DJS Vol. 40 (2019) 69-75

Delta Journal of Science

Available online at
https://djs.journals.ekb.eg/

Research Article

CHEMISTRY

Synthesis and Antibacterial Activity of Schiff base Compounds Based on Poloxamine

El-Refaie Kenawy, Mohamed M. Azaam* and Samar A. Khattab

Polymer Research Group, Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt

* ***Correspondence****: M. M. Azaam; Polymer Research Group, Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt; Email:* Mohmed.azam@science.tanta.edu.eg

KEY WORDS

Poloxamine;
Schiff base;
Antimicrobial
Polymers

ABSTRACT

The hydroxyl end groups of Tetronic T1107 were modified with chloroacetyl chloride. The chloroacetylated Tetronic T1107 was then modified to introduce amino group in the side chain by reacting it with ethylenediamine. New Schiff bases were synthesized by the reaction of aminated Tetronic 1107 with different aromatic aldehydes such as p-hydroxybenzaldehyde, vanillin, p-chlorobenzaldehyde, and p-dimethylamino benzaldehyde. The chemical structure of the obtained Schiff bases was confirmed by FT-IR and 1H-NMR spectroscopy. The antibacterial activity of Schiff base compounds was investigated by well diffusion method against gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris) and grampositive bacteria (Staphylococcus aureus). The results showed that the polymeric Schiff base compounds have a significant inhibiting effect on all the tested microorganisms. It was also found that the antibacterial activity of the Schiff base was higher than that of unmodified tetronic 1107.

1. Introduction

Due to the growing global concern of emerging infectious diseases, research activities on polymeric biocides have been greatly increased [1–6]. Low molecular weight biocides are used for sterilization of water and medical applications. However, they have the problems of residual toxicity of the agents [7]. To resolve these problems, antimicrobial polymers are proposed because they have advantages such as chemical stability, non-volatility and presenting long-term activity [8-10].

Schiff bases are proved to have a variety of biological activities such as antibacterial, antifungal, anticancer and herbicidal activities [11-18].

Tetronics are polyethers with four PPO-PEO arms bonded to a central ethylene diamine linker. They are widely used in many applications such as antifoaming agents, wetting agents, dispersants, thickeners, and emulsifiers for different industrial purposes [19, 20]. In the recent years, they were applied in biomedical and pharmaceutical fields such as drug delivery, genetic immunization, and membrane biochemistry [21-24].

The present work describes the synthesis of some Schiff bases based on tetronic T1107 and testing their antibacterial activities against selected Gram-positive bacteria and Gram-negative bacteria using well diffusion method.

