Evaluation of Novel Quaternary Phosphonium salts Asn an Active Biological Agent Against Microorganism

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Abstract:

In this study, Decyl derivatives (Ia, Ib, Ic), were prepared as cationic biocidal salts that lower the tensioactif and have anti-microbial properties. These salts have single or double alkyl chains with varying lengths (C8= Ia, C12 = Ib, C16 = Ic). Because of their molecular structures, phosphonium salts with long alkyl chains, such as Decyl triphenyl phosphonium bromide C16 = Ic have shown potent antibacterial activity.It demonstrated bactericidal efficacy against the fungi *A. flavus* is (25 mm), *C. albicans* (30 mm), *S. aureus* (35 mm) and *P. aerognosa* (40 mm), grew as the length of the alkyl chain rose at doses of 2.8 and 28 uM, respectively.It has been observed that phosponium salt with the longest alkyl chain (C16) killed all bacterial cells about (110 cells per milliliter) within 30 minutes of contact.The fact that the double decyl groups in the phosphonium biocide demonstrated the broadest range of activity against tested pathogens and the highest bacteriostatic activity against MRSA (MIC = 0.98 ug/ml) is noteworthy.When measuring against the same cationics in copper Cu (II), cobalt Co (II) complexes, it exhibit a significant increase in biocidal activity towards the targeted bacterial strains and fungi, comparing their parent cationic ion to those connected with cobalt, it is evident that the inhibitory zone diameter steadily increases such as fungi A. flavus (41 mm) and C. albicans (35 mm) as well as bacteria found in Pseudomonas aeruginosa (42 mm) and Staphylococcus auerus (60 mm), respectively.

Keyword: Quaternary phosphonium salts, Surface active agent, Biological activity

Introduction:

Surfactants are surface-active agents consisting of double molecules, a non-polar hydrophobic part consisting of a straight or branched hydrocarbon or fluorocarbon chain containing 8-18 carbon atoms and a polar or ionic (hydrophilic) part that gives a special ability for adsorption at the interfaces which acquired them biocidal activity against microorganisms.(1).Tartaric acid polyamide derivatives with ammonium and phosphonium salts showed complex and antimicrobial activity(2).The Phosphonium salts reactions are unstable in chloroform, acetone and methanol but stable in DMSO at ambient temperature (3).The cations are presumed to interact with ioodin to give adduct dissociates into trimethyltin iodide (4).Tributylphosphonium salt prepared from polyamide is characterized by high activity against all Gram-positive and negative bacteria compared to triethyl ammonium and triphenylphosphonium salts prepared from polyamide (5).Cationic phosphonium salts manufactured in a laboratory feature lipophilic molecules that accumulate on the surface of mitochondria and help to kill cancer cells in humans and rodents (6). Microorganisms' cell membranes are made up of many layers of lipids and proteins organized in a particular configuration known as a bilayer (7). The hydrophobic nature of cell membranes is attributed to the lipids that comprise (8).The primary factor regulating biological responses within the cell is the selective permeability of these lipoprotein membranes,therefore every element that affects permeability severely harms the microbe and ultimately results in its death (9).The lower surface tension activity, the greater the bactericidal power. For example, the pentadecyl derivative has a lower surface tension(10). Tetra decyl trimethyl- and di decyl dimethyl phosphonium chlorides killed all S. aureus organisms (ca. 107 cells per ml) within (60 – 30) min of exposure at 28 and 2.8 ,uM, respectively, while tetra decyl trimethyl- and di decyl dimethyl ammonium chlorides which are representative of the existing cationic disinfectants did not kill all the bacteria even at the longest exposure time (120 min) (11). Recently, a facile and efficient biomimetic is synthesis of thiazolines accomplished by treating N-acylated cysteines with bis(triphenyl)oxo-bis-phosphonium trifluoromethane sulfonate (12).The outer wall of living organisms consists of several layers of protein with selective hydrophobic permeability that controls the molecular elements. Therefore, any substance that affects this permeability leads to its death (13). Although quaternary ammonium and phosphonium groups are harmful to the environment and have a limited half-life, they exhibit strong biological activity against microorganisms(14).Polymers carrying ammonium salts showed high activity against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sp*, and *Salmonella typhus*), and Gram-positive bacteria (Bacillus subtillus and Bacillus cereus), giving no results with the fungus (Trichophytum Rubrum (15). The results showed that quaternary phosphonium salt (tetradecyl tributyl phosphonium bromide, TDTB)inhibited the growth of *E. coli* and S. aureus, and the MICs were 200 ± 20 and 80 ± 15. mg/L, respectively. The amount of TDTB released into the broth was 3.42 ± 0.71%.(16). mixed quaternary ammonium and phosphonium salts bound to macromolecular supports for removal bacteria from water, where insoluble carriers could be used in repeated cycles, only it needs sterilization prior to use.These products showed antibacterial activity against (*Staphylococcus auerus*, *Escherichia coli* and *Pseudomonas aeruginosa*(17).Systematically investigated differences in bactericidal activity between the phosphonium salts and ammonium salts having the same hydrophobic structure, it was observed that the phosphonium salts showed an advantage features over corresponding ammonium salts in bactericidal activity and killing rate (18).

