# Supplementary Materials: Chitosan Loaded into a Hydrogel Delivery System as a Strategy to Treat Vaginal Co-Infection

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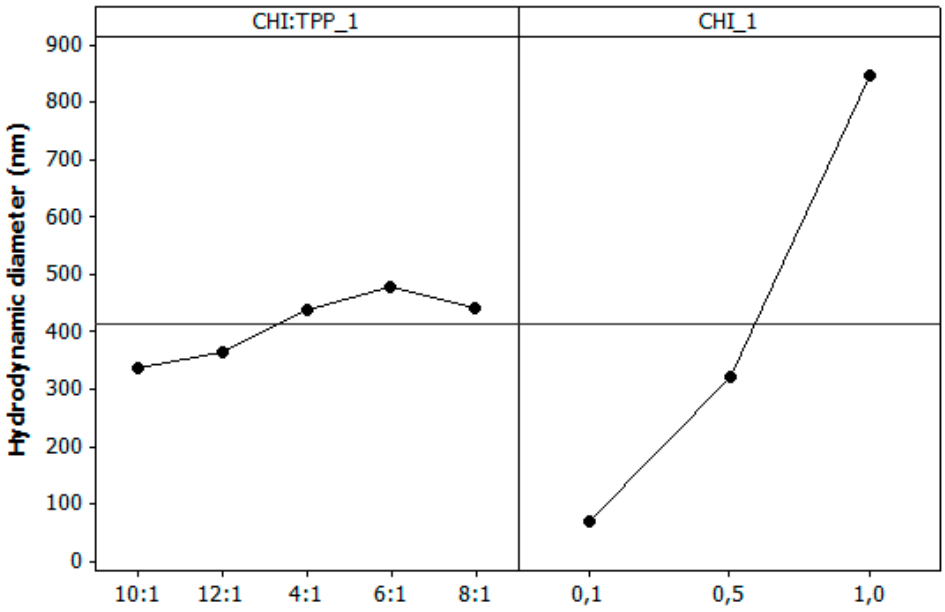


