Biocidal Polymers Active by Contact. IV. Polyurethanes Based on Polysiloxanes with Pendant Primary Alcohols and Quaternary Ammonium Groups

J. HAZZIZA-LASKAR, G. HELARY, and G. SAUVET*

Laboratoire de Recherches sur les Macromolécules (U.A. 502), Université Paris-XIII, Avenue J.-B. Clément, 93430 Villetaneuse, France

SYNOPSIS

Functional polysiloxanes bearing both primary alcohols and quaternary ammonium salts (QAS) as lateral substituents were prepared. The synthesis involves a cohydrosilylation of allylic derivatives (*N*,*N*-dimethylallylamine and allyloxytrimethylsilane) with various poly(dimethylsiloxane-*co*-hydrogenomethylsiloxane)s. During the quaternization of the tertiary amino groups the alcohol functions are also deprotected. The hydroxyl groups allow the polysiloxane to be incorporated in polyurethane films whereas the QAS impart biocidal properties to the coating. In the case of a QAS bearing a hexadecyl substituent, a very high activity was found against *Escherichia coli* without any observable diffusion. The mode of action by contact between the solid polymer and the microorganisms was confirmed by the excellent durability of the biocidal power after 1 month of immersion in water. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

In many domains (food manufacturing, biomedical devices, sanitary equipments, outdoor or marine paints, etc.), polymers presenting a surface free of microorganisms (bacteria, fungi, algae) are required or would represent a strong advantage. This property is usually imparted by the addition of a low molecular weight biocide that inhibits the growth of the microorganisms by slowly diffusing out of the polymer. In some cases (antifouling paints for example), a better control of the rate of leaching may be obtained by linking the toxic group to the matrix through a chemical bond sensitive to hydrolysis. However, the drawbacks are the same in both cases: a loss of activity with time and environmental problems due to the high toxicity of the liberated compounds. For some medical applications, a controlled rate of release of a bactericide may be an objective,[1] but this is not a satisfactory solution if a permanent protection of a polymer surface against microorganisms is the goal.

Quaternary ammonium salts (QAS) belong to the class of cationic disinfectants. They have been known and widely used for half a century.[2] Interestingly, they kill bacteria and fungi by interaction with the constituents of the cell envelop: 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.[3,4] Studies about QAS-functionalized polymers are much more recent. Water-soluble polymers such as poly(trialkylvinylbenzylammonium chloride)s[5] or poly(*N*-benzyl-4-vinyl pyridinium bromide)[6] were found to be more active than the corresponding monomers. It was also shown that insoluble polymers such as crosslinked poly(*N*-benzyl-4-vinylpyridinium bromide) were able to remove bacteria from water, but the mechanism was an adhesion, not a destruction of the microorganisms.[7] Much attention has been paid to the antimicrobial and algicidal properties of surface-bonded organosilicon QAS.[8-10] Biocidal activity was claimed to occur by

* To whom correspondence should be addressed.

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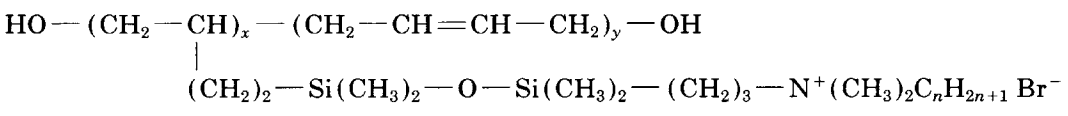
contact between the microorganisms and the treated surface, but a decrease of activity was noted on washing,[8] which might be due to the leaching of nonbonded QAS or to the splitting of a weak bond (particularly when Si—O—C linkages are involved).