Material and Methods:

1. Materials:

2.1.Synthesis of Quaternary Phosphonium Compounds ($I-_{a,b,c}$):

The most common synthesis of quaternary phosphonium salts containing at least one or two alkyl group (19).

$$R_3P + R'X \rightarrow R_3P^+R'X^-$$

R = alkyl or aryl, R' = alkyl, X =halide or other leaving group

The available alkyl halides are generally employed, with order of reactivity being:

RI > RBr > RCl.

Quaternary phosphonium salts are prepared as follows (20):

- Adding equal amounts of triphenylphosphineosyl-tetradecyl- and reflux octadecyl bromide in the presence of acetonitrile as a solvent for 5 hours.
- The reaction mixture was then allowed to precipitate over night.
- After the products were filtered at lower pressure, the residue crystallized when di-isopropyl ether was added.
- vacuum-filtered, dried, and cleaned with di-isopropyl ether.
- Decyl derivative (Ia), tetra decyl derivative (Ib), and octadecyl derivative (Ic) were the designations given to the products shown in table (1) and figure (1).

2.2. Synthesis of of Cu (II) and Co (II) Metal Complexes.

A metal ion solution of anhydrous copper and cobalt in a 2:1 molar ratio,(0.005 mole) in ethanol (50 ml), was added separately to the solution of compounds (Ia-C) (4.84, 5.40, and 5.96 g, 0.01 mole) in ethanol (100 ml). Then the reaction mixtures were left to precipitate overnight.The resulting metal were filtered, thrice with an ethanol /petroleum ether combination (50–50 vol.), and vacuum-dried at 40 ^{o}C (21).

2.3. Biological and Anti-microbial activity:

The agar diffusion method was used for surfactants to detect anti-bacterial and antifungal activities. (22)

2.3.1. Growing of Microorganisms:

The fungal strains were cultivated on malt medium, while bacterial strains were grown on nutrient agar.The broth media were cultured for a full day for bacterial growth . Regarding the fungus, the broth media were incubated for about 48 hours. After that, the culture was filtered through a thin layer of sterile Sintered Glass to get rid of any leftover mycelia, where spore-containing solution was used to inoculate the culture (22).

2.3.2. Bacterial suspensions:

Aseptically placed onto the nutrient agar slope, one-milliliter aliquots of a 24-hour broth culture of Bacillus subtilis (NCTC 8236), Staphylococcus aureus (ATCC 25923), Eschericha coli (ATCC 25923), and Pseudomonas aeruginosa (ATCC 9763) were incubated for a full day at 37°C. After the bacterial growth, it was rinsed and suspended in 100 milliliters of steriel normal saline to yield a suspension with approximately 108–109 colony-forming units per milliliter. Before being used, the suspension was kept in a refrigerator at 4 °C.

