Research article

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Antimicrobial activity evaluation of a novel triblock cationic copolymer (PHEMA-*b*-PNIPAM-*b*-PVEAH)

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Abstract

Background and objective: Antimicrobial compounds are considerably interested because of their importance in control of biological contaminants. Several techniques have been used for fabrication of novel and efficient antimicrobial compounds. The aim of present work was to develop a durable cationic antimicrobe based on triblock copolymer.

Materials and methods: Novel poly(2-hydroxyethyl methacrylate)-*b*-poly(*N*-isopropyl acrylamide)-*b*-poly (*N*-4-vinylbenzyl), *N*,*N*-diethylamine hydrochloride (briefly named PHEMA-*b*-PNIPAM-*b*-PVEAH or ABC) cationic triblock copolymer was synthesized via reversible addition fragmentation transfer (RAFT) polymerization as antimicrobial compound. Chemical structure of ABC triblock copolymer was characterized by Fourier Transform Infrared and ^{1}H Nuclear Magnetic Resonance spectroscopy. Furthermore, the antimicrobial activity of cationic triblock copolymer was examined on four micro-organisms including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

Results and conclusion: Molecular weight of blocks in the copolymer including PHEMA, PHEMA-*b*-PNIPAM and PHEMA-*b*-PNIPAM-*b*-PVEAH was 10950, 17103 and 26165 g mol^{-1}, respectively. Results showed remarkable antibacterial activity so that inhibition diameter of *B. cereus* and *S. aureus* were 25, 29, 34 mm and 32, 37, 41 mm, respectively. Low inhibition was detected against *E. coli* (14, 17 and 21 mm) and the highest inhibition was observed for *C. albicans* (48, 81 and 98 mm). Acceptable antibacterial and high anti-yeast activity was observed for ABC. Therefore, it could be used in therapeutic purposes and microbial suppression.

Keywords: Antimicrobial activity, *Bacillus cereus*, *Candida albicans*, cationic triblock copolymer, *Escherichia coli*, *Staphylococcus aureus*

Abbreviations
A= PHEMA: poly(2-hydroxyethyl methacrylate)
B = PNIPAM: poly(*N*-isopropylacrylamide)
C = PVEAH: poly ((*N*-4-vinylbenzyl), *N*,*N*-diethylamine hydrochloride)
ABC = PHEMA-*b*-PNIPAM-*b*-PVEAH

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1. Introduction

Antimicrobial agents are widely used in disease prevention or therapeutic purposes through which they suppress or inhibit microbial growth [1-3]. Inorganic antibacterial are extensively adopted by physical or chemical treatments such as nano-Ag, Ag-carrying composites, ZnO Cu_2O, SiO_2 and TiO_2 [4-6]. Some synthetic polymeric antimicrobial compounds are popular due to their advantages such as they are less toxic to the environment, non-volatile and chemically stable [7-9]. They include phosphonium, quarternary ammonium, imidazolium and pyridinium [10, 11]. Among investigated polymer-based materials, quaternary ammonium polymers such as [(*N*-4-vinylbenzyl, *N*,*N*-diethylamine hydrochloride): (VEAH)] [12] have demonstrated a sharp and instant inhibitory impact on wide range of microorganisms. It interacts with negatively charged sites of bacterial surface [13]. Moreover, polyacrylic acid (PAA) grafted on polyethylene (PE) showed antimicrobial effect against *Staphylococcus aureus (S. aureus)* [14]. In accordance, Sui et al. investigated antifouling and antibacterial alteration of poly (vinylidene fluoride) membrane prepared by surface functionalization [15]. Furthermore, PVDF-*b*-PHEMA-*b*-PDMAE MA copolymer was effective against *Escherichia coli* (*E. coli*) and *S. aureus* [16], and PHEMA-b-PVEA copolymer showed antimicrobial activity against *Bacillus cereus* (*B. cereus*), *S. aureus*, *E. coli* and *Candida albicans* (*C. albicans*) [17]. In this regard, we fabricated a novel triblock copolymer of poly(2-hydroxy ethylmethacry-late)-*b*-poly (*N*-isopropyl acrylamid)-*b*-poly((*N*-4-vinylbenzyl), *N*,*N*-diethylamine hydrochloride) by reversible addition fragmentation transfer (RAFT) polymerization technique. Then, we characterized the product and examined its antimicrobial activity against four microbial species of *B. cereus*, *S. aureus*, *E. coli* and *C. albicans* as models of spore forming bacteria, gram positive bacteria, gram negative bacteria and yeast, respectively.

