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Quaternary Ammonium Salts (QAS) Modified Polysiloxane Biocide Supported on Silica Materials

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Abstract Three types of silica particles modified with vinyl groups were obtained: (i) xerogel formed by hydrolytic polycondensation of the mixture of tetramethoxysilane (TMOS) and 1,1,1,7–tetramethoxy-3,5,7-trimethyl-3,5,7-trivinyltetrasiloxane, (ii) mesoporous silica obtained from the same precursors in the presence of the cetyltrimethylammonium bromide (CTAB), and (iii) commercial Fluka silica gel 60A with a vinyltriethoxysilane-treated surface. Vinyl groups on these silica materials were transformed into silyl chloride by hydrosilylation with HMe_2SiCl. These groups were used to graft living polysiloxane that was synthesized by anionic ring-opening polymerization of 2,4,6-tri(3-chloropropyl)-2,4,6-trimethylcyclotrisiloxane and initiated by BuLi. Chloropropyl groups on the grafted polymer were used to quaternize *N,N*-dimethyl-*n*-octylamine. Silica particles with grafted polysiloxane having quaternary ammonium salt (QAS) groups pendant to polymer chains were obtained. Silica material with QAS groups directly attached to the surface were generated by the action of *N,N*-dimethyl-*n*-octylamine on particles obtained by the sol–gel process involving tetraethoxysilane (TEOS) with 3-chloropropyltriethoxysilane. The bacteriocidal properties of all these materials were tested in water suspension against five representative strains for Gram-positive and Gram-negative bacteria. Some of the silica–polysiloxane hybrid materials have good antibacterial properties against Gram-positive strains, but not as good as the non-tethered QAS-substituted polysiloxane in water solution. The QAS groups that are directly bonded to the silica material surface are inactive.

Keywords Silica hybrids · Sol–gel silica · Silica materials · Functionalized polysiloxanes · Biocidal polymers · QAS · Biocides · Functionalized silica

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1 Introduction

There has been a growing interest in polymers with covalently bonded biocidal quaternary ammonium salt (QAS) groups used as effective polymeric antimicrobial agents [1–10]. A QAS having one long hydrocarbon chain bonded to nitrogen, such as *n*-octyl or *n*-dodecyl, exhibits a high bacteriostatic activity. These groups covalently attached to polymers were demonstrated to be able to kill bacteria by contact [11–14]. These polymeric biocides show permanent bacteriocidal activity and do not release toxic compounds to the environment. An attachment of biocidal polymers by a chemical bond to the surface of silica particles would provide useful organic–inorganic hybrid antimicrobial materials, which could find application in the purification of water from micro-organisms. They could be also used as fillers in composite polymeric materials thereby preventing them from their biological corrosion, which has recently been identified as an important problem [15].

Immobilization of antimicrobial agents on the surface of various solid materials has often been practiced [16–21]. Rather little effort has so far been concerned with grafting to surface of polymers having attached biocidal groups to their chains [22–24]. If a water-soluble polymer is tethered to a particle suspended in water, biocidal groups attached

to this polymer would behave as they were dissolved in the water phase. Thus, they are expected to be effective in killing bacteria in water.

In this paper we describe the generation of silica-based hybrid materials with QAS-modified siloxane polymers grafted to the surface of silica particles. Polysiloxane shows very high static and dynamic flexibility of the chain, which makes the QAS groups attached to it readily available for the destructive interaction with bacterial walls causing the bacterial death [25]. In addition, polysiloxane is physiologically inert, chemically stable and, by modification with the ionic QAS groups, is water-soluble. As a result, if the hybrid particles are suspended in water the biocidal active groups will extend into the water phase.

A high biocidal activity of polysiloxanes and polysilsesquioxanes bearing (dimethyl-*n*-octylammonio)propyl groups attached to silicon atoms was demonstrated [25–27]. The purpose of this study was to check how the immobilization of the chain end of these polymers on silica or silica-hybrid materials affects their biocidal activity. We believed that exploring materials of various chemical structure and morphology can shed more light on this problem. Testing of the antibacterial behavior of the QAS-polysiloxane-inorganic on two common Gram-positive and three Gram-negative strains should show how broad is the spectrum of bacteriocidal ability of these hybrid materials.

2 Experimental

2.1 Materials

The THF was refluxed over sodium for 24 h and distilled under an argon, stored over Na/K alloy in an ampoule fitted with a rotaflo stopcock, and distilled under vacuum into the reactor shortly before use. Toluene, dimethylformamide (DMF), dichloromethane, methanol and ethanol are products from POCh (analytical grade) and were purified according to standard methods [28]. Tetramethoxysilane, TMOS, 98% and tetraethoxysilane, TEOS 98% (ABCR-Gelest) were refluxed with sodium and distilled. 3-Chloropropyltriethoxysilane, 97%, vinyltriethoxysilane, 98%, dimethylchlorosilane, 96% and trimethylchlorosilane, 99%, were reagent grade products from ABCR-Gelest and were used without further purification. *N*,*N*-dimethyloctylamine, cetyltrimethylammonium bromide (CTAB), *n*-butyllithium, 2.5 M in *n*-heptane, (Aldrich) and platinum(0) Karstedt catalyst (GE), 11 wt.% in xylene, were used without further purification. Silica gel 60A, (35–70 mesh), particle size 0.2–0.5 mm, pore volume 0.68 $cm^3\ g^{-1}$, pore surface 675 $m^2\ g^{-1}$, (Fluka) was silylated with vinyltriethoxysilane as described below.