**Figure S1.** "Main effect" plot relative to the factors (CS % and CS/TPP ratio) influencing the size of nanoparticles.

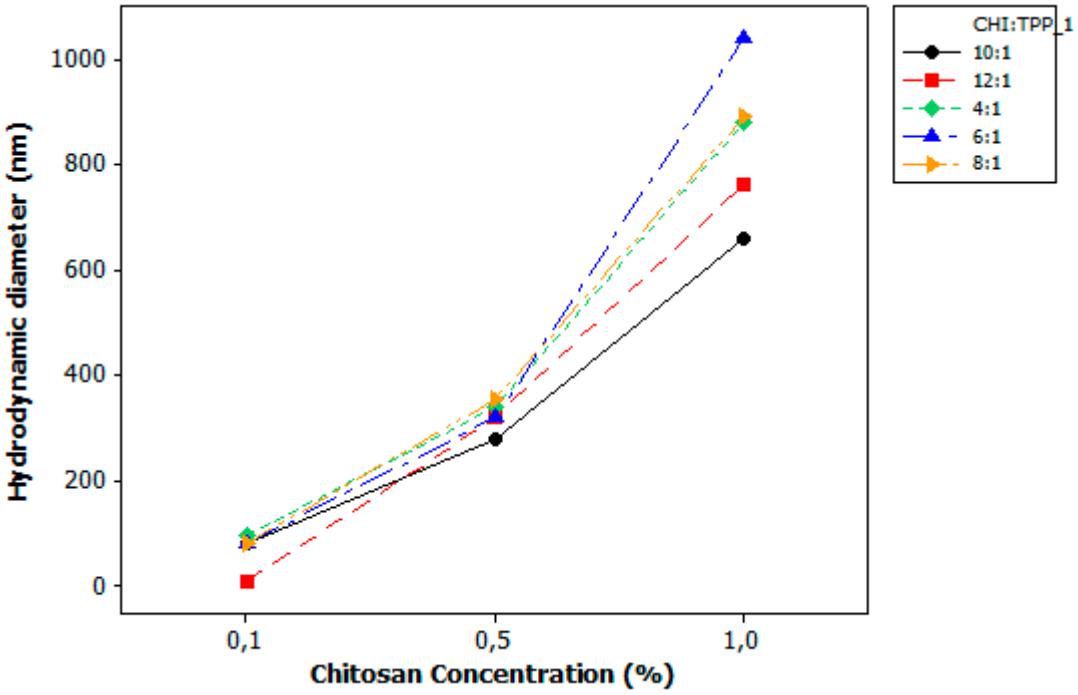


**Figure S2.** "Interactions plot" between the factors (CS % and CS/TPP ratio) influencing the size of nanoparticles.

**Characterization of CS nanoparticles Prepared in 200 mM Acetate Buffer pH 5 and pH 5.5**

The pH value of the buffer used for the preparation of CS nanoparticles has a strong effect on nanoparticles size. By analysing CS nanoparticles prepared at the same concentration, a progressive rise of size occurred by increasing the pH from 4.5 to 5 and 5.5. For chitosan 0.1% *w/w* nanoparticles, size increased from approximately 90 nm at pH 4.5 to around 200 nm at pH 5 and 300-400 nm at pH 5.5. For 0.5% CS nanoparticles, size increased from 300 nm at pH 4.5 to around 400-500 nm at pH 5 and 600-700 nm at pH 5.5. 1% CS nanoparticles were obtained only at pH 4.5. By increasing the pH to 5 and 5.5, colloidal systems with a size above 1000 nm were produced.

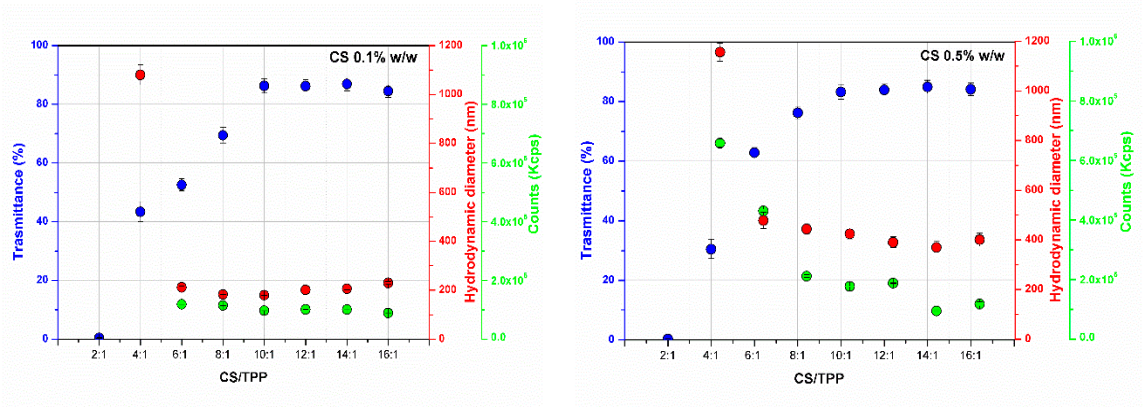


**Figure S3.** Hydrodynamic diameter (nm), counts (Kcps) and transmittance (%) of nanoparticles at different CS concentrations (0.1%, 0.5% and 1%; *w/w*) and different CS/TPP ratio prepared in acetate buffer pH 5.

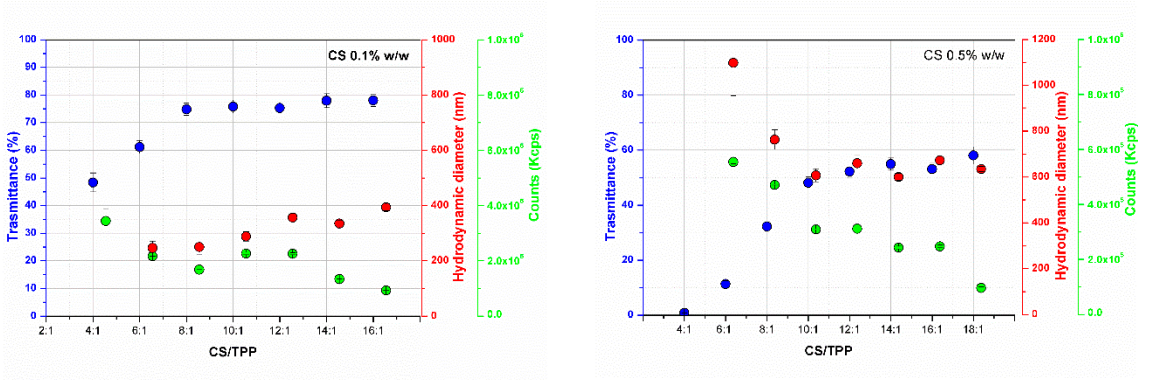


**Figure S4.** Hydrodynamic diameter (nm), counts (Kcps) and transmittance (%) of nanoparticles at different CS concentrations (0.1%, 0.5% and 1% *w/w*) and different CS/TPP ratios prepared in acetate buffer pH 5.5.

