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An Antibacterial Activity effect of a Novel AB Block Copolymer

Aliyeh Ghamkhari[1,*] **Hossein Samadi-Kafil**[2]

[1]Institute of Polymeric Materials and Faculty of Polymer Engineering, Sahand University of Technology, Tabriz, Iran
[2]Drug Applied Research Center, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Correspondence to: Ghamkhari A. (E-mail: aliyeh_ghamkhari@yahoo.com)

Abstract

Various approaches are being developed for the explore of novel and powerful antimicrobial agents, in the form of synthetic polymeric.
Novel poly(2-hydroxyethyl methacrylate)-*b*-[(*N*-4-vinylbenzyl),*N*,*N*-diethylamine) PHEMA-*b*-PVEA diblock copolymer was prepared via reversible addition fragmentation transfer (RAFT) polymerization to investigate antibacterial behavior. The structure of the AB diblock copolymer was investigated by means of Fourier transform infrared (FTIR), and ^{1}H nuclear magnetic resonance (NMR) spectroscopies. The molecular weights of PHEMA and PHEMA-*b*-PVEA segments were calculated to be 10300 and 24000 gmol^{-1} by GPC, respectively. Furthermore, the antibacterial activity was verified by selecting four types of antibacteria subsuming *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Candidaalbicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) as Gram-positive and Gram-negative bacteria models. Results exhibited remarkable fine antibacterial activity. High antibacterial activity effects were observed for *C. albicans* with PHEMA-*b*-PVEA diblock copolymers having 44, 75, and 90 mm diameter halo of bacterial inhibition. PHEMA-*b*-PVEA copolymers could be considered in nanoparticles and antibacterial applications due to their excellent behavior.

Keyword: diblock copolymer, antibacterial activity, S. aureus, B. cereus, C. albicans,

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1. Introduction

Recently, Antimicrobial resistance has been revealed as a critical level worldwide [1]. In the past decades, inorganic antibacterial materials have more attention because of antibacterial behavior such as Ag-carrying composites, nano-Ag, TiO2 and ZnO [2-3]. Although, the applications of inorganic materials are limited due to a heap of heavy metals in the high metal concentration may be had environmental problems and harmful to humans [4, 5].

Cationic materials are known as well candidates for antibacterial properties, including peptides, surfactants and cationic polymers [6-9].
Recently, synthetic polymeric materials with antibacterial properties have found a lot of attentions because of their advantages such as nonvolatility, less toxicity to the environment, and chemically stability. Most of materials including the aromatic and heterocyclic structures like polystyrene and poly (vinylpyridine) (P4VP) have antimicrobial properties [10-13]. Quaternized P4VP with different alkyl

bromides kills drug resistant staphylococcus epidermids, P. aeruginosa and Escheria coli bacteria, being more propounded with N-hexylated P4VP [14]. Also, poly(acrylic acid) (PAA) copolymers have demonstrated an antimicrobial effect. In one study, cold-plasma-grafted acrylic acid on a poly(ethylene)(PE) surface creates inhibition zones for Staphylococcus aureus (S. aureus) [15,16].

The antimicrobial polymers properties were examined against E. coli and S. aureus, revealing control effectiveness. Lu et al synthesized four quanternary ammonium salt monomers with DMAEMA. Their bactericidal activities were investigated and inhibitory zone diameters against E. coli and S. aureus [17]. Also, the antibacterial activity of HEMA-DMAEMA copolymer against S. aureus and E. coli was considered by zone of inhibition. The results depict that quaternized monomer has caused the antibacterial activity increase [18].

Herein, we strategically synthesized a library of novel poly(2-hydroxyethylmethacrylate)-*b*-poly[(*N*-4-vinylbenzyl), *N*,*N*-diethylamine) PHEMA-*b*-PVEA diblock copolymer created by a highly efficient polymerization technique reversible addition fragmentation transfer (RAFT) polymerization technique. Among reported approaches, RAFT can be carried out in more moderate conditions to afford polymers with controlled molecular weights, narrow dispersities, and complex macromolecular architectures [19-21], and screened against a range of bacteria.

Furthermore, the bactericidal properties of synthesized diblock copolymer were studied by selecting four types of antibacteria, i.e., *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Candidaalbicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) as Gram-positive and Gram-negative bacteria models, respectively. The antibacterial properties were successfully verified for PHEMA-*b*-PVEA diblock copolymer.

