Study of Pyridinium-Type Functional Polymers. II. Antibacterial Activity of Soluble Pyridinium-Type Polymers

GUANGJI LI, JIARUI SHEN, YINLAN ZHU

Department of Polymer Science and Engineering, South China University of Technology, Guangzhou 510641, People's Republic of China

Received 19 November 1996; accepted 27 August 1997

ABSTRACT: The antibacterial activity of a series of soluble pyridinium-type polymers with different compositions against *Escherichia coli* (*E. coli*) suspended in sterilized distilled water was investigated by a colony count method. The results show that the antibacterial activity of the soluble pyridinium-type polymers is characterized by their activity to kill bacterial cells and this activity can be enhanced as the content of the pyridinium group (C_q) in the polymers increases. The species of the bacteria has a great influence upon the antibacterial activity of the soluble pyridinium-type polymers. The polymers possess a strong ability to kill Gram-positive and Gram-negative bacteria, and yeasts, excepting *Bacillus subtilis,* having gemmae and fungi. The toxicity of this kind of polymer has also been appraised. In the acute stimulation and allergy experiments, the red maculae, edema, and abnormal phenomena of an allergy on the skin of the tested animals were not observed. The acute toxicity experiment shows that the LD_{50} of the polymer is 2330 mg/kg, implying that this kind of polymer has only very weak toxicity. This is significant for the application of soluble pyridinium-type polymers. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci **67:** 1761–1768, 1998

INTRODUCTION

By the antibacterial activity of polymers, we mean a kind of interaction between polymers and bacteria, i.e., bactericidal or bacteriostatic action of polymers against bacterial cells, or capturing bacterial cells by the polymers. In general, antibacterial agents like bactericides or disinfectants are low molecular weight compounds. Phenols and cationic compounds are two main groups of compounds which are used almost exclusively for disinfectants.[1] The latter covers many kinds of compounds differing considerably in chemical structure. Their common features are the presence of strongly basic groups attached to a fairly massive nonpolar molecule.[1] Among them, quaternary ammonium (QA) salts and biguanides are the best and most widely used antibacterial agents. Interestingly, they kill bacteria and fungi by interaction with the constituents of the cell envelope: interaction with the negative charges of the cell wall, destabilization, and weakening of the cytoplasmic membrane (thanks to their lipophilic moiety) leading to a loss of cytoplasm constituents due to the very high osmotic pressure.[2] On these grounds, it is considered that electrostatic interaction of the positive charges on the molecules of the antibacterial agents with the negatively charged species present in the cytoplasmic membranes (such as acidic phospholipids and membrane proteins) can play an important role in course of the killing of bacteria using these bacterial agents.

Therefore, polycations containing QA salts or biguanides, which are similar to QA salts or biguanides in the molecular structure, are expected to be capable of interacting strongly with the species

Correspondence to: G. Li.

Contract grant sponsor: National Natural Science Foundation (China).

Journal of Applied Polymer Science, Vol. 67, 1761–1768 (1998)

 CCC 0021-8995/98/101761-08

bearing the opposite charges in the cytoplasmic membrane of bacterial cells. This implies that polycations could be the best candidates for effective antibacterial agents. From these viewpoints, some researchers studied the antibacterial activities of polycations like poly[trialkyl(vinylbenzyl) ammonium chloride]s,[3,4] poly(hexamethylene biguanide hydrochloride),[5] polyurethane coatings with pendant QA salts,[6] and polymeric pyridinium salts with various main-chain structures[7,8] and found that these polycations possessed much higher antibacterial activity than did the corresponding monomers. This result undoubtedly provides an important and scientific basis for developing a new generation of polymeric antibacterial agents.

As compared with conventional antibacterial agents of low molecular weight, polymeric antibacterial agents have the advantages that they are nonvolatizable, chemically stable, and hard to permeate through the skin of a man or an animal. So, they can significantly reduce losses associated with volatilization, photolytic decomposition, and transportation. Moreover, increased efficiency, selectivity, and handling safety are additional benefits which may be realized. Polymeric antibacterial agents offer great promise for enhancing the efficacy of some existing antibacterial agents as well as reducing the environmental problems associated with others.[9] For this reason, an investigation on polymers with antibacterial activity represents a new developing direction in the field of antibacterial agents.