In fact, the concept that the surface of a solid polymer may exert a biocidal activity *merely* by contact has not been satisfactorily demonstrated. We have recently addressed this problem in the case of polyurethane (PU) films based on a hydroxytelechelic polybutadiene in which an organosilicon QAS was attached to the main chain through a silicon–carbon bond (**1**).[11]



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The bactericidal activity of these films was extremely high (6–8 orders of magnitude in bacteria decay after 1 h of contact).[12] Samples obtained from different polyols **1** (with n = 8, 12, 14, or 16) were immersed in water for different times and their biocidal activity against *Escherichia coli* was tested at intervals by contact and by diffusion.[13] Several conclusions were drawn from this series of experiments and correlated with NMR characterizations: the diffusion of a QAS of low molecular weight (synthesis residue) was partly responsible for the very high activity observed during the first days; this small amount of soluble QAS was able to kill a very large number of bacteria; the presence of a nonbonded low molecular weight QAS was also revealed by the presence of a zone of inhibition in the case of $-N^+(CH_3)_2C_8H_{17}$; no growth inhibition was observed with $-N^+(CH_3)_2C_{16}H_{33}$ although a low molecular weight QAS was also present in this case; the nonbonded QAS was rapidly extracted (within a few days whatever the nature of the substituents of the ammonium); the subsequent activity, remaining at a good level, was only due to QAS attached to the surface of the polymer; and a very slow drop of activity was observed after hundreds of days in water (this was not due to a splitting of the arm joining the QAS to the main chain, but probably to the formation of a tertiary amine with elimination of a slightly soluble alkyl bromide).

These results show that a negative answer obtained in a test of diffusion on gelose does not allow the conclusion that a polymer is biocidal by contact, particularly when the active groups are QAS with very lipophilic substituents. The durability of the biocidal efficiency after repeated washing or long water immersion should be shown simultaneously.

This article presents a new class of insoluble polymers, based on polysiloxanes functionalized with QAS, that present a high biocidal power that remains remarkably constant after a long period of contact with water.

EXPERIMENTAL

Materials

Octamethylcyclotetrasiloxane (Aldrich), 1,3,5,7-tetramethylcyclotetrasiloxane (Petrarch), and 1,1,3,3-tetramethyldisiloxane (Janssen) were distilled under vacuum. The gas chromatography (GC) purity of these compounds was, respectively, 99.6, 99, and 99.8%. Tonsil® (clay containing 1% HCl, Süd Chemie AG) was used as received.

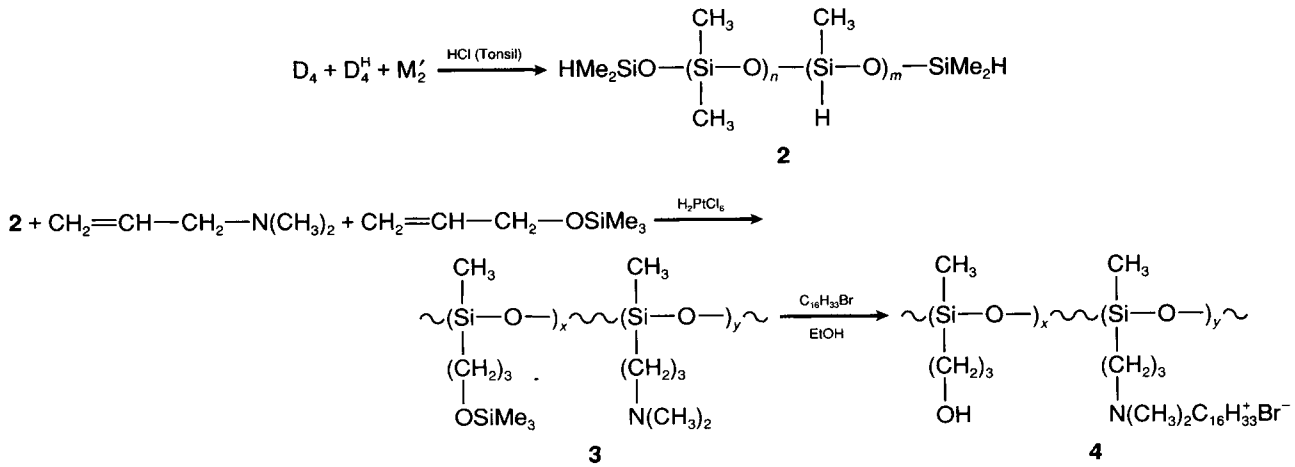
Allyloxytrimethylsilane (Aldrich) was distilled over CaH_2 (bp = 102°C, GC purity = 99.9%). *N*,*N*-Dimethylallylamine (Merck) was distilled over sodium (bp = 63°C, GC purity = 99.9%).