Monomers, 2,4,6-tri(3-chloropropyl)-2,4,6-trimethylcyclotrisiloxane [29] and 1,1,1,7-tetramethoxy-3,5,7-trimethyl-3,5,7-trivinyltetrasiloxane, {TMOS-V_3} were synthesized and purified as described earlier [30].

2.2 Synthesis

Siloxane-silica particles, modified by vinyl groups, were prepared as described earlier [30]. In the synthesis of the mesoporous gel, a mixture of {TMOS-V_3} (4.12 g, 0.01 mol) and TMOS (6.08 g, 0.04 mol) was added to a solution of NaOH (2.0 g, 0.050 mol) and cetyltrimethylammonium bromide (7.8 g, 0.0214 mol) in distilled water (150 mL) containing dimethylformamide (50 mL). Stirring at room temperature was continued for 24 h. The mixture was then heated at 35 °C for 10 h. The product was collected by filtration, washed with water, and dried in air at room temperature. The surfactant was removed by a solvent extraction. For this purpose about 4 g of hybrid was refluxed in a Soxhlet apparatus with 400 mL of methanol containing 5 g of 6 N HCl for 36 h, then filtered and washed with methanol. Finally the product was dried under vacuum (10^{-3} mmHg) for 12 h. The yield of gel was 5.16 g (92.2%). The properties of the gel are given in Table 1 (sample number I).

In the synthesis of xerogel, {TMOS-V_3} (12.32 g, 0.03 mol) and TMOS (9.13 g, 0.06 mol), were mixed with 4.95 g of distilled water and 0.075 mL of 1 N HCl. The mixture was stirred vigorously for 2 h at room temperature. Water (4.95 g) and NH_4OH (0.016 mL of 25% solution) was introduced and stirring was continued for 10 min. The monolith was left to age at room temperature for 24 h and at 35 °C for an additional 24 h. The crushed product was washed with water, methanol and dried for 36 h under vacuum (10^{-3} mmHg); Yield, 12.98 g (98%) of white xerogel. The gel was heated under argon at 220 °C for 6 h. The properties of the product are given in Table 1 (sample number II).

2.3 Modification of Silica Gel Surface

Silica gel 60A, (Fluka) 12 g was dried in vacuum at 80 °C, then filled under argon and suspended in 120 mL of anhydrous toluene. As the suspension was gently stirred, 4.8 g of water was introduced. After 1 h vinyltriethoxysilane (20.93 g, 0.11 mol) was added and the suspension was heated to reflux for 18 h. Approximately 90 mL of liquid was removed by azeotropic distillation and the functionalized gel was collected by filtration, washed with toluene and ethanol and dried in vacuum at 40 °C for 12 h. Finally 14.7 g of functionalized gel was obtained. Its characteristics are summarized in Table 1 (sample number III).

Table 1 Characteristics of precursor silica materials

Symbol of material (see Fig. 1)	Starting materials/ precursors/[a]	Method[b]	BET surface area [$m^2 g^{-1}$]	Pore volume [$cm^3 g^{-1}$]	Average pore diameter [nm]	Shape, diameter [µm]	Chemical analyses (wt.%)			Functional groups	Density of funct.group [$mmol g^{-1}$]
							%C	%H	%Cl		
I	{TMOS-V_3} +TMOS 1:4	Sol–gel CTAB, NaOH, H_2O, DMF	799	0.40	1.99	Ellipsoidal 0.9–1.8	14.46	3.09		$-CH=CH_2$	4.02
II	{TMOS-V_3} +TMOS, 1:2	Sol–gel HCl, NH_4OH, H_2O	34	0.15	18.22	Irregular[c] 1–1.5	23.16	4.40		$-CH=CH_2$	6.43
III	Silica gel 60A, Fluka	Functionalization of surface with $ViSi(OEt)_3$	675	0.68	6.00	Irregular 200–500	4.02	1.01		$-CH=CH_2$	1.67
IV	$(EtO)_3Si(CH_2)_3Cl$ + TEOS 1:3	Sol–gel CTAB, NaOH, H_2O, DMF	430	0.33	2.23	Ellipsoidal 1.5–2.5	15.57	3.03	9.10	$-(CH_2)_3Cl$	2.56

[a] TMOS = $(CH_3O)_4Si$; TEOS = $(C_2H_5O)_4Si$,

{TMOS-V_3} = 

[b] CTAB = $CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$

[c] Small particles, about 1 µm, aggregated in larger particles

2.4 Synthesis of Siloxane-Silica Particles Modified by Chloropropylsilyl Groups

The mixture of 3-chloropropyltriethoxysilane (7.22 g, 0.03 mol) with TEOS (18.75 g, 0.09 mol) was introduced to the solution of NaOH (4.8 g, 0.12 mol) and CTAB (18.22 g, 0.05 mol) in the mixture of distilled water (360 mL) with DMF (95 mL) during vigorously stirring of the solution. The stirring was continued for 24 h at room temperature then for additionally 10 h at 35 °C. The surfactant was removed by the solvent extraction in a typical procedure described above. A white powder (9.4 g; Yield 100%) was obtained and analyzed. Its characteristics are summarized in Table 1 (sample number IV).

