Effect of Different Surfactants on the Efficacy of Triclosan against *Bacillus subtilis*

A

DISSERTATION REPORT

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Submitted by

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Shelja Sharma

Place: Patiala
Dated: 12/7/13

CANDIDATE'S DECLARATION

I, hereby declare that the work presented in the dissertation entitled **"Effect of Different Surfactants on the Efficacy of Triclosan against *Bacillus subtilis*"** in partial fulfillment of the requirements for the award of the degree of Master of Science in Chemistry, School of Chemistry and Biochemistry, Thapar University, Patiala, is an authentic record of my own work during the period of six months from January 2013 to July 2013, under the supervision of Dr. Manmohan Chhibber, Assistant Professor, School of Chemistry and Biochemistry, Thapar University. The report has not been submitted for the award of any other degree or certificate in this or any other university.

Place: Patiala

Dated: 12/7/13

Shelja Sharma.

Shelja Sharma

This is to certify that the above statement made by the candidate is correctand true to the best of our knowledge.

Dr. Manmohan Chhibber
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CERTIFICATE

This is to certify that the dissertation entitled **"Effect of Different Surfactants on the Efficacy of Triclosan against *Bacillus subtilis*"** submitted by Ms. Shelja Sharma, Roll No. 301102015 in partial fulfillment of the requirements for the award of degree of Master of Science in Chemistry to School of Chemistry and Biochemistry, Thapar University, Patiala, is the work carried out under my supervision. No part of the work has been submitted for the award of any other degree or certificate in this or any other University.

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INTRODUCTION

The major challenge with the design of oral dosage forms lies with their poor bioavailability. The oral bioavailability depends on several factors including aqueous solubility, drug permeability, dissolution rate and susceptibility to efflux mechanisms. The most frequent causes of low oral bioavailability [1] are attributed to poor solubility and low permeability. Low aqueous solubility is the major problem encountered with formulation development of new chemical entities as well as generic development. Any drug to be absorbed must be present in the form of an aqueous solution at the site of absorption. Water is the solvent of choice for liquid pharmaceutical formulations. Most of the drugs are either weakly acidic or weakly basic having poor aqueous solubility [2].

There are various techniques available to improve the solubility of poorly soluble drugs for instance pH adjustment[3], self-emulsifying drug delivery system[4], particle size reduction[5], co-solvency[6,7] and solid dispersions[8,9]. Also surfactants are widely used as complexing agents for lipophilic and amphiphilic substances and functional excipients that have gained widespread use and attention because of their ability to solubilize, and in some instances stabilize, poorly water-soluble drug candidates enabling both oral and parenteral formulation [10, 11].

Among all the solubility enhancement techniques inclusion complex formation technique has been employed more precisely to improve the aqueous solubility, dissolution rate and bioavailability of drugs that are less soluble in water [12]. However solubility enhancing agents should not mask the activity of the drug. Recent reports have shown that the nature of surfactant and its concentrations has significant effect on growth of the microorganisms [13, 14] both individually and in presence of some antibacterial agent. For instance *Streptococcus agalactiae*, a causative agent of neonatal meningitis, can survive the activity of both triclosan and cerulenin (inhibitors of FabI and FabF/B, respectively) when culture media is supplemented with long-chain fatty acids [15].

Therefore, there is need to study the effect of surfactant on the activity of certain drug molecules. In this study, we examined the antimicrobial activity of triclosanin combination with various surfactants and compared it to antimicrobial activity of triclosan alone against *Bacillus subtilis*.

REVIEW OF LITERATURE

The use of surfactants to improve the dissolution performance of poorly soluble drug products is probably the basic, primary, and the oldest method. Surfactants reduce surface tension and improve the dissolution of lipophilic drugs in aqueous medium. They are also used to stabilize drug suspensions. Both nonionic and anionic surfactants increase the solubility of hydrocarbons by forming micelles [16 - 20]. The surfactants begin to assemble into micelles at the critical micelle concentration (CMC), and the interiors of the micelles provide a hydrophobic environment to solubilize nonpolar compounds, such as hydrocarbons [21]. Compounds of poorly soluble that use miceller solubilization are antidiabetic drugs, gliclazide, glyburide, glimepiride, glipizide, repaglinide, pioglitazone, and rosiglitazone [22].

