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Supplementary Information for:

Dual enzyme responsive microcapsules simulating an "OR" logic gate for biologically triggered drug delivery applications

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Materials

Chondroitin sulphate A sodium salt from bovine trachea, poly (styrene sulfonate) (PSS, Mw =70kDa), Protamine sulphate salt from salmon (PRM, Mw ~ 5.1kDa), trypsin (Mw ~ 23.3 kDa), calcium chloride, sodium carbonate, ethylene diamine tetra-acetic acid (EDTA), dextran-TRITC (Mw ~65-85kDa), and Phosphate Buffer Saline (PBS) were all purchased from Sigma Aldrich, India. All the chemicals were used as received.

Fabrication of $CaCO_3$ (PSS) microparticles

PSS doped $CaCO_3$ particles were fabricated by mixing $CaCl_2$ and Na_2CO_3 solutions in the presence of PSS. Briefly, 0.33 M $CaCl_2$ solution was mixed with 2mg/ml PSS, into which equal volume of 0.33 M Na_2CO_3 was added rapidly at room temperature under magnetic agitation. After the reaction, $CaCO_3$ precipitate was collected and washed with water for three times using membrane filtration (with a cellulose filter having pore size of 0.45 μm).

Characterisation

The microelectrophoresis of the polyelectrolyte coated microparticles dispersed in water was measured using a Zetasizer Nanoseries NanoZS90 (Malvern Instruments, UK). Each value was averaged from three parallel measurements. Scanning electron microscope (FEI sirion, Eindhoven, Netherlands) was used to characterize the morphology of calcium carbonate particles and microcapsules. The EDX spectra of the microparticles and microcapsules were taken to confirm $CaCO_3$ core dissolution of microcapsules. Confocal microscopy images were taken using Zeiss LSM 510 META confocal scanning system (Zeiss, Germany) equipped with a 100× oil immersion objective with a numerical aperture of 1.4.

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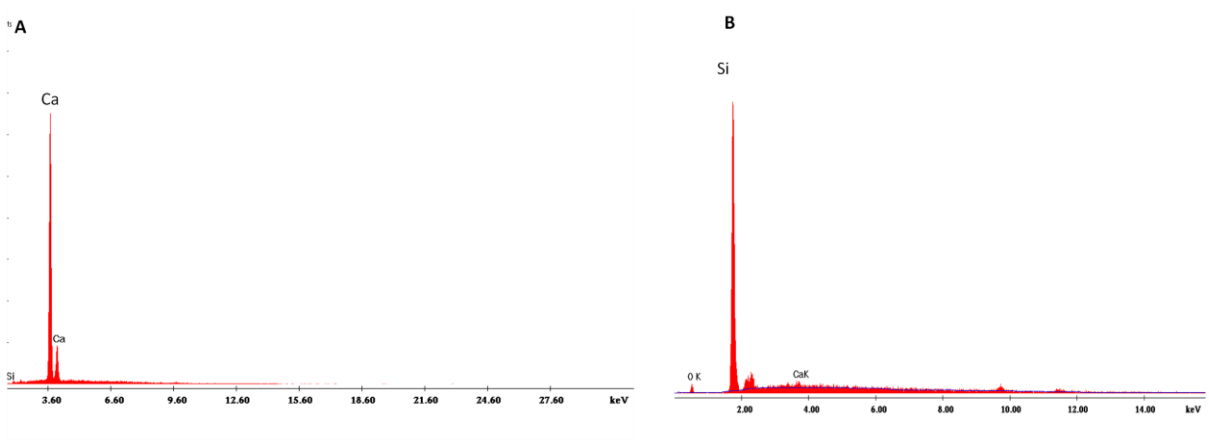

Figure S1. EDX spectra (A) before and (B) after core dissolution.

Dextran-TRITC loading and enzyme mediated release experiment

Loading of Dextran-TRITC into microcapsules was done at acidic pH**. D**extran-TRITC was added to 500µl of pH adjusted PR/CS microcapsules suspension in an eppendorf tube. The solution was incubated for overnight while maintaining the acidic pH. Enzyme mediated release was observed by adding dextran-TRITC loaded microcapsules into a solution of trypsin in PBS buffer pH 7.4. The samples were then viewed under confocal microscope after the required time.