2. Materials and Methods

2.1. Materials

4-chloromethyl styrene (CMS), diethyl amine (DEA), anhydrous potassium carbonate (K_2CO_3) and 2,2´-azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich (USA). 4-cyano-4-[(phenylcarbothioyl)sulfanyl] pentanoic acid (RAFT) agent was synthesized in our laboratory. *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Candidaalbicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) were obtained from Hangzhou Microbe Reagent Co., Ltd. (China). All other reagents were purchased from Merck and purified according to the standard methods.

2.2. Synthesis of *N*-(4-vinylbenzyl)-*N*, *N*-diethylamine (VEA)

2 mL of DEA, 4.2 mL of CMS, 27.6 g of K_2CO_3 were added to the reactor and dissolved in 30 mL $CHCl_3$. The solution was stirred under an argon atmosphere for about 24 hours at 50 °C. The crude product was filtered and extraction, and then the solvent were transferred by a rotary evaporator. The crud product was segregated using petroleum ether with silica-gel column chromatography (Scheme 1A).

2.3. Synthesis of PHEMA by RAFT technique

In an ampoule, HEMA (5 mL, 40 mmol), RAFT agent (46.0 mg, 0.16 mmol) and AIBN (1.7 mg, 0.01 mmol), 5 mL DMF were poured and degassed with several freeze–pump–thaw cycles. Then, the mixture moved in an oil bath at 70 °C for about 18 hours. Finally, the ampoule was quenched and precipitated in cold diethyl ether (50 mL). The product was dried under vacuum at room temperature (Scheme 1B).

2.4. Synthesis of PHEMA-*b*-PVEA diblock copolymer

In an ampoule, PHEMA (700 mg, 0.07 mmol), VEA (1 mL, 5.2 mmol) and AIBN (1.7 mg, 0.01 mmol), 5 mL DMF were poured and degassed with several freeze–pump–thaw cycles. Then, the mixture moved in an oil bath at 70 °C for about 18 hours. Finally, the ampoule was quenched and precipitated in water/methanol mixture cold (70 mL). The product was dried under vacuum at room temperature (Scheme 1C).

2.5. Antibacterial activity test

Antibacterial activity of samples was investigated against *Staphylococcus aureus* (*S. aureus*), *Bacillus*

cereus (*B. cereus*), *Candidaalbicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) by the Kirby bauer disc diffusion method according to Clinical laboratory standard institute recommendations [22]. The materials were tested in Muller hinton agar plates (*Merck KGaA*, Darmstad, Germany). A diblock copolymer with 10, 25, and 50 mg mL^{-1} concentration and 1 mL of the bacterial suspension were transferred into each plate. Plates were incubated at 37 ºC for 24 h in the static mode conditions, and then results were expressed as diameter of inhibition zone and compared with control bacteria [23].

2.6. Characterization

Fourier transform infrared (FTIR) spectra of the samples were taken on a shimadzu 8101 M FTIR (Shimadzu, Kyoto, Japan) between the wavenumbers of 400 and 4000 cm^{-1}. The samples were prepared by grinding the dry powders with potassium bromide (KBr), and compressing the mixture into disks. The spectra were recorded at room temperature. Proton nuclear magnetic resonance (1H-NMR) spectra were recorded at 25°C using a FT-NMR (400 MHz) Bruker spectrometer (Bruker, Ettlingen, Germany). The samples for 1HNMR spectroscopy was prepared by dissolving about 10 mg of sample in 1 mL of deuterated dimethyl sulfoxide (DMSO-d6) and chemical shifts were reported in ppm units with tetra-methylsilane (TMS) as an internal standard. Size exclusion analyses were carried out using a Waters 1515 (USA) gel permeation chromatography (GPC) instrument equipped with Breeze 1515 isocratic pump and 7725 manual injectors.

