

Antibacterial and Anti-Fungal Activity of Copper and Nickel Nanoparticles Stabilized by Cationic Thiol Polyurethane Surfactants

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Abstract: Metal nanoparticles have attracted considerable interest particularly because of the size dependence of physical and chemical properties and its enormous technological potential. Among different metal nanoparticles, Copper and Nickel nanoparticles have attracted great attention. Grinding method is used to synthesize Copper and Nickel nanoparticles. In this paper, the new cationic Thiol polyurethane surfactants with different alkyl chain length were synthesized (PQ8, PQ10 and PQ12). The chemical structure of the synthesized surfactants was confirmed using infra-red spectroscopy (IR) and proton nuclear magnetic resonance spectroscopy (¹H-NMR). The nanostructure of the synthesized surfactant with Copper and Nickel nanoparticles with diameters ranging from 10 to 55 nm was prepared and characterized using ultra violet spectrophotometer (UV), infra-red spectroscopy (IR) and transmission electron microscope (TEM). The results declare formation and stabilization of Copper and Nickel nanoparticle using synthesized cationic surfactants. Antimicrobial activity of the synthesized cationic surfactants and their nanostructure with Copper and Nickel nanoparticles were evaluated against pathogenic bacteria and fungi. The antimicrobial activity showed the enhancement in the antimicrobial activity of the synthesized cationic surfactants in the nanostructures form.

Keywords: Polyurethane, Cationic Surfactants, Copper Nanoparticles, Nickel Nanoparticles, Antimicrobial Activity, Transmission Electron Microscope

1. Introduction

Copper, Nickel and the compounds of Au, Ag, Pd and Pt are widely used these days. Copper and Nickel have an excellent electrical conductivity. Due to relatively low costs, this metal plays a significant role in modern electronic circuit [1]. Because of its excellent electrical conductivity, catalytic behaviour, good compatibility and surface enhanced Raman scattering activity. Cu and Ni nanoparticles have drawn the attention of scientists to be used as essential component in the future nano-devices [2]. Cu and Ni nanowires are used in nanoelectronics and have application possibilities for magnetic devices, nanosensors, electron emitters and other electronic applications. Cu and Ni nanoparticles have been

explored to be used as nanoprobe in medicines and bio-analytical areas [3-4].

The emergence of nanoscience and nanotechnology in the last decade presents opportunities for exploring the bactericidal effect of metal nanoparticles. The bactericidal effect of metal nanoparticles has been attributed to small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and not merely due to the release of metal ions in solution [5].

In the present study stable Copper and Nickel nanoparticles with narrow size and homogenous distribution were synthesized by grinding method and cationic Thiol polyurethane surfactants used as a capping agent. Even today, the exact mechanism of antimicrobial

action of the CuNPs remains unknown. The general view seems to be a combination of several factors: releasing Cu^{+2} ions, their penetration and disruption cell membrane and biochemical pathway by chelating cellular enzymes and DNA damage [6-7].

Previously reported antibacterial activity of Copper nano particle, it was found that it has significant potency to act as bacteriocidal agent than gold, silver, zinc nano particles. Combination of different Nano particles such as Silver and Copper may show more significant effect on bacterial growth. Gram-positive bacteria have a thick cell wall containing multiple layers of peptidoglycan, while gram-negative bacteria have a relatively thin cell wall consisting of a single layer of peptidoglycan. Surfaces of copper nano particles interact directly with the bacterial cell wall & outer membrane, leads to rupture of cell wall and killing bacteria [8-9].

2. Experimental Methods

2.1. Materials

Fatty alcohols (Octanol, Decanol and Dodecanol) were purchased from Sigma, Germany. Toluene diisocyanate TDI (97%) was purchased from DOW, USA. Mercapto acetic acid, Bromoacetic acid and Sodium borohydride were purchased from Aldrich, Germany. Copper Chloride and Nickle chloride were purchased from BDH, British. Triethanol amine and solvents were obtained from ADWIC chemicals company, Egypt.