Hexachloroplatinic acid (H_2PtCl_6, $6H_2O$, Janssen) was dissolved in isopropanol (dried on CaH_2 and distilled). Solutions (0.5 M) were stored in a refrigerator. Pt(0)-divinyltetramethyldisiloxane complex (Petrarch) in xylene solution (0.15 M) was used as received.

1-Bromohexadecane (Aldrich, GC purity $\approx$ 98%) was used as received.

Synthesis of Copolymers 2

The polymerizations were carried out under stirring and nitrogen flow (cf. Scheme 1). Reactants and catalyst were mixed at room temperature in a vessel and rapidly heated to 70°C. At the end of the reaction, the mixture was filtered on a Büchner funnel with a Fluoropore 0.5-μm filter. The polymer dissolved in hexane was washed with diluted sodium hydrogen carbonate to neutrality, washed with wa-



Scheme 1 Synthesis of the functional polysiloxanes **4**.

ter, and hexane was evaporated. Cyclic oligomers (about 12–13%) were removed by distillation at 150°C under reduced pressure (<0.5 mm Hg) during 30 min.

Synthesis of Copolymers 3 by Hydrosilylation

To a mixture of allyloxytrimethylsilane and *N*,*N*-dimethylallylamine in hexane, hexachloroplatinic acid was added. The mixture was heated to 78°C for 15 min before the addition of copolymer **2** dissolved in hexane. Samples for IR analysis were withdrawn at intervals. Volatile compounds were finally evaporated at 50°C under vacuum. The polymer dried under high vacuum was a slightly yellow viscous oil.

Synthesis of Copolymers 4 (Quaternization and Deprotection)

Copolymer **3** and 1-bromohexadecane (20% excess with respect to the amine) were mixed in 95% ethanol and the mixture was heated at 80°C for 4 h. The solvent was evaporated and the polymer dried at 100°C under vacuum. In these conditions, the bromide in excess was very difficult to eliminate from the viscous oil.

PU Films

An aliphatic triisocyanate (Tolonate–HDB from Rhone–Poulenc, a biuret from hexamethylene diisocyanate) was added to the functional copolysiloxane **4** diluted with xylene. No catalyst was necessary. The mixture was spread on plates of polyester (Mylar) with a hand coater of 90 μm and the films were dried at 50°C. Films obtained from copolymers **4d** and **4e** dried in 6 h, whereas the others remained sticky after 24 h.

Characterizations

^{1}H-NMR spectra were recorded on a Bruker ACE 200 spectrometer. Molar masses were determined by size exclusion chromatography (SEC; Waters instrument) with microstyragel columns using polystyrene standards. FT-IR (Perkin–Elmer 1600) was used to follow the consumption of the silane during hydrosilylation. The stretching vibration at 2161 cm^{-1} perfectly obeys Beer–Lambert's law between 0.15 M and 7×10^{-3} M.

Bacteriological Assessment

Two types of bacteriological tests were performed with *E. coli* (ATCC 10536). The first one, called "test by contact," consisted of laying down a known number of bacteria on the sample to be tested and counting the surviving cells after a given contact time. The second test, called "test by diffusion," consisted of placing the sample on a seeded gelose and noting the presence of a zone around the sample, in which the growth of bacteria was inhibited. The details of the procedures have been previously described.[13]

RESULTS AND DISCUSSION

Copolymers Poly(dimethylsiloxane-*co*-hydrogenomethylsiloxane) 2

Copolymers **2** were prepared by cationic ring-opening polymerization of octamethylcyclotetrasiloxane (D_4) and 1,3,5,7-tetramethylcyclotetrasiloxane (D_4^H) in the presence of 1,1,3,3-tetramethyldisiloxane (M'_2) as end blocker (Scheme 1).

