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Supporting Information

High-throughput nanoprecipitation of the organic antimicrobial Triclosan and enhancement of activity against *Escherichia coli*

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A high-throughput approach was developed was an automated liquid handling robot (Epmotion) was developed to rapidly and simultaneously produce 96 different formulations for nanoprecipitation within each screen. Initially we used this process to screen rapidly for viable nanoprecipitates for further study, Figure SI1. Hits were defined as having a particle distribution with z-average diameters ≤ 500 nm and polydispersity indexes ≤ 0.5. Four of the stabilizers, PVA (Figure 1A), PVP (Figure 1B), F68 (Figure 1C) and F127 (Figure 1D) produced nanoprecipitates of which approximately >50 % were defined as hits. Much fewer PEG nanoprecipitates were defined as hits ~ 34 % due to a large number of the resulting nanosuspensions having a mean diameter above 500 nm, suggesting inefficient stabilization of the growing triclosan nuclei in this case.

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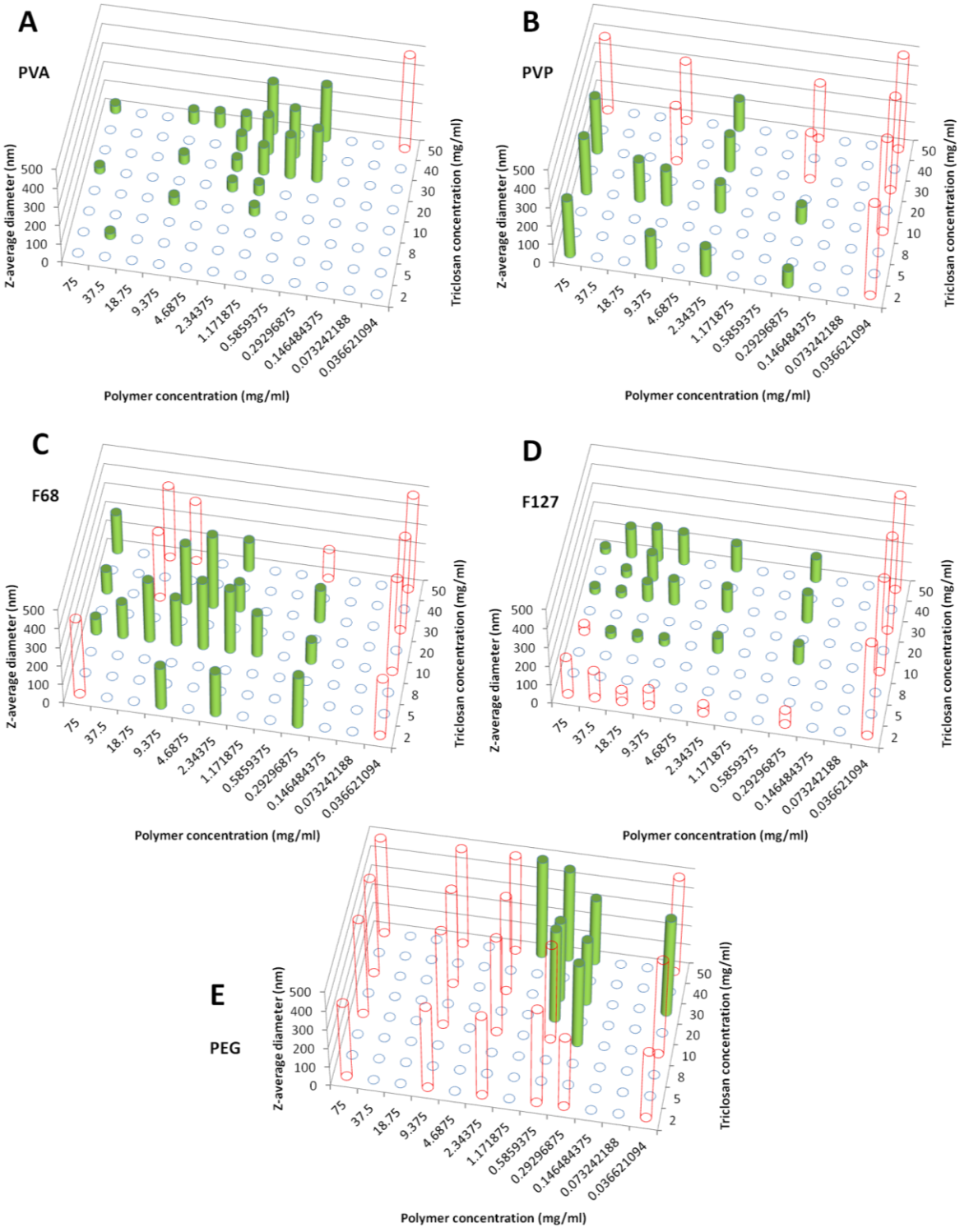