3. Results and Discussion

However, the development of antimicrobial synthetic polymeric have been extended due to the various structure of polymers such as star, hyparbranched polymers, and inorganic-polymer hybrids in other targeted applications like drug/gene delivery [24-26]. The FTIR spectra of VEA monomer, PHEMA and PHEMA-*b*-PVEA are demonstrated in Fig. 1. The FTIR spectrum of the VEA monomer shows the stretching vibrationsas a strong band of aromatic group and C-N at 1663 cm^{-1} and 1066 cm^{-1}, respectively. The FTIR spectrum of the PHEMA

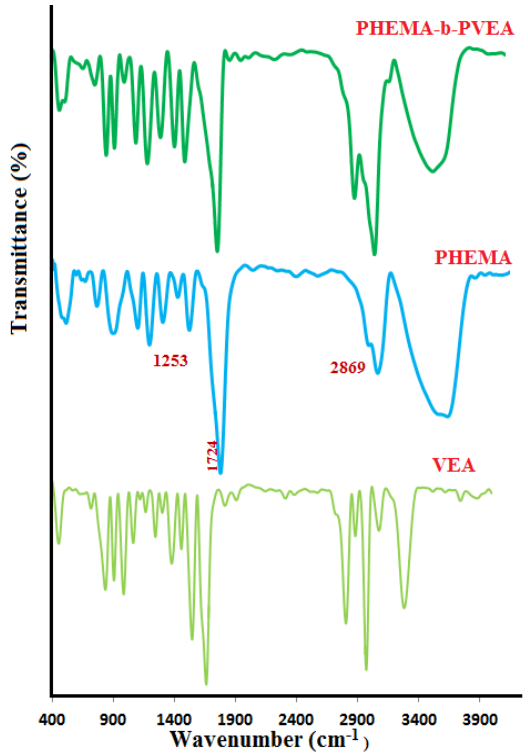


Figure 1. The FTIR spectra of the VEA, PHEMA and PHEMA-*b*-PVEA samples.

shows the aliphatic stretching vibrations at 2918 and 2958 cm^{-1}, the stretching vibration of carbonyl group, C-O and C-O-C at 1724 cm^{-1}, 1373 cm^{-1} and 1271 cm^{-1}, respectively. The hydroxyl group centered at 3509 cm^{-1}. The FTIR spectrum of the PHEMA-*b*-PVEA diblock coplolymer exhibits the main band aromatic C=C stretching vibration and aromatic ring at 1456 cm^{-1} and 831 cm^{-1}, respectively.

The ^{1}HNMR spectrum of the VEA monomer is shown in Fig 2A. The chemical shifts at 1.20, 2.40 and 3.60 ppm are related to the CH_3, -CH_2 and N–CH_2 protons of the VEA, respectively. The chemical shifts at 5.20-5.80 and 6.50-7.10 ppm are allied to the vinyl and aromatic protons of the VEA monomer.

The successful preparation of the PHEMA homopolymer is confirmed by ^{1}HNMR in Fig. 2B. The chemical shifts of O$\underline{C}H_2$-CH_2-OH, OCH_2-$\underline{C}H_2$-OH, OCH_2-CH_2-O$\underline{H}$ protons and RAFT agent backbone are observed at 3.56, 4.02, 4.83 and 0.85-2.10 ppm, respectively.

The successful preparation of the PHEMA-*b*-PVEA diblock copolymer is confirmed by ^{1}HNMR in Fig. 2C. The chemical shifts of CH_3, -CH_2 protons and

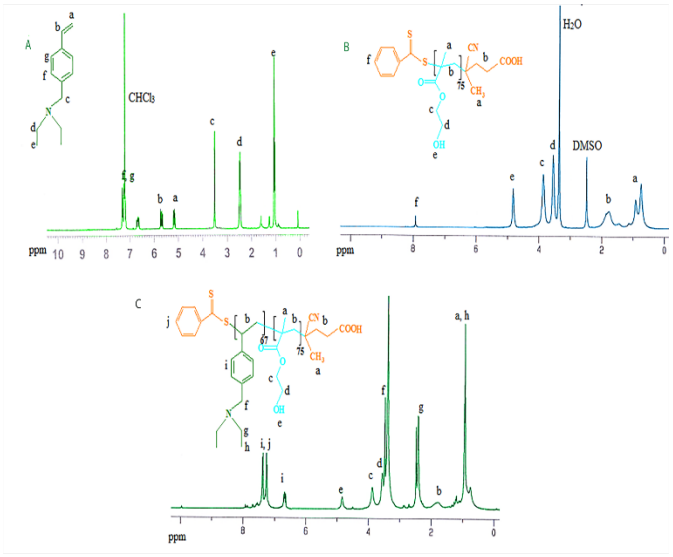


Figure 2. The ^{1}H NMR spectra of the VEA, PHEMA and PHEMA-*b*-PVEA.