The catalyst was HCl adsorbed on clay (known as Tonsil), which is easily eliminated at the end of the reaction. With the concentration of catalyst used in these experiments (0.5% weight), the thermodynamic equilibrium between linear polymer and cyclics was reached in 4 h at 70°C and the molecular weight of the polymer did not vary for longer reaction times. However, a study by ^{29}Si-NMR showed that D and D^H units were not randomly distributed in these conditions.[14] The mean length of D^H blocks is about 1.5 times larger than expected from a statistical distribution. The composition of the copolymers was almost identical to that of the feed, which indicates that the cyclics eliminated by distillation are not significantly enriched in one of the units. Three copolymers **2a–2c** with different compositions were prepared for this study (Table I).

Cohydrosilylation of *N,N*-Dimethylallylamine and Allyloxytrimethylsilane by Copolysiloxanes 2

The reaction of hydrosilylation catalyzed by platinum derivatives needs relatively high platinum concentration when tertiary amino groups are present, due to the formation of an inactive complex.[15] To suppress the induction period observed when all reactants are added at once, the catalyst was mixed with the allylic compounds for 15 min before the addition of the copolymer.

Different conditions and different catalysts [H_2PtCl_6 in isopropanol known as Speier's catalyst and a complex of Pt(0) with 1,3-divinyltetramethyldisiloxane known as Karstedt's catalyst] were first compared in the case of *N,N*-dimethylallylamine alone. Kinetics were followed by IR (Fig. 1) and some typical results are reported in Table II. As expected, the reaction was much faster with Karstedt's catalyst than with Speier's catalyst, but the final yield was not significantly better. About the same platinum concentration was necessary in both cases to get high conversion (at least 1×10^{-3} with respect to *N,N*-dimethylallylamine). NMR analysis of the

Table I Poly(dimethylsiloxane-*co*-hydrogenomethylsiloxane)s Obtained by Ring-Opening Copolymerization of D_4 and D_4^H

Ref.	D—D^H Comp.	M_n (SEC)	M_w/M_n
2a	55/45	12500	1.94
2b	52/48	48000	1.81
2c	74/26	15500	2.40

Tonsil, 0.5% weight; T = 70°C; t = 4 h. Elimination of ca. 12% cyclics (150°C, 30 min).

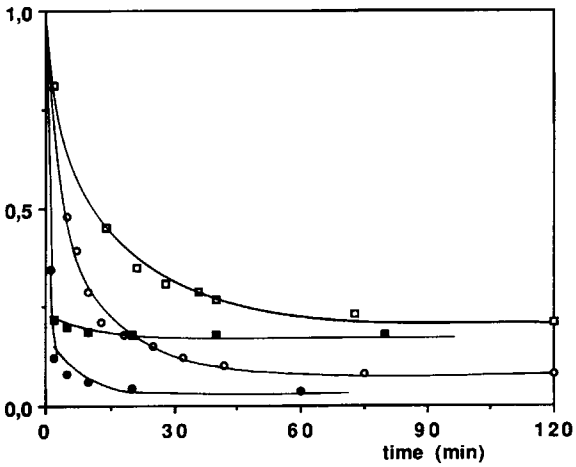


Figure 1 Kinetics of hydrosilylation of *N,N*-dimethylallylamine by copolysiloxane **2a**. [SiH] = [allylamine] = 0.27 mol L^{-1}. (□) [H_2PtCl_6] = 2.1 10^{-4} mol L^{-1}, *n*-hexane, 78°C; (○) [H_2PtCl_6] = 5.8 10^{-4} mol L^{-1}, *n*-hexane, 78°C; (■) [Pt(0), DVS] = 1.9 10^{-4} mol L^{-1}, toluene, 60°C; (●) [Pt(0), DVS] = 5.8 10^{-4} mol L^{-1}, toluene, 60°C.

polymers showed that both catalysts gave about the same proportion of α-isomers (about 30%).

