

Supplementary Materials: Compartmentalized Polymeric Nanoparticles Deliver Vancomycin in a pH-Responsive Manner

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HPLC method

The mobile A phase was water and the mobile B phase was acetonitrile, both containing 0.1% TFA. Chromatographic separation of VCM was performed at room temperature and elution was performed at a rate of 1 mL/min in gradient elution mode (Table S1). The injection volume was 10 μ L.

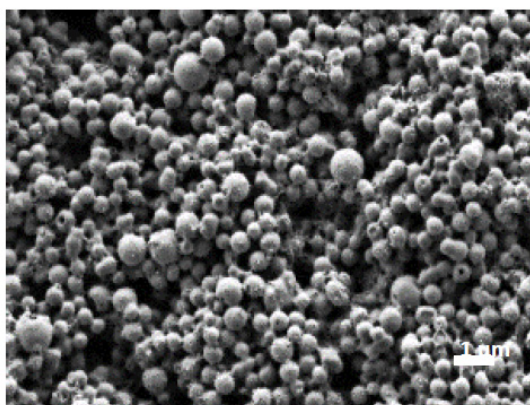
Table S1. HPLC gradient elution program.

| Time (min) | A (%) | B (%) |
|------------|-------|-------|
| 0 | 90 | 10 |
| 6 | 55 | 45 |
| 10 | 90 | 10 |

PVA quantification

1.3 g of NP suspension containing around 20 mg of PLGA₁₅C or PLG₈C NPs was centrifuged at 17,000 \times g for 15 min and the supernatant was discarded. The NP pellet was hydrolyzed overnight in 5 mL of 1N KOH and then neutralized with 1N HCl. To 0.1 mL of neutralized sample, 0.375 mL of 3.7% *w/v* boric acid solution, 0.01 mL of iodine solution (1.66% potassium iodide and 1.27% iodine in distilled water) *w/v* and 0.7 mL of 1N HCl were added. The absorbance of this solution was further measured in UV spectrometer at 620 nm. All samples were analyzed in triplicate.

A)



B)

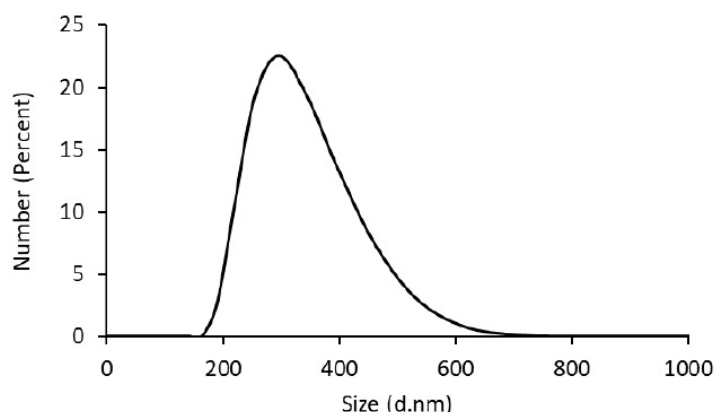


Figure S1. (A) Typical SEM image and (B) size distribution of PLGA₁₅C NPs loaded with 14 wt% VCM.

Effect of preparation parameters on DL, EE and mean diameters

The influence of drug to polymer ratio on the DL and EE was investigated (Table S2). DL and EE increased with the drug/polymer ratio until reaching a plateau at the ratio of 0.4/1 (DL=14±4 wt%). In contrast, the drug/polymer ratio had no effect on the size of the NPs.

Table S2. Effect of drug/polymer mass ratio on DL, EE and mean diameter on VCM loaded PLGA₁₅C NPs.

| Formulations (drug/polymer ratio) | DL (wt% ± SD) | EE (wt% ± SD) | Mean diameter*† (nm) |
|--------------------------------------|------------------|------------------|-------------------------|
| 0.2/1 | 9 ± 2 | 50 ± 11 | 324 |
| 0.3/1 | 10 ± 1 | 37 ± 4 | 330 |
| 0.4/1 | 14 ± 4 | 36 ± 2 | 320 |
| 0.5/1 | 14 ± 2 | 33 ± 3 | 327 |

* Standard deviations (SD) are 25 nm for all the formulations. † Measured by DLS.