2. Materials and methods

2.1. Chemicals

Diethyl amine (DEA), anhydrous potassium carbonate (K_2CO_3), 4-chloromethyl styrene (CMS) and 2,2´-azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich (USA). 4-cyano-4-[(phenylcarbothioyl) sulfanyl] pentanoic acid (RAFT) [18] and PHEMA and PHEMA-*b*-PNIPAM [19] was synthesized in our laboratory. *C. albicans*, *E. coli*, *S. aureus*, and *B. cereus* were purchased from Hangzhou Microbe Reagent Co., Ltd. (China).

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

4.2 ml of CMS, 2 ml of DEA, 4.2 ml of CMS, 27.6 g of K_2CO_3 and 30 ml $CHCl_3$ were charged to flask. The mixture was stirred under argon atmosphere for 20 h at 50±5°C. Final product was extracted and the solvent was removed. The crud was separated using silica-gel column chromatography with petroleum ether as solvent. Then, VEA monomer was acidified by hydrochloric acid and tertiary amine of VEA converted to quaternary ammonium salt (Figure 1a) [20].

2.3. Synthesis of PHEMA-*b*-PNIPAM-*b*-PVEAH cationic triblock copolymer

VEAH monomer (1 ml, 5.2 mmol), PHEMA-*b*-PNIPAM (700 mg, 0.04 mmol), AIBN (1.7 mg, 0.01 mmol) and dimethylformamid (8 ml) were added to flask and degassed. The mixture was heated in oil bath at 75°C for 20 h. Final product was precipitated by addition of 100 ml cold water:methanol mixture (75:25). The product was dried under vacuum at ambient temperature (Figure 1b) [20].

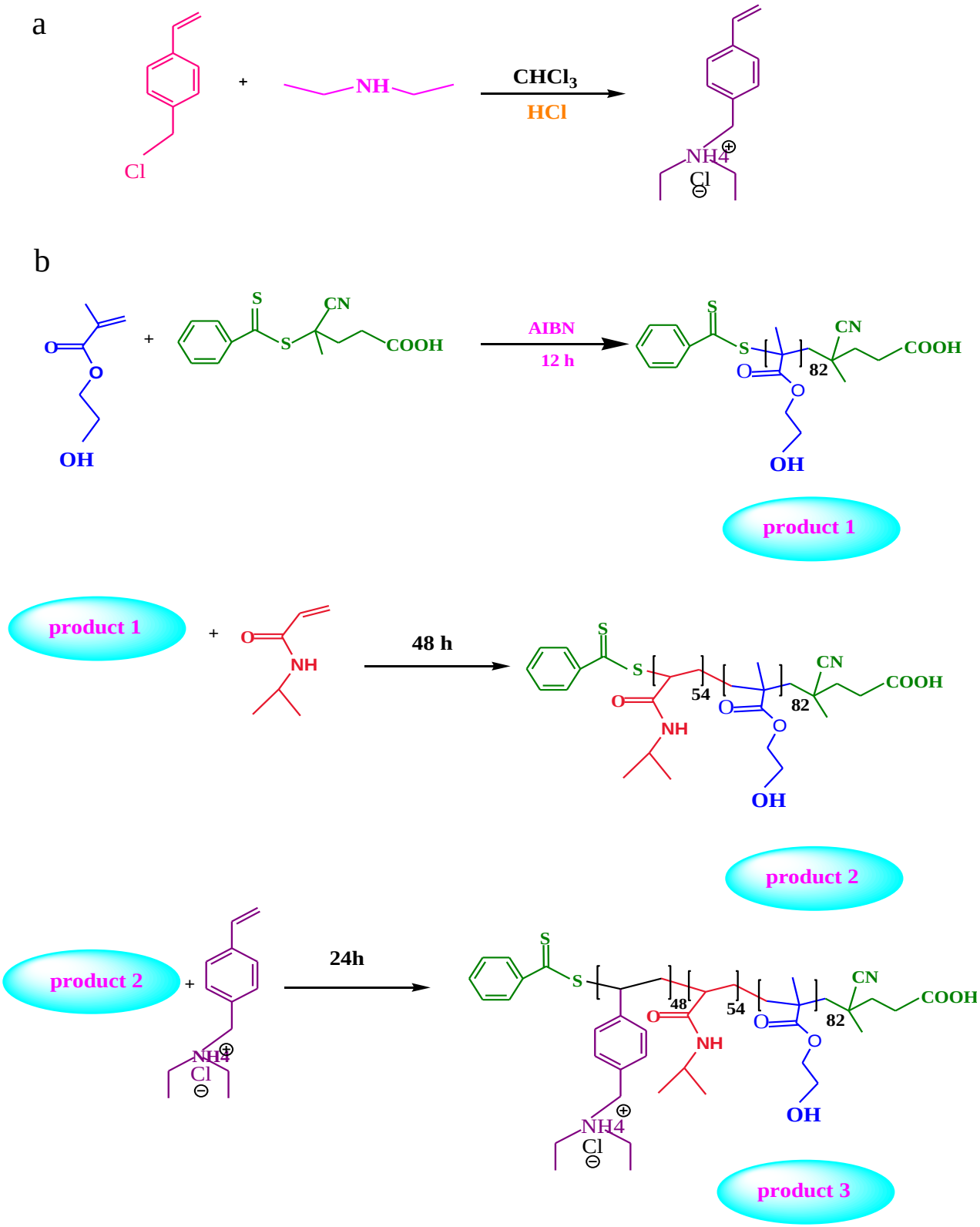

Figure 1- Synthesis steps of a) VEAH and b) PHEMA-*b*-PNIPAM-*b*-PVEAH cationic triblock copolymer