Cohydrosilylations were carried out in hexane solution at 78°C using H_2PtCl_6 as catalyst and a constant molar ratio [catalyst]/[silane] = 10^{-3}. In these conditions, the yields were relatively high (Table III). The polymers were characterized by NMR (Fig. 2). Characteristic signals of the two isomers resulting from β and α addition were identified. The proportion of α-isomers was 25–30% in each experiment, as in the case of *N,N*-dimethylallylamine alone. Dimethylamino groups were identified by two unresolved signals near 2.15 ppm attributed to the two methyl groups and to the adjacent methylene group. The trimethylsiloxy group was most conveniently identified by a triplet at 3.45 ppm corresponding to the CH_2 linked to the oxygen atom. In most cases, a small signal at 4.6 ppm due to Si—H indicated that the reaction was not complete. Integrations allowed a quantitative characterization of the functionalized copolymers (Table III).

In one experiment, the two allylic compounds were used in excess and in equal porportions to determine their relative reactivities (copolymer **3a** in Table III). The proportion of dimethylamino groups was found slightly higher than that of trimethylsiloxy, but the difference was probably not significant. In other synthesis, both allylic compounds were incorporated in a ratio approximately equal to their ratio in the reacting mixture (Table III).

Table II Hydrosilylation of *N,N*-Dimethylallylamine by Copolysiloxane 2a

Nature of Catalyst	[Pt] 10^4 mol L^{-1}	Solvent	T (°C)	Time (h)	Max. Yield (%)	α-Isomer (%)
H_2PtCl_6	2.7	*n*-Hexane	60	120	71	28
H_2PtCl_6	2.1	*n*-Hexane	78	120	78	34
H_2PtCl_6	3.4	*n*-Hexane	78	120	86	—
H_2PtCl_6	5.8	*n*-Hexane	78	120	93	30
Karstedt	1.9	Toluene	60	20	82	31
Karstedt	5.8	Toluene	60	20	97	39

[SiH] = [allylamine] = 0.27 mol L^{-1}.

Quaternization of Amine and Deprotection of Alcohol

The quaternization of polysiloxanes **3b–3e** was carried out with 1-bromohexadecane at 80°C in ethanol containing a small quantity of water. In these conditions, the deprotection of the alcohols was expected to take place simultaneously. At the end of the reaction, the unreacted bromide was difficult to eliminate by distillation or by selective precipitation. It was identified in the NMR spectra by a triplet at 3.35 ppm ($C\underline{H}_2Br$) overlapping with the signal of the methyl groups of the quaternary ammonium salt (3.3 ppm) (Fig. 3). Unfortunately, the signal at 3.45 ppm attributed to the CH_2 directly bound to the nitrogen atom of QAS could not be used for the determination of the quaternization yield, because it overlaps with the $C\underline{H}_2-OSi(CH_3)_3$ groups and the deprotected alcohols ($C\underline{H}_2OH$). The yield of quaternization was deduced from the relative decrease of the dimethylamino groups at 2.2 ppm (Table IV). On the other hand, the efficiency of the deprotection was also determined indirectly by the decrease of the $Si-C\underline{H}_3$ signal using the signal at 0.5 ppm as a reference (invariant before and after reaction). Measurements were not accurate when the proportion of trimethylsiloxy groups was lower than 10% (copolymers **4b** and **4c**). In the case of copolymers **4d** and **4e**, the deprotection was found to be incomplete in these experimental conditions (about 50% in both cases).