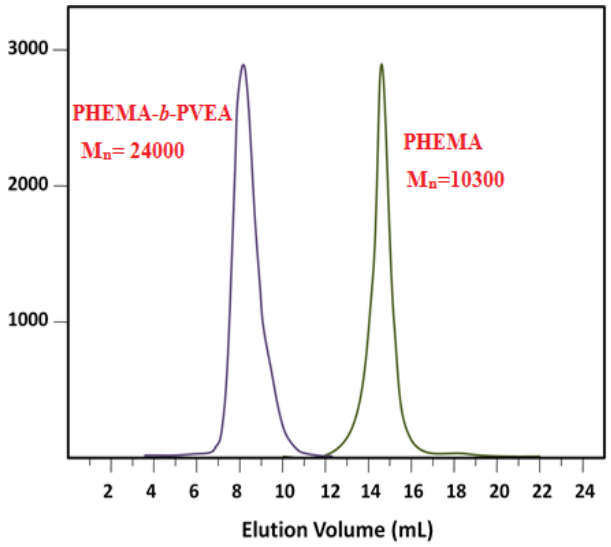


Figure 3. GPC traces of PHEMA homopolymer and PHEMA-*b*-PVEA diblock copolymers.

RAFT agent backbone are observed at 0.85-2.10 ppm, respectively. The protons of the N-CH_2 , –CH_2OH, and –OCH_2 groups are appeared at 2.37, 3.50 and 3.55 ppm, respectively. The aromatic proton is shown at 6.70-7.30 ppm.

Herein, a novel PHEMA-*b*-PVEA diblock copolymer was prepared with RAFT polymerization method. The GPC chromatograms of PHEMA and PHEMA-*b*-PVEA are shown in Fig. 3. The polydispersity index (*PDI*) and M_n values of PHEMA = 1.14, M_n=10300 and PHEMA-*b*-PVEA (*PDI* = 1.19), M_n=24000.

3.1. Antibacterial activity analysis of PHEMA-*b*-PVEA

Most of cytotoxic materials also possess antibacterial properties. The plate images of PHEMA-*b*-PVEA are exhibited after antimicrobial assays with *S. aureus* in 10, 25 and 50 mg/mL concentration per disc. These images showed 27, 31 and 35 mm diameter halo of bacterial inhibition. The same effects were detected against *B. cereus*, which showed 22, 26 and 30 mm diameter halo of bacterial inhibition (Fig. 4). The high antibacterial activity effects were observed against *C. albicans* with PHEMA-*b*-PVEA diblock copolymers. The bacterial and fungal results demonstrated that the highest effect was acquired for *C. albicans* with 44, 75 and 90 mm diameter halo of fungal inhibition, and inversely, the lowest effect was detected for *E. coli* having 12, 14 and 17 mm diameter halo of bacterial inhibition. The antibacterial and anti-fungal properties of these materials were very good for Gram-positive (Fig. 4), however, they had a little effect on Gram-negative (Fig. 5). The antibacterial activity of PHEMA-*b*-PVEA against four bacteria are reported in Table 1, in which the determination of inhibitory expressed in concentrations of 10, 25, 50 mg/mL.

4. Conclusion

The structure activity relationship of synthetic antimicrobial polymers has been investigated due to possibility of discovering new alternatives for combating the rise of multidrug resistance in bacteria. The antimicrobial assays of PHEMA-*b*-PVEA were also exhibited with *S. aureus*, *B. cereus*, *C. albicans*