2. Materials and Methods

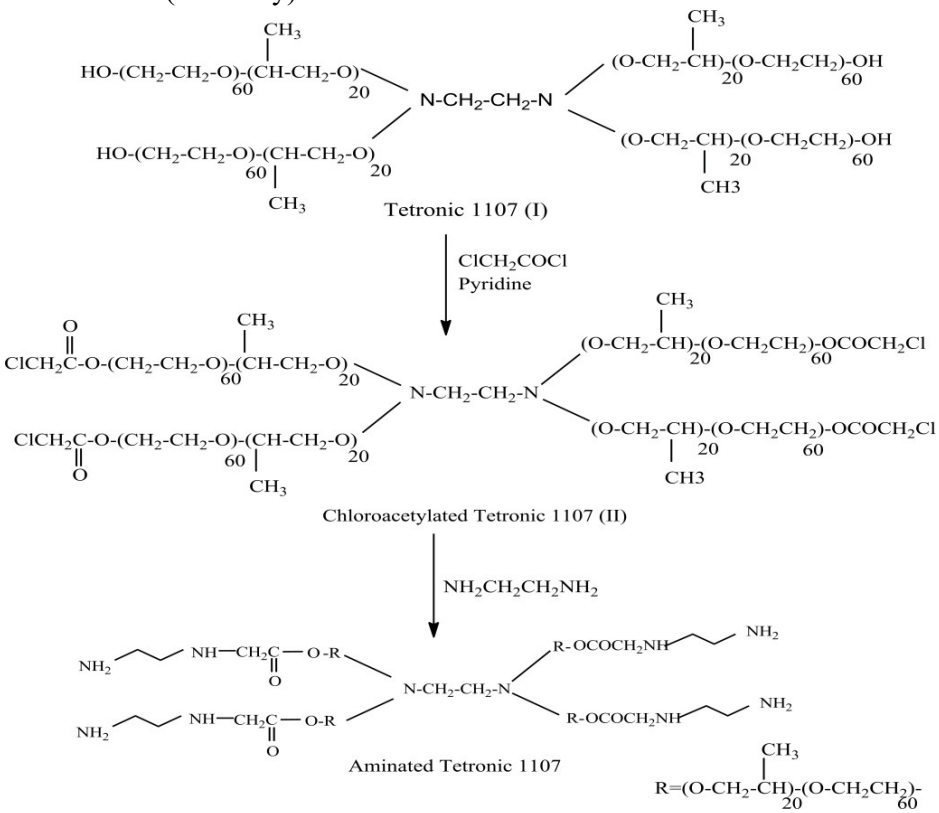
2.1. Material and Instruments

Tetronic T1107 (Average molecular weight: 15000) was obtained from BASF Corporation (New Jersey, USA). Chloroacetyl chloride and Glacial acetic acid were purchased from El-Gomhouria Chemicals Company (Cairo, Egypt). Pyridine was purchased from El-Nasr Pharmaceutical Chemicals (Cairo, Egypt). Ethylene diamine (EDA) was purchased from Acros (Belgium). Vanillin, *p*-Chlorobenzaldehyde, *p*-Hydroxybenzaldehyde and *p*-Dimethyl aminobenzaldehyde was purchased from Aldrich (USA). Diethyl ether was received from El-Nasr Pharmaceutical Chemicals Company (Cairo, Egypt) and dried using molecular sieve (4A) before use. Absolute ethanol was purchased from Merck-Schuchardt, Hohenbrunn (Germany).

FT-IR spectra were recorded on a Perkin–Elmer 1430 ratio (KBr pellets.) **Elemental microanalyses** were determined on Heraeus, elemental analyzer mode 1106 Carlo Erba Strumentazione. **^{1}H-NMR spectroscopy** (400 MHz) was recorded on Varian Mercury-300BB NMR Spectrometer. **Vacuum oven** was supplied from BINDER, Germany.

2.2. Chloroacetylation of Tetronic T1107 (II)

The chloroacetylation of tetronicT1107 was carried out as following: Pyridine (19 mL, 240 mmol) was added to a solution of tetronic T1107 (10 g, 20 mmol) in dry ethanol and the mixture was cooled to 0 ºC. Chloroacetyl chloride (19 mL, 240 mmol) was added to the cold mixture dropwise with vigorous stirring. The reaction mixture was stirred at 0 ºC for 3 hours and at room temperature for further 48 hours. The excess ethanol was removed by rotary evaporator. The precipitate was washed with diethyl ether, and the product was dried under vacuum oven for 10 hours to give 91% yield (Scheme 1).



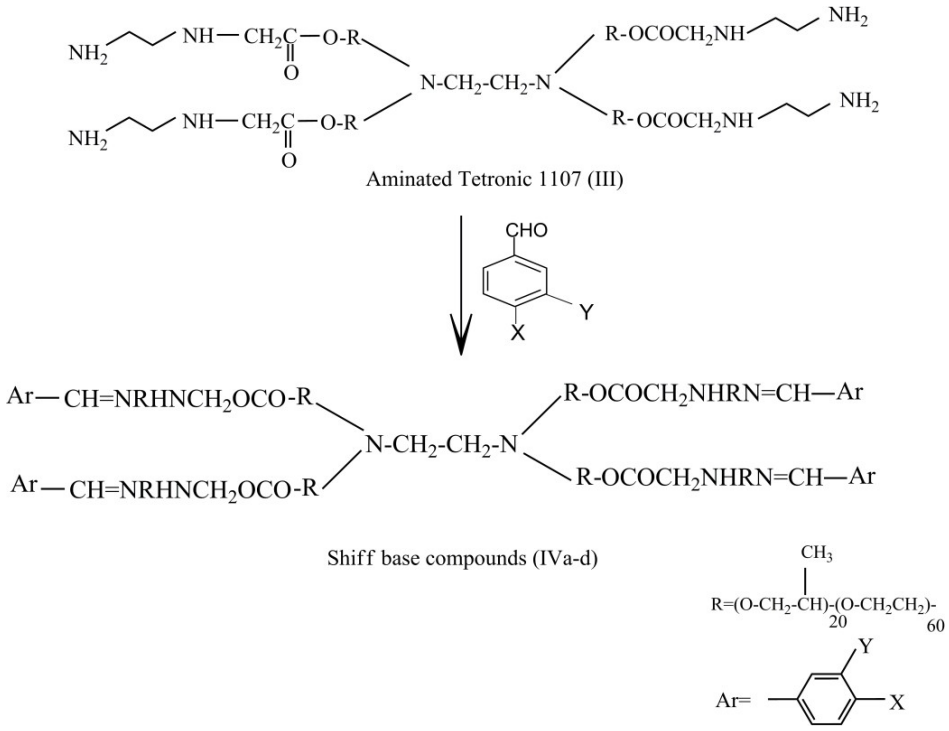
Scheme 1. Amination of chloroacetylated tetronic 1107