PU Films

PU films were prepared by mixing a solution of the hydroxylated polysiloxane with an aliphatic triisocyanate (Tolonate HDB). The amount of isocyanate functions was calculated with respect to the theoretical number of OH in the polymer. Because the real number was found lower when it was possible to evaluate, isocyanate was probably in excess in all cases. The films obtained from copolymers **4b** and **4c** that contained the lowest proportions of hydroxy groups and the highest proportions of QAS remained sticky even after prolonged drying at 50°C. Their biocidal activity could not be evaluated because the films swelled rapidly and broke up in water. On the contrary, excellent films (not sticky and withstand-

Table III Cohydrosilylation of *N*-*N*-Dimethylallylamine (DMMA) and Allyloxytrimethylsilane (ATMS) with Copolysiloxanes 2b and 2c

		Allylic Reactants/Silane		Copolymer Composition			
Ref.	Polysiloxane	[ATMS]/[SiH]	[DMAA]/[SiH]	$(CH_3)_3SiO-$ (%)	$-N(CH_3)_2$ (%)	$-SiH$ (%)	Overall Hydrosil. Yield (%)
3a	**2b**	1	1	21.6	24.5	1.9	96
3b	**2b**	0.015	0.985	0.7	35.2	12.1	75
3c	**2b**	0.06	0.94	2.5	42.9	2.6	95
3d	**2b**	0.5	0.5	20.4	24.8	2.7	94
3e	**2c**	0.5	0.5	11.5	9.9	4.6	82

$[H_2PtCl_6] = 3.55\ 10^{-4}$ mol L^{-1}; [SiH] = 0.355 mol L^{-1}; T = 78°C; solvent, *n*-hexane.

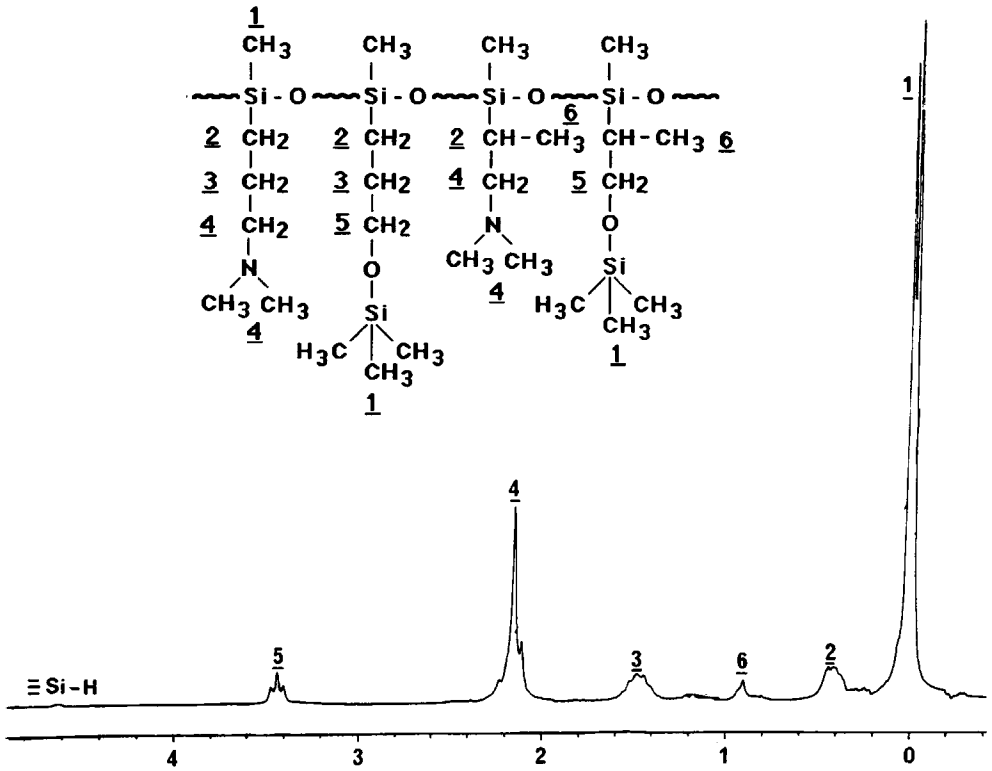


Figure 2 ^{1}H-NMR spectrum of copolysiloxane **3d** (solvent $CDCl_3$).