2.2. Synthesis

2.2.1. Preparation of Triethanol Amine Mono Mercaptoacetate

Triethanol amine (0.1 mole) and mercaptoacetic acid (0.1 mole) were charged in 250 mL round flask in presence of xylene (75 mL) as a solvent and *p*-toluene sulfonic acid (0.1 g) as a dehydrating agent. The completion of the reaction was monitored by obtaining 0.1 mole of H_2O (1.8 mL) [10]. At the end of the reaction, the solvent was removed by evaporated by the effect of evacuation, while *p*-toluene sulfonic acid was eliminated from the reaction medium by take out the obtained esters by dissolving in ether (diethyl ether). The evaporated solvent was recovered and purified to reuse. The unreacted and excess reactants were eliminated from the products by successive sanitization of the crude products to afford triethanol amine mono mercaptoacetate in a yield of 96%.

2.2.2. Preparation of Thiol Polyurethane (P)

Thiol polyurethane polymerization reaction was carried out in a suitable flat bottom glass reactor (500 mL) connected to a mechanical rotor, dropping funnel, thermometer and

condenser. Inside the reaction vessel, a mixture of toluene diisocyanate (TDI, 0.1 mole) dissolved in methyl ethyl ketone (50 mL), triethanol amine mercaptoacetate (0.2 mole) and five drops of triethylene diamine dropped during 20 min was mixed [11]. The reaction medium was continuously mixed at 30°C for 30 min to obtain the pre-polymer. The ratio of isocyanates (NCO) to reactive hydroxyl group (OH) was reserved at 1:2 in polyurethane reaction polymerization. The prepared polymer was washed twice using appropriate amounts of methyl ethyl ketone and finally dried (yield 92%).

2.2.3. Preparation of Fatty Esters Bromoacetate (Q8, Q10 and Q12)

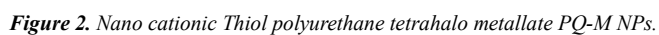
Fatty esters bromoacetate (Q8, Q10 and Q12) were prepared throughout reacting different fatty alcohols (0.1 mole) namely: Octanol, Decanol and Dodecanol and bromoacetic acid (0.1 mole) in the presence of xylene (100 mL) as a solvent and *p*-toluene sulphonic acid (0.01 wt%) as dehydrating agent under heating and stirring conditions until the expected amount of water (1.8 mL) is produced. At the end of the reaction, xylene was stripped off using reduced pressure; *p*-toluene sulphonic acid was eliminated by extracting the product from diethyl ether and the solvent was removed [12] to afford the different fatty esters bromoacetate (yield 95-98%).

2.2.4. Preparation of Cationic Thiol Polyurethane Surfactants (PQ10, PQ12 and PQ14)

Cationic Thiol polyurethane surfactants were prepared by refluxing equimolar amounts of Thiol polyurethane (P) and octyl, decyl, dodecyl esters (Q8, Q10 and Q12) individually in a suitable amount of dimethyl formamide (DMF) for 20 h. The produced compounds were filtered off, washed by excess DMF, and dried under reduced pressure at 50°C to afford yellow to brown viscous liquids designated as: PQ8, PQ10 and PQ12 (yield 82-89%) [13, 14] (Figure 1).

2.2.5. Synthesis of Nano Cationic Thiol Polyurethane Tetrahalo Metallate PQ-M NPs

By ball mill grinding (0.1 mole) of Copper chloride dihydrate $[\text{CuCl}_2 \cdot 2\text{H}_2\text{O}]$ and Nickel chloride hexahydrate [after heating the hexahydrate in the range 66-133°C gives the yellowish dihydrate, $\text{NiCl}_2 \cdot 2\text{H}_2\text{O}$] with (0.2 mole) of cationic Thiol polyurethane surfactants in the mortar until all components mixed well. After then ethanoic solution of the previous mixture was refluxed under magnetic stirring for about 2 hours to produce tetra halo metallate complexes and this indicate by change the color of mixture. The product was poured into a flat plate and dried in the hood, washed with alcohol twice and dried. The product is believed to have the structure in (Figure 2) [15, 16].