Figure SI 1. Screening for viable triclosan nanosuspensions by nanoprecipitation with a high-throughput methodology. Z-average diameter for five different polymers each across a range of polymer and triclosan concentrations. The ethanol to water fraction was constant at 0.2. The pass

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criteria (green bars) was a z-average diameter ≤ 500 nm, a polydispersity index ≤ 0.5 and a pass on the DLS quality result. Transparent bars with red outline represent poor nanosuspensions: when the diameter > 500 nm, a polydispersity index > 0.5 and/or when the DLS quality report showed poor data. Unfilled blue circles represent a formulation that was not analysed by DLS.

Table SI 1. Details of formulation and DLS characterization for triclosan nanosuspension produced from scale-up experiment.

Sample	Triclosan concentration (mg/ml)	Polymer concentration (mg/ml)	Surfactant	Surfactant concentration (mg/ml)	Theoretical Loading (weight %)	Size (nm)	PDI	Zeta (mV)
1	40	63.75	Hyamine	4.5	13	168	0.12	40
2	40	3.98	Hyamine	4.5	33	322	0.36	41
3	40	0.25	Hyamine	4.5	54	Failed to disperse		
4	40	63.75	Tween 20	4.5	13	202	0.05	-28
5	40	3.98	Tween 20	4.5	33	270	0.35	-39
6	40	0.25	Tween 20	4.5	54	203	0.16	-36
7	40	63.75	Sodium deoxycholate	4.5	13	161	0.09	-34
8	40	3.98	Sodium deoxycholate	4.5	33	189	0.01	-33
9	40	0.25	Sodium deoxycholate	4.5	54	Failed to disperse		

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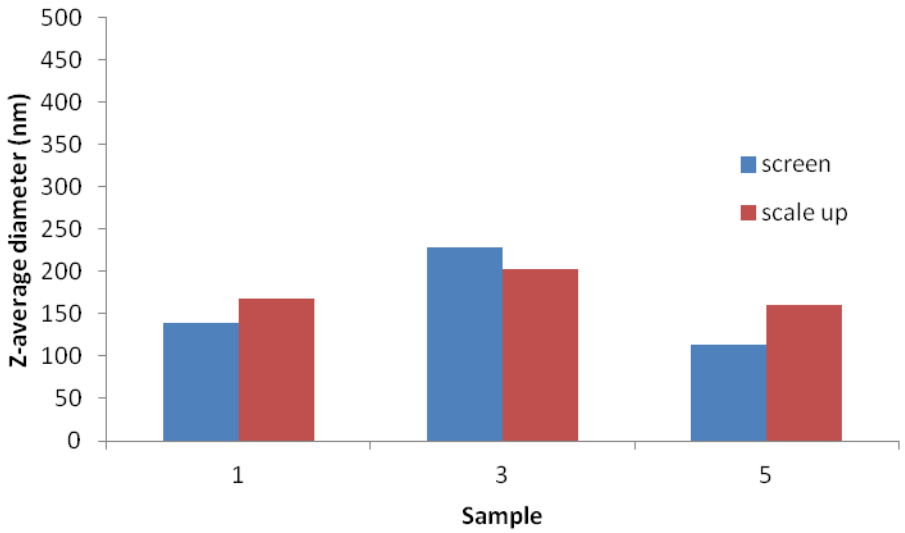

Figure SI 2.Comparison of mean size by DLS for formulations measured in the screen screen and then scaled up to a larger volume.

Table SI 2. Triclosan content of the nanosuspensions used in the antimicrobial experiments as quantified by UV/Vis.