Surface-active agents (surfactants) have two regions in their molecular structures, one a hydrocarbon, water-repellent (hydrophobic) group and the other a water-attracting (hydrophilic or polar) group. Depending on the basis of the charge or absence of ionization of the hydrophilic group, surfactants are classified into cationic, anionic, nonionic, and ampholytic (amphoteric) compounds. It has been known for many years that QACs are membrane active agents i.e., with a target site predominantly at the cytoplasmic (inner) membrane in bacteria or the plasma membrane in yeasts. Salton [23] proposed the following sequence of events with microorganisms exposed to cationic agents: (i) adsorption and penetration of the agent into the cell wall; (ii) reaction with the cytoplasmic membrane (lipid or protein) followed by membrane disorganization; (iii) leakage of intracellular low-molecular-weight material; (iv) degradation of proteins and nucleic acids; and (v) wall lysis caused by autolytic enzymes. There is thus a loss of structural organization and integrity of the cytoplasmic membrane in bacteria, together with other damaging effects to the bacterial cell [24]. A novel non-ionic surfactant nanoemulsion designated 8N8 has been tested for its biocidal activity by Baker et al [25]. One percent 8N8 produced effective bactericidal activity against *Bacillus cereus, Bacillus subtilis, Haemophilus influenzae, Neisseria gonorrhoeae, Streptococcus pneumoniae*, and *Vibrio cholerae* in 15 minutes. The quaternary ammonium compound dodecyldimethylammonium Chloride (DDDMAC) has bactericidal activity and has been used for cattleshed disinfection, wood preservation [26] and cellulosic string protection [27]. Bartolo et al [28] also reported that didecyldimethylammonium Chloride was bactericidal against all organisms tested *viz E. coli,*

Staph. aureus and *Ps. aeruginosa* and all compounds had some bacteriostatic action. Low level static effects on bacterial growth were seen with sub-MIC concentrations. The surfactant C10–C16 alkyldimethyl amine N-oxides (ADMAO) has been shown to have antimicrobial activity against *Staph. aureus* and *Saccharomyces cerevisiae* [29]. The activity of ADMAOs increases with chain length up to a cut-off point of 14 [30] and is thought to be linked to interaction with the cell membrane [31]. Surfactin exhibits antifungal and moderate antibacterial properties [32,33]. Similar results were reported by Vater et al in which they had shown an improvement in proliferation rates and changes in the morphology of mycoplasma contaminated mammalian cells after treatment with this drug. A single treatment over one passage led to complete removal of viable *Mycoplasma hyorhinis* cells from various adherent cell lines, and *Mycoplasma orale* was removed from non-adherent human T-lymphoid cell lines by double treatment [34]. The non-ionic detergent Triton X-100 (TX-100) reduces methicillin resistance in a range of *S. aureus* strains [35, 36], with resistant strains showing the greatest increase in antibiotic sensitivity. These effects do not correlate with changes in *mecA* gene expression or in the ability of penicillin-binding protein 2a to bind antibiotic. Transposon mutagenesis of another gene, labelled *fmt*, which encodes a product with protein sequence similarity to penicillin-binding proteins, has been shown to further increase *S. aureus* methicillin sensitivity in the presence of TX-100 [37]. TX-100 stimulates autolysis and the release of acylated LTA and strains that show the greatest reduction in methicillin resistance also release significantly more LTA. These effects appear to be independent of known autolysins [38, 39], since mutants deficient in these enzymes also show increased susceptibility to methicillin in the presence of TX-100. Similarly CTAB and L14 were found to potentiate the activity of chlortetracycline on strains of *Escherichia coli*, *Proteus mirabilis*, and *Klebsiella pneumonia* but not Tween-80 or alpha-(2,4,5-trichlorophenoxy)propionic acid. Studies on the inhibition of protein synthesis by chlortetracycline in cells of a strain of *E. coli* suggested that the surfactants increased the uptake of antibiotic into the cells. CTAB and L14 almost completely sensitized strains of *P. mirabilis*, *Serratia marcescens*, *K. pneumoniae*, and *E. coli* to PmB. With the exception of *K. pneumoniae*, alpha-(2,4,5-trichlorophenoxy) propionic acid was also effective in potentiating the activity of polymyxin B on the above strains whereas Tween-80 showed potentiation only with a strain of *E. coli*. CTAB and L14 but not alpha-(2,4,5-trichlorophenoxy) propionic acid or Tween-80 potentiated the activity of penicillin G but not methicillin on strains of staphylococci. Studies of