2.5 Transformation of Vinyl Groups and Grafting of Linear Functionalized Living Polysiloxane

Synthesis of SiCl functionalized silica gels by hydrosilylation of vinyl groups with dimethylchlorosilane was performed according to the description in [30, 31] under argon in a reactor connected by a rotaflo-stopcock to the other reactor in which living chloropropyl-substituted polysiloxane was obtained. For example, in the reaction of 1 g of gel II containing 6.43 mmol vinyl groups with dimethylchlorosilane (1.87 g, 17.25 mmol) using Karstedt catalyst (2 mg, 1.13×10^{-6} mol) in 8 mL of toluene, 1.17 g (yield, 26.40%) of the gel product containing 1.70 mmol of Si-Cl group in 1 g of gel was obtained.

2,4,6-Tri(3-chloropropyl)-2,4,6-trimethylcyclotrisiloxane (8.06 g, 19.66 mmol) was placed under vacuum into a reactor equipped with a Teflon stopcock. THF (8.11 g) was distilled into the reactor. The vacuum line and the reactor were then filled with argon and of butyllithium (2.5 M solution in *n*-heptane; 1.03 mL, containing 2.57 mmol of *n*-BuLi) was added to initiate anionic ring-opening polymerization. Conditions of polymerization were selected on the basis of earlier experiments [29]. The reaction was carried out at room temperature with stirring for 90 min. Then the reaction mixture was introduced, under argon, to the reactor in which the freshly chlorosilylated gel had been prepared. The mixture was shook for 2 h at room temperature, then Me_3SiCl (0.27 g, 2.50 mmol), was added and the mixture was stirred for additional 30 min. Solvent and excess TMS were evaporated under reduced pressure. The hybrid was mixed with toluene and filtered, washed again with toluene, methanol, toluene, and dried under vacuum (10^{-3} mm Hg) at room temperature for 20 h. Grafted hybrid (1.31 g; yield 16.0%) containing $CH_2CH_2CH_2Cl$ (2.27 mmol per 1 g of product) was obtained (Table 2, sample II). The excess poly-3(chloropropyl)methylsiloxane that was isolated after the grafting reaction was characterized by ^{1}H-NMR and GPC analysis, M_n(GPC) = 1,500, M_w/M_n = 1.51.

Table 2 Preparation of precursor polysiloxane–silica hybrid materials

Symbol of silica precursor	Hydrosilylation			Grafting of polysiloxane functionalized with $Si(CH_2)_3Cl$			
	SiCl wt.%Cl	Density of SiCl [mmol g^{-1}]	Yield of hydrosilylation [%]	$Si(CH_2)_3Cl$ wt.%Cl	Density of $Si(CH_2)_3Cl$[mmol g^{-1}]	Yield of grafting [%]	Polymer weight fraction in hybrid
Ia	2.53	0.71	17.7	3.12	0.88	12.6	0.12
Ib	3.41	0.96	23.9	4.19[a]	1.18[a]	16.3[a]	0.25[a]
II	6.03	1.70	26.4	8.07	2.27	16.0	0.31
III	2.00	0.56	33.5	2.31	0.65	11.8	0.09

[a] Grafted with copolymer of (3-chloropropyl)methylsiloxane with dimethylsiloxane M_n = 2,200 containing 50 mol% of (3-chloropropyl)methylsiloxane units distributed in gradient way with a higher density of functional group out of grafting point

Copolymer of (3-chloropropyl)methylsiloxanedimethylsiloxane was prepared in a similar way by using of 1:1 mol/mol mixture of 2,4,6-tri(3-chloropropyl-2,4,6-trimethylcyclotrisiloxane and hexamethylcyclotrisiloxane. Since living polymerization is used, a gradient distribution of siloxane units [32] with decreasing density of 3-chloropropyl substituted units as the chain grows [M. Cypryk and B. Delczyk in preparation] is observed.

2.6 Synthesis of QAS Containing Gels

The suspension of the precursor silica hybrid obtained from gel II by grafting chloropropyl substituted polysiloxane (0.736 g, 1.67 mmol of $-(CH_2)_3Cl$) in the mixture of *N,N*-dimethyl-*n*-octylamine (3.92 g, 24.87 mmol) and DMF (3 mL) was placed in a 20 mL glass reactor fitted with a magnetic stirrer in an oil bath. The suspension was stirred for 1 h at room temperature and then at 60 °C for 10 days. The volatile components were distilled on the vacuum line. The crude product was washed with CH_2Cl_2 and dried to constant mass on the vacuum line. Powder material (0.886 g) was obtained and subjected to elemental analysis, ^{29}Si-MAS-NMR and ^{13}C-MAS-NMR spectroscopy and thermogravimetric analysis (TGA). These analyses showed that 48.9% of chloropropyl groups were transformed to the QAS group. All other QAS-containing gels were synthesized in analogous way.