Sample	Expected mass triclosan (mg)	Actual mass of triclosan (mg)	% Loss
1	8	5.9	25.9
2	8	5.7	28.3
3	8	5.7	28.2
4	8	5.0	37.8
5	8	5.6	29.5
6	8	5.8	27.8

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Table SI 3. Investigating the effect of different solvents on the loss of triclosan during freeze drying as quantified by UV/Vis. 1 ml of the specified triclosan solution was frozen in liquid nitrogen and then freeze dried for 2 days.

Solvent	Melting point	mg/ml	Expected mass (mg)	Actual mass (mg)	% loss
Ethanol	-114	50	50	7.8	84.4
Methanol	-98	50	50	6.1	87.8
Propan-1-ol	-126	50	50	7.1	85.8
Propan-2-ol	-89	50	50	7.8	84.5

A

Sample	E. coli MC1061 IC50 (μg ml)	Standard deviation	IC50 graph
Aqueous triclosan*	11.17	3.4×10^{-4}	B
1	0.87	3.6×10^{-3}	C
2	1.02	3.2×10^{-3}	D
3	1.15	2.4×10^{-3}	E
4	1.21	2.2×10^{-3}	F
5	1.51	4.7×10^{-3}	G
6	2.87	2.8×10^{-3}	H

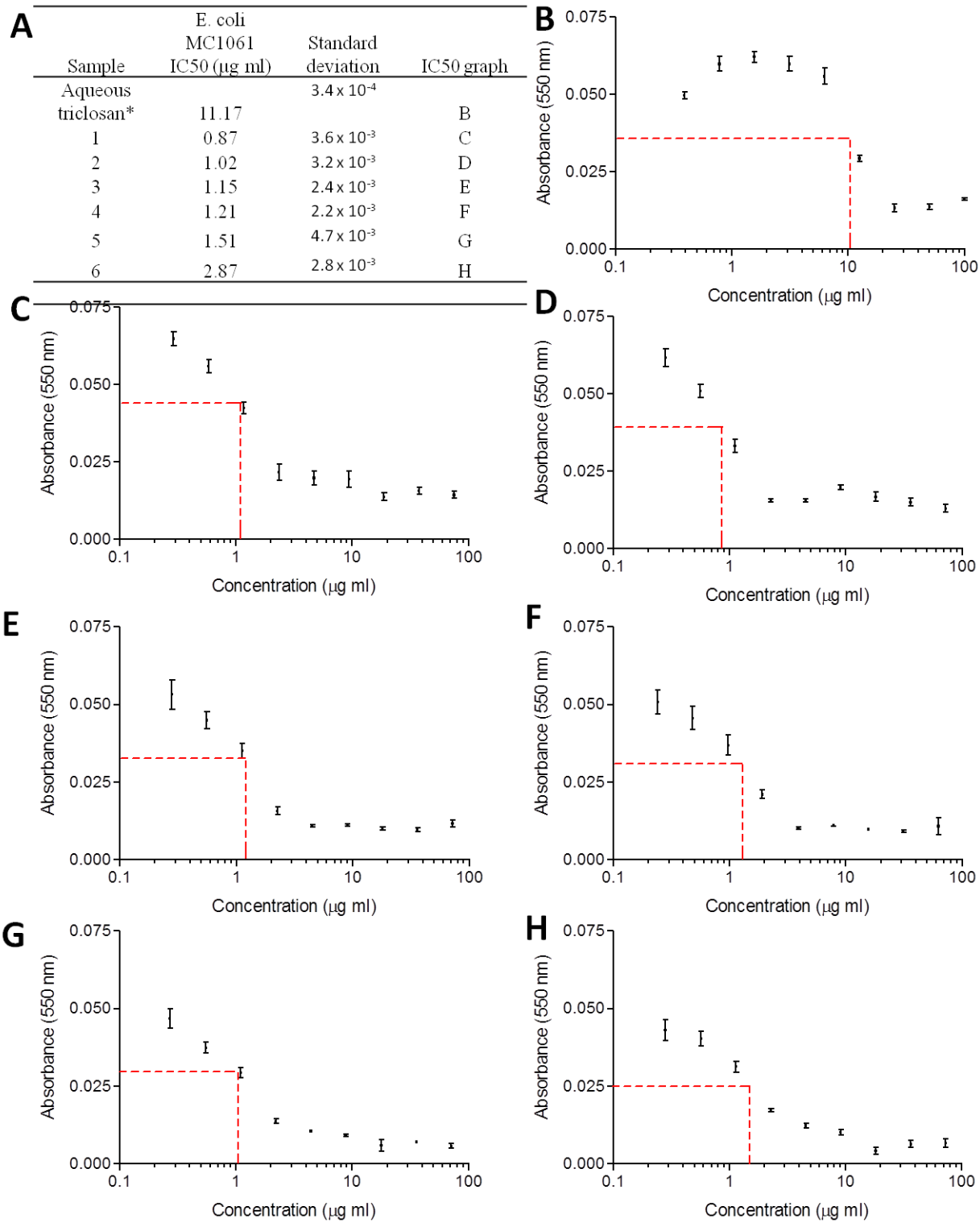

Figure SI 4. IC_{50} against *E.coli* graphs for all the triclosan nanosuspensions. A: Table with sample information showing which samples are represented by which graphs. B – H: IC_{50} graphs for the nanosuspensions.

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A

Sample	*S. aureus* IC50 (μg ml)	Standard deviation	IC50 graph
Aqueous triclosan	4.23	7.4×10^{-3}	B
1	0.89	2.9×10^{-3}	C
2	0.71	2.0×10^{-3}	D
3	0.94	4.6×10^{-3}	E
4	2.32	1.9×10^{-3}	F
5	1.34	2.9×10^{-3}	G
6	1.76	4.2×10^{-3}	H