penicillinase in the cells suggested that the surfactants inhibited the formation of this enzyme possibly at the level of induction. None of the surfactants were found to potentiate the activity of streptomycin [40]. Tween 80 has been shown to enhance the activity of polymyxin B on a strain of Pseudomonas aeruginosa [41] and *E. coli* [42]. Recently, it has been shown that use of an exogenous pulmonary surfactant labelled with a radioactive technetium sulfur colloid and mixed with pentamidine results in a more peripheral and uniform distribution pattern in the lungs compared with that obtained with a combination of pentamidine and saline [43]. The influence of surfactant on the bactericidal activity of amoxicillin was tested against *Staphylococcus aureus* and *Streptococcus pneumoniae*, and the influence of surfactant on the activities of ceftazidime and tobramycin was tested against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S. aureus*, and *S. pneumoniae*. Amoxicillin and ceftazidime activities were not changed in the presence of surfactant, except for a decreased killing rate of *S. pneumoniae* by ceftazidime in medium with additional rabbit serum. In contrast, killing curves with low concentrations of tobramycin showed a decreased level of activity of tobramycin against all pathogens tested in the presence of surfactant [44]. Tween-80 was added to rumen fluid at the level of 0.05 and 0.1 per cent (v/v), the total and specific activities of most of the cell-free enzymes were significantly ($P<0.01$) increased, but those of cell-bound enzymes did not follow a definite trend. The growth rates of rumen noncellulolytic bacteria *Ruminobacter amylophilus*, *Megasphaera elsdenii*, *Preuotella ruminicola* and *Selenomonas ruminantium*) were significantly ($P<0.01$) increased by the addition of Tween 80 at both the concentartions tested. However, the growth rate of ruin en cellulo1ytic bacteria (*Fibrobacter succ inogenes*, *Ruminococcus albus*, *Ruminococcus flauefaciens* and *Butyriuibrio fibrisoluens*) were generally not affected by Tween 80. In general, Tween 80 appears to affect Gram-negative bacteria more than Gram-positive bacteria; and non-cellulolytic bacteria more than cellulolytic bacteria [45]. The MIC of cetrimide against resistant AR *E. coli* was 312.5 µg/ml. The cetrimide also resulted in ultra-cellular structural changes in treated AR *E. coli* revealed vacuole formation, disaxilization of nuclear material, loss of cytoplasmic granularity, bleb formation and cell lysis [46]. *Staphylococcus aureus* mutants resistant to the non-ionic detergent Triton X-100 could grow and divide in broth containing 5% (vol/vol) Triton X-100, while growth of the parental strains was markedly inhibited above the critical micellar concentration (0.02%) of the detergent. Growth-inhibitory concentrations of Triton X-100 killed wild-type cells without

demonstrable cellular lysis. Treatment with either Triton X-100 or penicillin G in the growth medium stimulated release of predominantly acylated intracellular lipoteichoic acid and sensitized *staphylococci* to Triton X-100-induced autolysis. The resistant mutant TXR1, derived from *S. aureus* H, had a higher level of L-a-glycerophosphate dehydrogenase-I activity, and its oxygen uptake was more resistant to inhibition by a submicellar concentration (0.008%) of Triton X-100 [47]. Polysorbate 80 (PS80) which is also known as tween-80 commonly added to food and medicines, is able to inhibit biofilm formation by *Pseudomonas aeruginosa* on a variety of surfaces, including contact lenses [48].The effect of deoxycholate on the growth parameters of *Escherichia coli* showed an increase in the lag time constant and generation time and a decrease in the growth rate constant and total cell yield of this microorganism. Cell fractionation studies indicated that sodium deoxycholate at levels used in bacteriological media interferes with the incorporation of [U-14C] glucose into the cold-trichloroacetic acid-soluble, ethanol-soluble, and trypsin-soluble cellular fractions of *E. coli*.. Also sodium deoxycholate interfered with the flagellation and motility of *Proteus mirabilis* and *E.coli*. It would appear then that further improvement of the deoxycholate medium may be in order [49]. Triton X-100 at low concentration (0.024 mM or 0.09 CMC) inhibited the rate of growth of either a *Mycobacterium sp*. or a *Pseudomonas sp*. on solid anthracene as sole carbon source. Recovery of microbial growth rate could be achieved by dilution of surfactants, while addition of more surfactant gave an immediate decrease in growth rate [50]. The lipopeptide daptomycin was found to be ineffective for treating pneumonia during clinical trials since its binding to lung surfactant made it unavailable to act against the infecting bacteria [51].

MATERIALS AND METHODS

Materials

All the experiments were performed with analytical-grade chemicals and solvents. Tween-80, Tween-20, Triton X-100, γ-Cyclodextrin, Sodium deoxycholate, CTAB, Triclosan (antibacterial agent) and Dimethyl sulphoxide were obtained from Sigma-Aldrich. Doubly distilled conductivity water was used for solution preparation.

Media: Nutrient broth of Milar, Himedia was taken as microbial growth medium consisted of casein enzyme hydrolysale, yeast extract and sodium chloride.

Bacterial Strain: *Bacillus subtillus* (MTCC: 441) was used.

Instruments: The spectroscopic measurements were performed on a double-beam UV-Vis spectrophotometer Specord-205 equipped with a matched pair of cuvettes of 10.0mm path length. The accuracy for λ_{max} of the spectra was ±0.1nm. All measurements were done in triplicate.

Laboratory equipment: All the plastic sterile disposable equipment was purchased from Tarson Plastic Ware. All the glassware, plastic tips, 1.5 ml micro centrifuge tubes and culture media were sterilized at 120^{o}C for 2 hours by steam sterilization.

Methods:

Microbial growth: Microbial strain *B. subtilis was* transferred from glycerol stocks at −80^{o}C to nutrient agar plates by streaking. The plates were incubated for 24 hours at 37^{o}C. After incubation, the plates were stored at 4^{o} C. Single colony was picked up from the culture plate and inoculated in 5 mL of Luria Bertani broth at 37^{o}C for 12 hours for the preparation of primary culture.