2.7 Studies of Antibacterial Activities

The silica hybrid materials were tested against bacterial strains: *Enterococcus hirae* (ATCC 10541), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Proteus vulgaris* (NCTC 4635) and *Pseudomonas aeruginosa* (ATCC 9027). Tests were performed by the standard broth dilution technique [33] with inoculums of approximately 1×10^5 CFU mL^{-1}. Suspensions of tested compounds were diluted in geometric progression and dispensed into the wells of a micro-plate. Overnight bacterial culture was diluted with Mueller-Hinton broth to the proper density and dispensed into wells. The growth of bacteria in the wells was examined after the incubation of micro-plates for 24–36 h at 37 °C. The minimum inhibitory concentration (MIC) was taken as a lowest concentration of polymer that inhibits visible growth of bacteria. Three independent measurements were performed to determine each of the MIC values.

2.8 Analyses

The composition of the hybrids was determined by elemental analysis of C, H, N and Cl on a Euro EA elemental analyser made by Euro Vector Instruments & Software. TGA was performed with Hi-Res TGA 2950 thermal analyser in a nitrogen atmosphere with a heating rate of 10 °C min^{-1} from 30 to 960 °C.

Nitrogen adsorption–desorption isotherms were determined using an ASAP 2010 Micrometries sorption analyser. Samples were degassed for 6 h at 150 °C under vacuum prior to analysis. The BET surface areas were evaluated using adsorption data in a relative pressure range from 0.05 to 0.9 and calculated by the Brunauer-Emmett-Teller (BET) method. The mesopore size distribution was calculated on the basis of adsorption branches using the Barrett-Joyner-Halenda (BJH) model.

Solid-state NMR spectra were obtained using a Brucker DSX 300 spectrometer with samples contained in 4 mm zirconia rotors. ^{13}C spectra were recorded at 75.48 MHz using cross polarization (CP) and magic angle spinning (MAS) at rate of 8 kHz applying 90° pulses and 6.0 s pulse delays. ^{29}Si spectra were recorded at 59.63 MHz using MAS at rate 8 kHz applying 90° pulses, 6 s pulse delays, and 3 ms contact time. The assignment of the NMR signals was performed on the basis of earlier papers.

3 Results and Discussion

Synthesis of various types of silica gels modified with vinyl groups were described and discussed in our earlier papers

[30, 31]. These silica materials were used here to graft polysiloxane-containing 3-chloropropyl groups to obtain precursor hybrids of the biocidal materials. For this purpose vinyl groups were converted to silyl chloride groups, which were used further for the termination of the functionalized living polysiloxane. Chloropropyl groups on the grafted polymer were transformed into QAS groups by quaternization of dimethyl-*n*-octylamine. 3-Chloropropyl groups bonded to silicon were also introduced directly to silica by sol–gel co-polycondensation of tetraethoxysilane (TEOS) with chloropropyltriethoxysilane. The simplified scheme of the syntheses of these hybrid materials is presented in Fig. 1.

3.1 Generation of the Vinyl Group Modified Particles

The silica materials containing vinyl groups, prepared according to route 1 (Fig. 1), were obtained by the sol–gel process of a mixture of TMOS with 1,1,1,7-tetramethoxy-3,5,7-trimethyl-3,5,7-trivinyltetrasiloxane [30]. Two variants of this process were practiced. In the first variant the sol–gel process was performed in the presence of *n*-cetyltrimethylammonium bromide (CTAB) as the structuralization agent to obtain mesoporous silica-type material with a high surface area and regular ellipsoidal shape of particles. In the second mode no surfactant was used to generate xerogel-type, low porous material having a small area of pores and irregular shape of particles.

Other vinyl-modified silica particles were generated using route 2 (Fig. 1) by the treatment of commercial Fluka 60A silica with triethoxyvinyl silane. The morphology of the particles was studied by sorption BET and BJH methods and scanning microscopy. The properties of all three vinyl modified materials are presented in Table 1.

3.2 Preparation of the Precursor Hybrid Materials

The vinyl-modified silica particles were subjected to hydrosilylation with dimethylchlorohydrosilane. A Karstedt Pt(0) catalyst of high catalytic activity was used. Under the experimental conditions used here, the yield of the addition of the hydrosilane to vinylsiloxane in a homogeneous reaction is usually quantitative. However, the yield of the addition to the vinyl groups on the silica particle is low (Table 2, column 4), and appears to be a general feature [30]. A considerable number of vinyl groups that were introduced into the sol–gel material is trapped in the silica framework, therefore, these groups are not available to interact with the hydrosilane and catalyst. The penetration of the catalyst and reactant into the silica pores may also be impeded.