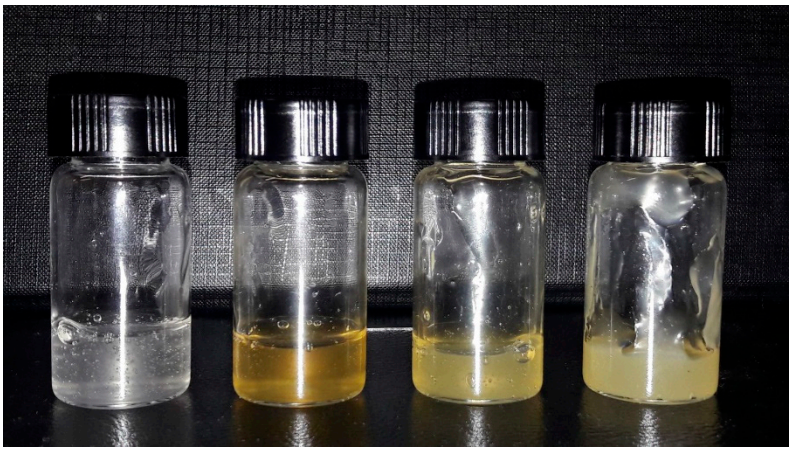

**Figure S5.** Image of the prepared HPMC/CS mixed hydrogels in 200 mM acetate buffer pH 4.5. From left to right: 5.5% HPMC (control); 5.5% HPMC/1%CS; 5.5% HPMC/1%CS NPs 12:1; 5.5% HPMC/1%CS NPs 6:1.

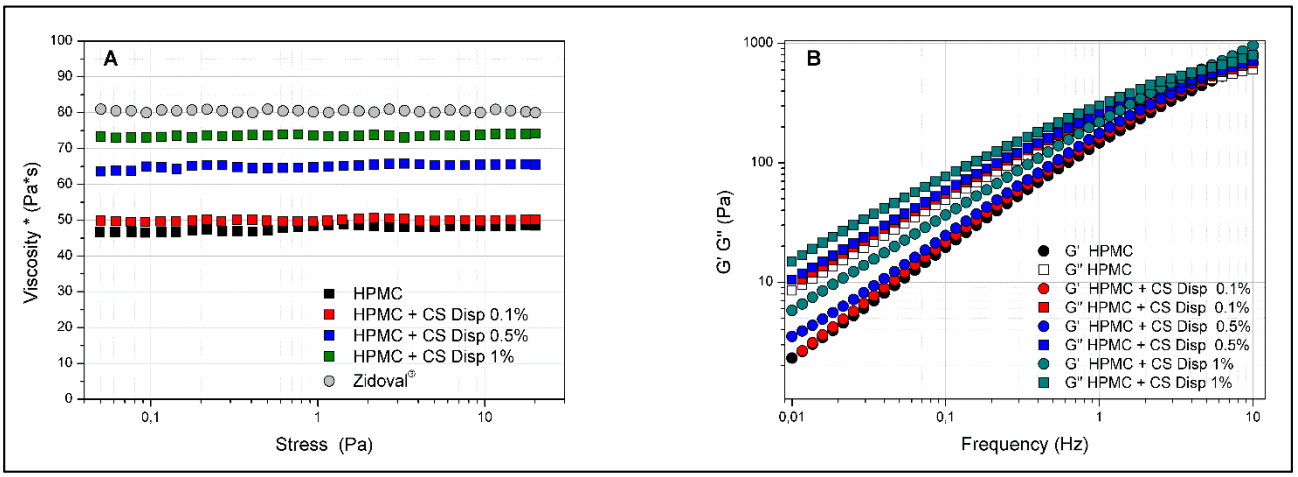


**Figure S6.** Stress sweep (**A**) and frequency sweep (**B**) results obtained from HPMC/CS mixed hydrogels at 37 °C. CS was dispersed into HPMC as a free polymer.

**Table S1.** Growth inhibition diameter (in mm) of Fluconazole (25 and 50 μg/mL) tested against the different *Candida* spp strains. Values are reported as the mean ± SD of two replicates.

| Antifungal | Inhibition growth diameter (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *albicans* strains | | | | *non-albicans* strains | | | |
| | *C. albicans* 11/01 | *C. albicans* 18/01 | *C. albicans* 4940 | *C. albicans* 360923 | *C. glabrata* 104/1 | *C. glabrata* 104/22 | *C. glabrata* 49/55 | *C. lusitaniae* 360804 |
| **Fluconazole 25 μg/mL** | 0 | 0 | 0 | 20±1.3 | 0 | 0 | 0 | 0 |
| **Fluconazole 50 μg/mL** | 0 | 0 | 0 | 28±0.8 | 0 | 0 | 0 | 0 |