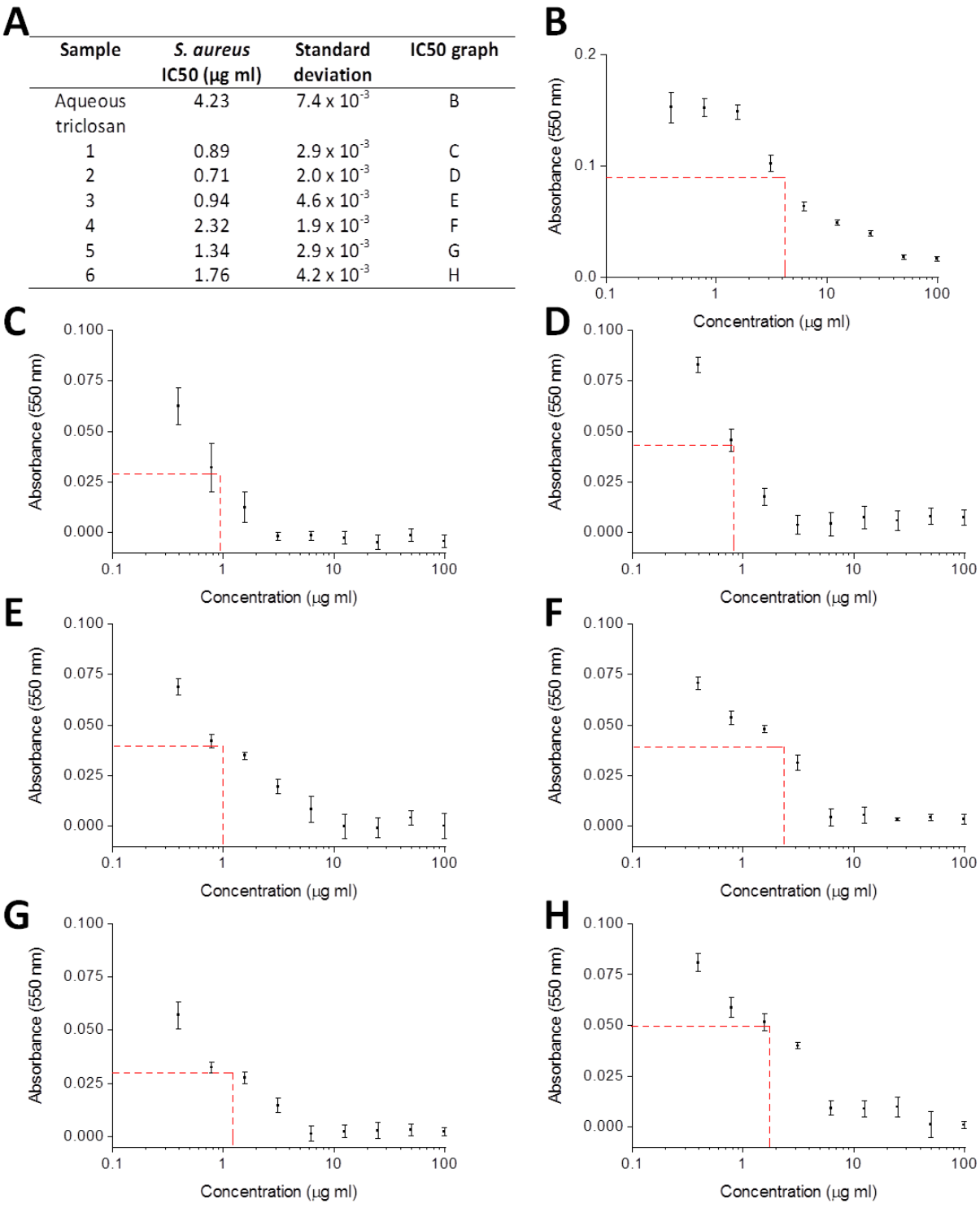

Figure SI 5. IC_{50} against *S. aureus* for aqueous and nanoparticle formulated triclosan. A: Sample details and IC_{50} values. B – H: IC_{50} graphs for each of the test nanosuspensions. The

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antimicrobial activity of the triclosan nanosuspensions against *S. aureus* were