In this work, we conducted a systematic investigation into the behavioral features of antibacterial activity of soluble pyridinium-type polymers against *Escherichia coli* (*E. coli*) suspended in sterilized distilled water and the factors influencing it. In addition, we made a preliminary research on their toxicity. We describe and discuss here the results.

EXPERIMENTAL

Materials

Soluble Pyridinium-Type Polymers

Using the procedure described elsewhere,[10] a series of copolymers of 4-vinylpyridine (4VP) and styrene (St) with different ratios of 4VP and St chain units, i.e., P(4VP–St), was first synthesized, and then soluble pyridinium-type polymers in the bromide form were prepared by quaternizing each P(4VP–St) using benzyl bromide (BzBr). The quaternized P(4VP–St) using BzBr were designated as Q-P(4VP–St). The results of the composition analysis of these soluble pyridinium-type polymers[10] are given in Table I. C_1, C_q, C_{ion}, and DQ, respectively, stand for the content of 4VP, the pyridinium group content, the ion content, and the degree of quaternization in each Q-P(4VP–St) sample. C_q was determined by the manner of back titration in argentometry. From C_q and the mol fraction of 4VP in the corresponding precursor copolymer (which has been also determined by the method of nonaqueous titration), C_1, C_{ion}, and DQ can be calculated.[10] DQ is the percentage of the quaternized 4VP to the total 4VP in each Q-P(4VP–St) sample. Therefore, DQ is equal to C_{ion}/C_1.

Table I Composition Analysis of the Soluble Pyridinium-Type Polymers

Sample No.	C_1 (mol %)	C_q (mmol/g)	C_{ion} (mol %)	DQ (%)
Q-A1	17.7	1.11	14.2	80.1
Q-A2	25.0	1.69	24.8	99.3
Q-A3	36.0	2.15	35.6	98.9
Q-A4	45.0	2.48	44.7	99.4
Q-A5	52.8	2.70	52.5	99.5
Q-A6	62.2	2.92	61.1	98.2
Q-A7	69.9	3.09	68.9	98.5
Q-A8	78.1	3.24	76.1	97.4

It should be indicated that the prepared Q-P(4VP–St) polymers are mostly water-soluble, but the sample with only 14.2% of the pyridinium groups (Q-A1) can be hardly dissolved in water and can be swelled. The solubility depends strongly on the composition of the polymers.

Bacteria

Bacillus subtilis MIG 1.19 and MIG 1.22, *Staphylococcus aureus* MIG 1.55, *Escherichia coli* (*E. coli*) 44113 and MIG 1.45, *Pseudomonas geniculata* MIG 1.48, *Pseudomonas pudida* MIG 1.57, *Saccharomyces cerevisiae* MIG 2.75, *Saccharomyces cerevisiae var. ellipsoideus* MIG 2.83, *Penicillium funiculosum* MIG 3.104, and *Aspergillus niger* CAM 01 were used as test bacteria for this work. All these strains were provided by the Microorganism Institute of Guangdong, except for *E. coli* 44113, which was provided by the University