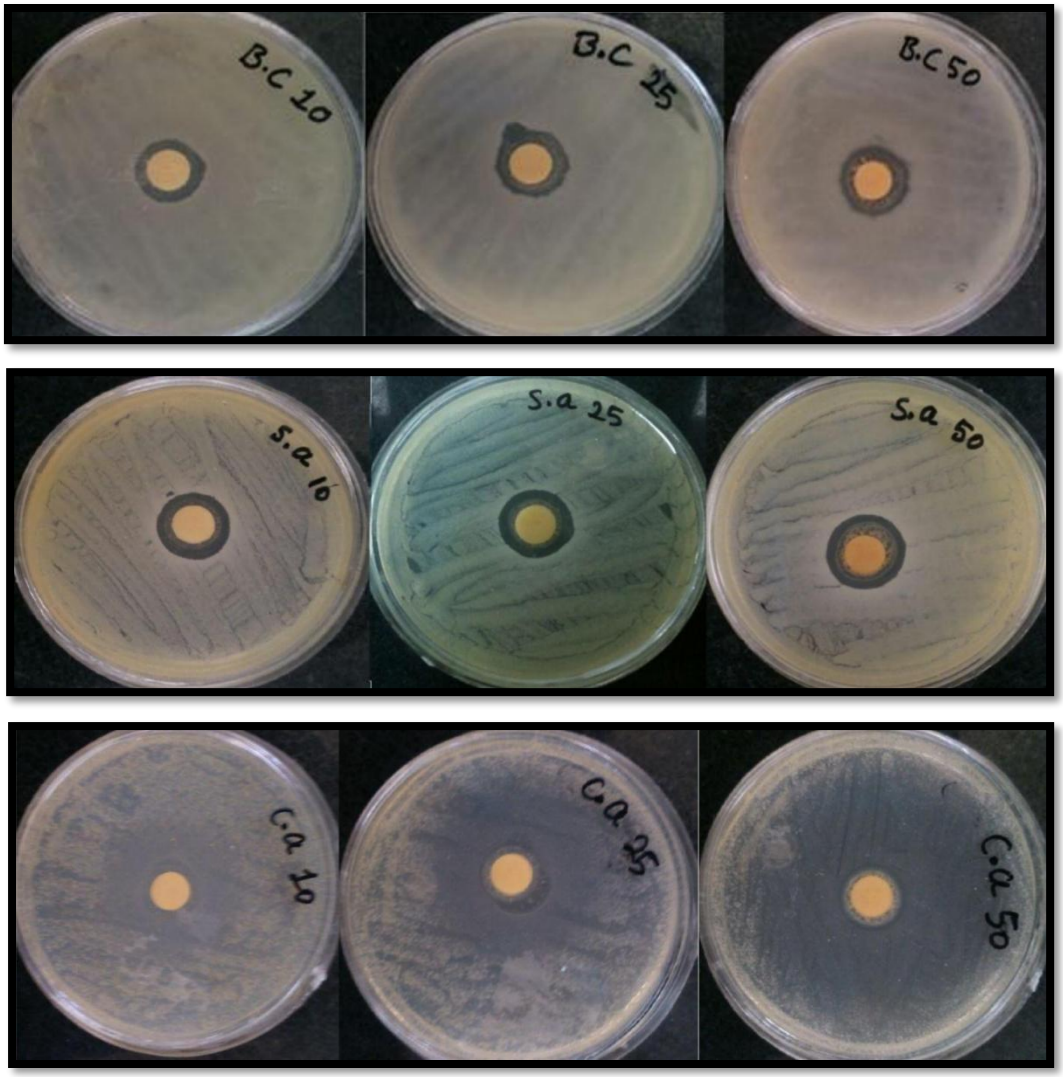
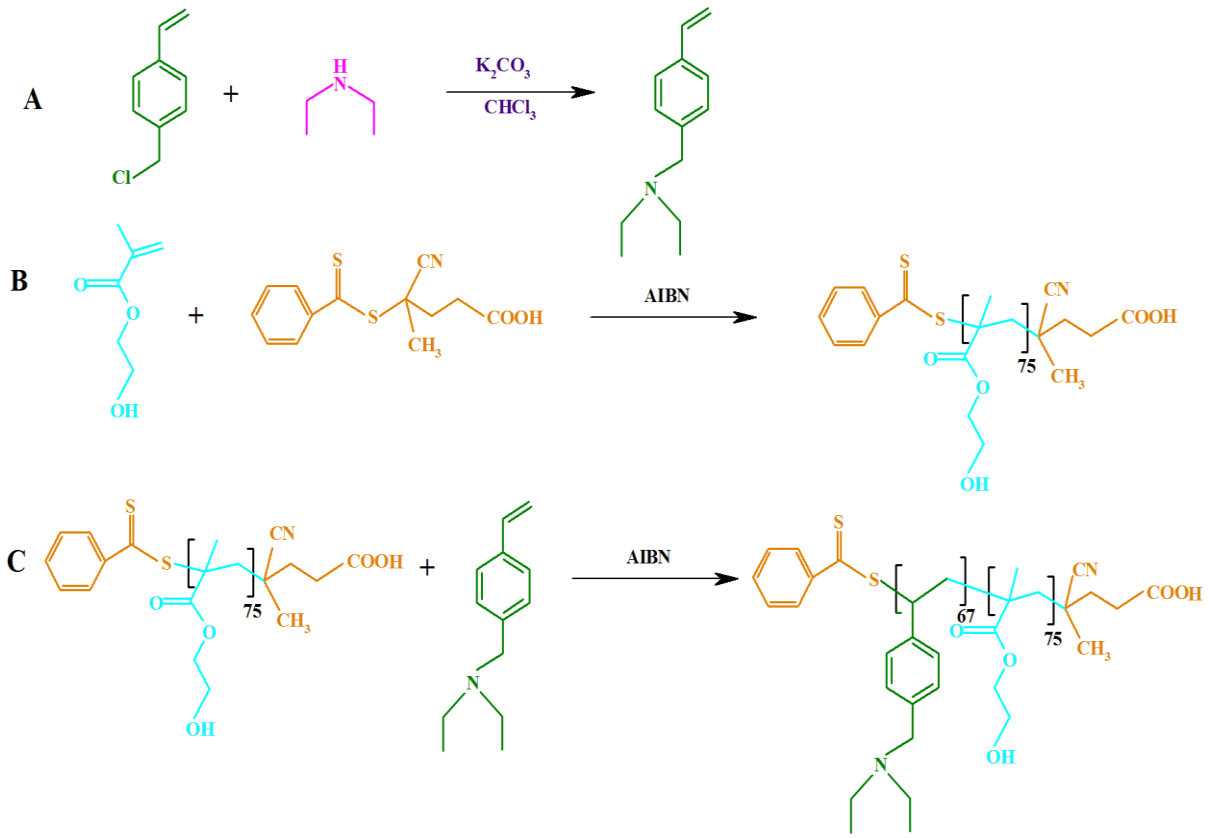


