Electronic Supplementary Material (ESI) for RSC Advances.

Understanding and controlling the release mechanism of *Escherichia coli* in double $W_1/O/W_2$ emulsion globules in the presence of NaCl in the W_2 phase

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Table S1. The rotational speeds of the rotor used to homogenize the different formulations of W_1/O and $W_1/O/W_2$.

W/O_1 percentage	Tween80 percentage	Rotor speed (rpm) for W/O_1	Rotor speed (rpm) for $W_1/O/W_2$
20	1	3000	2000
20	5	5000	1800
40	1	3000	2300
40	5	5000	2600

Table S2. The encapsulation efficiency (%) of *E. coli*-GFP and D (4,3) of different $W_1/O/W_2$ emulsions. Results are taken from a minimum of 3 independent experiments.

DE Formulations	20% W_1, 1% Tw80	20% W_1, 5% Tw80	40% W_1, 1% Tw80	40% W_1, 5% Tw80
Encapsulation efficiency (%)	99.95±0.02	99.9±0.03	99.93±0.02	99.9±0.09
D (4, 3)	67±1.37	64.1±0.85	70.9±0.17	70±1

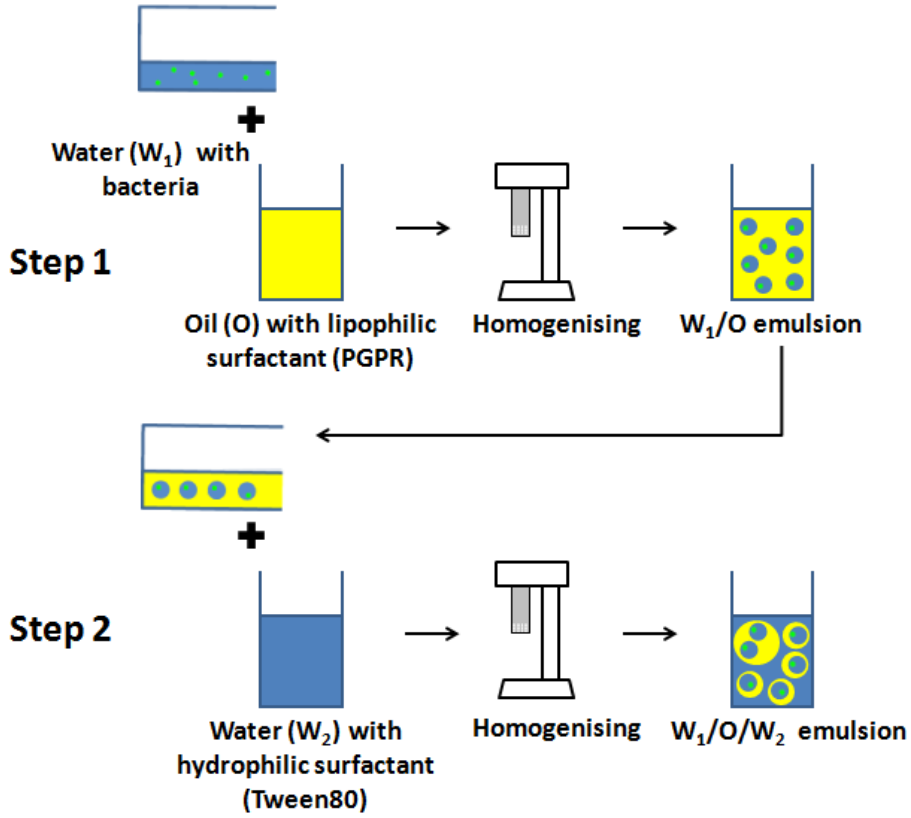

Figure S1. Schematic illustration of the two-step emulsification of a $W_1/O/W_2$ emulsions and encapsulation of bacteria.