2.3.3. Resistance and Susceptibility for bacteria:

50 milliliters of 40°C agar medium were combined with 1.0 milliliter of inocula to prepare the discs and inoculation. After filling 120 mm Petridishes, the agar was allowed to cool to ambient temperature. Using the appropriate sterile tubes, wells (6 mm in diameter) were cut out of the agar plates. then added 0.1 ml of the artificial surfactants dissolved in DMF (1 mg/mL) until the agar was completely filled. After the plates were incubated for 24 hours at 30 degrees Celsius for bacteria and 48 hours for fungi on a level surface, the diameters of the inhibitory zones were measured. The synthesized compounds' anti-bacterial properties were ascertained by the inhibitory zone that these compounds created against the specific strain of bacteria . Each sample's zone of growth inhibition was determined by using the average value acquired from three separate duplicates. The outcomes were contrasted with a comparable batch of commercially available biocide (Glokill-77), used as a benchmark for anti-bacterial and anti-fungal properties. The anti-fungal and anti-microbial activities were determined by calculating the average of three replicates.

2.4. Biocidal activity and surface properties:

The produced cationic triphenyl phosphonium derivatives' biocidal activity is closely correlated with their surface activity. A close examination of the biocidal activity and maximum surface excess values shows that rising ((Γ_{max}) values are correlated with rising biocidal activity. Additionally, reducing the produced cationics' minimal surface area significantly improves their biocidal activity; similar results were found by (23–24). The produced cationic compounds undergo alterations when the adsorption free energy becomes more negative, and this is accompanied by an increase in their biocidal activity.There is an increase in adsorbed cationic surfactant molecules (biocide molecules) at the level of the cellular membrane Furthermore, the robust adsorption of these compounds at the targeted membranes is confirmed by the significant negativity of the typical free energies of adsorption. Biocides' powerful effectiveness against tested bacteria and fungi is enhanced by an increase in their density at the cellular membrane.

2.5.Statistical analysis:

As (25) the utilization of ANOVA for one-way classified data followed by Duncan's multiple range test to enabled the comparison of the significance variances between means at each point in time.

Results and Discussion:

The present study was undertaken to elucidate type of cationic surfactants which were synthesized based on quaternization of triphenyl phosphine with alkyl halides including decyl bromide, tetradecyl bromide and octadecy1 bromide as long chain derivatives contain different kinds of atoms to produce quaternary phosphonium salts (Ia-8 Lb.12 & Ic-16). Shows in table (1) and figure (1&2), but recently synthesis pathway for dimethyl-substituted tertiary phosphines was discovered (26), and a new class of cationic biocides known as phosphonium-salt derivatives has been created using different kinds of phosphonium salts (27).

Table (1): Analysis of Synthesis of quaternary phosphonium salts compounds (Ia-c).

Name	C%	H%	P%	Br%	Chemical formula
Decyl triphenyl phosphonium bromide (Ia)	69.45	6.85	6.39	16.48	**Ia:** $C_{28}H_{36}PBr$
Tetradecyl triphenyl phosphonium bromide (Ib)	71.18	7.62	5.57	14.79	**Ib:** $C_{32}H_{44}PBr$
Octadecyl triphenyl phosphonium bromide (Ic)	72.50	8.20	5.22	13.40	**Ic:** $C_{36}H_{52}PBr$