The reactive silyl chloride groups introduced by the hydrosilylation were used for grafting the polymer onto the silica particle surface. The graft was living poly(3-chloropropyl)methylsiloxane-terminated with an *n*-butyl group at one chain end and the living lithium silanolate propagation center at the other chain end. The latter served for polymer grafting by its reaction with silyl chloride. The polymer was synthesized by polymerization of 2,4,6-tri(3-chloropropyl)-2,4,6-trimethylcyclotrisiloxane, which was initiated by *n*-BuLi [29]. Although the polymer was used in a significant excess relative to the silyl chloride group, the graft-yield was low (10–20%) (Table 2, column 7). During the washing procedure, the remaining silyl chloride groups were transformed to silanol groups, which were then partly silylated by treatment with Me_3SiCl. The molar mass (MM) of the polymer from the stoichiometry (monomer to initiator ratio), allowing for the monomer conversion

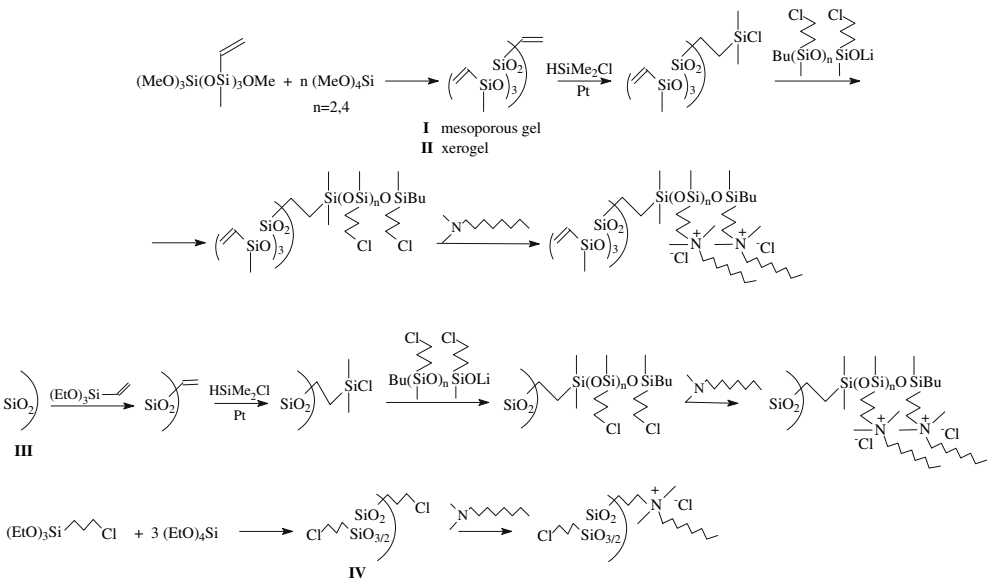


Fig. 1 Synthesis of hybrids I–IV

(80%), should be 2,500 g/mol and the polydispersity (PD) should be 1.1. However, the unreacted polymer, recovered from the grafting reaction, had an MM 1,500 g/mol and a PD of 1.5. It is suggested that the lower MM and higher PD is a result of chain transfer, which occurs during grafting. Chain transfer may be accelerated by interaction with the particle surface, which may also supply the end groups.

The ^{29}Si-NMR-MAS analysis of gels was performed and a representative spectrum is shown in Fig. 2. The most intensive signals, as expected, are those of Q and D units. The signal of the D units in the grafted polymer appears at about –19 ppm, instead, and the signal near –32 ppm results from unconverted MeViSiO groups in hydrosilylation. The intense signal at +10 ppm is attributed to M units of $–CH_2(CH_3)_2SiO–R$ [R = H, CH_3, $Si(CH_3)_3$], BuMe[Cl $(CH_2)_3$]SiO and Me_3SiO. Magnetic resonances of the silicon nuclei of these M units appear at similar fields therefore signals of these groups are superimposed to each other. A small outstanding signal at the lower field wing of the M group signals may be attributed to the $–CH_2(CH_3)_2SiOH$ structure. A considerable number of the $–CH_2(CH_3)_2SiO–R$ group originates from $–CH_2(CH_3)_2SiCl$ that was unconverted in grafting and transformed into $–CH_2(CH_3)_2$ SiO–R [R = H, CH_3, $Si(CH_3)_3$] during further treatment of the material. Signals that unexpectedly appear at –57 ppm and –65 ppm disclose the presence of T-units, $CSi(OSi)_2$ OH and $CSi(OSi)_3$, respectively. These T-units should not be formed in a sol–gel process producing xerogel as the cleavage of vinyl groups were only observed in the presence of surfactants at higher temperature, i.e. in calcinations

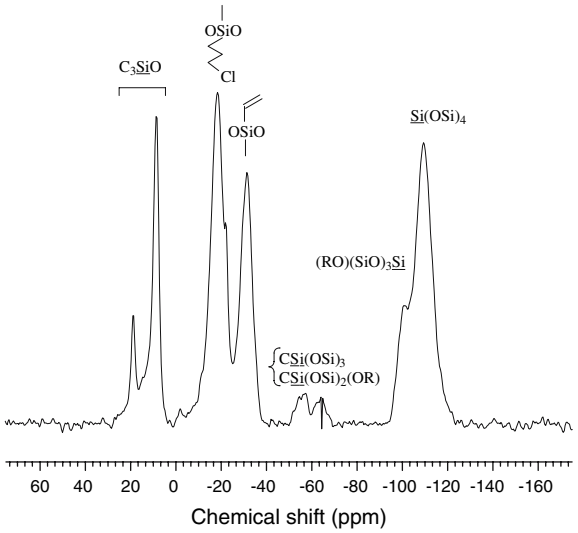
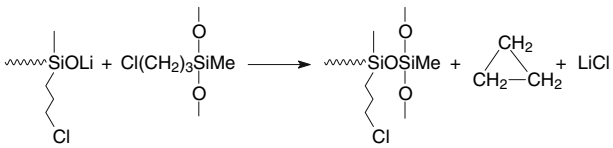