2.3. Amination of chloroacetylated Tetronic T1107 (III)

Chloroacetylated tetronic 1107 (4 g, 4.76 mmol) was added portionwise to ethylene diamine (6.5 mL, 95.2 mmol) and was refluxed at 80 ºC for 72 hours with continues stirring. The product was washed with dry diethyl ether to remove the unreacted ethylene diamine followed by drying under vacuum oven at room temperature for 8 hours (scheme 1).

2.4. Modification of the aminated tetronic T1107 (III) with aromatic aldehydes

To a solution of different aldehydes (8 mmol) in 20 mL absolute ethanol, aminated tetronic T1107 (III) (1 mmol) and 1 mL glacial acetic acid were added. The reaction was stirred at room temperature for 48 hours, and then was refluxed at 80 ºC for 10 h. The product was washed with dry methanol to remove the excess aldehyde and acetic acid was dried under vacuum oven for 10 hours (Scheme 2).



Schiff base R= CH_2CH_2 (III)	Aldehyde	X	Y
IVa	*p*-dimethylaminobenzaldehyde	$(CH_3)_2N$	H
IVb	Vanillin	OH	OCH_3
IVc	*p*-chlorobenzaldehyde	Cl	H
IVd	*p*-hydroxybenzaldehyde	OH	H

Scheme 2. Schiff base formation of tetronic T1107

2.5. Antibacterial activities of the modified tetronic T1107

2.5.1. Tested Microorganisms

The test microorganisms included were Gram negative bacteria; *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and the Gram-positive bacteria; *Staphylococcus aureus*. The microorganisms were obtained from Tanta University Educational Hospital, Tanta, Egypt. The bacterial strains were maintained on nutrient agar which contained 3 g peptone, 5 g NaCl, 5 g beef extract, and 20 g agar per liter.

2.5.2. Screening of antibacterial activity for tested compounds

The antibacterial activities of the tested samples were determined using well diffusion method.

Powder test samples (20 mg/1mL) were dissolved in dimethyl sulphoxide (DMSO). Wells were then created and 50 µl of the samples were pipetted into each well to detect the most sensitive microorganisms against investigated polymers. DMSO was used as negative control. The plates were incubated at 37 °C for bacteria were examined for inhibition zones development. Three replicates were carried out at least. The inoculum's concentrations were approximately 10^7 CFU for bacteria. Plates were incubated at 37 ºC for 24 h then the average diameters of inhibition zones were recorded in millimeter.

3. Results and discussion

3.1. Chloroacetylation of Tetronic T1107

Chloroacetylated tetronic T1107(II) was synthesized by treatment of tetronic T1107 (I) with excess chloroacetyl chloride in the presence of pyridine as a base in ethanol (Scheme 1). The amount of chloroacetyl chloride was large excess compared with the amount of tetronic T1107 to ensure the complete chloroacetylation of all the terminal hydroxyl groups. Similar chloracetylation method was described by Kenawy et al. for polyvinyl alcohol [25]. The FT-IR spectrum of the product (II) showed peaks at 757 cm^{-1} for (-CH_2Cl), 1750 for (-C=O) and 2906 cm^{-1} for (aliphatic CH) as shown in (Fig.1).