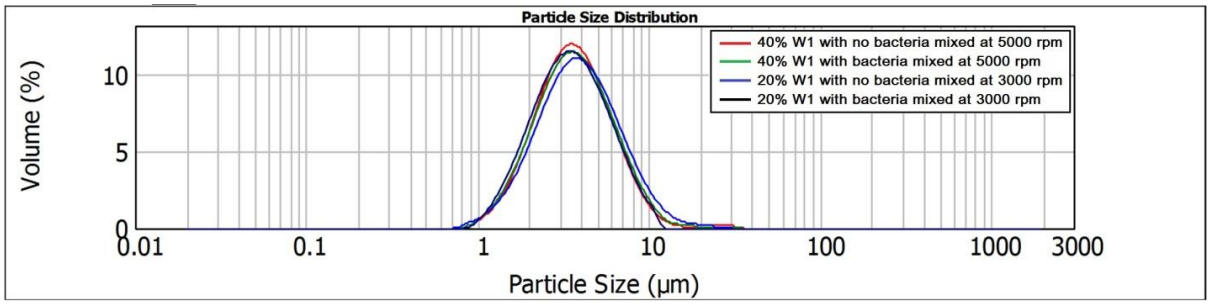

Figure S2. Particle size [D (4, 3)] analysis of the W_1 droplets from W_1/O emulsion. W_1/O emulsion is prepared with 20% or 40% W_1 with or without bacteria stabilized with 2% lipophilic surfactant. Measurements were taken at refractive index of 1.33.

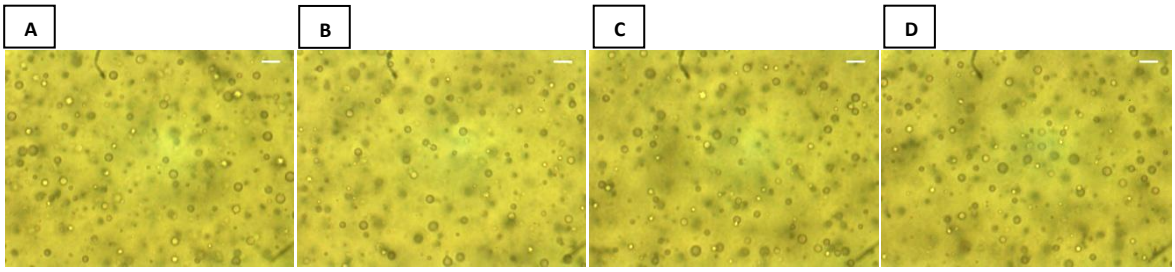

Figure S3. Optical images of W_1/O emulsions. W_1/O emulsions were prepared with water (W_1) volume percentage of 20% or 40% with or without bacteria and stabilized with 2% PGPR. The formulations were as follows: A) 40% W_1 with no bacteria mixed at 5000rpm; B) 40% W_1 with bacteria mixed at 5000 rpm; C) 20% W_1 with no bacteria mixed at 3000 rpm; D) 20% W_1 with bacteria mixed at 3000rpm. Scale bar: 10µm.

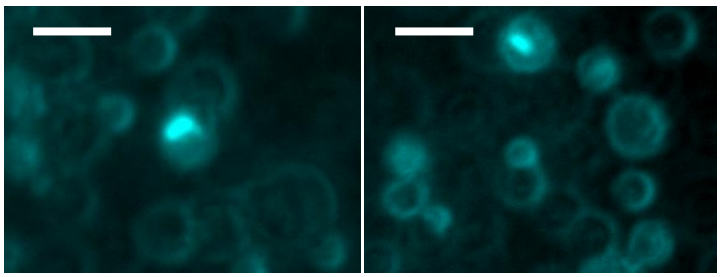

Figure S4. Fluorescent images of W_1 droplets encapsulating GFP-*E.coli* within a W_1/O emulsion. Scale bar: 5 µm.

Video S1. Optical video-microscopy showing oil globule bursting and release of W_1 droplets into W_2 after adding NaCl in W_2. The $W_1/O/W_2$ emulsion was prepared with inner W_1 phase volume percentage of 40% containing bacteria and stabilized with 1% Tween80 containing 0.085 M NaCl in the W_2 phase.

Video S2. Fluorescence video-microscopy showing bursting of oil globule and release of *E. coli*-GFP after altering the osmotic balance. The double emulsion was prepared with inner W_1 phase volume percentage of 40% containing bacteria and stabilized with 1% Tween80 containing 0.085 M NaCl in the W_2 phase.