investigated in the same way as described in the methods section for *E. coli.*

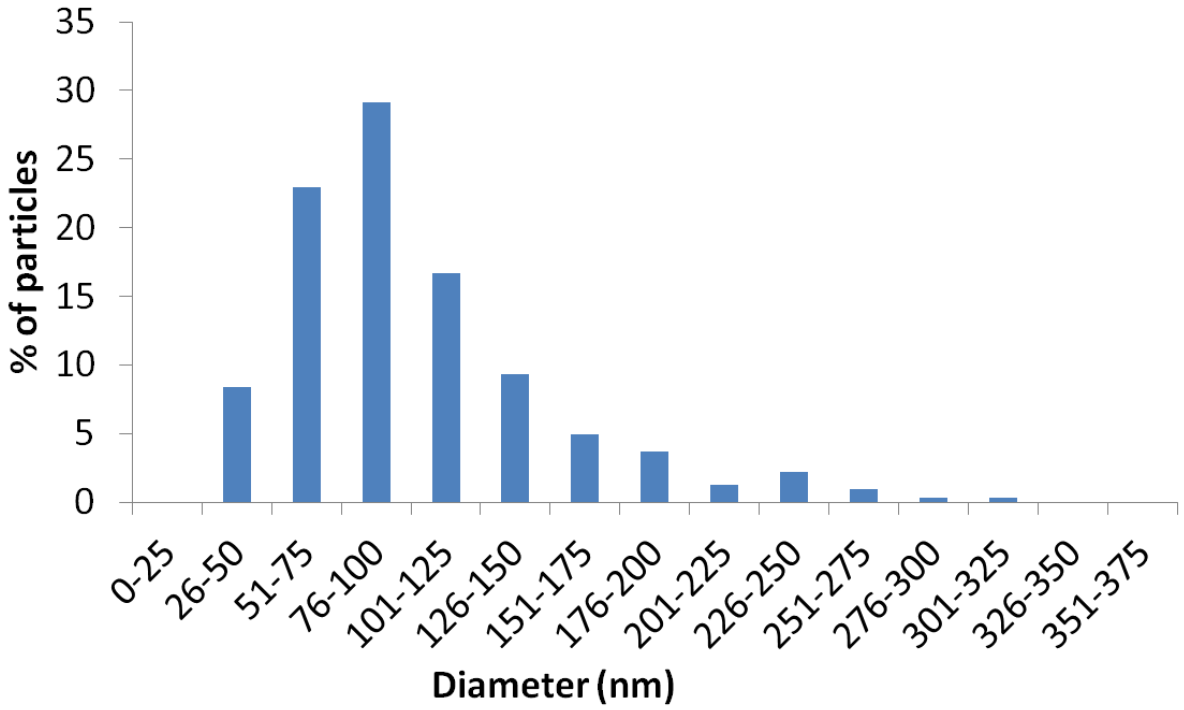

Figure SI 6. Frequency distribution graph of nanoparticles from dried triclosan nanosuspension stabilized with F68 and hyamine (sample 2 (table 1)) as measured by SEM. A total of 323 particles were measured to obtain a representative distribution.