Fig. 2 ^{29}Si-CP/MAS solid state NMR spectrum of the gel II obtained by sol–gel process of the mixture of TMOS and {TMOS-V_3}, (2:1) grafted with poly-(3-chloropropyl)methylsiloxane (M_n = 1,500, M_w/M_n = 1.51, 0.31 g of polymer in 1 g of hybrid, 2.27 mmol chloropropyl group in 1 g) R = H or CH_3

process [30]. T-units in the xerogel materials, therefore, must have been produced in the grafting process. The polymerization of cyclic siloxanes bearing 3-chloropropyl group at silicon on lithium silanolate centers occurs without cleavage of the 3-chloropropyl groups [29]. However, such a cleavage by lithium silanolate, according to Eq. (1) [34], may occur during the grafting process because this side reaction may be assisted on the surface of the silica material. In addition, the grafting process is slow, so the contact time of living polymer with the grafted polymer is long.



Although the yield of grafting was small, considerable amounts of 3-chloropropyl groups were introduced into the hybrid because many 3-chloropropyl groups were located in the polymer chain. Since the number average MM of the grafted polymer was assumed to be equal to that of the mass of unreacted polymer, each of grafted chain bore on average of approximately eleven 3-chloropropyl groups.

The living polysiloxane bearing pendant 3-chloropropyl groups, which were obtained under identical conditions, was also grafted on the Fluka silica gel 60A after the surface was modified by treatment with $(EtO)_3SiVi$ (see route III, Fig. 1). Vinyl groups were transformed into silyl chloride groups, which in turn were used to attach the living polysiloxane. The density of vinyl groups introduced to the Fluka silica was lower than that in materials obtained by the sol–gel procedure. However, all the vinyl groups were on the particle surface, therefore, the yield of grafting was relatively high (Table 2, entry III).

Finally, a silica hybrid material having 3-chloropropyl groups was also prepared directly in the sol–gel process using as precursors the mixture of tetraethoxysilane with 3-chloropropyltriethoxysilane. The properties of this hybrid are compared with the other sol–gel products in Table 3, entry IV.

3.3 Generation of QAS Groups on the Silica Hybrids

3-Chloropropyl groups on hybrids were used for quaternization of *N,N*-dimethyloctylamine. The reaction was carried out under the same conditions for all the silica hybrids studied using a large excess of the amine, DMF as a solvent, elevated temperature (60 °C), and a long time of reaction. The quaternization of amines by the 3-chloropropyl group pendant to the polysiloxane chain has been studied earlier [25, 26, 35] and the decomposition and cleavage of the QAS groups were not observed even under more severe

Table 3 Generation of QAS functionalized hybrid materials

Symbol of functionalized silica precursor	Chemical analyses (wt.%)			QAS content [mmol g^{-1}]	Yield of CH_2Cl transformation %	Weight fraction of polymer in hybrid
	%C	%H	N%			
I a	22.12	4.37	0.96	0.71	89.5	0.21
I b	24.70	5.10	1.22	0.97	94.7	0.35
II	32.08	6.70	0.81	0.97[a]	48.9	0.41
III	15.05	3.20	1.08	0.30	48.3	0.13
IV	26.30	5.28	3.06	1.60	78.1	

[a] Data obtained using wt.% of C in chemical analyses

conditions than those used here. The properties of the QAS-substituted hybrids are given in Table 3. Information about the structure of these hybrids was obtained from elemental analysis, ^{29}Si-MAS-NMR, ^{13}C-MAS-NMR and TGA. Unfortunately, the resonance signal of the ^{29}Si nucleus in the [3-(*n*-octyldimethylammonio)propyl]methylsiloxy unit overlaps with the signal in $OSi[(CH_2)_3Cl]CH_3$. Clearer changes were observed after quaternization in the ^{13}C-MAS spectra. Thus the ^{13}C-MAS-NMR spectra of the gel obtained by copolycondensation of 3-chloropropyltriethoxysilane with TEOS before and after quaternization are relatively simple (Fig. 3a, b). After quaternization new broad signals appear at 60–70 ppm and about 50 ppm, which are attributed to resonances of $–CH_2N^+CH_2–$ and $CH_3N^+CH_3$, respectively. Four other sharp signals at 15.9, 22.2, 28.7 and 31.2 ppm belong to the *n*-octyl group. The same signals of the dimethyl-*n*-octylammoniopropyl group appear in the ^{13}C-MAS-NMR spectra of the polymer–silica hybrids after quaternization (an example presented in Fig. 4), but these signals are broadened and obscured by the resonance of other C-atoms being in the grafted polymer chains.