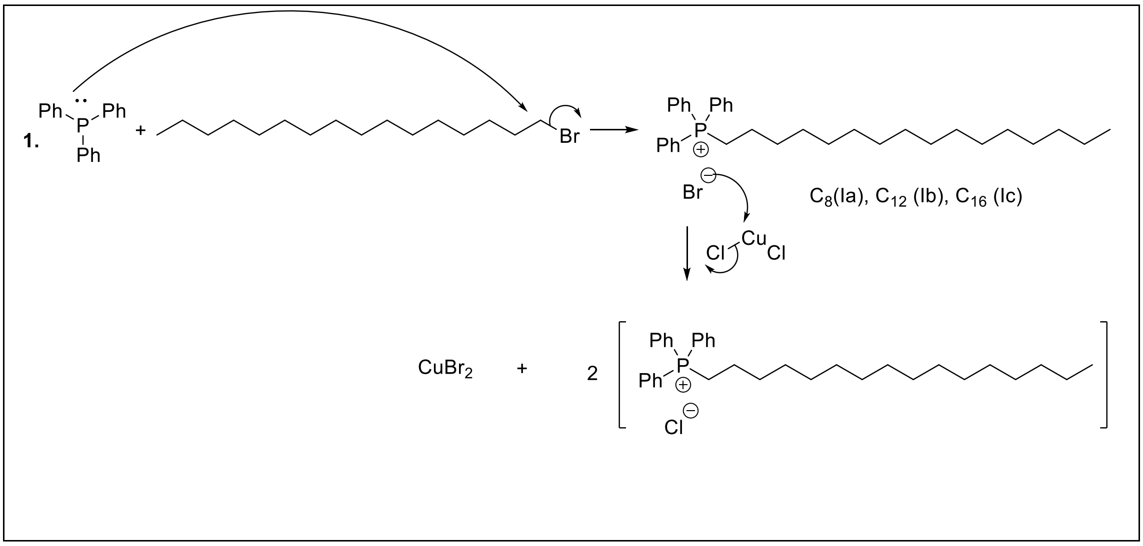

Figure (1). Synthesis of copper (II) bromide phosphonium chloride

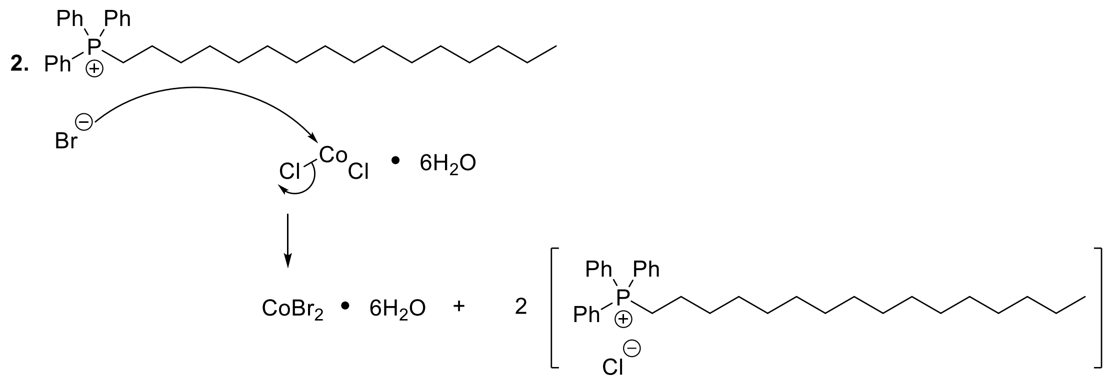

Figure (2). Synthesis of cobalt (II) bromide hexahydrate phosphonium chlori

3.1. Biocidal activity of cationic surfactants (Ia-c):

Shows in Table (2) and figures (3,4&5).The produced cationic quaternary triphenyl phosphonium compounds (Ia-c) have been shown to have anti-microbial activity against a variety of bacterial strains, including gram +ve and gram -ve, as well as fungi. In comparison to the standard (Glokill-77), the inhibition zone widths of the evaluated cationic surfactants range from (16– 40) mm. which gave 18 mm inhibition zone diameter. It is clear that the inhibition zone diameter is increased gradually by increasing the hydrophobic chain length to reach to the maximum diameter by using Ic as biocide represent the bacterial growth and the inhibition zones obtained in case of Pseudomonas aeruginosa (40mm) and for Staphylococcus auerus (35mm) where in fungi A.flavus (25mm),C.albicans (30 mm) respectively.

Table(2): Biocidal efficacy of the synthesized quaternary triphenyl phosphonium salts.

Compound	**Inhibition zone diameter (mm/mg)**			
Organism	***P.aeruginosa*** **(Gram –ve)**	***S.auerus*** **(Gram+ve)**	***A.flavus*** **(Fungi)**	***C.albicans*** **(Fungi)**
Control	0.0	0.0	0.0	0.0
Ia	20	19	16	16
Ib	23	20	17	19
Ic	40	35	25	30
***Glokill-77**	18	18	18	18