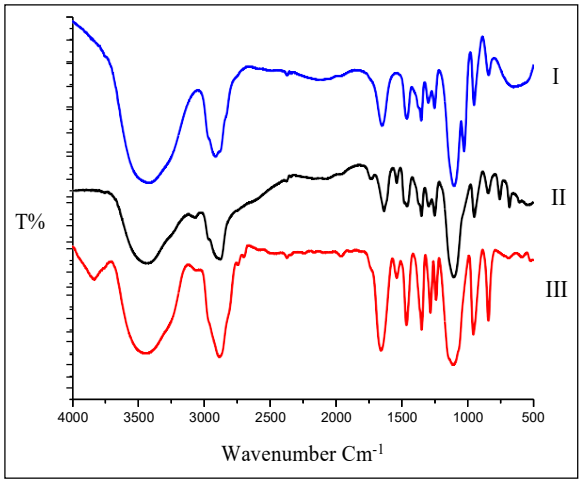


Figure 1. FT-IR spectra of tetronic T1107 (I), chloroacetylated tetronic T1107 (II), Aminated tetronic T1107 (III).

The ^{1}H-NMR spectrum of the chloroacetylated tetronic T1107 showed a characteristic band of protons of chloroacetate group at 4.06 ppm. There are also bands at 3.55 ppm, which belong to protons of –CH_2- in the main chain, and a signal of protons of CH_3 group at 1.12 ppm.

3.2. Amination of chloroacetylated tetronic T1107

The chloroacetylated tetronic T1107 was reacted with ethylene diamine in absolute ethanol as a solvent to give aminated tetronic T1107 (III) (Scheme 1). It was found when the molar ratio was 1:20; the reaction gave the highest degree of amination as previously reported be Kenawy et al., [26].

The FT-IR spectrum of compound (III) showed peaks at 3445 cm^{-1} for (NH_2), 1740 cm^{-1} for (C=O), 2887 cm^{-1} for (CH aliphatic) and peak at1538 cm^{-1} for –NH secondary amine as shown in Fig. 1.

^{1}H-NMR spectrum of aminated tetronic T1107 (III) is characterized by the appearance of signal at 3.3 ppm (CH_2N), 3.85 ppm for (CH_2). There are also bands at 3.27 ppm for (CH_2) and at 1.12 ppm for (CH_3).

3.3. Modification of the aminated tetronic T1107 (III) with aromatic aldehydes

The reaction of aminated tetronic T1107 with different aldehydes (vanilline, *p*-hydroxybenzaldehyde, *p*-chlorobenzaldehyde and *p*-dimethyl aminobenzaldehyde) in absolute ethanol in the presence of glacial acetic acid as catalyst in an oil bath at 80–90 ºC yielded Schiff base compounds (Scheme 2). The Schiff bases were formed at room temperature, but heating was used to ensure the condensation and to increase the reaction yield [27].

The FT-IR spectra of the polymers ($IV_{a–d}$) showed strong band at 1591–1598 cm^{-1} due to (C=N) which confirm formation of Schiff base. Polymer (IV_b) showed an absorption bands at 2615 cm^{-1} due to the methoxy group (OCH_3) of vanilline. Polymer (IV_c) showed strong band at 703 cm^{-1} due to the C-Cl group of *p*-chlorobenzaldehyde as in (Table 1) and (Fig. 2).

Table 1. FT-IR Analysis of Schiff base compounds

Polymer code	C-H (aliph)	C=N	C-H (arom)
IVa	2922	1591	810
IVb	2962	1598	819
IVc	2918	1591	827
IVd	2920	1596	840

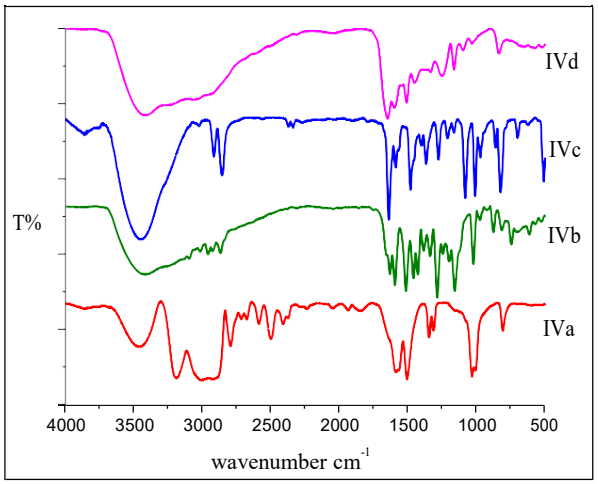


Figure 2. FT-IR spectra of Schiff bases compounds (IVa-d)**.**

The ^{1}H-NMR spectra of Schiff base compounds (IVa–d) were characterized by the appearance of peak at 8.2-8.6 ppm (s, CH=N) which confirms the formation of the Schiff base of tetronic T1107 as shown in (table 2).

