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Biostatic Behavior of Side Chain Charged Polycations and Polymer-Ag Complexes

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Summary

Poly[(3-methacryloylamino)propyl]trimethylammonium)chloride (PMPTA), and poly (2-acrylamido-2-methyl-1-propane sulfonic acid) (PAPSA) were synthesized by radical polymerization. Three copolymers of (3-methacryloylamino)propyl] trimethylammonium chloride and 2-acrylamido-2-methyl-1-propane sulfonic acid P(MPTA-co-APSA) with different feed monomer mol ratios were also synthesized by radical polymerization. These polymer materials and the commercial poly(vinylpyrrolidone-*co*-2-dimethylaminoethyl methacrylate) quaternized P(VP-*co*-DMAEM) were purified by ultrafiltration membranes and subsequently their complexes with Ag(I) were prepared. Antibacterial activity of all these polymers, was investigated against *Escherichia coli* (6538P), and *Staphylococcus aureus* (ATCC 28922), using the National Comittee for Clinical Laboratory Standards method [**1**]. None of these compounds exhibited biocidal or biostatic action against *E. coli*, and only PMTA and P(VP-*co*-DMAEM) exhibited some action against *S. aureus*.

Introduction

Contamination of surfaces by microorganisms is a great concern in several areas, particularly in medical devices, health care products, water purification systems, hospital and dental office equipment, food storage, *etc.* Usually disinfectants or antibacterial agents are liquids or gases of low molecular weight. With these agents, the problem of residues cannot be avoided, bringing about more serious consequences. Therefore, biocidal polymers have been received increasing attention [2-4]. Among the disinfectants possessing the most relevant characteristics are water-soluble and crosslinked polymer materials. The latters are called *insoluble polymeric contact disinfectants* (IPCD) [5-7]. Because bacteria bear negative charges on their cell surfaces under usual conditions, insoluble polymers containing cations or bearing positive charges on their surfaces can be good materials to be considered as IPCDs [8-14].

New soluble biocidal materials generally require extensive and expensive procedures before approval is granted by regulatory agencies. Considerable work by Sun et al. has been led to the development of a several series of soluble N-halamine biocidal compounds [15], and N-halogenated hydantoin derivative of polystyrene can be prepared, which is completely insoluble in water and releases less than 1 mg/L of free chlorine into flowing water [16].
Accordingly, we report in this paper some experiment test using *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) as a test bacteria and sterilized and distilled water as the suspending medium of the bacterial cells. The results of these experiments are analyzed and discussed, and the behavior features of the antibacterial activity of ammonium and sulfonic-type polymers and polymer-Ag(I) complexes are summarized.

Experimental

Reagents

[3-(methacryloylamino) propyl] trimethyl ammonium chloride (MPTA) (Aldrich), poly(vinylpyrrolidone-*co*-2-dimethylaminoethyl methacrylate) quaternized P(VP-*co*-DMAEM) (Aldrich), 2-acrylamido-2-methyl-1-propane sulfonic acid (APSA) (Aldrich), and ammonium persulfate (APS) (Aldrich), were used as received.

Preparation of the homopolymers

Poly[(3-methacryloylamino)propyl]trimethylammonium)chloride (PMPTA), and poly(2-acrylamido-2-methyl-1-propane sulfonic acid) (PAPSA) were synthesized by radical polymerization as previously described [**17**]. 0.023 mol of each monomer (MPTA or APS) and 1.4 mmol of the initiator ammonium persulfate (APS) were dissolved in 50 mL of water. The reaction was kept at 70 °C for 24 h. The products were completely soluble in water and dried under vacuum until constant weight. The fractionation was carried out by ultrafiltration membranes.

