

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL POLYURETHANE IONOMERS

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ABSTRACT

This study is concerned with the removal of Staph-aureus from water by polyurethane ionomers. All the samples are quaternary ammonium salts which possess a positive charge on the surface. The ratio of the soft segment to diisocyanate and chain extender is 1:2:1 and the ionization degree is 100%. The ionomers strongly capture microbial cells on their surfaces in a living state and the hydrophilicity of polymer matrix used in the study enhanced the affinity of the polymer for bacterial cells. The adhesion of the microorganism to polymer was due mainly to electrostatic interaction between them. The calculation of the viable cell count showed that D value for Staph-aureus is not proportional to the nitrogen concentration. The microorganism capture ability of pyridinium-containing polymer is greater than that of piperazine based samples.

INTRODUCTION

Water disinfecting processes include the removal and destruction of micro-organism by both physical and chemical means. It has been widely known that chlorine can be used to disinfect and sterilize water for domestic supply. However the reaction between chlorine and organic substances in the water will produce halo methane analogues and other carcinogens thus its use should be limited. In recent years, studies have been made on the anti microbial activity of polymers^[1,2] and adsorption of bacterial cells onto such polymers^[3]. The capture of micro-organisms by a polymer appears to be a new idea of water treatment in microbiology and biotechnology. Previous study proved that the quaternary ammonium salt groups, amines, alcohol and cationic and non-ionic surfactants have anti microbial activity towards bacteria and fungi, etc. To the author's knowledge, there is no report on the capture of viruses using polyurethane ionomers yet.

Polyurethane is a block-copolymer consisting of alternating soft and hard segments. This kind of material exhibits good biocompatible and mechanical properties and has many potential applications^[5]. In the previous studies of polyurethane cationomers, materials incorporating the tertiary amine-containing diols as chain extenders were synthesized. Ionization was accomplished by quaternization of the tertiary amines of the chain extender. It was found that ion incorporation increases phase-separation and hard-domain cohesion because of the aggregation of ionic groups in the sample^[6].

This study is concerned with the removal of *Staph.aureus* from water by polyurethane ionomers derived from the reactions of pyridinium and piperazine groups containing polyurethane with γ -propane sultone and iodoethane.

EXPERIMENTAL

The polyurethanes in this paper were synthesized by two step addition reaction. Poly(tetramethylene glycol) (PTMG, $MW = 1000$) or poly(ethylene glycol) (PEG, $MW = 1000$) N,N-dimethylacetamide (DMAC) solutions, containing 0.15wt% stannous octoate catalyst, were slowly added to the stirred 1,6-hexane diisocyanate(HDI) (Aldrich Co.) or methylene diphenyldiisocyanate (MDI)/DMAC solution at 60–65°C under a dry argon purge. After 1h, the chain extender 1,4-bis (2-hydroethyl) piperazine (N) or N,N'-bis (2-hydroxyethyl) isonicotinamide (P), which were obtained from Aldrich Co., in DMAC solution was added dropwise to the stirred prepolymer solution followed by 4h reaction at 75–80°C to complete this polymerization. The polyurethane was then dried in a vacuum oven at 70°C for about 2 days. An appropriate amount of γ -propane solution and iodoethane in DMAC was added to the polyether polyurethane DMAC solution respectively and the mixture was stirred at 65°C for 1h to allow quaternization. The ratio of the soft segment to diisocyanate and chain extender is 1:2:1 and the ionization degree is 100%. All quaternary ammonium salts possess a positive charge on the surface and are widely used as effective antibacterial agents.