Elemental analysis: Vario Elementar Analyzer; FTIR

bromide compressed thin pellet technique. The nuclear magnetic resonance spectra were measured by Varian NMR-300, Mercury 300 MHz spectrometers using CDCl_3 as solvent and trimethyl silane (TMS) as a reference to determine the different chemical shifts $\delta(\text{ppm})$; GPC measurements were performed using GPC-7890A instrument equipped with DB-23 column, 60 mm x 0.25 mm, i.d. of 0.25 μm . TEM images were performed using TEM-JEOL JEM-2000, Germany.

2.4. Biological Activity

The antimicrobial activity of synthesized cationic Thiol polyurethane surfactants and their nanostructures with copper nanoparticles were measured against a wide range of test-organisms comprising: (bacteria and fungi)

2.4.1. The Media

The following media used in the antimicrobial activity of synthesized products, the bacterial species grow on nutrient agar, while fungi mold grow on Czapek's dox agar. (a) Nutrient agar: Nutrient agar consists of Beef extract (3.0 g/l); peptone (5.0 g/l), sodium chloride (3.0 g/l) and agar (20.0 g/l), then, completes the volume to 1 l, heated the mixture until the boiling and sterilizes the media by autoclave. (b) Czapek's Dox agar: Czapek's Dox agar consists of sucrose (20.0 g/l), sodium nitrate (2.0 g/l), magnesium sulfate (0.5 g/l), potassium chloride (0.5 g/l), ferrous sulfate (0.01 g/l) and agar (20.0 g/l), then, complete the volume to 1 l, heated the mixture until the boiling, and sterilize the media by autoclave.

2.4.2. Microorganisms

The different species of tested organisms were obtained from the unit of Operation Development Center, Egyptian Petroleum Research Institute. The used microorganisms were Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus*

aureus), Gram-negative bacteria (*Salmonella typhimurium* and *Escherichia coli*), Yeast and Fungi (*Candida albicans* and *Aspergillus niger*). An assay is made to determine the ability of an antibiotic to kill or inhibit the growth of living microorganisms, the technique which used is: filter-paper disk-agar diffusion (Kirby-Bauer) are as the following [17]:

1. Inoculate flask of melted agar medium with the organism to be tested.
2. Pour this inoculated medium into a Petri dish.
3. After the agar has solidified, a multilobed disk that impregnated with different antibiotics laid on top of agar.
4. The antibiotic in each lobe of disk diffuses into medium and if the organism is sensitive to a particular antibiotic, no growth occur in a large zone surrounding that lobe (clear zone).
5. The diameters of inhibition zones were measured after 24–48 h at 35–37°C (for bacteria) and 3–4 days at 25–27°C (for yeast and fungi) of incubation at 28°C.
6. Measure each clear zone and compare between them to determine the antibiotic which is more inhibitor.

3. Results and Discussion

3.1. Chemical Structure

Figure 1 represents the preparation of polyurethane cationic surfactants.

Figure 2 represents the preparation of Nano cationic Thiol polyurethane tetrahalo metallate.

The elemental analysis of the prepared cationic polyurethane surfactants (Table 1) showed the comparable values of the predicted and obtained ratios of the different elements in their chemical structure. The chemical structures of the prepared cationic polyurethane surfactants were confirmed using molecular weight measurements, elemental analysis, FTIR spectroscopy and ^1H -NMR spectroscopy.

Table 1. Elemental analysis of the synthesized cationic Thiol polyurethane surfactants.

Compound	M.wt [*] g/mole	Formula	C%		H%		N%		S%		Br%	
			Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
PQ8	20500	$(\text{C}_{45}\text{H}_{78}\text{O}_{14}\text{N}_4\text{S}_2\text{Br}_2)_n$	46.47	46.42	6.65	6.66	5.56	5.53	6.35	6.30	15.89	15.85
PQ10	21400	$(\text{C}_{49}\text{H}_{86}\text{O}_{14}\text{N}_4\text{S}_2\text{Br}_2)_n$	48.54	48.50	7.05	7.06	5.27	5.25	6.02	5.98	15.05	15.01
PQ12	22300	$(\text{C}_{53}\text{H}_{94}\text{O}_{14}\text{N}_4\text{S}_2\text{Br}_2)_n$	50.36	50.31	7.41	7.43	5.00	4.97	5.71	5.68	14.28	14.25

* Obtained molecular weight from GPC measurements, $n \approx 18$.