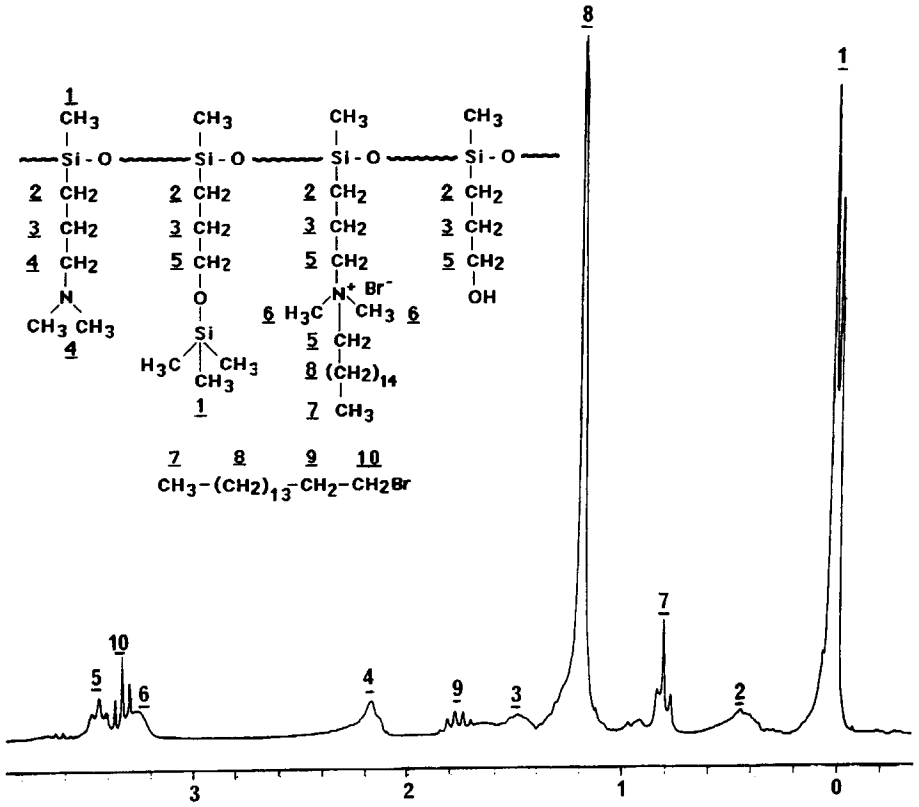


Figure 3 ^{1}H-NMR spectrum of copolysiloxane **4d** (solvent $CDCl_3$).

Table IV Quaternization of Tertiary Amino Groups of Copolysiloxanes 3 by 1-Bromohexadecane and Deprotection of Alcohols in Ethanol/Water

Ref.	Copolymer	RBr Excess	$-N^+(CH_3)_2R$ (%)	$-N(CH_3)_2$ (%)	$-CH_2OH$ (%)	$-CH_2O-Si(CH_3)_3$ (%)
4b	52/48	1.28	28.6	6.6	≤ 0.7	
4c	52/48	1.35	34.6	8.3	≤ 2.5	
4d	52/48	1.29	12.4	12.4	10.3	10.1
4e	74/26	1.17	3.9	6.0	~ 5.5	~ 6.0

$T = 80°C$; $t = 4$ h.

ing long water contact without swelling) were obtained in the case of copolymers **4d** and **4e**.