Key* Reference

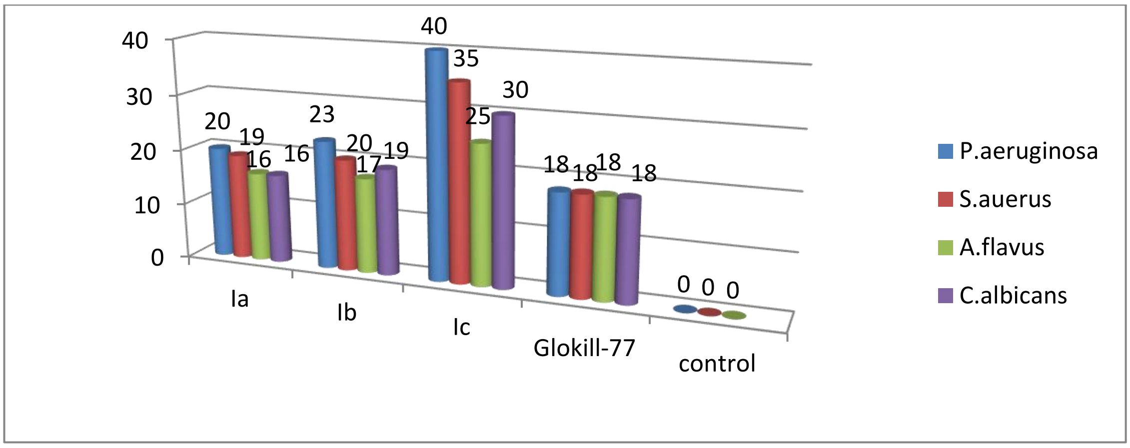

Figure (3): Biocidal effectiveness of the synthesized quaternary triphenyl phosphonium against microorganisms.

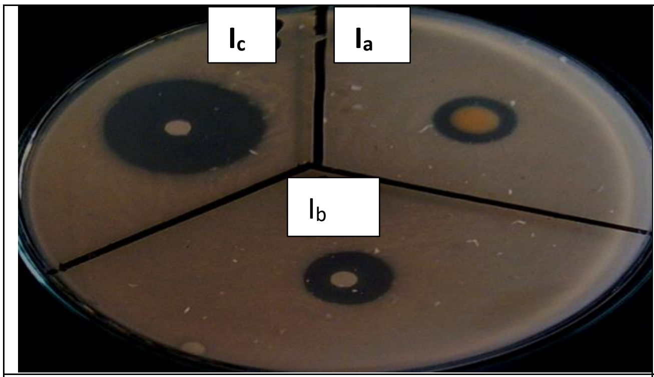

Figure (4): Inhibition zone of *Pseudomonas aeruginosa* in percentage (%) of the parent cationic surfactants.

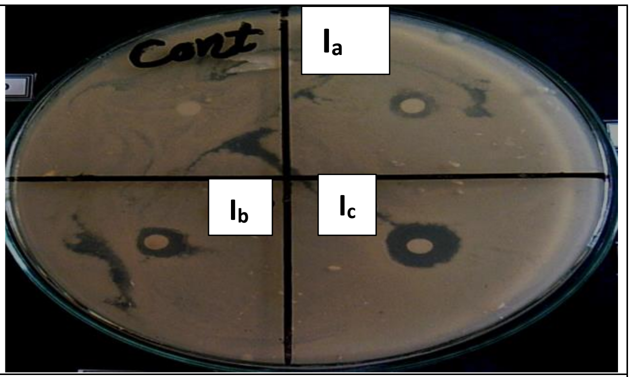

Figure (5): Inhibition zone of *Staphylococcus auerus* in percentage(%) of the parent cationic surfactants

3.2. Biocidal efficacy of metal Cu (II) & cobalt Co (II) complexes resulting from cationic surfactants:

The biocidal activity of the synthesized triphenyl phosphonium cationic surfactants was significantly enhanced when they were connected by copper shown in table (3) and figure (6), where is cobalt shown in table (4) and figure (7) ions against the tested strains of bacteria (gram +ve and gram -ve) and fungi. notice increased trend in the biocidal activities of the metal complexes compared to their parent cationics. The type of metal ions plays an important role in the activity of the different metal complexes. Generally cobalt complexes show a major increase in the biocidal activity towards the targeted bacterial strains and fungi compared to the copper complexes of the same cationics. shown in figure (8&9)represent the inhibition zone against the bacterial growth for complexes of copper and cobalt which synthesized from cationic phosphonium surfactants. It is clear that the inhibition zone diameter is increased gradually when conected with cobalt compared to their parent cationics. to reach to the maximum diameter by using Ic as biocide represent the bacterial growth and the inhibition zones obtained in case of *Pseudomonas aeruginosa* (42mm) and for *Staphylococcus auerus* (60mm) where in fungi *A.flavus* (41mm),*C.albicans* (35 mm) respectively.Copper of *Pseudomonas aeruginosa* (40mm) and for *Staphylococcus auerus* (42mm) where in fungi *A.flavus* (30mm),*C.albicans* (36 mm) respectively. The more biocidal activity of the copper and cobalt complexes of the synthesized cationics is attributed to the large maximum surface excess (Γ_{max}) low or minimum surface area and high depression in their ΔG_{ads}. Almost similar results were obtained by (28) .