Video S3. Fluorescence video-microscopy showing *E. coli*-GFP cells located within oil globules after loss of W_1 phase with no bursting of the oil globules occurring after osmotic balance alteration. The $W_1/O/W_2$ emulsion was prepared with inner W_1 phase volume percentage of 20% containing bacteria and stabilized with 5% Tween 80 containing 0.085 M NaCl in the W_2 phase.

Video S4. Fluorescence video-microscopy showing *E. coli*-GFP cells located within two oil globules before and after coalescing with each other during loss of W_1 phase due to the presence of NaCl in W_2. The $W_1/O/W_2$ emulsion was prepared with inner W_1 phase volume percentage of 40% containing bacteria and stabilized with 1% Tween 80 containing 0.085 M NaCl in the W_2 phase.

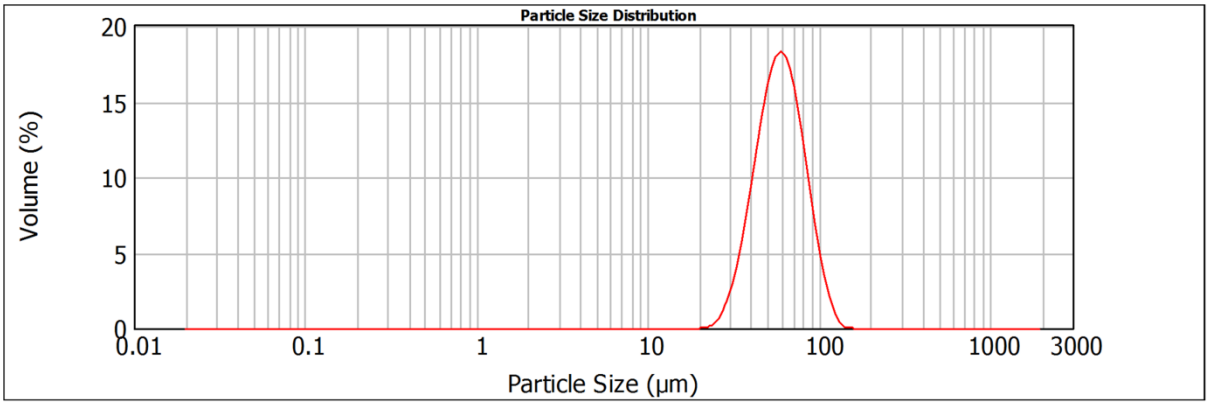

Figure S5. Particle size [D (4, 3)] analysis of the oil globules from freshly made $W_1/O/W_2$ emulsion before altering the osmotic balance. The $W_1/O/W_2$ emulsion is prepared with 40% W_1 containing no bacteria stabilized with 1% Tween80 and no NaCl added to the W_2 phase. Measurements were taken at refractive index of n_D^{22} 1.396.

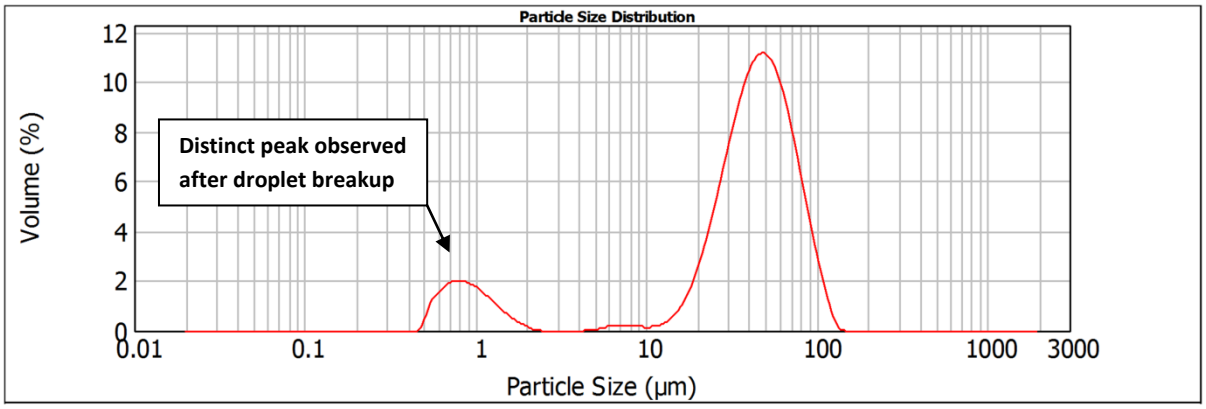

Figure S6. Particle size [D (4, 3)] analysis of oil globules from $W_1/O/W_2$ double emulsion after altering the osmotic balance. The $W_1/O/W_2$ emulsion is prepared with 40% W_1 containing no bacteria stabilized with 1% Tween80 and 0.085 M NaCl added to the W_2 phase. Measurements were taken at refractive index of n_D^{22} 1.396.