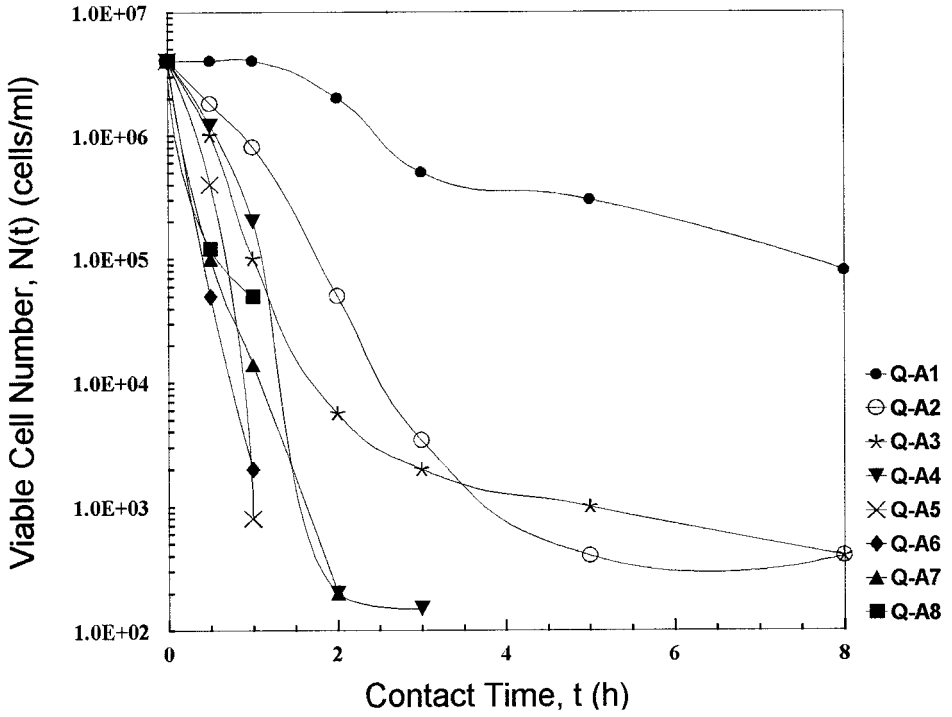

Figure 1 Decrease in viable cell number with the lapse of time in contact of *E. coli* (44113) cells with soluble pyridinium-type polymers Q-P(4VP–St) in sterilized distilled water.

of the First Military Medicine. In these bacteria, Gram-positive and Gram-negative bacteria were cultured at 37°C for 24 h on a nutrient agar plate; yeasts and fungi were cultured at 28°C for 48 h, respectively, on a malt extract agar plate and on a potato dextrose agar plate before use.

Antibacterial Tests for Soluble Pyridinium-Type Polymers

All procedures in the antibacterial tests for soluble pyridinium-type polymers were carried out under aseptic conditions and performed using a batch method.

One loopful of fresh bacterium was mixed with an appropriate amount of sterilized distilled water to prepare a bacterial cell suspension, which was immediately used for antibacterial tests for soluble pyridinium-type polymers. In a glass container with a cotton stopper were placed a certain weight of each soluble pyridinium-type polymer and a certain volume of the bacterial cell suspension, the viable cell number which was controlled over a range of the order of 10^6 cells/mL. The mixture was continually shaken. At prescribed time intervals, 0.5 mL of the treated cell suspension was pipetted out from the container and quickly mixed with 4.5 mL of sterilized physiological saline, and then decimal serial dilutions were prepared from this by taking 0.5 mL into 4.5 mL of sterilized physiological saline and mixing. The viable cell number in each of the treated cell suspensions was determined by the surface-plate method or by the pour-plate method. The colonies were counted after the inoculated plates were incubated at 35–37°C for 24–48 h, except for the case of yeasts and molds, whose colonies were counted after the inoculated plates were incubated at 28–30°C for 72 h. The counting was done in triplicate every time.

Scanning Electron Microscopy

The sample which was brought into contact with the *E. coli* cell suspension in a batch system was collected and repeatedly rinsed with sterilized physiological saline. The rinsed sample was fixed with glutaraldehyde and osmium oxide solutions, and then the sample was dehydrated with a graded ethanol series. After dehydration, the

sample was dried in a critical point dryer (Model DX-1, EIKO Co.), mounted on a sample stand, and coated with gold with an ion fine coat (Model JFC-1100, JEOL). Observation of the sample was done with a scanning microscope (Model JSM-T300, JEOL).

RESULTS AND DISCUSSION

Behavioral Features of Antibacterial Activity of the Soluble Pyridinium-Type Polymers

In a glass container with a cotton stopper were placed 1.0 g of each soluble pyridinium-type polymer and 10.0 mL of the *E. coli* cell (44113) suspension, in which the initial viable cell number is $N(0)$. Supposing that the viable cell number at the contact time t is $N(t)$, the decreased behaviors of $N(t)$ can be seen in Figure 1.

It can be seen from Figure 1 that the soluble pyridinium-type polymers Q-P(4VP–St), except Q-A1 of the lowest C_q, all possess the function which makes the viable cell number in the bacterial suspension, $N(t)$, decrease greatly, showing a strong antibacterial activity against *E. coli.*

Owing to the relatively great amount of the polymer used in this test, there existed a part of the swelled but undissolved polymer in the Q-P(4VP–St)/cell suspension system, and the system exhibited a light yellow color. The color of the system became deep with increase in the C_q of the polymer, i.e., in this system, the polymer was only partly dissolved and its dissolvability increased with increase in its C_q. Therefore, a decrease in $N(t)$ can only be contributed to two causes: One is that some chemical or physical action may take place between the swelled polymer and the bacterial cells; the other is that the dissolved polymer may effectively destroy the structure of the cells and inactivate the cells. Both can lead to a decrease in $N(t)$.