Table (3): Biocidal characteristics of copper complexes with cationic triphenyl phosphonium.

Compound	**Inhibition zone diameter (mm/mg)**			
Organism	***P.aeruginosa*** **(Gram –ve)**	***S.auerus*** **(Gram+ve)**	***A.flavus*** **(Fungi)**	***C. albicans*** **(Fungi)**
Control	0.0	0.0	0.0	0.0
I$_a$	20	20	20	19
I$_b$	25	24	23	22
I$_c$	40	42	30	36
Reference*	**18**	**18**	**18**	**18**

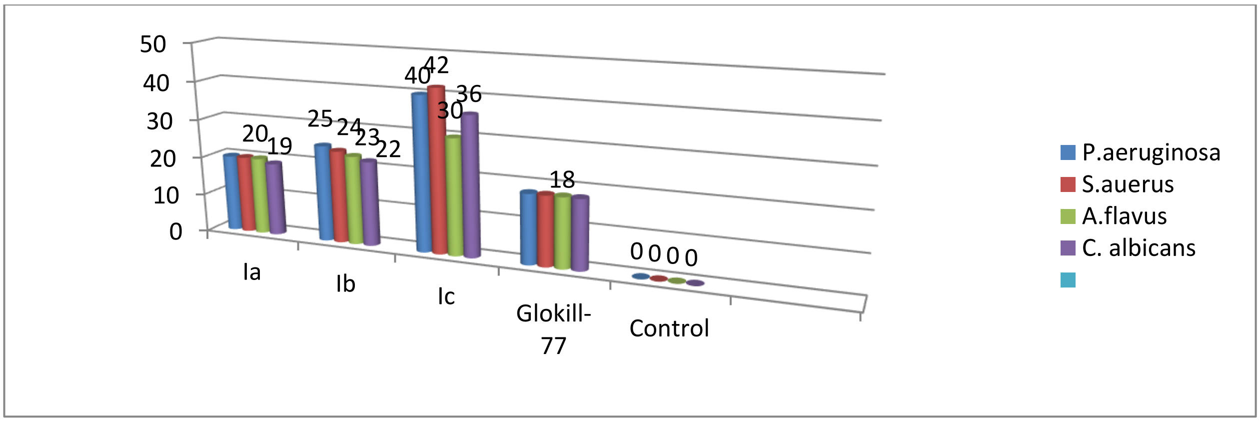

Figure (6): Inhibition zone of microorganisms in percentage (%) of the copper and cobalt complexes of the cationic surfactants.

Table (4): Biocidal characteristics of cobalt complexes with cationic triphenyl phosphonium.

Compound	Inhibition zone diameter (mm/mg)			
Organism	*P. aeruginosa* (Gram –ve)	*S. auerus* (Gram +ve)	*A. flavus* (Fungi)	*C. albicans* (Fungi)
Control	0.0	0.0	0.0	0.0
I_a	21	20	22	19
I_b	25	27	24	24
I_c	42	60	41	35
Reference*	**18**	**18**	**18**	**18**