3.1.1. FTIR Spectroscopic Analysis

The chemical structures of the prepared cationic surfactants and their intermediates were confirmed using FTIR spectroscopic analysis.

FTIR spectra of Triethanol amine mercaptoacetate showed the following absorption bands: broad absorption band centered at 3435 cm^{-1} corresponds to -OH stretching; weak absorption band at 2550 cm^{-1} corresponds to stretching of S-H group; absorption band at 2932 cm^{-1} attributed to symmetric stretching of C-H group; 1018 cm^{-1} corresponds to C-N stretching group of aliphatic amine and absorption band at 1732 cm^{-1} corresponds to C=O ester group.

Thiol polyurethane compound (P) showed the following absorption bands: 1663 cm^{-1} corresponds to C=O of urethane group; 1458 and 1462 cm^{-1} corresponds to N-H of urethane group; 1508-1510 cm^{-1} described for the double bonds in the phenyl moiety (C=C) in toluene diisocyanate moiety (TDI).

FTIR spectra of alkyl bromoacetate esters (Q8, Q10 and Q12) represented the disappearance of the broad band at 3400 cm^{-1} of alcoholic hydroxyl groups of the reacted fatty alcohols, the appearance of new absorption bands at: 1736-1738 cm^{-1} corresponds to carbonyl ester indicates the ester (Q8, Q10 and Q12) formation; 1275-1277 cm^{-1} corresponds to ether linkages C-O-C; 2920-2922 cm^{-1} and 2849-2850 cm^{-1} corresponded to symmetric and asymmetric stretching

of C-H groups. The characteristic absorption band of C-Br bond was appeared in the range of 663-667 cm^{-1} .

FTIR spectra of the prepared cationic polyurethane surfactants showed similar absorption bands to the absorption bands of triethanol amine mercaptoacetate, thiol

polyurethane and alkyl bromoacetate esters. Furthermore, IR spectra recorded a disappearance of the absorption band at 660 cm^{-1} and the appearance of a new absorption band at 3040 cm^{-1} corresponded to C-N+ group (Figure 3).