Table 2. ^{1}**H-NMR of Schiff base compounds**

Polymer code	CH=N	H arom	CH_3	CH_2	OCH_3	OH
IV a	8.3	6.6-7.7	1.2	3.7	-	-
IV b	8.2	6.8-7.4	1.2	3.7	3.84	6.8
IV c	8.6	6.8-7.4	1.2	3.8	-	-
IV d	8.5	6.7-7.9	1.2	3.7	-	10.67

3.4. Antibacterial activities of the polymers

The antibacterial activity of tetronic T1107 and their Schiff bases (I-IVd) were initially evaluated by well diffusion method [28]. The screening of the antibacterial activity of tested polymers was carried out against different microorganisms (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Staphylococcus aureus*) as shown in (Table 3) and (Fig. 3). The results showed a very good inhibitory effect against all the tested bacteria. It was found that the diameter of the inhibition zone was varied according to the substitution and also, the microorganisms tested. The inhibition zones ranged from 13 to 40 mm. It was observed that tetronic T1107 had no effect on *P. vulgaris* (0 mm). The highest activity was recorded for chloroacetylated tetronic T1107 (II) against *S. aureus* (40 mm). Polymer (IVc) showed a potent inhibition against *K. pneumoniae* (27.5 mm), while polymer (IVd) showed a good inhibition against *P. vulgaris* (24.5 mm).

Table 3. Diameters of inhibition zones (mm) of tetronic 1107 and its Schiff bases

Microorganisms	Polymer code						
	I	II	III	IV_a	IV_b	IV_c	IV_d
E. coli	16	32	11	0	0	0	0
P. vulgaris	0	14	18	15	14.5	13.5	24.5
K. pneumoniae	16.3	25	11.5	19	21	27.5	20
S. aureus	11	40	15.5	0	10	10.5	12.5

Conclusion

New Schiff base compounds based on tetronic T1107 were successfully synthesized. The results indicated that Schiff base had a significant inhibiting effect on all the tested microorganisms. The antibacterial activity of the Schiff base was higher than that of unmodified tetronic T1107.