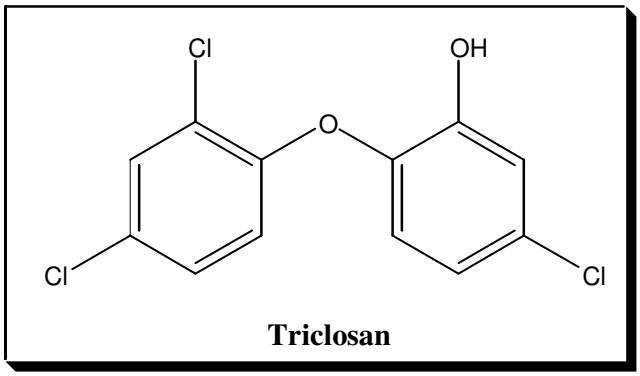
Secondary culture was prepared by taking 1% primary culture in nutrient broth grown under same conditions as that of primary culture. Secondary culture was used for the experiment when its optical density reached 0.5. 1% of secondary culture was inoculated to the each of culture tube having different concentrations of triclosan and surfactant in media. The culture tubes were incubated for 8 hrs and then optical density measured taking Luria Bertani broth as reference.

Dissolution of Antibacterial Agent: A 250 µM triclosan stock solution was prepared in 1 mL of AR grade DMSO. Triclosan was weighed on a glass butter paper and transferred to a 1.5 ml eppendorff and dissolved in 1 mL DMSO. The triclosan stock solution was stored at $4^{o}C$ up to 5 days. All subsequent dilutions of the 250 µM stock solution were prepared using DMSO.

The efficacy of triclosan in presence of various surfactants at different concentrations of the growth medium was determined by broth dilution method. IC_{50} values were calculated by plotting the absorption values at λ_{max} = 600 nM in origin software.

GAPS IN RESEARCH AND OBJECTIVES

Triclosan, a diphenyl ether, is an effective antibacterial agent used in a number of consumer products like mouthwashes, soaps, toys and others. It has been shown to inhibit Fab I, an essential enzyme, present in most of the bacteria thus locking their fatty acid biosynthesis. A number of derivatives of the compound have been synthesized recently that have shown excellent results with the enzyme.



Triclosan

However, when these compounds including triclosan are used for bacterial cells, more quantity of the molecule is required than what is required for the enzyme. This is because of poor solubility of these compounds in aqueous media and their inability to cross biological membranes. A number of surface active agents and organic solvents have been employed to facilitate the molecules reach their target. Recently, it has been observed that such surface active agents and solvents exert their effect on the efficacy of drug [15].

The objective of the present work is to study the effect of different surfactants on the efficacy of triclosan against *Bacillus subtillus*. Different surfactants chosen for the study were Tween-80, Tween-20, Triton X-100, γ-Cyclodextrin, Sodium Deoxycholate and CTAB.

RESULTS AND DISCUSSION

Triclosan is known to be a fatty acid biosynthesis inhibitor and that is why it is in use as an antibacterial agent. It has been reported recently that *Streptococcus agalactiae* can survive presence of triclosan when culture media is supplemented by long chain fatty acids [15]. We therefore chose to study the effect surfactants that contain long hydrocarbon chain (Tween-80, Tween-20, Triton X-100 and CTAB) and compare it with carbohydrate based (γ-Cyclodextrin) and steroid based (Sodium deoxycholate) ones. The bacterial system chosen one was *Bacillus subtillus.* A similar study using *E.coli* has already been carried out in our lab.