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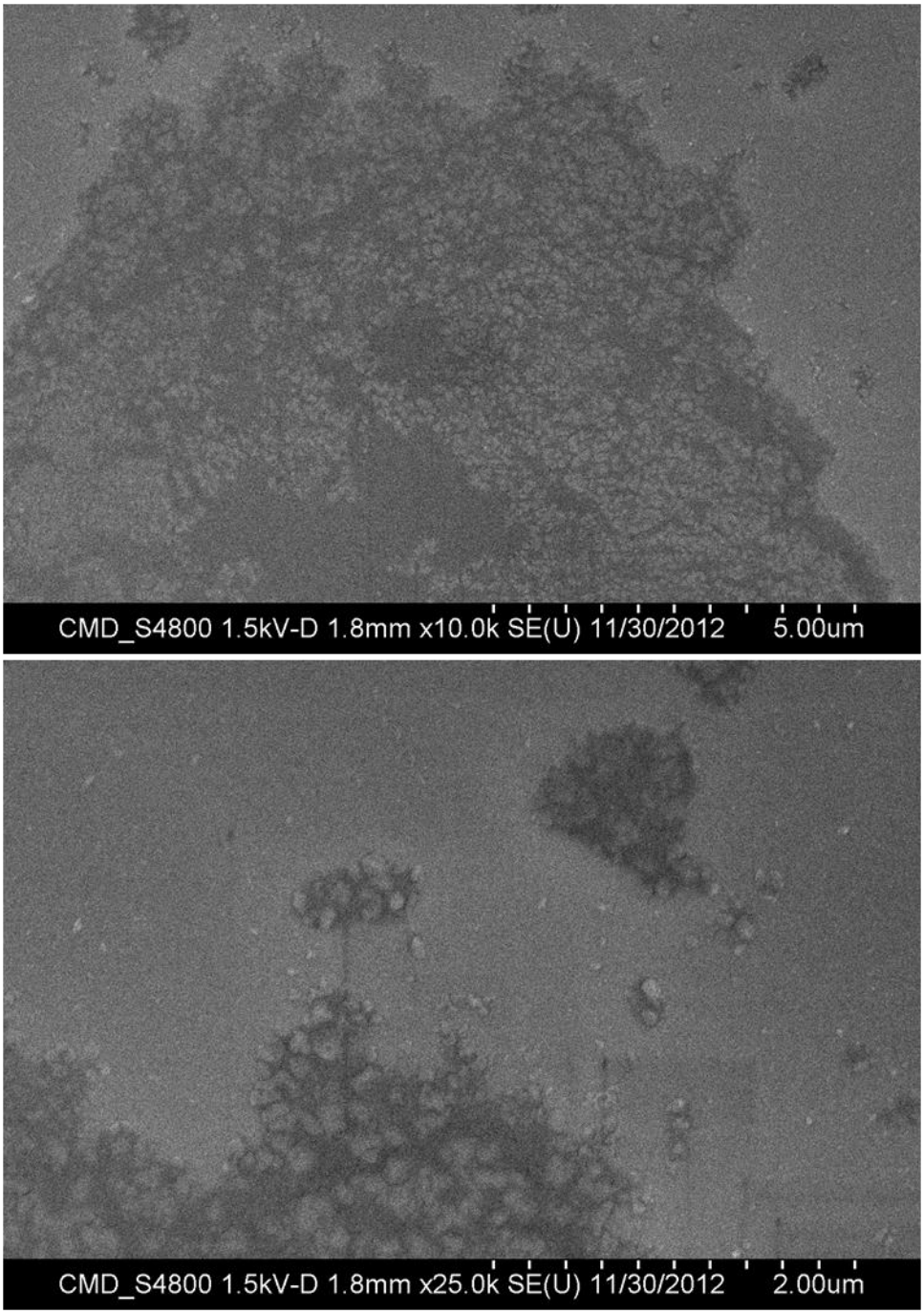

Figure SI 7. SEM images of the dried triclosan nanosuspension stabilized with F68 and hyamine (sample 2 (table 1)), displaying the presence of triclosan nanoparticles embedded in a film of the stabilizers.