2.4. Antimicrobial activity test

Antimicrobial activity of cationic triblock copolymer was tested against *B. cereus*, *S. aureus*, *E. coli* and *C. albicans* by Kirby Bauer disc diffusion method [17]. The materials were tested in Muller Hinton agar plates (Merck, Germany). Triblock copolymer in concentration of 15, 30 and 50 mg ml^{-1} and 1 ml of bacterial suspension were moved to wells of each plate. Plates were incubated at 37ºC for 24 h in static mode condition. Results were expressed as diameter of inhibition zone.

2.5. Characterization

Fourier Transform Infrared (FT-IR) spectra of the samples were achieved by Shimadzu 8101 M apparatus (Japan) in range of 400 and 4000 cm^{-1} wavenumbers at room temperature. The samples were prepared by grinding to dry powder with potassium bromide (KBr) and compressing to disks.

Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectra were recorded at 25°C by using FT-NMR (400 MHz) Bruker spectrometer (Ettlingen, Germany). Ten mg of sample was dissolved in 1 ml of deuterated dimethyl sulfoxide (DMSO-d6) and chemical shifts were reported per mg l^{-1} of tetramethylsilane (TMS) as internal standard.

3. Results and discussion

Cationic polymers exhibited significant antimicrobial effects against living cells of microorganisms. Therefore, several efforts have been made to develop and design novel antimicrobial agents [21].

FT-IR spectra of VEAH monomer and ABC triblock copolymer are displayed in Figure 2a and 2b, respectively. For VEAH monomer, functional groups are observed as follows: stretching vibrations of vinyl and aromatic C=C at 1663 cm^{-1}, C–N stretching vibration at 1066 cm^{-1}, aromatic group at 1475 and 1600 cm^{-1}, C–H bending vibrations at 1450–1370 cm^{-1}, γ(C–H) in aromatic ring at 823 cm^{-1} and aliphatic C–H at 2850-3100 cm^{-1}, respectively. For ABC triblock copolymer, stretching vibrations are observed at 1722 and 1641 cm^{-1} related to carbonyl groups of PHEMA and PNIPAM, respectively. N–H secondary amid and hydroxyl groups overlapped at 3475 cm^{-1} and C–H bending vibrations are visible at 1456 and 1372 cm^{-1}.

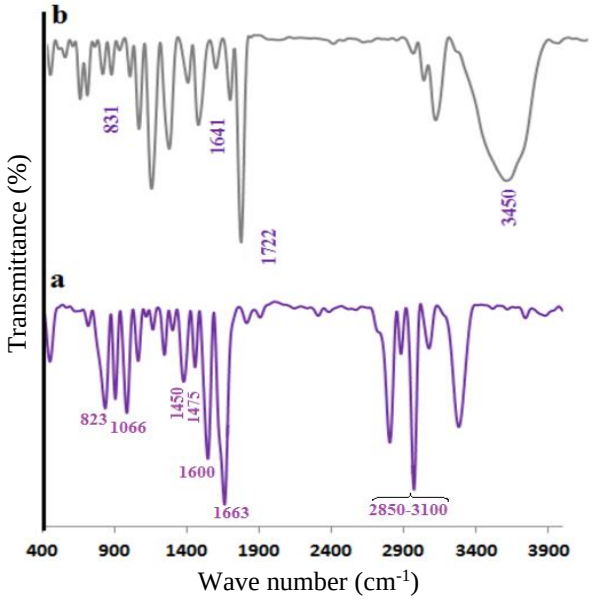

Figure 2- FT-IR spectra of a) VEAH and b) PHEMA-*b*-PNIAPM-*b*-PVEAH cationic triblock copolymer

^{1}H-NMR spectrum of VEAH monomer and ABC triblock copolymer are shown in Figure 3a and 3b, respectively. Chemical shift of VEAH protons are cleared at 1.23, 2.42 and 3.63 ppm related to CH_3, CH_2 and N–CH_2 protons, respectively. Chemical shift of vinyl and aromatic protons at VEAH monomer are cleared at 5.21-5.82 and 6.51-7.12 ppm. Successful preparation of ABC triblock copolymer shows chemical shifts of methyl and methylene protons of PHEMA backbone at 0.76-0.96 and 1.77-2.09 ppm, respectively. Chemical shifts of CH_2OH and OCH_2 protons and hydroxyl group of PHEMA can be seen at 3.58, 3.88 and 4.82 ppm, respectively (Figure 3b).