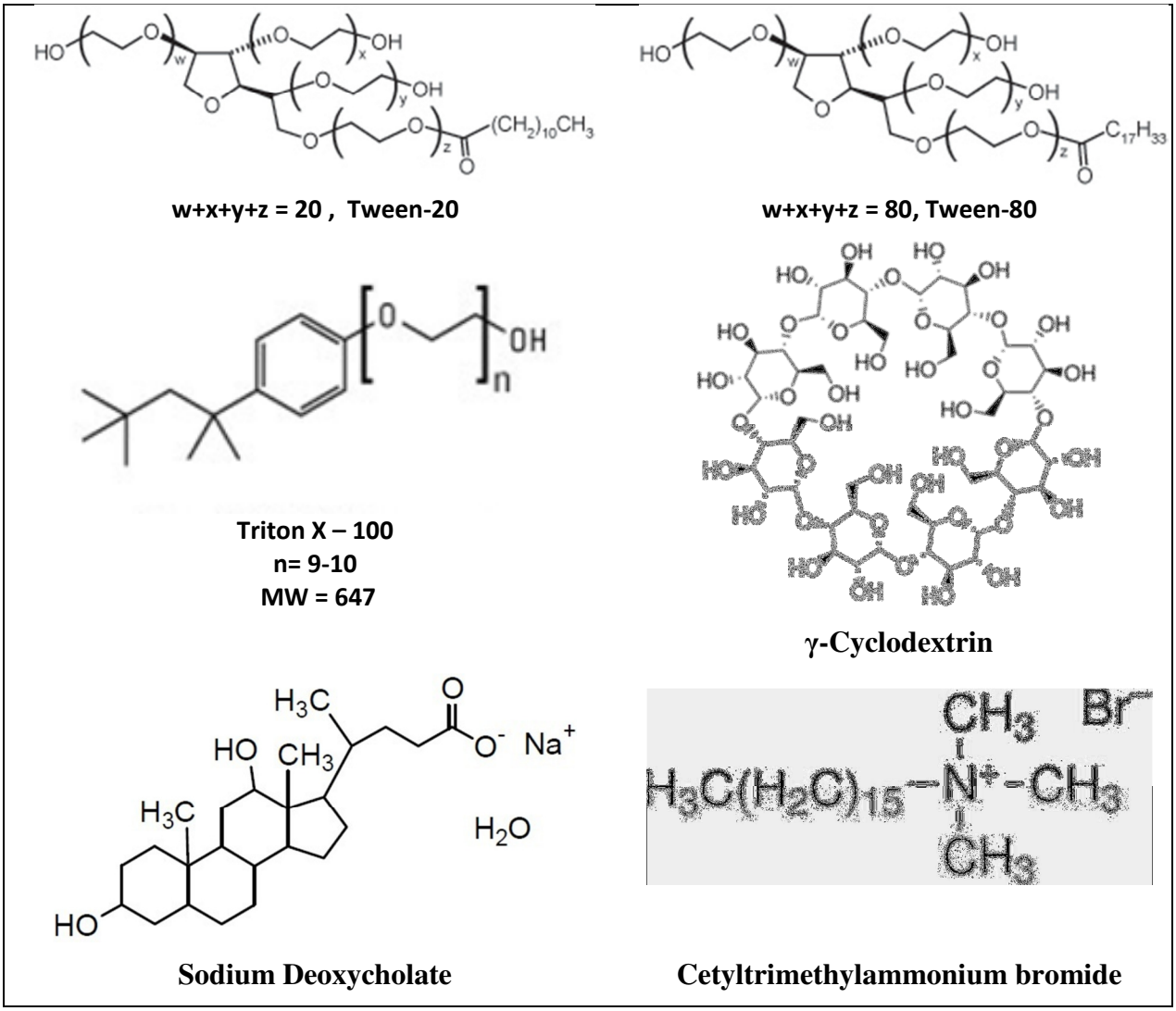


Figure-I : Structure and names of different surfactants used in the study

IC_{50} value of triclosan was determined for all the surfactants at their different concentrations. The criteria for choosing the surfactant concentration were its critical micelle concentration (CMC) values. Most of the surfactant concentrations were around their CMC value because it had been reported that the interiors of the micelles provide a hydrophobic environment to solubilize nonpolar compounds, such as hydrocarbons [21].

Tween-80 with a CMC value of 0.012 mM diminished the effect of triclosan thereby increasing its IC_{50} value from 1.9 µM for the blank to 3.9 µM at 0.25 mM concentration of the surfactant. The CMC and under- CMC (0.006 mM) values of the surfactants had similar influence on the IC_{50} value of the triclosan. **Figure –II (a)** below shows the growth curve for antibacterial activity of the organism at different concentration of the surfactants. The comparison of optical density without triclosan at different concentration of surfactant also showed growth of *subtilis*.