Synthesis of poly([(3-methacryloylamino)propyl] trimethylammonium chloride-co-2-acrylamido-2-methyl-1-propane sulfonic acid) P(MPTA-co-APSA)

The copolymers with different feed monomer mole ratios but keeping constant the total mole number (0.03 mol) were synthesized. APS (1 mol %) was used as initiator. The copolymerization reaction was kept at 70 °C for 24 h. The copolymers were soluble in water except that sinthesized with an equimol feed monomer ratio. The yield in all cases is higher than 90%. Finally, the products were purified and fractioned by ultrafiltration membranes. [17].
PMPTA, PAPSA, the commercial polymer poly(vinylpyrrolidone-*co*-2-dimethylaminoethyl methacrylate) was quaternized and the copolymers purified and fractionated through ultrafiltration membranes with molecular mass cut off 3,000, 10,000, 30,000, and 100,000 Da. The fraction lower than 3,000 Da was discarded. Then the polymers were liophilized, obtaining polymer fractions with different molecular weights, which are characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy.

Preparation of the Polymer-Ag Complexes

The polymer-Ag compounds were prepared using liquid-phase polymer-based retention (LPR) technique [18]. For this purpose poly([(3-methacryloylamino)propyl]

trimethylammonium chloride-*co*-2-acrylamido-2-methyl-1-propane sulfonic acid) P(MPTA-*co*-APSA)>100,000 Da, with different copolymer compositions and Ag^+ metal ions in different ratios were used. The optimum pH = 5, and other experimental conditions were taken from our previous metal ion retention studies [17].

Study of the bactericidal activity

The antibacterial activity of polymers, and polymer-Ag complexes were investigated for Escherichia coli (6538P), Gram(-) cell and Staphylococcus aureus (ATCC 28922), Gram(+) cell. Antibacterial activity was evaluated by the NCCL method [1]. According to that different aqueous solutions of the compounds were prepared, the concentrations of these solutions were 1, 2, 4, 8, 16, 32, 64, and 128 μg/mL. These solutions were inoculated with those corresponding to the bacteria and then incubated for 24 h at 37°C using a nutrient solution of tripticase soja. With this experiment it was possible to determine the minimum inhibitory concentration (MIC). From the tubes corresponding to dilutions that did not show bacteria development, a sample was taken and spread in agar-blood medium and incubated at 37°C for 24 h. From this experiment, the minimum bactericidal concentration (MBC) was obtained.

Results and Discussion

Poly[(3-(PMPTA)], was synthesized by radical polymerization with yield over 70%. The ^{1}H NMR and ^{13}C NMR are shown in figures 1 and 2 respectively.

The homopolymer poly(2-acrylamido-2-methyl-1-propane sulfonic acid) (PAPSA) was synthesized by radical polymerization with yield over 90%. The principal spectral data for PAPSA are: FT-IR (KBr pellets): 2930 (s C-H), 1647 (s C=O amide), 1400-1450 cm^{-1} (C-N amine). ^{1}H NMR (D_2O, TMS as reference, room temperature): δ= 3.2-3.5 (C-C**H**$_2$-SO_3H), 1.95 (H-C-C**H**$_2$), 1.45 ppm (C-(C**H**$_3$)$_2$).