Based on the above inference, Q-A3 treated with a bacterial suspension in a batch system was observed using SEM. From the SEM photographs shown in Figure 2, *E. coli* cells adhering to the surface of Q-A3 can be clearly seen. To clarify whether the bacterial cells on its surface were alive, the sample of Q-A3 which was brought into contact with the bacterial suspension was extensively washed with sterilized physiological saline and then inoculated into a nutrient broth. After the broth was incubated, no sign of the cell multiplication was observed. For the sake of prudence, the broth was spread on a nutrient agar plate and the plate was incubated. As a result, no colonies appeared on the incubated plate. These experiments indicate that the swelled polymer in the bacterial suspension has the ability to capture and inactivate bacterial cells.

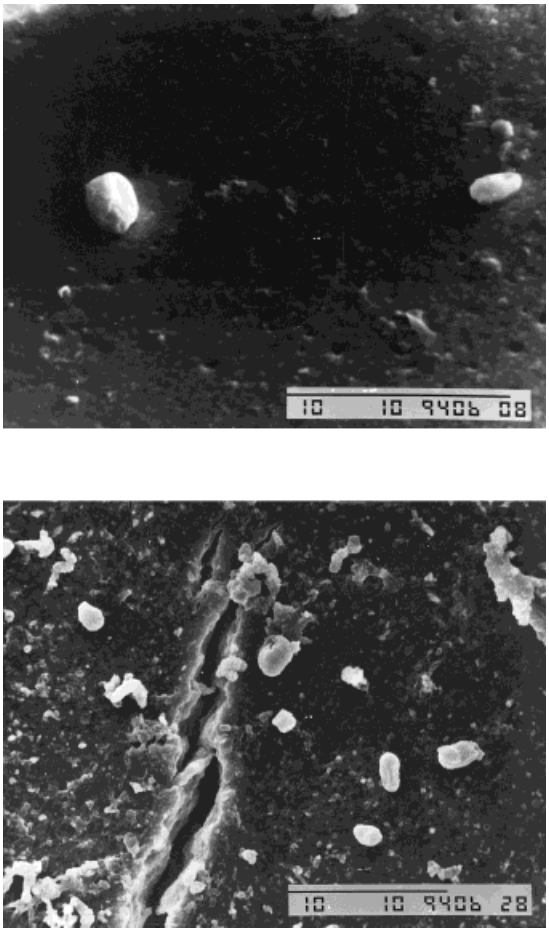

Figure 2 SEM photographs of the surface of Q-A3 which was brought into contact with the *E. coli* cell suspension.

On the other hand, sample Q-A6 was selected for another similar test, but the amount of it used in a Q-P(4VP–St)/cell suspension system was greatly cut down and it was fully dissolved prior to being brought into contact with the cell suspension so as to study the antibacterial activity of a soluble pyridinium-type polymer completely dissolved in the system. The results in Table II demonstrate that when the concentration of Q-A6 in the system is 100 mg/L $N(t)$ is reduced in 5 min from 1.80×10^6 to 2.01×10^3 cells/mL, or the percentage sterilization reaches 99.89%. In this

Table II Antibacterial Activity of Soluble Pyridinium-type Polymer Q-A6 Against *E. coli* (44113) in Sterilized Distilled Water

Polymer Concentration (mg/L)	Viable Cell Number $N(t)$ (cells/mL), at the Following Contact Times t (s)					
	0	5	10	30	60	300
100	1.80×10^6	3.20×10^5 (82.22%)	7.00×10^4 (96.11%)	3.70×10^4 (97.94%)	1.15×10^4 (99.36%)	2.01×10^3 (99.89%)

The figures in the parentheses are the percentage sterilization.

test, Q-A6 was fully dissolved, so the death of bacteria can only be attributed to the destruction of the cell structure caused by the dissolved Q-A6.

Thus, it can be seen that the swelled or the dissolved pyridinium-type polymer can produce the lethal action against *E. coli* cells, i.e., the antibacterial activity of soluble pyridinium-type polymers is characterized by their ability to kill bacteria.