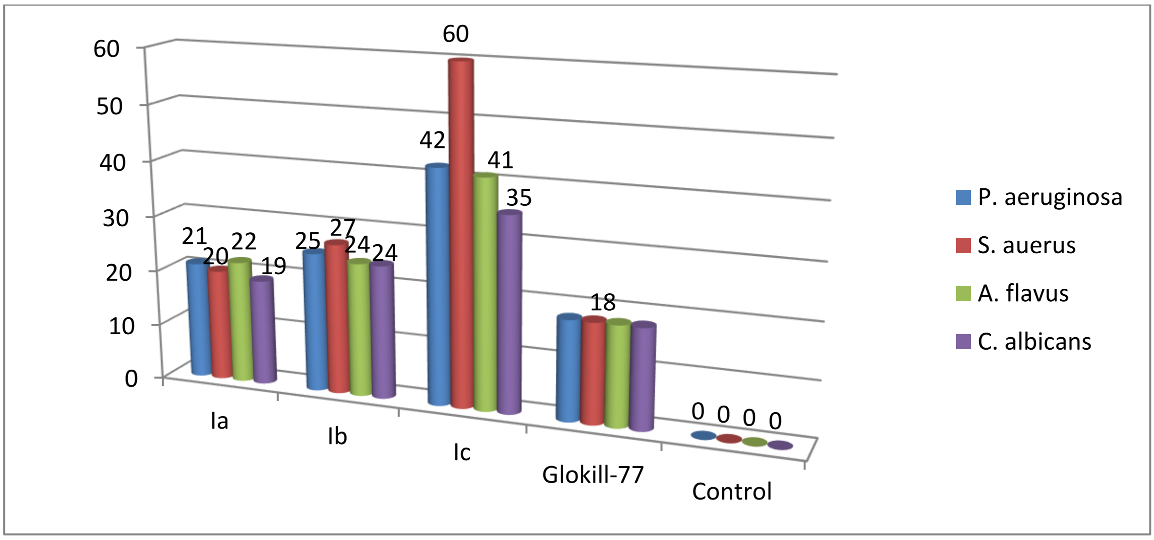

Figure (7): Inhibition zone of microorganism in percentage(%) of the metal complexes(Cu &Co) of the cationic surfactants

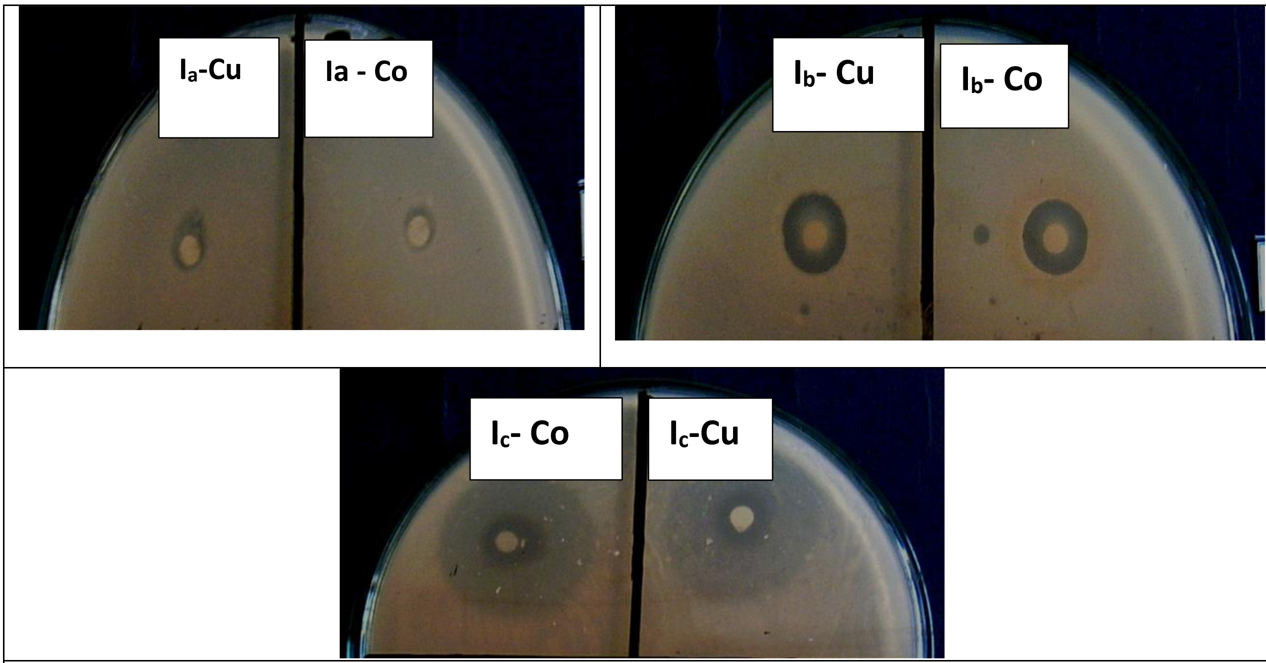

Figure (8): Inhibition zone of *Pseudomonas aeruginosa* in percentage(%) of the metal complexes (Cu &Co) of the cationic surfactants