Finally, average molecular weight (M_n) and polymerization degree (DP_n) of the synthesized copolymer were calculated by ^{1}H-NMR data [19] and are reported at Table 1.

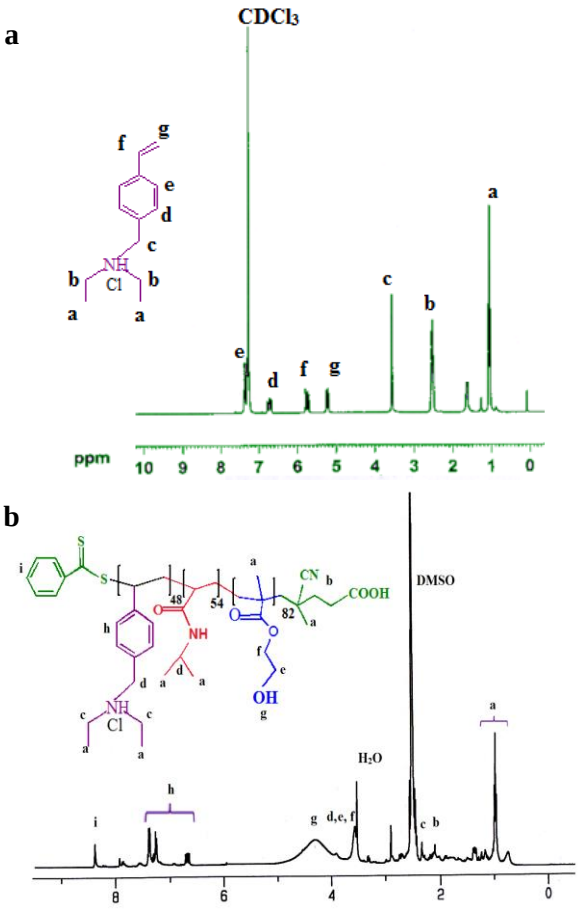

Figure 3- ^{1}H-NMR spectra of a) VEAH and b) PHEMA-*b*-PNIAPM-*b*-PVEAH cationic triblock copolymer

Table 1- Molecular weight and polymerization degree of PHEMA, PHEMA-*b*-PNIAPM and PHEMA-*b*-PNIAPM-*b*-PVEAH by ^{1}H-NMR

Sample	M_n (g mol^{-1})	DP_n
PHEMA	10950	48
PHEMA-*b*-PNIPAM	17103	54
PHEMA-*b*-PNIAPM-*b*-PVEAH	26165	82

Antimicrobial activity of ABC cationic triblock copolymer at different concentrations of 10, 25, 50 mg ml^{-1} was evaluated against viable cells of *S. aureus*, *B. cereus*, *E. coli* and *C. albicans* (Table 2). It displayed inhibition against *S. aureus* at three concentrations, which resulted in 32, 37 and 41 mm inhibition diameter, respectively. Same results were observed against *B. cereus* by inhibition diameter of 25, 29 and 34 mm. The highest antagonistic activity was observed against *C. albicans* with 48, 81 and 98 mm inhibition diameter. In contrast, the least inhibitory effect was observed for *E. coli* by 14, 17 and 21 mm inhibition diameter. According to the results, ABC triblock copolymer had significant anti-yeast impact and showed moderate inhibition against gram positive bacteria. However, gram negative bacteria were lower affected that might be due to their lipopolysaccharide outer membrane that is protective layer against some preservatives.

Table 2- Antimicrobial activity (mm) of PHEMA-*b*-PNIPAM-*b*-PVEAH cationic triblock copolymer against four microbial species

	PHEMA-*b*-PNIPAM-*b*-PVEAH concentration (mg ml^{-1})		
Microorganism	10	25	50
S. aureus	32	37	41
B. cereus	25	29	34
C. albicans	48	81	98
E. coli	14	17	21

4. Conclusion

Due to gradual resistance of microorganisms to antimicrobial agents, there is a need to new alternatives in the market. Characterization of ABC triblock copolymer showed several functional groups in the structure that can participate in chemical interactions to microbial active sites. With regard, acceptable anti-yeast and anti-bacterial effect were observed for the product in our study. Therefore, the prepared triblock copolymer could be applied as a candidate in control of tested microorganisms. However, more studies are required to find out its effectiveness against other pathogenic microbes.

5. Conflict of interest

There is no confilict of interest to be declared.

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