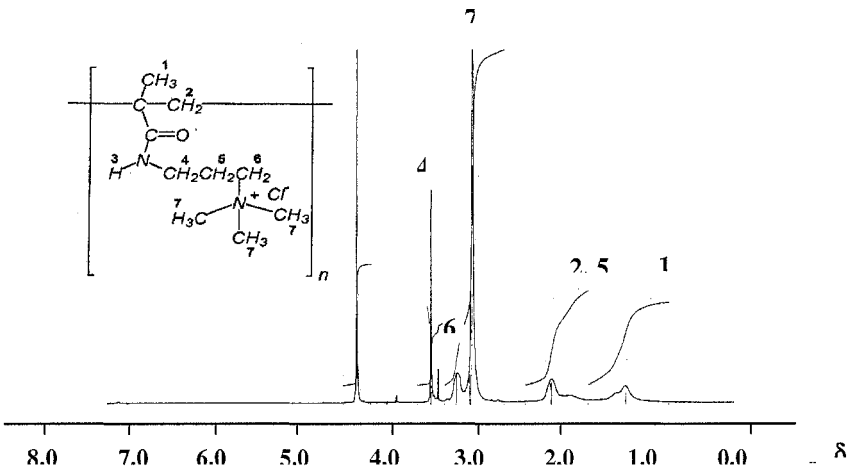


Figure 1. ^{1}H NMR spectrum of poly[(3-methacryloylamino) propyl] trimethyl ammonium) chloride (PMPTA), (250 MHz, D_2O, TMS as reference, room temperature).

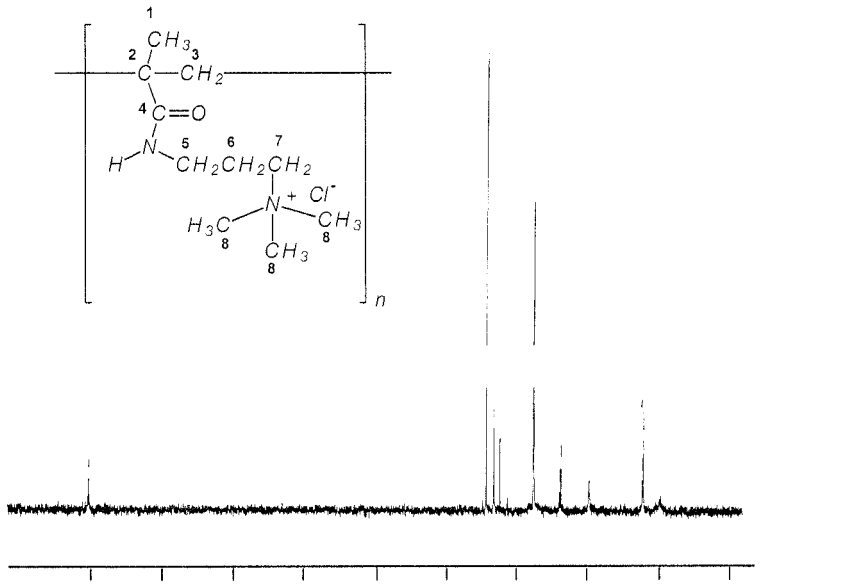
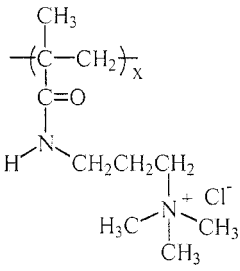
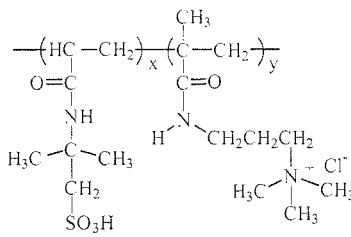


Figure 2. ^{13}C NMR spectrum of poly[(3methacryloylamino) propyl] trimethyl ammonium) chloride (PMPTA), (250 MHz, D_2O, TMS as reference, room temperature).

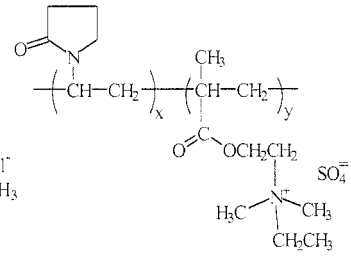
Three copolymers containing either, ammonium or sulfonic groups were synthesized. The copolymer composition was determined through ^1H NMR spectra by comparison of the integration area of the characteristic proton signal of each monomer [**17**]. The yield is higher than 90%. The copolymer with an equimol feed mol ratio was completely insoluble in water. It is due to the electrostatic interaction between the sulfonate and ammonium groups yielding an inter or intra polymer complexes. This complex will be neutral with probably crosslinked points. The other two copolymers were soluble in water. Moreover, copolymer-Ag complexes were prepared. The structure of the polymers is shown below.