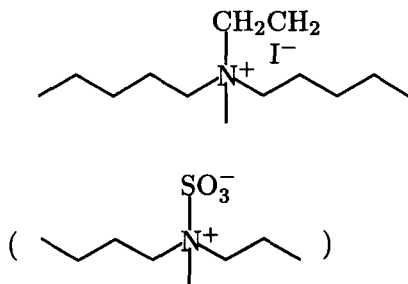
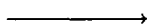
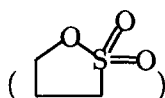
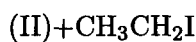
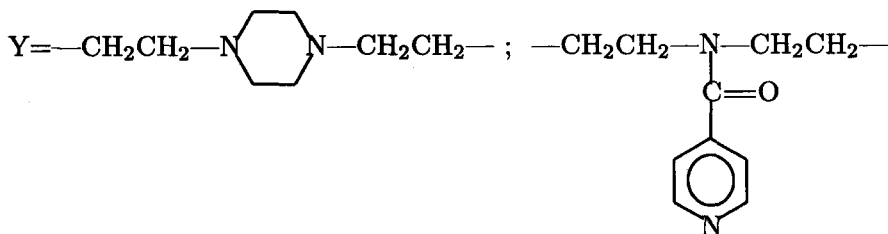
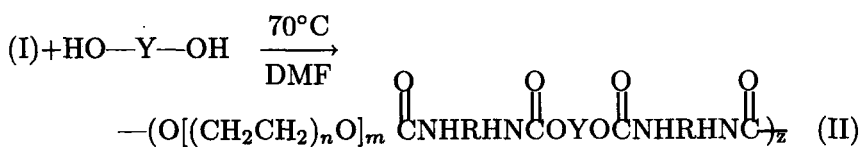
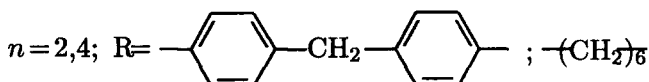
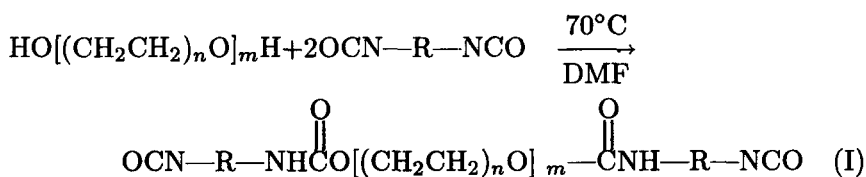
The synthetic route is briefly outlined in Scheme I and the composition of the samples are shown in Table 1.

Measurement of viable cell number in the constant suspension was carried out according to the conventional method. A small piece of polymer was placed in a round-bottomed flask and then 18ml of water was added into it. The polymer was completely wetted and stirred by using a magnetic stirrer. One ml of a cell suspension was added into the flask kept in a thermostat bath at 37°C. Before sampling, one-tenth millilitre of the constant suspension was pipetted out from the

flask at known time intervals, and consecutively diluted with the water. One-tenth millilitre of the diluted suspension was spread on an agar plate and the number of viable cells was counted from that of the colony formed on the plate.

Table 1 Composition of the samples synthesized

Sample code	Soft segment	Isocyanate	Chain Extender	Counter Ion
PEG-H-N	PEG1000	HDI	N	—
PEG-H-N-S	PEG1000	HDI	N	SO ³⁻
PEG-H-P-I	PEG1000	HDI	P	I ⁻
PEG-M-N-I	PEG1000	MDI	N	I ⁻
PTMG-M-N-I	PTMG1000	MDI	N	I ⁻
PTMG-H-N-I	PTMG1000	HDI	N	I ⁻
PTMG-H-N-S	PTMG1000	HDI	N	SO ³⁻
PTMG-H-P-I	PTMG1000	HDI	P	I ⁻



RESULTS AND DISCUSSION

Fig.1 and Table 1 show that the viable cell count obviously decreases in the presence of the ionomers. However the substantial charge in viable cell number decreased hardly at all during contact for 4 h, without the polymer. For a quantitative evaluation of the ability of the polymer to remove viable bacteria from water, a removal coefficient was defined based on the initial rate of decrease of the viable cell count (D) as follows.

$$D = V/(W \times t) \times \log N_0/N_t$$

In this ionomer system, V (volume of viable cell suspension) is 20ml, W (dry weight of the ionomer) is 0.25g and t (the contact time) is 2–4h, N_0 (the initial viable cell number) is 1.09×10^7 and N_t is the viable cell number at contact time.

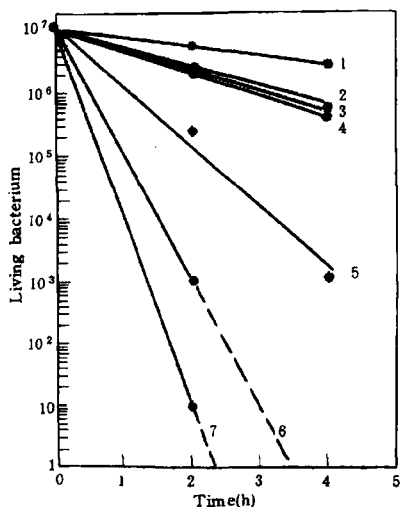


Fig. 1 Plot of viable cell number with lapse of time for Staph-aureus in contact with ionomers in sterilized water



Fig. 2 Scanning electron micrographs of the surface of PTMG-H-N-I bead after contact with a Staph-aureus suspension

- 1) Control; 2) PEG-M-N-I; 3) PTMG-H-N-I; 4) PTMG-H-N-S; 5) PTMG-H-P-I;
6) PEG-H-N-S; 7) PEG-H-P-I

In the early stage of contact, a linear correlation was observed between the logarithm of viable cell number and contact time. In this paper, the values of $V/W \times t$ and N_0 remain constant. From Fig.1 it can be seen that D values for Staph-aureus are decreased by ionomers in the order of PEG-H-P-I > PEG-H-N-S > PTMG-H-P-I > (PTMG-H-N-S, PTMG-H-N-I and PEG-M-N-I). The D values of the last three samples are on the same level.