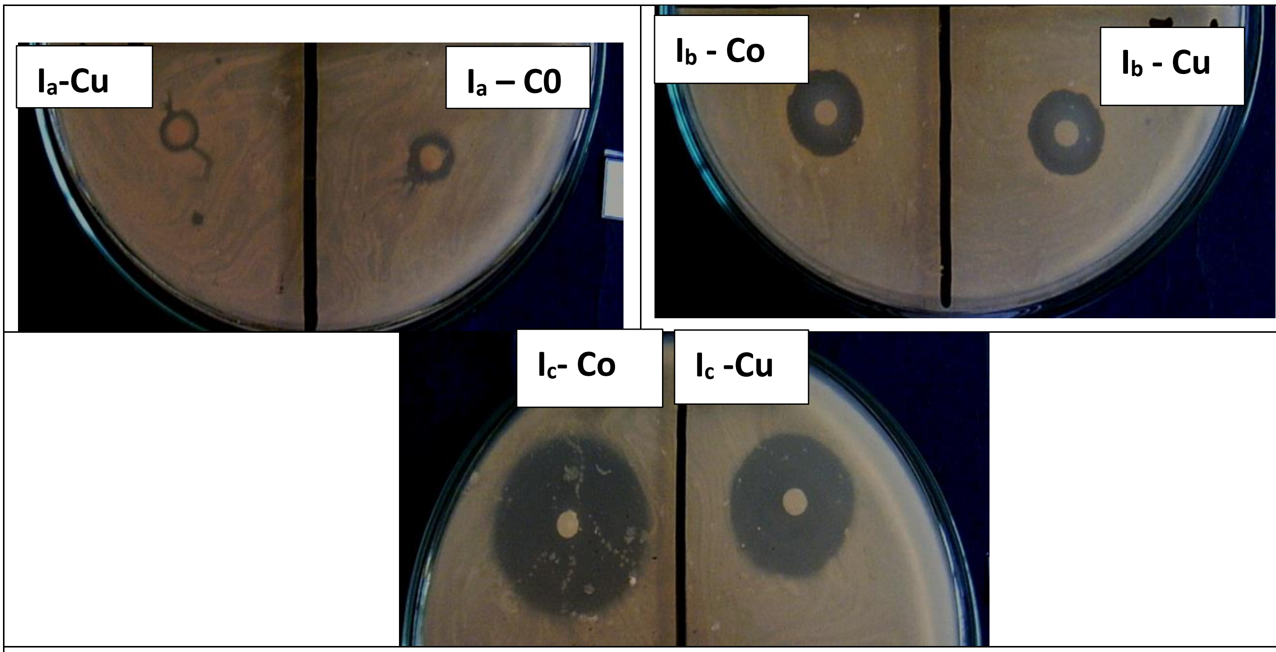

Figure (9): Inhibition zone of *Staphylococcus auerus* in percentage(%) of the metal complexes (Cu &Co) of the cationic surfactants

Conclusion:

In conclusion, decyl derivatives (Ia, Ib, Ic),which have contain single or double alkyl chains with varying lengths (C8= Ia, C12 = Ib, C16 = Ic), have anti-microbial properties. Because of their molecular structures, have shown potent antibacterial activity.It demonstrated bactericidal efficacy against the fungi. Notice that, the surface activity and thermodynamic factor affect the biocidal activity of the quaternary phosphonium-salt-produced derivatives (Ia-c) and their copper and cobalt complexes.Therefore, the combination of two mentioned factors above results in active biocidal action of these chemicals.Also Surface tension measurement showed good surface activity of these compounds in their long chain forms. Meanwhile, shorter chains exhibit lower surface activity.The synthesized cationic surfactants showed biocidal activity towards gram-positive and gram-negative bacteria. Complexion of cationic surfactants with Cu (II), Co (II) always increases their biocides activity.

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