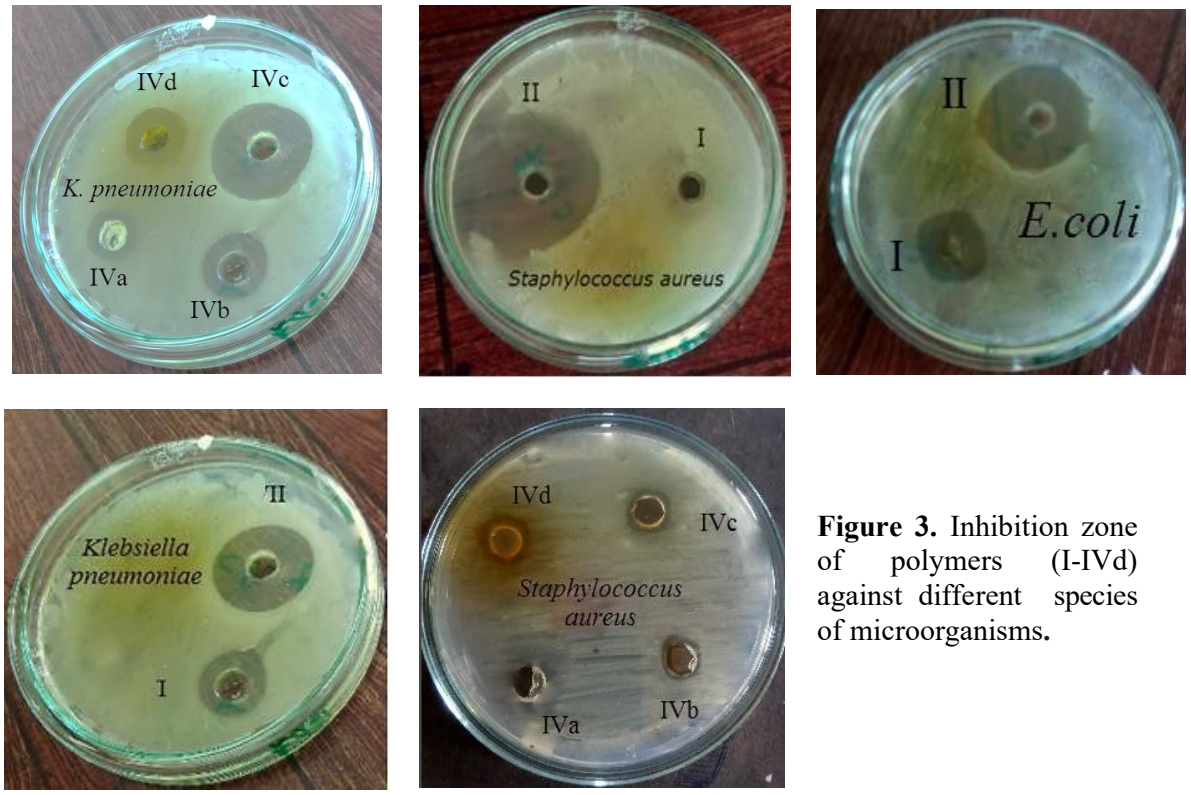


Figure 3. Inhibition zone of polymers (I-IVd) against different species of microorganisms**.**

References

1. Newehy MH, Kenawy E-R, Al-Deyab SS. Biocidal Polymers: Preparation and Antimicrobial Assessment of Immobilized Onium Salts onto Modified Chitosan. International Journal of Polymeric Materials and Polymeric Biomaterials. 2014;63(15):758-66.
2. Pasquier N, Keul H, Heine E, Moeller M, Angelov B, Linser S, et al. Amphiphilic branched polymers as antimicrobial agents. Macromolecular bioscience. 2008;8(10):903-15.
3. Eknoian M, Worley S, Bickert J, Williams J. Novel antimicrobial N-halamine polymer coatings generated by emulsion polymerization. Polym. 1999;40(6):1367-71.
4. Bansal R, Pathak R, Kumar B, Gautam HK, Kumar P. Enhanced antimicrobial activity of amphiphilic cationic polymers against a broad range of bacterial strains and skin microbes. Colloid. Polym. Sci. 2017;295(7):1177-85.
5. Kenawy E-R. Biologically active polymers. IV. Synthesis and antimicrobial activity of polymers containing 8-hydroxyquinoline moiety. Journal of Applied Polymer Science. 2001;82(6):1364-74.
6. Dizman B, Elasri MO, Mathias LJ. Synthesis and antimicrobial activities of new water-soluble bis-quaternary ammonium methacrylate polymers. J Appl Polym Sci 2004;94(2):635-42.
7. Kenawy E-R, Abdel-Hay FI, El-Shanshoury AERR, El-Newehy MH. Biologically active polymers. V. Synthesis and antimicrobial activity of modified poly (glycidyl methacrylate-co-2-hydroxyethyl methacrylate) derivatives with quaternary ammonium and phosphonium salts. J Polym Sci: part A: Polym Chem 2002;40(14):2384-93.
8. Ilker MF, Nüsslein K, Tew GN, Coughlin EB. Tuning the hemolytic and antibacterial activities of amphiphilic polynorbornene derivatives. J Am Chem Soc 2004;126(48):15870-5.
9. Dong C, Ye Y, Qian L, Zhao G, He B, Xiao H. Antibacterial modification of cellulose fibers by grafting β-cyclodextrin and inclusion with ciprofloxacin. Cellulose. 2014;21(3):1921-32.
10. Majumdar P, Lee E, Gubbins N, Stafslien SJ, Daniels J, Thorson CJ, et al. Synthesis and antimicrobial activity of quaternary ammonium-functionalized POSS (Q-POSS) and polysiloxane coatings containing Q-POSS. Polym. 2009;50(5):1124-33.
11. Przybylski P, Huczynski A, Pyta K, Brzezinski B, Bartl F. Biological properties of Schiff bases and