Antibacterial Activity

PU films based on copolymers **4d** and **4e** were evaluated as bactericides against *E. coli* in two ways. First, a test by diffusion on seeded gelose was found strictly negative. However, the absence of an inhibition halo around the sample is not absolute proof that a polymer is active by contact. We found in a previous study that a low molecular weight QAS (with a C12 or higher alkyl substituent), contained in a PU network, was effectively extracted by water in a few days, but *did not give rise to an inhibition zone* in a test by diffusion.[12,13]

A second test was made by directly dropping a concentrated suspension of bacteria ($2.5\ 10^5$ bacteria in 50 μL) on a small piece of the sample (≈ 1 cm^2). After 1 h of contact, the viable bacteria were counted (see Experimental). The biocidal activity expressed as the *logarithmic reduction ratio* (decimal log of the ratio of the initial number of bacteria to the number of survivors) was higher than 4 for both polymers (Table V). These values are interesting to compare with those previously obtained with PU films based on hydroxytelechelic polybutadiene **1** also quaternized with an hexadecyl substituent (HPBAQ16).[13] In this case, the initial value was larger (about 5.7) but fell rapidly by two orders of magnitude after a few days in water (Table V). It was shown that the large initial activity was partly due to the presence of a soluble QAS acting by diffusion, whereas the following plateau value (≈ 3.5) reflected the true activity by contact of the polymer.

In the present case, the activity of the silicon-based PU film was almost unchanged after 30 days of immersion in water, which is another way to show that there is no extractible toxic molecule in this polymer and that the bactericidal power is an intrinsic property of the polymer surface. It is noteworthy that the same conclusion had been previously drawn in the case of organosilicon QAS bonded to glass, but the reported data were not convincing (logarithmic reduction ratio decreasing from about 3 to 1.3 after washing for 400 min).[8]

Table V Bactericidal Activity of PU Films Prepared from Copolysiloxanes 4d and 4e and Comparison with HPBQ16PU

Film	QAS (mmol/g)	Time of Water Immersion (Days)	log (N_o/N)
4d-PU	1.0	0	4.4
4e-PU	0.4	0	4.1
4e-PU	0.4	30	3.7
HPBQ16PU	1.17	0	5.7
HPBQ16PU	1.17	14	3.8
HPBQ16PU	1.17	78	3.2

HPBQ16PU, PU films prepared from a polybutadiene bearing the same QAS.[13] Test by contact: $N_o = 2.5\ 10^5$ bacteria (*E. coli*) layered on the sample (1 cm^2); N = number of surviving cells after 1 h at 20°C.

CONCLUSION

PU films were prepared by curing with an aliphatic triisocyanate, a functional polysiloxane bearing in the same chain primary alcohols and quaternary ammonium groups $\sim N^+(CH_3)_2C_{16}H_{33}$. These films were characterized by a high biocidal activity against *E. coli* (reduction of the number of living bacteria by a factor of 10^4 in 1 h of contact). The activity remained practically constant after 1 month of immersion in water. In a test of diffusion, no inhibition zone was observed.

In our opinion, the absence of an inhibition zone in a diffusion test on seeded gelose similar to those

classically used for testing antibiotics is a necessary condition, but is not sufficient to demonstrate that a polymer is really biocidal by contact, particularly when the active group is a QAS with highly lipophilic substituents such as hexadecyl. The test should be completed by a quantitative assessment of the biocidal activity before and after a long period of contact with water to rule out the possibility of a soluble toxic compound acting by diffusion in water. In a test of diffusion on gelose, the bacterial growth may be faster than the rate of migration of QAS so that no inhibition is detected visually.

On the other hand, the silicon-based PU films synthesized in the present work confirmed the view that a logarithmic reduction ratio of about 4 may represent the true biocidal power of a solid polymer (against *E. coli* and for a concentration of active QAS of about 1 mmol/g). The performance of these films seems to be slightly better than that of the polybutadiene-based PU[13] and that of a glass-bonded organosilicon QAS.[8]

Owing to their high activity against *E. coli,* which is often considered as more resistant than Gram+ bacteria, these polymers might be useful as binders in paints, as additives for mastic compounds, or in other applications of elastomers. A closer examination of the biocidal properties of various polysiloxanes of this family is in progress.

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