Effect of the Pyridinium Group Content in Soluble Pyridinium-Type Polymers

In Figure 1, $N(t)$ always reduces with t and the degree of reduction in $N(t)$ varies with the polymers, which reflects the dependence of the bactericidal ability of polymers on their compositions. Roughly speaking, their bactericidal ability exhibits the tendency to be enhanced as the content of the pyridinium group in the polymers, C_q, increases.

For the sake of convenience, we adopted a parameter, i.e., the percentage sterilization P, to describe the ability of killing bacteria by each polymer. P can be calculated by the following equation:

$$P = \frac{N(0) - N(t)}{N(0)} \qquad (1)$$

When soluble pyridinium-type polymers are used as a bactericide, their ability to kill bacteria within a short time often attracts much attention. Thus, we calculated and listed in Table III the values of P when $t = 0.5$ h according to the experimental data used in Figure 1.

Pyridinium-type polymers are quaternary ammonium salts which possess a positive charge on the macromolecular chains or the surface. Quaternary ammonium salts are widely used as effective antibacterial agents. The method of the lethal action of these cationic disinfectants has been summarized as the following six steps[7]: (1) absorption onto the bacterial cell surface, (2) diffusion through the cell wall, (3) binding to the cytoplasmic membrane, (4) disruption of the cytoplasmic membrane, (5) release of the K^+ ion and other cytoplasmic constituents, and (6) precipitation of the cell contents and death of the cell. Owing to the fact that the molecular weight of them is much higher than that of conventional cationic disinfectants and they possess a long-chain structure, soluble pyridinium-type polymers inevitably exhibit completely different conformation and activity from those that the disinfectants do in a bacterial suspension. The macromolecular conformation and activity can affect the contact process of macromolecular chains with bacterial cells and their ability to diffuse through the cell wall. According to the method of the lethal action of cationic disinfectants, the inactivation of bacterial cells caused by the soluble pyridinium-type polymer can be taken as two processes at least: The first one is the contact process of the polymer with the cells, and the second one is the dying process of the cells, which resulted from some chemical or physical or biological changes in the cells.

The prepared Q-P(4VP–St) is practically a cationic polyelectrolyte which has a positive charge along its macromolecular chains. Also, bacterial cells usually have negative charges and behave as colloidal particles in aqueous systems, so the cells can be considered as living colloids.[11] Then, a Q-P(4VP–St)/bacterial suspension system is the system containing different species of charges. It should be pointed out that in such a system, even though the molecular weight of the cationic polyelectrolyte is very low, flocculation can also occur.[12] However, the mechanism of flocculation in this system is greatly different from that in the system containing the same species of charges. The flocculation occurring in the system containing differ-

Table III Data on *P* for Killing Bacteria by Contact of Each Q-P(4VP–St) Sample with *E. coli* Cell Suspension for 0.5 h

Sample No.	*P* (%)
Q-A1	0.00
Q-A2	55.00
Q-A3	75.00
Q-A4	70.00
Q-A5	90.00
Q-A6	98.75
Q-A7	97.50
Q-A8	97.00

ent species of charges is generally thought to be the result of dual actions of charge neutralization and bridging sorption.[12] In addition, there is the experimental fact that a polyelectrolyte can make bacterial cells bearing negative charges in a aqueous system flocculate.[13,14] Thus, the contact process of Q-P(4VP–St) with the bacterial cells (i.e., the first process) can be taken as the flocculation process.

Since in measuring the relation between $N(t)$ and t of each Q-P(4VP–St)/cell suspension system the polymer concentration was high enough to make the system contain undissolved polymer, it was impossible for the polymer chains to expand fully. In this case, the probability that the polymer chains result in the flocculating of the bacterial cells by bridging sorption is very little. Thus, the flocculation caused by charge neutralization is the main factor allowing the polymer to come into contact with the bacterial cells. When the polymer concentration in the system is given, the higher the C_q in the polymer, the higher is the charge density along the polymer chain and the more easily the polymer can come into contact with the bacterial cell by charge neutralization. For this reason, the changes in the curve of $N(t)$ vs. t shown in Figure 1 and the values of P presented in Table III all exhibit the tendency that the antibacterial activity of soluble pyridinium-type polymers is enhanced as the C_q in the polymers increases. As for the result shown in Figure 1, another important reason for it is that for a given excessive polymer the solubility of the polymer increases with an increase in C_q in it, which enhances the antibacterial effect.