PEG based ionomers have a higher hydrophilic portion so that PEG-H-P-I and PEG-H-N-S are able to be wetted easily and their D values are greater than that of PTMG-H-P-I and PTMG-H-N-S. It is postulated that hydrophilicity of polymer matrix can enhance the affinity of the polymer for bacterial cells. The proportion of hydrophilic to hydrophobic chains seems to have the contribution to adsorption of cells. On the other hand, the type of tertiary amine also has effect on the removal of viruses by ionomers. The D value of PTMG-H-P-I is higher than that of PTMG-H-N-I. This result showed that the micro-organism's capture ability of pyridinium-containing polymer is greater than that of piperazine based samples. As the pyridinium groups are highly hydrophilic, it is reasonable to believe that the difference in hydrophilicity between the two ammonium-type polymers appears to play an important role in the ability to capture bacterial cells. Comparing the curves of PTMG-H-N-S and PTMG-H-N-I in Fig.1, it is clear that the cationomer and zwitterionomer exhibit little effect on the ability of microorganism capture of quaternized ionomers.

Table 2 Decrease in viable cell numbers and coefficient value from water

	Sample code	Contact time	Viable cell number	D (ml/g.h)
1	Control	0	1.09×10^7	
	2h	5.74×10^6	11.14	
	4h	3.00×10^6	11.20	
2	PEG-H-N-S	0	1.09×10^7	
	2h	<1000	>161.5	
	4h	<1000		
3	PTMG-H-N-S	0	1.09×10^7	
	2h	2.55×10^6	25.2	
	4h	4.23×10^5	28.2	
4	PTMG-H-N-I	0	1.09×10^7	10^7
	2h	2.33×10^6	26.8	
	4h	5.24×10^5	26.3	
5	PTMG-H-P-I	0	1.09×10^7	10^7
	2h	2.18×10^5	67.95	
	4h	1045	80.3	
6	PEG-H-P-I	0	1.09×10^7	
	2h	<10	241.5	
	4h	none		
7	PEG-M-N-I	0	1.09×10^7	
	2h	2.72×10^6	24.1	

In addition, an examination was made to determine whether the increase in D value was due to the bactericidal action of substances leached from the polyurethane or by absorption of the cells to their surface. The photographs of STM proved that both insoluble ionomers based on P and N strongly capture microbial cells on their surfaces in a living state because insoluble polymer is unable to penetrate the cell

wall. In these cases, living bacterial cells would be captured on the surface of the ionomers. It is postulated that the adhesion of bacterial cell to solid surface is caused by electrostatic, hydrophilic interaction, and other forces of attraction. Quaternized nitrogen atoms behave as cations in water and bacterial cells have predominantly negative charges at physiological pH^[4]. It has been reported that the adhesion of bacterial cells to poly(lauryl-4-vinylpyridinium-co-divinylbenzene iodide) is due mainly to electrostatic interaction^[3].

REFERENCES

- [1] Nakagawa, Y.; Yamano, Y.; Tawaratani, T., et al., *Appl. Environ. Microbiol.*, **1983**, *43*: 1041.
- [2] Ikeda, T. and Tazuke, S., *Makromol. Chem. Rapid Commun.*, **1983**, *4*: 459.
- [3] Nakagawa, Y.; Tawaratani, T.; Konrai, H., et al, *Appl. Environ. Microbiol.*, **1984**, *47*: 88.
- [4] Daniels, S.L. in "Adsorption of Microorganisms to Surfaces", Wiley, New York, **1980**, Chap 8, p.163.
- [5] Erfle, D.J.; McDiamond, H., Santerra, J.P., et al., "The 20th Annual Meeting of the Society for Biomaterials", Boston, Massachusetts, USA, **1994**: 294.
- [6] Ding, Y.S.; Register, R.A.; Yang, C.Z., et al., *Polymer*, **1989**, *30*: 1204.