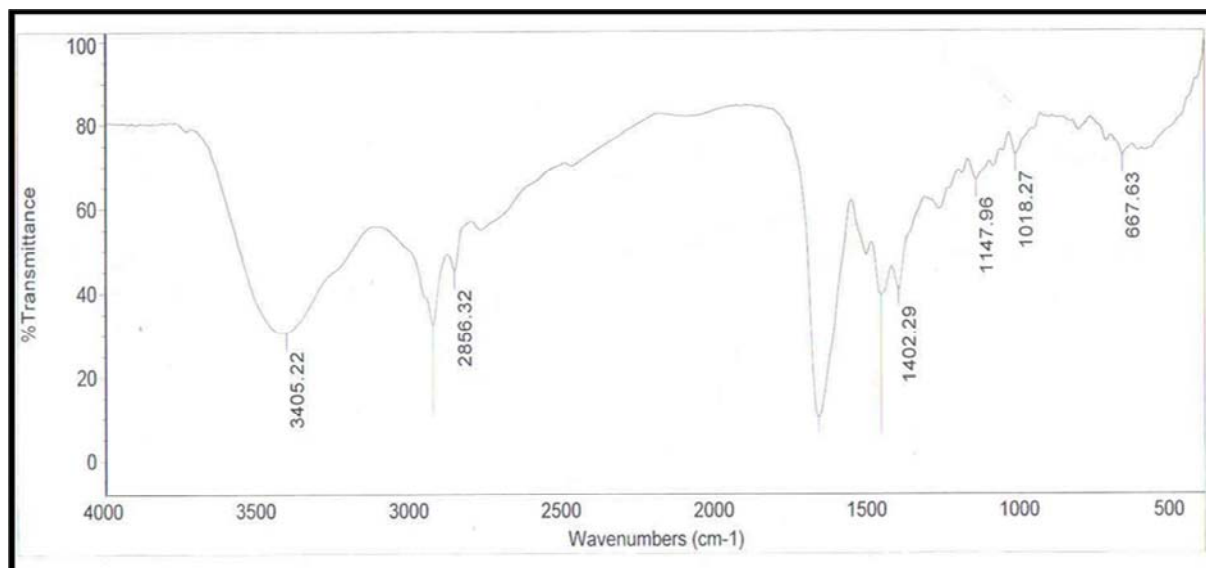


Figure 3. IR spectra of cationic Thiol polyurethane surfactant PQ10.

3.1.2. $^1\text{H-NMR}$ Spectroscopic Analysis

The $^1\text{H-NMR}$ spectra of the synthesized cationic surfactants (Figure 4 for PQ12 as representative for the prepared surfactants) showed the appearance of signals at: $\delta = 0.85$ ppm (t, 3H, CH_3) assigned to terminal methyl group;

1.25 ppm (m, nH, CH_2) attributed to the methylene groups of the fatty chains; 2.5 ppm (t, 3H, CH_3Ph) assigned to methyl group of toluene diisocyanate moiety; 7.8 ppm, 8.0 ppm and 8.2 ppm (m, 4H, phenyl group).