Figure 4. Antimicrobial activity of PHEMA-*b*-PVEA against *S. aureus*, *B. cereus*, and *C. albicans* as models of Gram-positive bacteria by the disc diffusion method with 10, 25, 50 mg mL^{-1} concentrations.

Figure 5. Antimicrobial activity of PHEMA-*b*-PVEA against *E. coli* as models of Gram negative bacteria by the disc diffusion method with 10, 25, and 50 mg mL^{-1} concentration.



Scheme 1. Synthesis of VEA, PHEMA and PHEMA-*b*-PVEA.

Table 1. Antibacterial activity of PHEMA-b-PVEA against four bacteria and determination of the inhibitory in concentrations expressed in 10, 25, 50 mg/mL.

PHEMA-*b*-PVEA concentration	10 mg/mL	25 mg/mL	50 mg/mL
S. aureus	27 mm	31 mm	35 mm
B. cereus	22 mm	26 mm	30 mm
C. albicans	44 mm	75 mm	90 mm
E. coli	12 mm	14 mm	17 mm

and *E. coli* in 10, 25, and 50 mg/mL concentration per disc. The diameter halo of *S. aureus* and *B. cereus* bacterial inhibition were 27, 31, 35 mm and 22, 26, 30 mm, respectively. The high antibacterial activity effects were observed against *C. albicans* with 44, 75 and 90 mm diameter halo of bacterial inhibition, and inversely, the low antibacterial activity effects were observed against *E. coli* having 12, 14 and 17 mm diameter halo of bacterial inhibition. The highest antibacterial activity was detected for *C. albicans* compared with the others. These diblock copolymers had a good effect on Gram-positive and a little effect on Gram-negative. The synthesized PHEMA-*b*-PVEA diblock copolymers could be used as an appropriate candidate for drug delivery and antibacterial activities. The significant anti-bacterial of the synthesized copolymer highlights them as promising copolymers for the development of highly specific anti-bacterial agents in other words, incorporating both properties in the same copolymer.

Acknowledgements

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