azo derivatives of phenols .Curr Org Chem 2009;13(2):124-48.
12. Silva Cd, Silva DL, Modolo L, Alves R, Resende Md, Martins C, et al. Schiff bases: A short review of their antimicrobial activities. J Adv Res. 2011;2(1):1-8.
13. Jin X, Wang J, Bai J. Synthesis and antimicrobial activity of the Schiff base from chitosan and citral. Carbohydr Res. 2009;344(6):825-9.
14. Al Zoubi W. Biological activities of Schiff bases and their complexes: a review of recent works. Int J Org Chem. 2013;3(03):73.
15. Chaudhary NK, Mishra P. Metal complexes of a novel schiff base based on penicillin: characterization, molecular modeling, and antibacterial activity study. Bioinorg ChemAppl. 2017;2017.
16. Creaven BS, Duff B, Egan DA, Kavanagh K, Rosair G, Thangella VR, et al. Anticancer and antifungal activity of copper (II) complexes of quinolin-2 (1H)-one-derived Schiff bases. Inorganica Chimica Acta. 2010;363(14):4048-58.
17. Hu G, Wang G, Duan N, Wen X, Cao T, Xie S, et al. Design, synthesis and antitumor activities of fluoroquinolone C-3 heterocycles (IV): s-triazole Schiff–Mannich bases derived from ofloxacin. Acta. Pharm. Sin. 2012;2(3):312-7.
18. Kenawy E-R, Azaam M, Saad-AllahK, El-Abd A. Preparation of organophilic montmorillonite-based dimethylamino benzaldehyde-Schiff-base as antibacterial agents. ARAB J CHEM 2016.
19. Mansur CR, Barboza SP, González G, Lucas EF. PLURONIC× TETRONIC polyols: study of their properties and performance in the destabilization of emulsions formed in the petroleum industry. J Colloid Interface Sci. 2004;271(1):232-40.
20. Chen Y, Liu T, Xu G, Zhang J, Zhai X, Yuan J, et al. Aggregation behavior of X-shaped branched block copolymers at the air/water interface: effect of block sequence and temperature. COLLOID POLYM SCI. 2015;293(1):97-107.
21. Y. Sun GS. Durable and refreshable polymeric N-halamine biocides containing 3-(4′ vinylbenzyl)-5, 5-dimethylhydantoin. . J PolymSci: part A: PolymChem 2001;19(39):3348-55.
22. Roques C, Fattal E, Fromes Y. Comparison of toxicity and transfection efficiency of amphiphilic block copolymers and polycationic polymers in striated muscles. J GeneMed 2009;11(3):240-9.
23. Cucchiarini AR-RaM. PEO-PPO-PEO Tri-Block Copolymers for Gene Delivery Applications in Human Regenerative Medicine—An Overview. Int J Mol Sci 2018;19:775.
24. Chiappetta DA, Alvarez-Lorenzo C, Rey-Rico A, Taboada P, Concheiro A, Sosnik A. N-alkylation of poloxamines modulates micellar assembly and encapsulation and release of the antiretroviral efavirenz. Eur J Pharm Biopharm. 2010;76(1):24-37.
25. Kenawy E-R, El-Newehy MH, Abdel-Hay FI, El-Shanshoury AE-RR. Synthesis and biocidal activity of modified poly (vinyl alcohol(.)Arab J Chem. 2014;7(3):355-61.
26. Kenawy E-R, El-Shanshoury AERR, Omar Shaker N, El-Sadek BM, Khattab AH, Ismail Badr B. Biocidal polymers: Synthesis, antimicrobial activity, and possible toxicity of poly (hydroxystyrene-co-methylmethacrylate) derivatives. J Appl Polym Sci. 2011;120(5):2734-42.
27. Kenawy E-R, Imam Abdel-Hay F, Abou El-Magd A, Mahmoud Y. Synthesis and antimicrobial activity of some polymers derived from modified amino polyacrylamide by reacting it with benzoate esters and benzaldehyde derivatives. Journal of Applied Polymer Science. 2006;99(5):2428-37.
28. Majid A, Azim A, WajidHussain ZI, HafsaHameed J, Malik AK, Ismail K, et al. Antibacterial effects of Cedrusdeodara oil against pathogenic bacterial strains in-vitro approaches. IJB 2015;6(1):185-91.