PMPTA



P(APSA-*co*-MPTA)



P(VP-*co*-DMAEM)

All the compounds synthesized and tested like bactericide are shown in table I.

Table I. Compounds synthesized and tested like bactericide.

Compound	*Copolymer composition*[a)]	*Polymer fractions (Da)*	*Polymer/metal ion mol ratio*
PMPTA	-	10,000-30,000	-
P(VP-co-DMAEM)	b)	>100,000	b)
PAPSA	-	>100,000	-
P(MPTA-co-APSA)1	MPTA:APSA 1.4:1.0	>100,000	*
P(MPTA-co-APSA)2	MPTA:APSA 1.0:3.47	>100,000	*
P(MPTA-co-APSA)1Ag1	MPTA:APSA 1.4:1.0	>100,000	21.6
P(MPTA-co-APSA)1Ag2	MPTA:APSA 1.4:1.0	>100,000	10.8
P(MPTA-co-APSA)2Ag1	MPTA:APSA 1.0:3.47	>100,000	21.6
P(MPTA-co-APSA)2Ag2	MPTA:APSA 1.0:3.47	>100,000	10.8

[a)] Determined by ^{1}H NMR spectroscopy. [b)] Is a commercial product.

In a previous paper [**19**] we reported that the polycations and polymer-Ag complexes that have a charged group in the main chain show a biocidal action against the Gram(+) bacteria. In the present paper we are interested in the biocidal action of some polymer, copolymer and polymer-Ag complexes that have the positive charge in the side chain.

The results about the bactericidal activity are summarized in table II. There was no compounds that show biocidal or biostatic action against *E. coli*, a Gram(-) bacteria and only PMTA and P(VP-*co*-DMAEM) show some action against *S. aureus*, a Gram(+) bacteria. The different behavior is due to the different structures of the bacteria cell wall. The Gram(-) bacteria has an additional membrane usually named outer membrane. This additional barrier has inserted some molecules like lipopolysacharides that give a high hydrophobicity to the bacteria cell wall.

PMTA and P(VP-*co*-DMAEM) have a MIC = 32 (μg/mL). This is the minimum concentration of compound that stoped the bacteria growth, however this does not mean necessary that the bacteria are dead. The MBC for both compounds is >128 (μg/mL). The MBC value gives the minimum concentration of the compound that is necessary to kill the bacteria. If the MIC has the same value than that of MBC, the compound is bactericide. If MIC<MBC, the compound is bacteriostatic. In this case, both, PMTA and P(VP-*co*-DMAEM) show a bacteriostatic behavior, *i.e.*, the compounds are not able to kill the bacteria *S. aureus*, but stop their growth. This fact is specially important in the treatment of some infectious diseases.

The first interaction between the compound and the bacteria will be at cell wall level through the electrostatic attraction of the positive charge of the polymers and the negative charge of the bacteria cell wall. Hence the bacteria is encircled by the polymers and stay at some distance due to that the charged groups are in a long side chain (see figure 3).

Table II. Minimum Inhibitory Concentration (MIC) and Minimum Bactericide Concentration (MBC).

Compound	*MIC (μg/mL)*		*MBC(μg/mL)*	
	S. Aureus 6538P	*E. Coli ATCC28922*	*S. Aureus 6538P*	*E. Coli ATCC28922*
PMPTA (10.000-30.000 D)	32	>128	>128	-
P(VP-co-DMAEM) (>100.000 D)	32	>128	>128	-
P(MPTA-co-APSA)1 (>100.000 D)	>128	>128	-	-
PMPTA-co-APSA)2 (>100.000 D)	>128	>128	-	-
P(MPTA-co-APSA)1Ag1 (>100K) (21,6)*	>128	>128	>128	-
P(MPTA-co-APSA)1Ag2 (>100K) (10,8)*	>128	>128	>128	-
P(MPTA-co-APSA)2Ag1 (>100K) (21,6)*	>128	>128	>128	-
P(MPTA-co-APSA)2Ag2 (>100K) (10,8)*	>128	>128	>128	-

This fact probably affected the ionic exchange between the inside and the outside of the bacterial cell producing the growth stop.