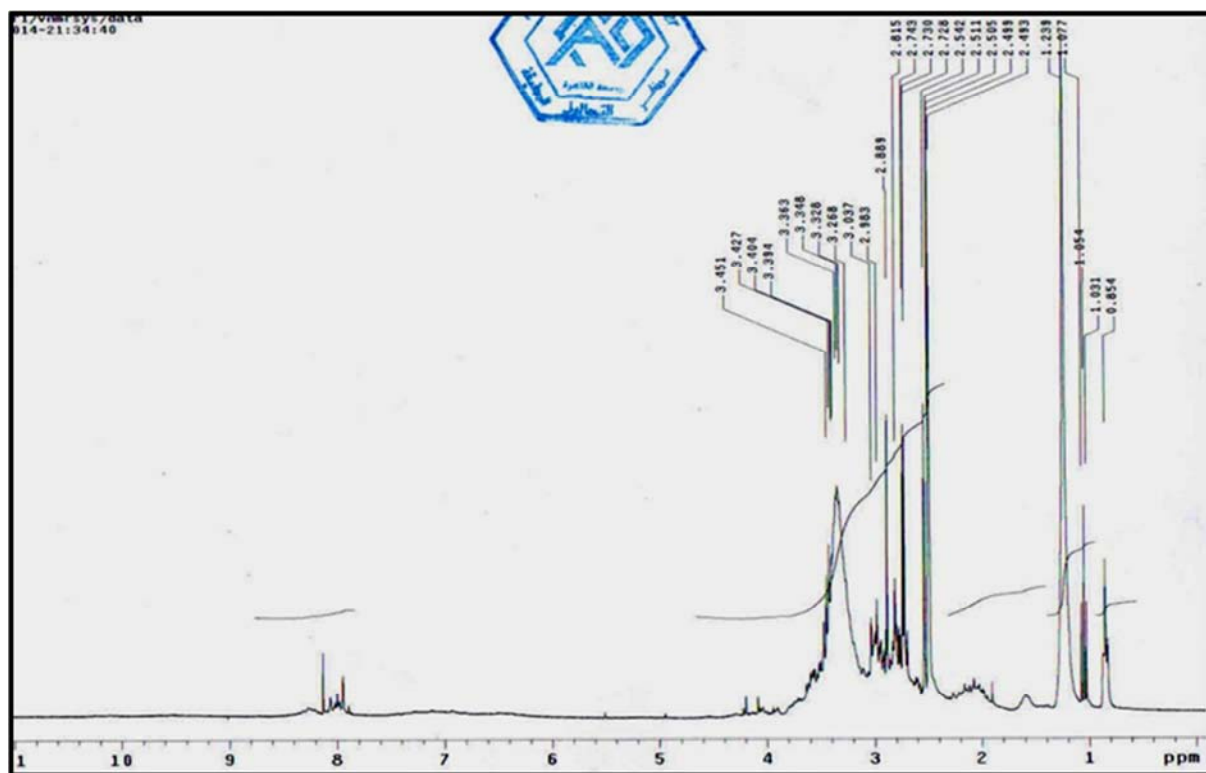


Figure 4. $^1\text{H-NMR}$ spectra of cationic Thiol polyurethane surfactant PQ12.

3.1.3. Molecular Weight Measurements

The molecular weights of the prepared cationic surfactants were determined using GPC chromatographic measurements according to the methodology of our colleague [18]. The results showed that the surfactant molecules contain an average of 18 repeated units (exactly 17.8 units). The obtained values of the molecular weights of the different surfactants were listed in Table 1.

3.1.4. UV-Vis Spectroscopy

UV-Vis absorption spectra were used to determine the formation of copper and nickel nanoparticles stabilized by different cationic Thiol polyurethane surfactants. UV-Vis absorption spectra of the prepared Copper and Nickel nanoparticles stabilized by different cationic Thiol polyurethane surfactants were recorded in water as a solvent in order to monitor their formation and stability. Cationic Thiol polyurethane surfactants with Copper and Nickel nanoparticles were confirmed by the appearance of new bands in UV spectra. UV-Vis spectroscopy is quite sensitive to the formation of Copper and Nickel nanoparticles due to surface Plasmon excitation.

UV spectrum of Copper nanoparticles for characterizing the metallic nature whose broad peak corresponds to the Cu range from 350–550 nm [5].

UV spectrum of Nickel nanoparticles for characterizing the metallic nature whose broad peak corresponds to the Ni range from 250–370 nm [19].

UV-Vis spectra shows absorption spectra of Cu NPs and Ni NPs capped by prepared cationic surfactants, which show absorption band listed in table 2 which an indication on formation Copper and Nickel nanoparticles, due to surface Plasmon resonance of Copper and Nickel nanoparticles.

Table 2. UV absorption maxima of Copper and Nickel nanoparticles with Cationic Thiol polyurethane surfactants.

Compounds	λ_{\max} (nm)
PQ8-Cu NPs	314, 389
PQ10- Cu NPs	310, 400
PQ12- Cu NPs	310, 402
PQ8-Ni NPs	224, 296
PQ10- Ni NPs	220, 316
PQ12- Ni NPs	210, 308

Bands at λ_{\max} range from 210 to 314 nm characteristic for the used capping agents, which matches with the band, appeared for aqueous solution of the used capping agents alone. It is known that the amount and size of Copper and Nickel nanoparticles are positively related with the adsorption peak intensity and the λ_{\max} on the UV-Vis spectra respectively.

3.1.5. TEM Spectroscopy

TEM spectroscopy determines the size and morphology of the formed nanoparticles. The size and morphology of the prepared Copper and Nickel nanoparticles stabilized by cationic Thiol polyurethane surfactants were investigated using transmission electron microscope (TEM), Figures 5 and 6 (as representative for the prepared Copper and Nickel nanoparticles). It is clear from TEM images that the Copper and Nickel nanoparticles stabilized by cationic Thiol polyurethane surfactants are predominantly spherical in shape and polydispersed. The TEM image showed the self-assembling of the prepared surfactant on the Copper and Nickel nanoparticles which causes the stabilization of the nano size of these nanoparticles due to the formation of nano shells with the used surfactant.