In complementary studies, it was found that the addition of NaCl during NP preparation had a high impact on VCM incorporation (Table S3). For example, DL increased from 9 ± 1 wt% to 14 ± 4 wt% in the presence of 1% NaCl and EE increased from 23 ± 1 % to 36 ± 2%, without having a significant effect on the NP size. Indeed, salts were reported to facilitate polymer precipitation and enhance DL. However, higher salt contents did not improve DL nor EE but led to much larger NPs (up to 460 nm).

Additionally, neither the use of more concentrated PVA solutions nor other emulsifiers lead to an improvement in DL. As presented in Table S3, doubling the concentration of PVA slightly affects the DL (from 12 ± 2 wt% to 14 ± 4 wt%). Noteworthy, the use of anionic surfactants such as sodium cholate instead of PVA failed to stabilize the emulsions, leading to phase separation.

Table S3. Effect of the concentrations of NaCl and PVA on the incorporation of VCM and mean diameters. NPs were prepared using PLGA₁₅C (co)polymer.

| Formulations PVA/NaCl of outer phase | DL (wt% ± SD) | EE (wt% ± SD) | Mean diameter*† (nm) |
|--|------------------|------------------|-------------------------|
| 0.25/0 | 9 ± 1 | 23 ± 2 | 318 |
| 0.25/1 | 12 ± 2 | 26 ± 2 | 320 |
| 0.25/5 | 11 ± 2 | 26 ± 2 | 496 |
| 0.5/0 | 9 ± 1 | 23 ± 1 | 320 |
| 0.5/1 | 14 ± 4 | 36 ± 2 | 320 |
| 0.5/5 | 14 ± 2 | 36 ± 2 | 460 |

* Standard deviations (SD) are 25 nm for all the formulations. † Measured by DLS.

NP Characterization by Solid-State NMR

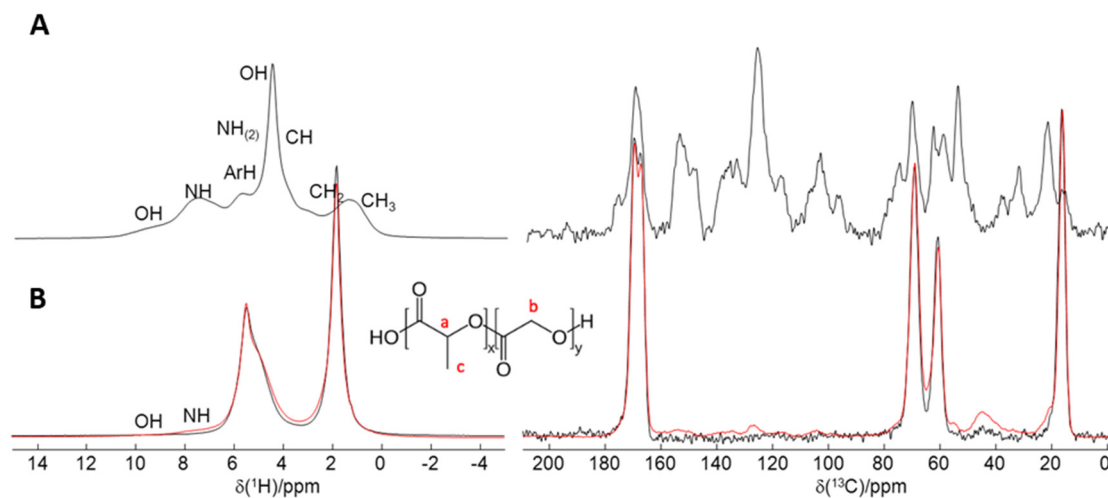


Figure S2. ^1H (left) and ^{13}C (right) MAS NMR spectra of (A) VCM and (B) empty PLGA₁₅C NPs (black line) and VCM loaded PLGA₁₅C NPs (red line).