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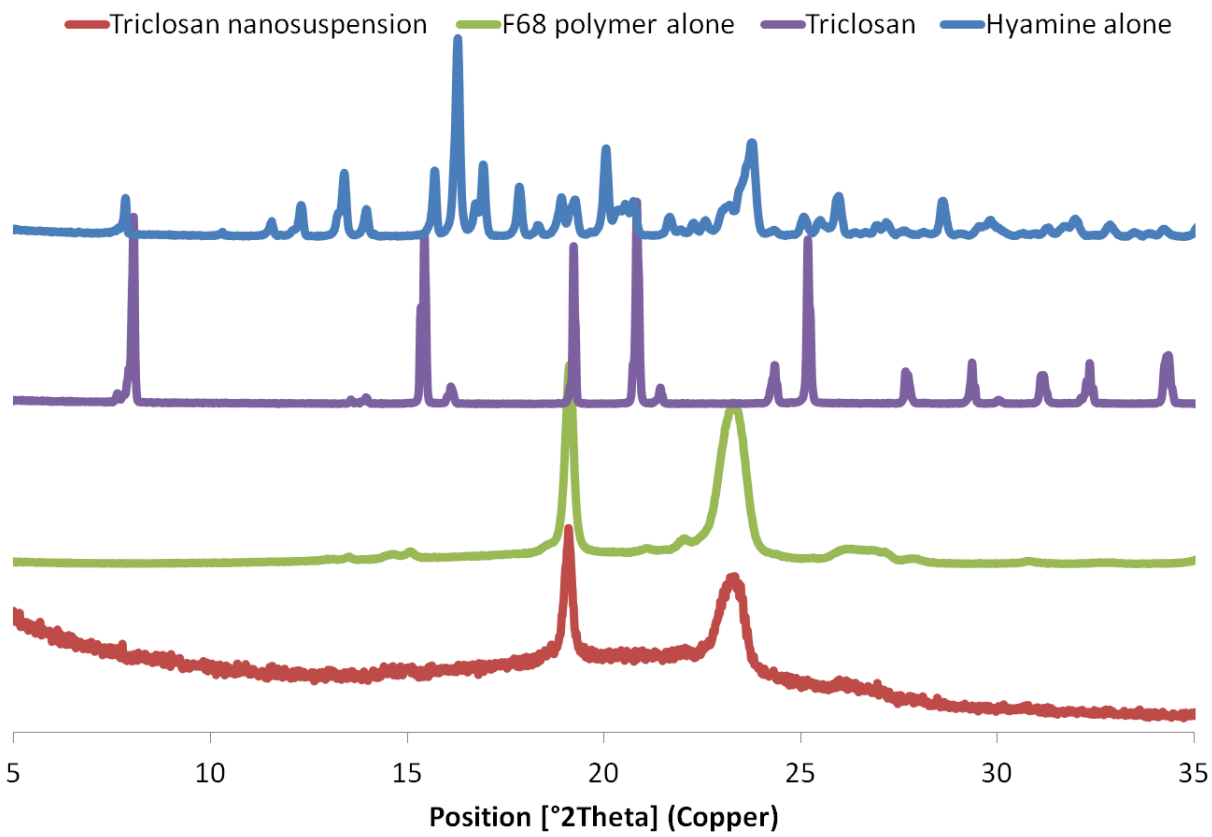

Figure SI 8. XRD analysis of the freeze-dried monolith of a triclosan nanosuspension containing F68 and hyamine (sample 2 (table 1)). The sharp peaks observed for the hyamine and triclosan samples were produced by long-range order in the material (i.e. crystallinity). The absent of these peaks in the freeze-dried triclosan nanosuspension indicate that it was amorphous.