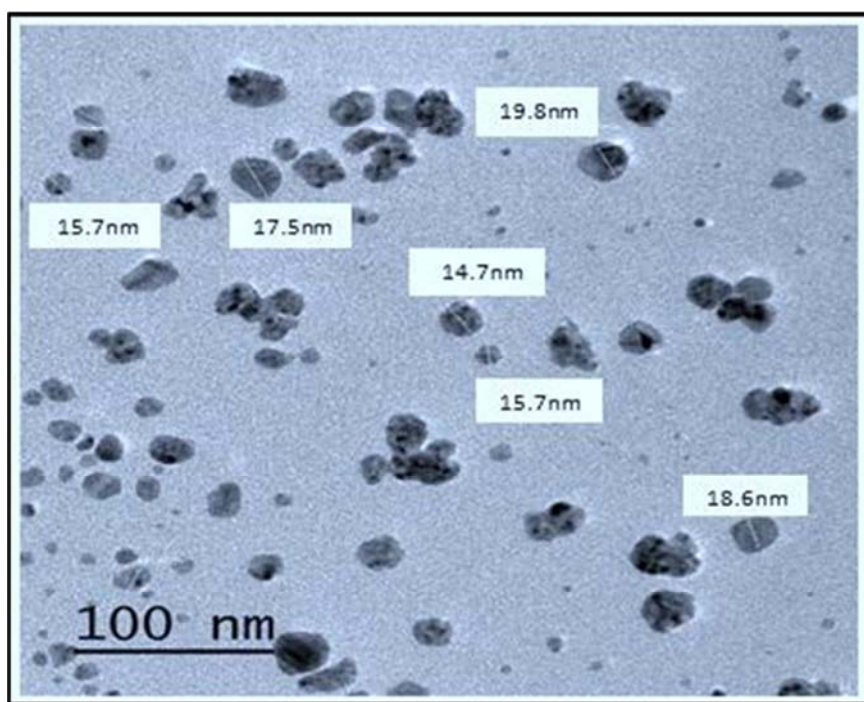


Figure 5. TEM images of Cu NPs with PQ12.

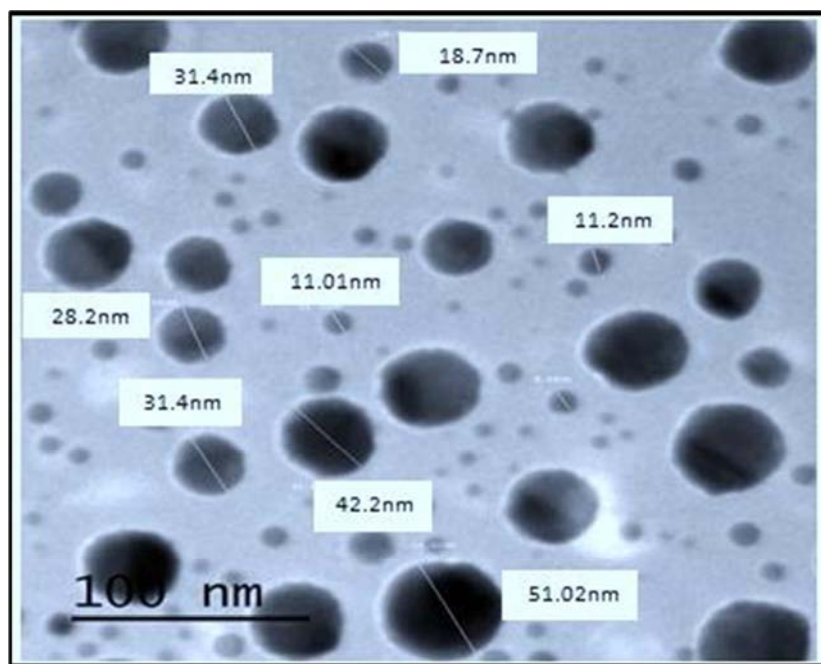


Figure 6. TEM images of Ni NPs with PQ12.