The thermal stability of the hybrids was studied by thermogravimetry. The onset of the pyrolytic process was ~180 °C. The decomposition occurred in two steps with maximum rates at 220 °C and 440 °C, respectively. In the first step the decomposition of the QAS groups occurred while in the second step other organic structures were cleaved. Thermograms for the hybrid before and after quaternization are compared in Fig. 5.

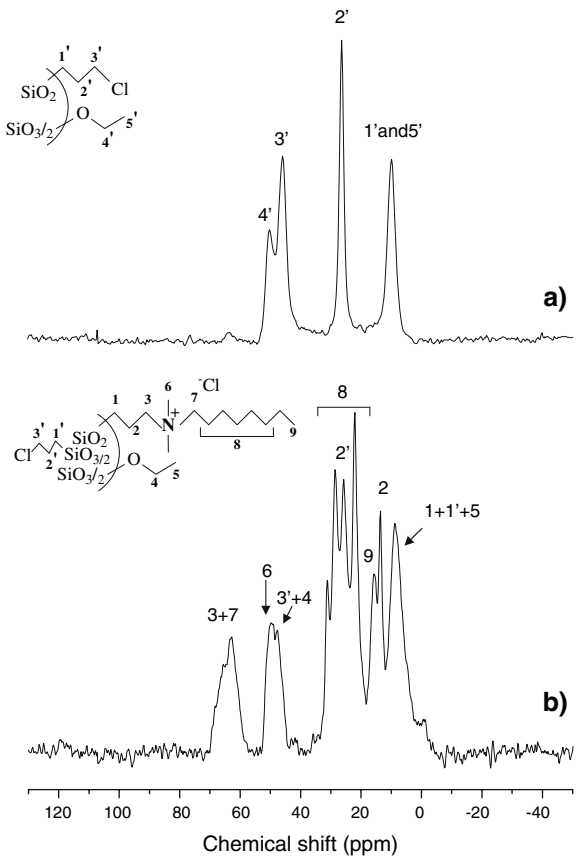


Fig. 3 Comparison of ^{13}C-CP/MAS solid state spectra (**a**) of the gel IV obtained by sol–gel process of the mixture of TEOS and 3-chloropropyltriethoxysilane (3:1), 2.56 mmol chloropropyl group in 1 g of hybrid, (**b**) this gel after quaternization of *N,N*-dimethyloctylamine (1.60 mmol QAS in 1 g of hybrid)

3.4 Bacteriocidal Activity of the QAS-Containing Hybrid Materials

Bacteriostatic properties of the QAS polysiloxane–silica hybrids were tested using five bacteria strains. Two strains, *Enterococcus hirae* and *Staphylococcus aureus*, are representative for Gram-positive bacteria, while *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* are Gram-negative. Tests were performed in a suspension of the hybrid particles in water. Values of the minimum inhibitory concentration (MIC), which is defined as the lowest concentration of the antimicrobial agent that is able to inhibit the growth of bacteria after overnight incubation [33], were determined. In the preparation of the hybrid particle suspensions for the bacteriocidal test, a standard doubling broth dilution procedure was practiced. Results for the four silica–organic hybrids bearing the bacteriocidal QAS groups are summarized in Table 4. The table includes also the data for the unimmobilized linear polysiloxane that bears QAS groups. Tests on the latter polymer were performed in the water solution. In all cases the MIC values

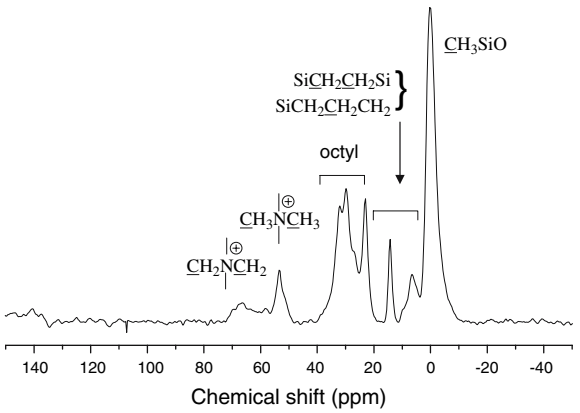


Fig. 4 ^{13}C-CP/MAS solid state spectrum of the silica-gel III grafted with poly(3-chloropropyl)methylsiloxane used for quaternization of *N,N*-dimethyloctylamine (0.30 mmol QAS in 1 g of hybrid)

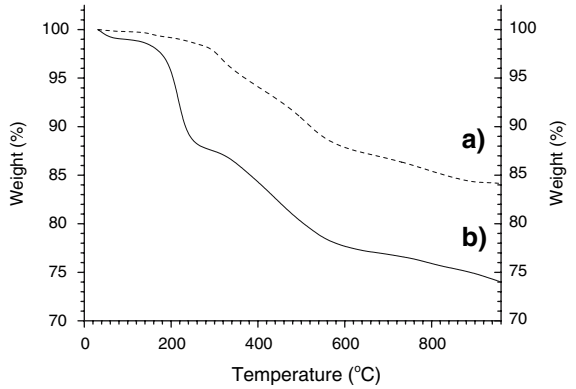


Fig. 5 TGA thermograms of the gel Ia grafted with poly(3-chloropropyl)methylsiloxane (0.12 g of polymer in 1 g of hybrid) (**a**) before quaternization (**b**) after the quaternization of *N,N*-dimethyloctylamine (0.71 mmol QAS in 1 g of hybrid)

were related to the weight of the grafted QAS polymer, not to the mass of all hybrid.