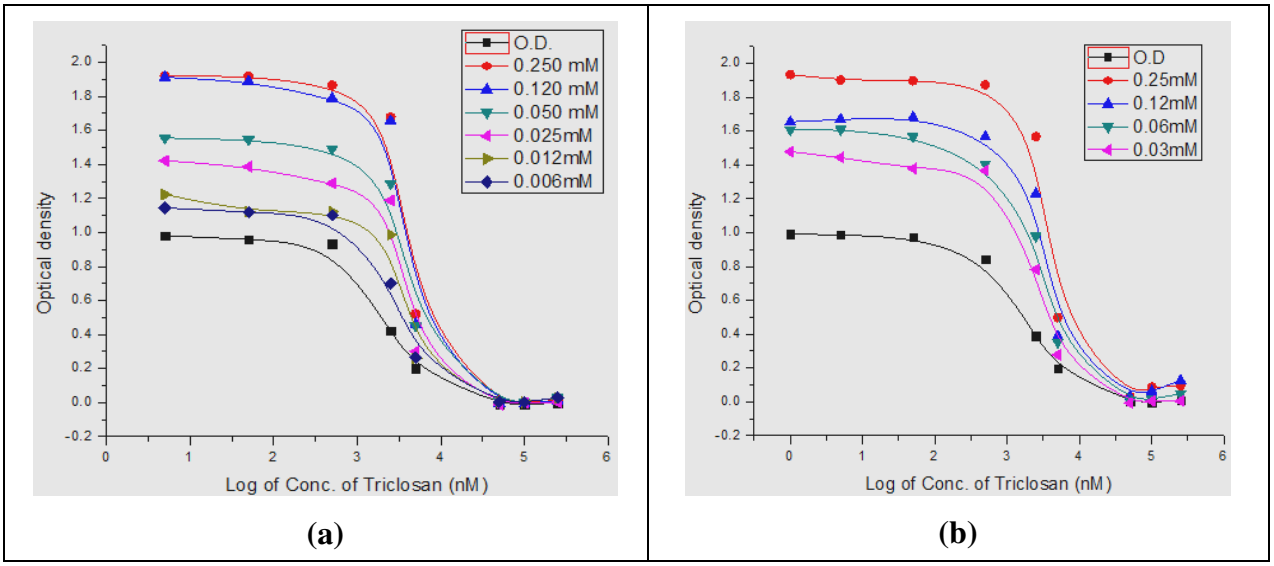


Figure- II : Growth curve of *Bacillus subtillusin* the presence of triclosan at different concentrations of **(a)** Tween-80 and **(b)** Tween-20.

Sr. No.	Tween-80 Conc. (mM)	IC_{50} Triclosan (µM)	Bacterial Growth	OD without Triclosan	
				No Tween-80	With Tween-80
1.	NIL	1.957	-	1.19	-
2.	0.006	3.017	Enhanced	1.16	1.15
3.	0.012	3.680	Enhanced	1.24	1.23
4.	0.025	3.726	Enhanced	1.22	1.49
5.	0.050	3.877	Enhanced	1.12	1.60
6.	0.120	3.902	Enhanced	1.23	1.92
7.	0.250	3.972	Enhanced	1.23	1.93

Table – 1: Effect on Growth of B. subtilis with and without Tween-80

However, this growth was more prominent at higer concentrations of triclosan than at near CMC ones. **Table-1** shows the comparative results for all the experiments done for Tween-80.

Tween-20 with a CMC value of 0.06 mM gave similar trend for IC_{50} value of tricolsan **[Figure-II (b)]** as observed in its higher molecular weight counterpart. Five concentrations used in this case were 0.25 mM, 0.12 mM, 0.06 mM and 0.03 mM. The growth of bacterial cell in media in presence of Tween-20 without triclosan was again in accordance with the amount of surfactant present in the aqueous media i.e. higher was the amount of surfactant higher was the growth in the media **(Table-2)**. Thus, it can be inferred that both Tween-20 and Tween-80 enhances the growth of *Bacillus subtillus.*

Table 2: Effect on Growth of *B. subtilis* with and without Tween-20

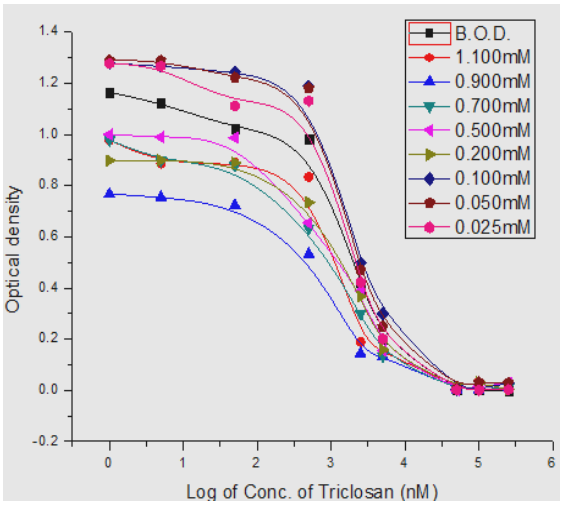
Sr. No.	**Tween-20 Conc. (mM)**	**IC_{50} Triclosan (µM)**	**Bacterial Growth**	**OD without Triclosan**	
				No Tween-20	**With Tween-20**
1.	NIL	1.840	-	0.991	-
2.	0.030	2.701	Enhanced	0.988	1.478
3.	0.060	3.010	Enhanced	0.976	1.607
4.	0.120	3.361	Enhanced	0.988	1.654
5.	0.250	3.677	Enhanced	0.982	1.934

Triton X-100 with a long alkoxy chain on one and hydrocarbon chain on the other side of benzene ring **(Figure-1)** has a wide range of its CMC value (0.2-0.9 mM). The expected results in this case were similar to Tween-80 and Tween-20. However, this surfactant inhibited the growth of bacteria thereby enhancing the activity of triclosan in its CMC range. The IC_{50} value ranged from 0.9 µM to 1.7 µM for a concentration ranging from 0.9 to 0.2 mM. The IC_{50} value of triclosan in this case, as in other cases, was 1.8 µM without any surfactant. Below CMC value that is 0.10 mM the surfactant masked the effect of triclosan as is evident from **Table-3**. Still lower concentrations of the surfactant were not observed in IC50 vale of drug as the values obtained were in comparison with blank sample without surfactant.