STEM-EDX

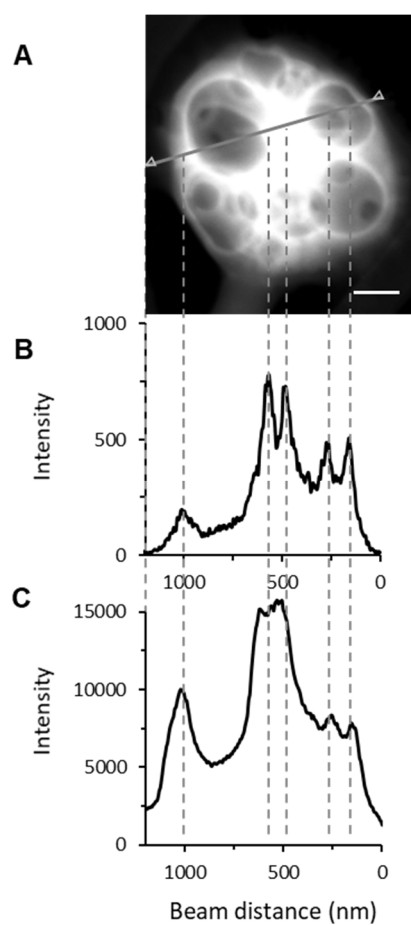


Figure S3. (A) STEM image of a VCM-loaded PLGA₁₅C NP. EDX line scanning profiles of (B) Nitrogen and (C) Carbon. Nitrogen intensity at its peak values can be followed by the dashed lines. Scale bar is 250 nm.

Degradation studies

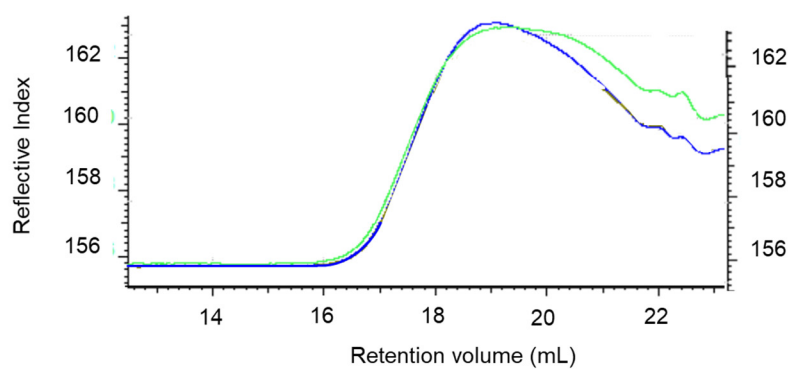


Figure S4. SEC analysis of loaded PLGA₁₅C NPs incubated in phosphate buffer at pH 5.3 during 5 min (light green) and 4 days (blue). No significant polymer degradation was observed.

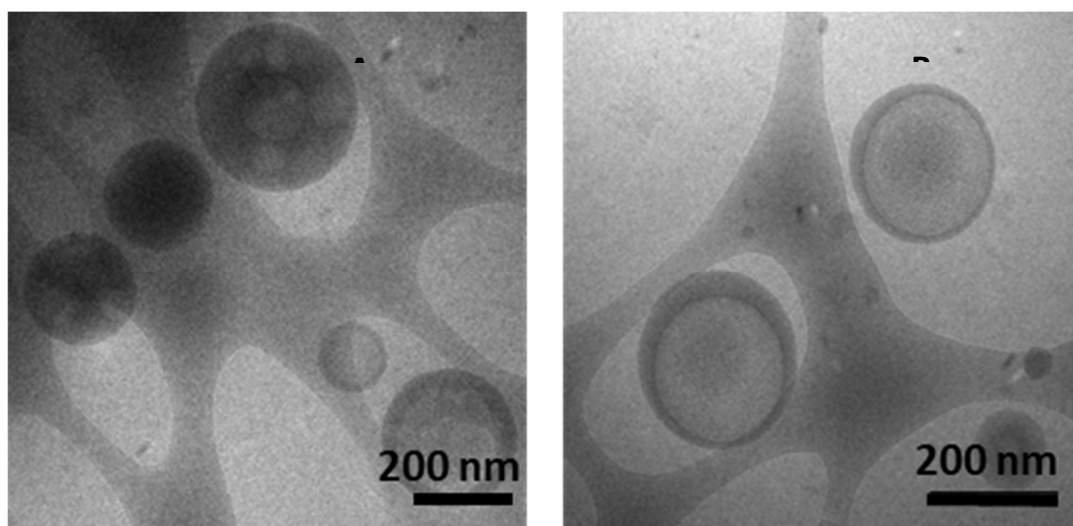


Figure S5. Typical Cryo-TEM images of VCM-loaded PLGA₁₅C NPs after 48h incubation at 37°C: (A) in phosphate buffer at pH 5 and (B) in water.