By comparing the above-mentioned results concerning the sample Q-A6, it can be noticed that the biocidal activity of Q-A6 at the low concentration (100 mg/L) is much stronger than that when the concentration was a thousand times higher. It seems to be inconsistent. After carefully analyzing the experimental method and procedures, we think that this enormous difference is certainly due to the complete dissolution of Q-A6 at the low concentration, in contrast to a slow and partial dissolution at the high concentration.

Effect of the Species of Bacteria

The antibacterial activity of Q-P(4VP–St) depends on the species of bacteria. Some of the preliminary experimental results are given in Table IV. The ability of Q-P(4VP–St) to kill bacteria is different according to the species. Q-P(4VP–St) exhibits a strong ability to kill Gram-positive and Gram-negative bacteria, and yeast, excepting *B. subtilis,* having gemmae and fungus. However, the ability to kill bacteria does not appear to depend upon the Gram-positive or Gram-negative character.

In the interaction between the substances bearing net electric charges, electrostatic forces should be very crucial. Therefore, the electrostatic interaction between the soluble pyridinium-type polymer chain bearing positive charges and the bacterial cell bearing negative charges was expected to play an important role in killing bacterial cells by the polymer. Information on surface charges of some bacterial cells has been reported. The surface charges of *S. aureus, Bacillus* sp., *P. aeruginosa,* and *E. coli* are 2.56×10^{-16}, 2.04×10^{-16}, 0.48×10^{-16}, and 0.24×10^{-16} g eq/g, respectively.[15] By analyzing the antibacterial activity of these four species of bacteria, we can draw the conclusion that the relation between the antibacterial activity of the polymer and the surface charges of bacterial cells is obscure. Thus, electrostatic interaction between the positive charge of the polymer and the negative charges of the cells is not the decisive factor making the polymer possess a strong ability to kill bacterial cells.

In addition, among the above-mentioned four species of bacteria, *S. aureus* is a bacterium of high hydrophobicity; *Bacillus* sp., of medium hydrophobicity; *P. aeruginosa,* of medium hydrophilicity; and *E. coli,* of high hydrophilicity.[15] According to the data presented in Table IV, there seems not to be a clear relationship between the ability of the polymer to kill bacteria and the hydrophobicity of bacterial cells. However, the affinity of bacterial cells for soluble pyridinium-type

Table IV Antibacterial Activity of Q-A6 Against Various Bacteria[a]

Bacteria	Viable Cell Number $N(t)$ (cells/mL), at the Following Contact Times, t (min)					
	0	1	3	5	10	20
Gram-positive bacteria						
Bacillus subtilis: MIG1.19	6.67×10^4	2.08×10^4	1.91×10^4	1.78×10^4	1.61×10^4	1.07×10^4
MIG1.22	1.03×10^5	4.98×10^4	4.92×10^4	4.80×10^4	4.91×10^4	4.51×10^4
Staphylococcus aureus: MIG1.55	8.43×10^5	3.80×10^3	3.07×10^3	1.26×10^2	1.00×10^2	66
Gram-negative bacteria						
Escherichia coli: strain 44113	1.31×10^6	8.37×10^3	3.82×10^3	1.47×10^3	9.33×10^2	1.87×10^2
MIG1.45	1.27×10^5	4.96×10^4	4.30×10^4	3.73×10^4	3.63×10^3	3.41×10^3
Pseudomonas geniculata: MIG1.48	4.43×10^5	6.90×10^3	2.30×10^3	2.65×10^3	1.47×10^3	1.50×10^3
Paeudomonas putida: MIG1.57	2.74×10^6	3.40×10^3	3.78×10^3	2.06×10^3	1.70×10^3	1.37×10^3
Yeasts						
Saccharomyces cerevisiae: MIG2.75	1.75×10^6	8.08×10^4	3.96×10^4	2.05×10^4	3.63×10^3	3.43×10^3
var. ellipsoideus: MIG2.83	1.71×10^6	4.33×10^2	67	0	0	0
Fungi						
Penicillium funiculosum: MIG3.104	4.73×10^5	4.23×10^5	3.77×10^5	3.87×10^5	3.53×10^5	2.80×10^5
Aspergillus niger: CAM01	5.37×10^5	5.11×10^5	4.83×10^5	4.37×10^5	3.97×10^5	3.70×10^5

[a] The concentration of the polymer Q-A6 is 100 mg/L.

polymers should be related to their hydrophobicity and will influence the contact process of the polymer with bacterial cells and then proceed to the antibacterial activity of the polymer against bacteria. Thus, on this subject, there is much work to be done.