3.2. Antimicrobial and Antifungal Activity of the Synthesized Cationic Surfactants and Their Nanostructures

The biological activities of the synthesized cationic surfactants (PQ8, PQ10 and PQ12) and their nanostructures with Copper and Nickel nanoparticles was screened against pathogenic Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative (*Salmonella typhimurium* and *Escherichia coli*), bacteria and also, some pathogenic fungi (*C. albicans* and *Aspergillus niger*) using the values of the inhibition zone diameter tests and the results are summarized in Table 3. Data in Table 3 indicating that the synthesized compounds have antimicrobial activity rang from

a moderate to slight high effect on Gram negative bacteria and Gram positive bacteria and moderate effect on fungi and high effect on yeast compared to the drug reference used.

It is clear that the antimicrobial activities are gradually increased by increasing the hydrophobic chain length. The PQ12 surfactant showed the maximum antimicrobial activities against the tested bacterial strains. That behavior is depending on the surface activities of these biocides. Increasing the hydrophobic chain length increases the adsorption tendency of the biocide molecules at the various surfaces (water or microorganism's membranes). Hence, the potent action of the molecules is increased due to their high population at the cellular membrane [20, 21].

Table 3. Antimicrobial activity of synthesized surfactants against pathogenic bacteria, yeast and fungi.

Compounds	Bacteria				Yeast	Fungi
	Gram positive		Gram negative			
	Bacillus subtilis	Staphylococcus aureus	Salmonella typhimurium	Escherichia coli		
Control	26	25	28	27	28	26
PQ8	15	0	0	10	17	0
PQ10	17	9	0	14	19	0
PQ12	22	14	0	16	24	0
PQ8-Cu NPs	20	20	17	18	15	0
PQ10-Cu NPs	29	21	21	26	18	16
PQ12-Cu NPs	35	41	33	32	23	16
PQ8-Ni NPs	23	26	22	21	23	16
PQ10-Ni NPs	27	29	27	25	24	14
PQ12-Ni NPs	32	25	34	20	25	18

By inspection data in Table 3, the biological activity of Copper and Nickel nanoparticle stabilized by synthesized cationic surfactants higher than corresponding synthesized cationic surfactants, this can be attributed to Copper and Nickel nanoparticle alone have biological activity, so prepared surfactant capped Copper and Nickel nanoparticles

have higher activity, this can be attributed to the higher surface area of prepared nanoparticles. In addition to the bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution.

A cell wall is present around the outside of the bacterial cell membrane and it is essential to the survival of bacteria.

4. Conclusion

The results of UV spectra and TEM indicate formation and stabilization of Copper and Nickel nanoparticle capped by synthesized cationic surfactants.

The synthesized cationic Thiol polyurethane surfactants and their nanoparticles were tested against different strain of bacteria using inhibition zone diameters and showed good antimicrobial activities against the tested microorganisms. Antibacterial and antifungal activity of the parent cationic Thiol polyurethane surfactants exhibited high efficiency against the tested microorganisms compared to the drug reference used. The antibacterial and antifungal activity of the synthesized compounds can be referred to several factors including chemical structure factors and surface factors. The antibacterial and antifungal activities are gradually increased by increasing the hydrophobic chain length of the synthesized compounds. That behavior is depending on the surface activities of these biocides. Increasing the hydrophobic chain length increases the adsorption tendency of the biocide molecules at the various surfaces (water or microorganism's membranes). Hence, the potent action of the molecules is increased due to their high population at the cellular membrane. Copper and Nickel nanoparticles capped by cationic Thiol polyurethane surfactants have higher antibacterial and antifungal activity than the parent cationic surfactants; this can be attributed to the higher surface area of prepared nanoparticles and their small size and high surface to volume ratio. In addition copper and nickel nanoparticles alone have biological activity.

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Biography



Mostafa Eid Hendawy is a Ph.D. student working as a chemist in the Foam Factory. His interests are in synthesis and applications of environmentally friendly compounds and their uses as biocides.



Mohamad Fahmy Zaky is a professor in the Petrochemicals Department of the Egyptian Petroleum Research Institute. He received his Ph.D. for a thesis in the field of surfactant applications from Ain Shams University, Cairo, Egypt in 2005. He is interested in surfactants applications, mainly corrosion and biological activity.