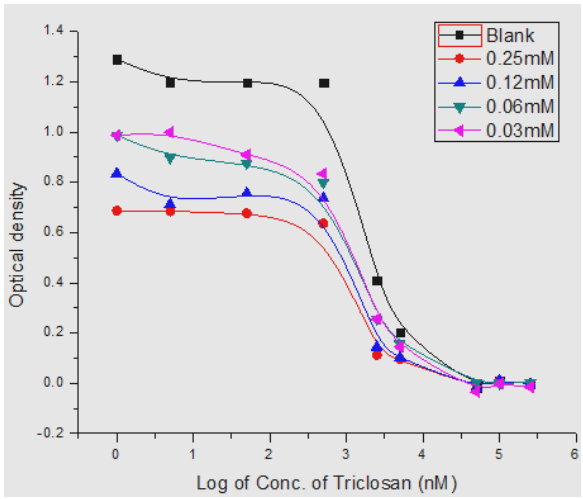
The fact that Triton X-100 is a surfactant that inhibits the growth of bacteria was also proved in experiments done without presence of inhibitor, triclosan. Table-3 below clearly shows decrease in optical density of the culture media when triton-100 is used then when it is not used without inhibitor being introduced in the reaction. Thus, it can be inferred that Triton X-100 is the surfactant of choice for development of commercial products with dual advantage. It increases both the solubility and efficacy of triclosan.

Table 3: Effect on Growth of *Bacillus Subtilis* with and without Triton X-100

Sr. No.	Triton X-100 (mM)	IC_{50} Triclosan (µM)	Bacterial Growth	OD without Triclosan	
				No Triton X-100	With Triton X-100
1.	NIL	1.844	-	1.164	-
2.	0.025	1.805	Same	1.020	1.277
3.	0.050	1.872	Same	1.092	1.289
4.	0.100	2.030	**Enhanced**	1.114	1.278
5.	0.200	1.703	Inhibited	1.102	0.898
6.	0.500	1.149	Inhibited	1.201	0.999
7.	0.700	1.150	Inhibited	1.133	0.978
8.	0.900	0.936	Inhibited	1.145	0.767
9.	1.100	1.338	Inhibited	1.102	0.978



(a)



(b)

Figure- III : Growth curve of *Bacillus subtillusin* the presence of triclosan at different concentrations of **(a)** Triton X-100 **(b)** γ- Cyclodextrin.

γ- Cyclodextrin, a cyclic oligosaccharide, enhanced the effect of triclosan by inhibiting *Bacillus Subtilis*. The concentrations used for this surfactant were from 0.03mM to 0.25mM. All its concentrations exhibited a lower IC_{50} value than that of the blank sample. **Figure-III (b)** shows all the growth curves below blank sample. γ- Cyclodextrin, otherwise also demonstrated decrease in optical density without the use of triclosan. **Table- 4** below shows decreased values of optical density when *Bacillus Subtilis* is grown in the presence of this surfactant without triclosan. There are litrature reports that indicate γ- Cyclodextrin to be a toxic surfactant for

other microorganisms also. Thus, the surfactant always shows enhancement of the IC_{50} vales in combination with triclosan.

Table 4: Effect on Growth of *B. subtilis* with and without γ-cyclodextrin

Sr. No.	γ-Cyclodextrin (mM)	IC_{50} Triclosan (µM)	Bacterial Growth	OD without Triclosan	
				No γ-Cyclodextrin	With γ-Cyclodextrin
1.	NIL	1.863	Expected	1.251	-
2.	0.250	1.052	Inhibited	1.300	0.687
3.	0.120	1.279	Inhibited	1.267	0.834
4.	0.060	1.469	Inhibited	1.216	0.987
5.	0.030	1.466	Inhibited	1.228	0.987

Other two surfactants namely **Sodium deoxycholate** with a CMC value of 2-6 mM and cetyl ammonium bromide, **CTAB**, with CMC value of 1 mM also inhibited the growth of bacteria thereby enhancing the effect of triclosan. Both surfactants exhibited high level of toxicity for *B. subtilis* **(Table-5 and 6).**

