{-6}$ g.

% released is calculated as: ($7.93*10^{-6}$ g / $1.73*10^{-5}$ g) × 100 = 46%.

- **ho-MSP-CLZ$_h$** (10 ± 0.1 mg),

10.1% CLZ w/w correspond to 1.010 mg in 50 mL of water pH 4.1.

in 1 mL corresponds to: (1.010/50) = 0.0202 mg = $2.02*10^{-5}$ g.

At 2 minutes of stirring:

$A_{(493\ nm)}$ = 0.12338, and ε (493nm) = 34000 $M^{-1}cm^{-1}$.

Concentration of CLZ is C= $3.6*10^{-6}$ M (in 2 mL of MA).

Moles of CLZ in 2mL of MA: ($3.6*10^{-6}$ M × 0.002 L) = $7.2*10^{-9}$ mole.

2mL of MA have same moles as 800 µl H_2O.

CLZ moles in 1 mL of H_20: ($7.2*10^{-9}$ mole / 0.8 mL) × 1 mL = $9*10^{-9}$ mole.

Amount of CLZ released is: m= mol × MW = ($9*10^{-9}$ mole × 473.4 $gmol^{-1}$) = $4.26*10^{-6}$ g.

% released is: ($4.26*10^{-6}$ g / $2.02*10^{-5}$ g) × 100 = 21%.

At 150 minutes of stirring:

$A_{(493\ nm)}$ = 0.16888, and ε (493nm) = 34000 $M^{-1}cm^{-1}$.

Concentration of CLZ molecules is C= $4.97*10^{-6}$ M (in 2 mL of MA).

Moles of CLZ in 2mL MA: ($4.97*10^{-6}$ M × 0.002 L) = $9.93*10^{-9}$ mole.

2mL of MA have same moles as 800 µl H_2O.

Moles in 1 mL of H_20: ($9.93*10^{-9}$ mole / 0.8 mL) × 1 mL = $1.24*10^{-8}$ mole.

Amount of CLZ released is: m ($1.24*10^{-8}$ mole × 473.4 $g.mol^{-1}$) = $5.88*10^{-6}$ g.

% released: ($5.88*10^{-6}$ g / $2.02*10^{-5}$ g) × 100 = 29%.