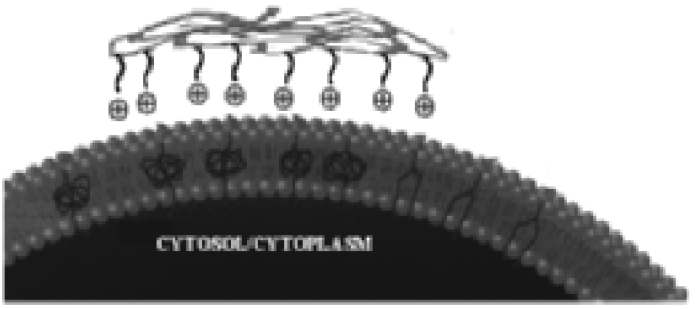


Figure 3. Interaction mechanism between side charged polycations and bacteria cell wall.

On the other hand, the copolymer P(MPTA-*co*-APSA) in both compositions, did not show, either, bactericidal or bacteriostatic activity. This is due to that the APSA co-monomer is negatively charged and hence the electrostatic interaction will be a repulsive force. This behavior is obviously observed also with the homopolymer PAPSA. The reason of synthesized copolymers with the MPTA and APSA co-monomers was to obtain compounds that were able to interact with the bacteria cell wall through the positive charged groups and interact with Ag^{+} ions through the negative charged groups due to that the antibacterial activity of silver ions is well known. However, all the copolymer-Ag complexes did not show biocidal or biostatic action probably due to that the APSA ratio in the copolymers is very high and there no interaction with the bacteria occurs.

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References

1. National Comittee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically-Second Edition (1990) Document N7-A2 **10** : 8: 31
2. Vigo TL (1992) Manmade Fibers: Their Origin and Development; Seymour, R.B.; Porter, R.S. (Eds.); Elsevier Appl Sci New York 214
3. Vigo TL (1994) Biotechnology and Bioactive Polymers; Gebelin, C.; Carraher, C. (Eds.), Plenum Press, New York 225
4. Sun Y, Sun G (2001) J Appl Polym Sci 80:2460
5. Janauer GE, Gerba CP, Ghiorse WC, Costello M, Heurich EM (1981) Chemistry in water Reuse; Ann Arbor Science: Ann Arbor, MI Vol 1
6. Gerba CP, Janauer GE, Costello M (1984) Water Res 18:17
7. Li G, Shen J (2000) J Appl Polym Sci 78: 676
8. Zhu M (1994) Technol Water Treatment 20:146
9. Dong B, Wang S, Yang Y (1988) Ion Exch Adsorption 4:277
10. Zhu Z, Huang Y (1992) Ion Exch Adsorption 8:81
11. Tashiro T (1991) J Appl Polym Sci 43:1369
12. Lambert JL, Fina GT, Fina LR (1980) Ind Eng Chem Prod Res Dev 19:259
13. Wallis C, Melnick JL, Fields JE (1971) Appl Microbiol 21:703
14. Sun G, Chen TY, Worley SD (1996) Polymer 37:3753
15. Barnella SB, Worley SD, Williams DE, (1987) J Pharm Sci 76:245
16. Sun G, Wheatly WB, Worley SD (1994) Ind Eng Chem Res 33:168
17. Rivas BL, Pereira ED, Horta A, Renamayor CS Eur Polym J (accepted).
18. Geckeler KE, Lange G, Eberhardt H, Bayer E (1980) Pure Appl Chem 52:1883
19. Rivas BL, Pereira ED, Mondaca MA, Rivas RJ, Saavedra MA (2003) J Appl Polym Sci 2003:45.