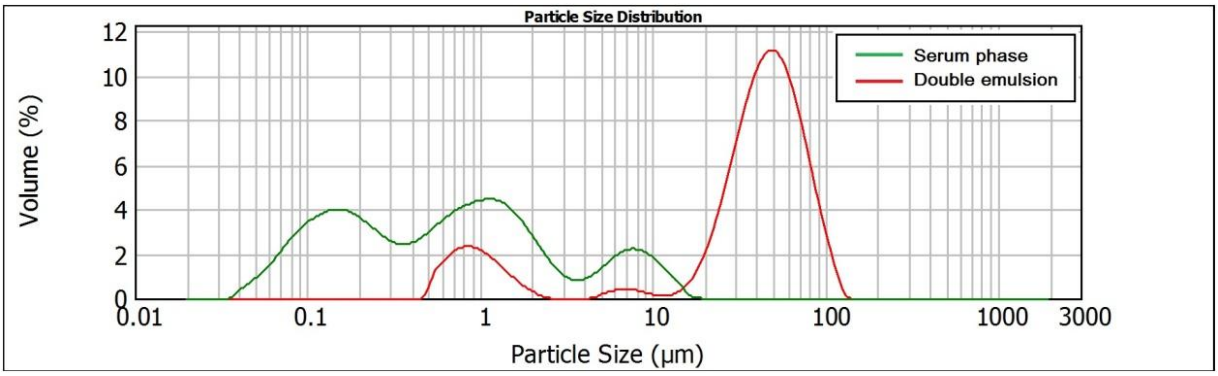

Figure S7. Particle size [D (4, 3)] analysis of W_1/O globules of serum phase and $W_1/O/W_2$ emulsion after altering the osmotic balance. The $W_1/O/W_2$ emulsion is prepared with 40% W_1 containing no bacteria stabilized with 1% Tween80 and 0.085 M NaCl in the W_2 phase. Measurements were taken at refractive index of n_D^{22} 1.396.

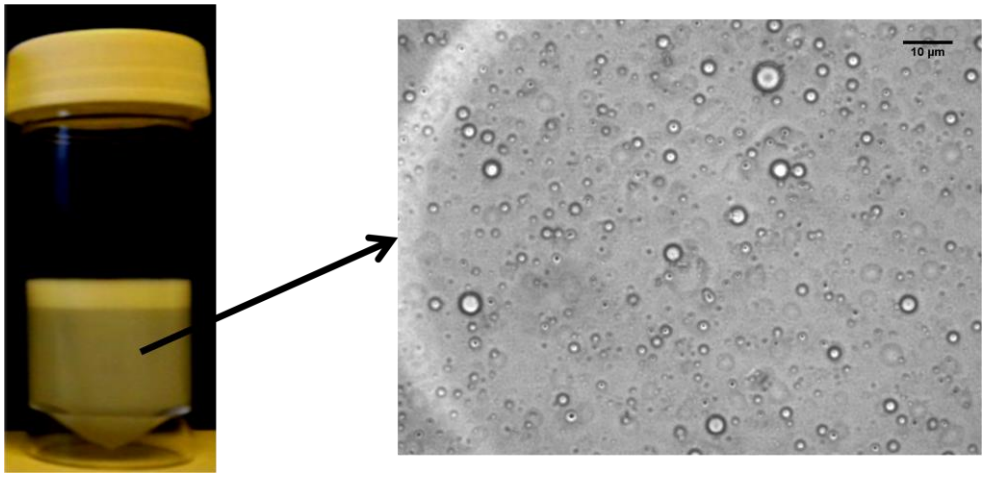

Figure S8. Optical observation of the serum phase. W_1 droplets persisted in the serum phase after adding 0.085 M NaCl. Scale bar = 10 µm.