Toxicity of Pyridinium-Type Polymers

When pyridinium-type polymers are used, especially the soluble polymers, their toxicity will certainly be taken into consideration. Therefore, we selected the soluble pyridinium-type polymer Q-A6 possessing a high content of the functional groups and a strong bactericidal ability as the sample for toxicity appraisement and consigned the experiment in the toxicity appraisement of Q-A6 to the Municipal Health & Anti-Epidemic Station of Guangzhou.

The acute stimulation and allergy experiments on the skin of guinea pigs using sample Q-A6 were carried out according to the test standard GB 7919-87. The results indicated that red maculae, edema, and abnormal phenomena of allergies on the skin of guinea pigs used in the experiments were not observed; the polymer can not cause an allergy or stimulate the skin.

The acute toxicity experiment was made by the Horn method. LD_{50} obtained from the experiment results is 2330 mg/kg. According the graded standard of acute toxicity, the polymer has only lower toxicity.

CONCLUSIONS

Several important results have been obtained in the study on the antibacterial activity of soluble pyridinium-type functional polymers:

1. The antibacterial activity of the soluble pyridinium-type polymers is characterized by their activity to kill bacterial cells and this activity can be enhanced as the content of pyridinium group (C_q) in the polymers increases.
2. The species of bacteria has a great influence upon the antibacterial activity of the soluble pyridinium-type polymers. The prepared polymers possess a strong ability to kill Gram-positive and Gram-negative bacteria, and yeast, excepting *B. subtilis,* having gemmae and fungi.
3. The soluble pyridinium-type polymers cannot basically cause the skin of the contacted animal or man to produce an allergic reaction or other metamorphosis reactions. The LD_{50} of the polymer is 2330 mg/kg, implying that this kind of polymer has only very weak toxicity.

We are grateful to the National Natural Science Foundation for its financial support.

REFERENCES

1. T. J. Franklin and G. A. Snow, *Biochemistry of Antimicrobial Action,* Chapman and Hall, London, 1981, Chap. 3.
2. J. Hazziza-Laskar, G. Helary, and G. Sauvet, *J. Appl. Polym. Sci.,* **58,** 77 (1995).
3. T. Ikeda and S. Tazuke, *Makromol. Chem. Rapid Commun.,* **4,** 459 (1983).
4. T. Ikeda and S. Tazuke, *Makromol. Chem.,* **185,** 869 (1984).
5. T. Ikeda, S. Tazuke, and M. Watanabe, *Biochimica et Biophysica Acta,* **735,** 380 (1983).
6. N. Nurdin, G. Helary, and G. Sauvet, *J. Appl. Polym. Sci.,* **50,** 663 (1993).
7. N. Kawabata and M. Nishiguchi, *Appl. Environ. Microbiol.,* **54,** 2532 (1988).
8. N. Kawabata, T. Hayashi, and T. Matsumoto, *Appl. Environ. Microbiol.,* **46,** 203 (1983).
9. S. T. Oh, C. S. Ha, and W. J. Cho, *J. Appl. Polym. Sci.,* **54,** 859 (1994).
10. G. Li, J. Shen, and Y. Zhu, *J. Appl. Polym. Sci.,* **62,** 2247 (1996).
11. G. Bitton and K. C. Marshall, *Adsorption of Microorganisms to Surfaces,* Wiley, New York, 1980, Chap. 1.
12. N. Man and T. Sho, Eds., *Polymeric Agents of Water Treatment* (Z. X. Chen, Translator), Chemical Industry Press, Beijing, 1985, Vol. 2.
13. B. A. Pethica, *Exp. Cell Res. Suppl.,* **8,** 123 (1961).
14. R. H. Harris and R. Mitchell, *Annu. Rev., Microbiol.,* **27,** 27 (1973).
15. N. Kawabata, Y. Ueno, K. Torii, and T. Matsumoto, *Agric. Biol. Chem.,* **51,** 1085 (1987).