Free QAS polymer showed a higher activity in preventing the growth of bacteria relative to polymers attached to the surface of the silica-hybrid particles. Free polymer, which is not engaged in the interaction with surface of a solid support, may interact with the bacteria wall more efficiently. As further support of this conclusion, there is a small ability of highly porous material to destroy bacteria (Table 4, entries Ia and Ib). Material Ib, which bears the same number of active groups in the chain and has twice as many siloxane units compared to Ia, is more active against Gram-positive bacteria. Thus, the length of the chain may by important. Instead, xerogel (Table 4, entry II), which has a small surface area and larger pores, is effective on all the bacteria studied; i.e., shows a high activity against Gram-positive bacteria, moderate activity against Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* and weak but still significant activity against *Proteus vulgaris*. The QAS polysiloxane attached to Fluka 60A silica, which is highly porous but has larger pore diameters, shows remarkably strong activity against *Staphylococcus aureus* and is able to impede the growth of *Enterococcus hirae*, although its activity against Gram-negative bacteria is weak. In contrast to the antibacterial property of the immobilized QAS-containing polymers, the QAS group that is directly attached to the solid support does not exert any significant bacteriocidal activity (Table 4, entry IV). The interaction of this QAS group with the silica hybrid framework is so strong that contact with the bacteria wall precludes antimicrobial action. Instead, a significant number of QAS groups attached to the polymer chains tethered to silica materials do not interact directly with the material

Table 4 Antimicrobial activity of silica gel hybrid containing $[n\text{-OctMe}_2(CH_2)_3N]^+Cl^-$ groups

Starting silica gel materials		MIC [μg mL^{-1}][a]				
		Gram-positive		Gram-negative		
Symbol	Type	*Enterococcus hirae*	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Ia	Sol–gel {TMOS-V_3} +TMOS precipitated from H_2O and DMF	>2,100 (>1,370)	>2,100 (>1,370)	>2,100 (>1,370)	>2,100 (>1,370)	>2,100 (>1,370)
Ib		1,750 (930)	220 (120)	1,750 (930)	>3,500 (>1,870)	>3,500 (>1,870)
II	Xerogel {TMOS-V_3} + TMOS	130 (59)	33 (15)	510 (230)	2,050 (930)	1,025 (470)
III	Silica gel 60A modified	160 (72)	5.2 (2.3)	650 (290)	1,300 (580)	>1,300 (>580)
IV	Sol–gel[b] $(EtO)_3Si(CH_2)_3Cl$ + TEOS	>10,000 (>3,600)	5,000 (1,800)	>10,000 (>3,600)	>10,000 (>3,600)	>10,000 (>3,600)
V	Linear polysiloxane containing QAS[c]	<5.0 (<2.2)	<5.0 (<2.2)	80 (35)	80 (35)	80 (35)

[a] MIC values are related to the weight of pure polymer. Data related to the weight of the $[n\text{-OctMe}_2(CH_2)_3N]^+Cl^-$ groups are given in parentheses

[b] MIC values are related to the weight of all hybrid. Data related to the weight of the $[n\text{-OctMe}_2(CH_2)_3N]^+Cl^-$ groups are given in parentheses

[c] Linear statistical copolymer, Mn = 5,600 g mol^{-1}, containing 50% by weight of [3-(dimethyl-*n*-octylammonio)propyl chloride]methylsiloxane and 50% of dimethylsiloxane units [U. Mizerska et al. in preparation]

surface when the material particles are suspended in water. These QAS groups may therefore efficiently interact with bacterial wall. A weaker antibacterial activity of the QAS-containing materials against the Gram-negative bacteria strains, such as *Pseudomonas aeruginosa*, is a general feature and is explained by the double membrane structure of the Gram-negative bacteria wall. The outer membrane may act as a barrier preventing the action of the QAS polymer on cytoplasmic membrane [23, 36].

4 Conclusions

Silica–organic hybrid particles of various morphologies with antimicrobial QAS groups were synthesized by grafting to the particle surface of a polysiloxane that bears 3-chloropropyl groups pendant to the polymer chain. These groups were used to quaternize *N,N*-dimethyloctylamine. Another hybrid material was obtained by quaternization of this amine by 3-chloropropyl groups bonded directly to the particle.

The antibacterial activity of these hybrid materials strongly depends on their morphology and the manner in which the antimicrobial QAS groups are bonded to the particle surface. QAS groups directly bonded to the particle surface do not exhibit any significant antimicrobial behavior. Polysiloxane with the QAS groups tethered to low porous particles of small surface area shows higher antibacterial activity than when attached to the mesoporous silica material. The significant biocidal activity was also shown by a highly porous silica hybrid. Antibacterial activity of the QAS that bears a polysiloxane tethered to the silica particle suspended in water is lower than that of free QAS-substituted polysiloxane in water solution. The materials studied here show a higher antibacterial activity against Gram-positive than against Gram-negative bacteria.

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