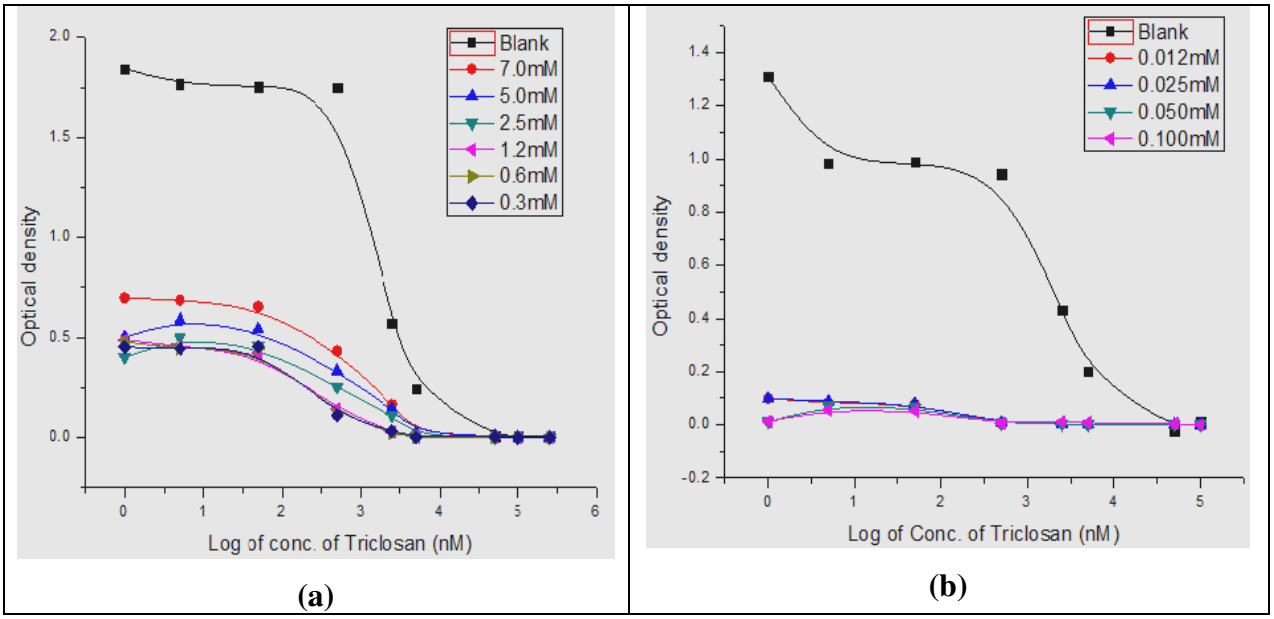


Figure- IV : Growth curve of *Bacillus subtillusin* the presence of triclosan at different concentrations of **(a)** Sodium deoxycholate **(b)** Cetylammonium bromide (CTAB).

Quartnary ammonium salts, that CTAB is, are known to be fantastic antibacterials agents and the IC_{50} values suggest that use of triclosan with CTAB or sodium deoxycholate is waste of the drug. Such quartnary ammonium salts are already in use for many commercial products for both cleanzing and antibacterial use. The growth curve of both the surfactants **(Figure- IV)** well

below the curve of blank and their optical density values in **Table-5** and **6** clearly suggest that theses surfactants cannot be used to study the inhibition of triclosan.

Table 5: Effect on Growth of *B. subtilis* with and without Sodium deoxycholate

Sr. No.	Sodium deoxycholate (mM)	IC_{50} Triclosan (µM)	Bacterial Growth	OD without Triclosan	
				No Sodium deoxycholate	With Sodium deoxycholate
1.	NIL	1.815	-	1.843	-
2.	0.30	0.167	Inhibited	1.823	0.454
3.	0.60	0.249	Inhibited	1.799	0.479
4.	1.20	0.258	Inhibited	1.801	0.489
5.	2.50	0.672	Inhibited	1.855	0.401
7.	5.00	0.274	Inhibited	1.777	0.501
8.	7.00	0.308	Inhibited	1.756	0.698

From the above study, it can be concluded that both Tween-80 and Tween-20 inhibit the activity of triclosan that is both the surfactants aid *B. subtilis* both individually and in combination with triclosan. However, Triton X-100 in its CMC range and above it inhibits bacterial growth individually and in combination with triclosan. This is the surfactant of choice for development of any commercial formulations.

Table 6: Effect on Growth of *B. subtili*s with and without CTAB

Sr. No.	CTAB (mM)	IC_{50} Triclosan (µM)	Bacterial Growth	OD without Triclosan	
				No CTAB	With CTAB
1.	NIL	1.827	Inhibited	1.31	-
2.	0.012	0.124	Inhibited	1.34	0.099
3.	0.025	0.118	Inhibited	1.31	0.099
4.	0.050	0.388	Inhibited	1.26	0.011
5.	0.100	0.181	Inhibited	1.32	0.013

Rest all the surfactants that is γ-Cyclodextrin, Sodium deoxycholate and CTAB inhibit the bacteria themselves and the effect of surfactant alone is more profound in case of Sodium deoxycholate and CTAB. Thus among six surfactants chosen Triton X-100 and γ-Cyclodextrin are surfactants of choice to solubilize triclosan and study their antibacterial activity.

In conclusion, a study was carried out to understand the effect of six different surfactants on the IC_{50} value of triclosan against Bacillus Subtilis. Four of the six surfactants contained long alkyl or alkoxy chains namely, Tween-80, Tween-20, TritonX-100 and CTAB. The other two

surfactants were carbohydrate and steroid based namely γ-cyclodextrin and sodiumdeoxycholate. Tween-80 and Tween-20 enhanced the growth of bacteria in presence of triclosan thereby inhibiting the effect of drug. TritonX-100 marginally inhibited the growth of bacteria around its CMC values there by suggesting it to be the best surfactant among six to dissolve triclosan. All other surfactants themselves inhibited the growth of bacteria and there by masked the effect of triclosan.

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