

Synthesis, Characterization, and Bactericidal Properties of Composites Based on Crosslinked Resins Containing Silver

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ABSTRACT: Two different commercial crosslinked resins (Amberlite GT73 and Amberlite IRC748) were employed for anchoring silver. The $-SH$ and $-N(CH_2COOH)_2$ groups, respectively, present on these resins were used for Ag^+ chelation from an aqueous solution. The Ag^+ ions were reduced with three different reductants: hydrazine, hydroxylamine, and formaldehyde (under an alkaline pH). The produced composites were characterized with thermogravimetry/differential thermogravimetry and scanning electron microscopy combined with a backscattered scanning electron detector. Energy-dispersive X-ray spectroscopy coupled to scanning electron microscopy allowed the observation of submicrometer particles of silver, and chemical

microanalysis of emitted X-rays revealed the presence of metal on the internal and external surfaces of the composite microspheres. The amount of incorporated silver was determined by titration. The antibacterial activity of the silver/resin composites was determined toward 10^3 – 10^7 cells/mL dilutions of the auxotrophic AB1157 *Escherichia coli* strain; the networks containing anchored submicrometer silver particles were completely bactericidal within a few minutes because of the combined action of silver and functional groups of the resins. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 107: 1879–1886, 2008

Key words: biomaterials; composites; synthesis

INTRODUCTION

Conventional bactericidal agents are usually liquids or gases of a low molecular weight. However, the use of these disinfectants may bring about recontamination problems.^{1–3} For example, in the case of water treatment for a domestic water supply, the most popular method is to use chlorine and other related chemicals to sterilize water. These compounds may react with organic substances in the water, yielding halomethane analogues, which are believed to be

carcinogenic, and chlorophenols, which confer odor to the water. These drawbacks may be overcome with insoluble bactericidal agents, which do not release small, molecular-size chemicals.

In this context, insoluble cationic polymers that can inactivate, kill, and/or remove targeted microorganisms by mere contact without releasing any reactive agent are a very promising alternative.^{1,3,4} These polymers, known as insoluble polymeric contact disinfectants, are mainly crosslinked anion-exchange resins, including quaternary ammonium type,⁵ polyiodide-type,^{6,7} and pyridium-type resins.¹ The polymers exhibit many unique properties with respect to the capture, or isolation, of bacteria and viruses and the removal of organic pollutants from an aquatic environment. Their antimicrobial and purifying effects have been studied,^{1,3–7} providing the basis for the development of a new antiseptic treatment method.

Copolymer networks with controlled porous structures are widely employed as starting materials for ion exchangers, specific adsorbents, packing for gel permeation chromatography, and also catalyst supports.^{8–10} The morphology of the copolymer beads

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and their porous structures are advantageous for the removal of metal ions and other pollutants from wastewater. These characteristics have a strong influence on the removal kinetics and flow properties.¹⁰ Porous, solid materials based on copolymer networks are of practical importance as supports mostly because of their small pores, interconnected pore structure, large surface area, and good mechanical and thermal properties.¹¹ To take full advantage of those porous materials, functional groups are usually introduced by chemical modification of the base polymer or by the use of a polymer that contains functional monomeric units.

It is also possible to prepare insoluble polymeric contact disinfectants by the anchoring of bactericidal agents to the polymeric materials. Composites containing metal particles have received attention in many fields, such as separation and purification, catalysis, and water treatment, because of their distinctive physicochemical properties.¹¹⁻¹³ Actually, the high performance of these composites is attained by the tailoring of their phase structure in the micro-scale range. A versatile methodology involves producing them in the presence of a solid carrier, such as a polymeric material.^{11,14,15}

The production of colloidal metal particles with a controlled morphology and size is of paramount significance because of the strong dependence of the physical and chemical properties of these particles on their shapes and sizes.^{16,17}

Silver powders containing ultrafine and uniformly distributed sizes particles are largely used in the chemical industry, electronics, and medicine because of singular properties, such as high electrical and thermal conductivity, high resistance to oxidation, and bactericidal activity (colloid silver). Silver is a long-lasting biocide with high temperature stability and very low volatility in comparison with other usual biocide agents, such as chlorine, bromine, and iodine, thus driving great recent attention to nanosized powders and silver colloidal dispersions.

From a practical point of view, chemical reduction from aqueous solutions may be regarded as the most suitable method for obtaining nanosized powders and silver colloidal dispersions.¹⁵ Composites based on crosslinked polymers containing silver combine the excellent high thermal stability of the polymeric carrier with the intrinsic antimicrobial property of silver. Besides the innovative aspect, silver-based antimicrobials (i.e., active silver) are non-toxic to human cells^{11,15} and so have great potential to be used as functional polymer networks with bactericidal activity for sterilizing water.

This article focuses on the evaluation of commercial porous copolymer networks with surface functionalities (i.e., thiol and iminodiacetic acid groups) containing submicrometer silver particles as a disin-

fecting medium for aqueous solutions containing *Escherichia coli* cells.

EXPERIMENTAL

Materials

The employed commercial resins were kindly supplied by Rohm and Haas Co. (São Paulo, Brazil): Amberlite GT73 (a resin containing thiol groups; exchange capacity $\cong 1.5$ mmol/g of resin) and IRC748 (a resin containing iminodiacetic acid groups; exchange capacity $\cong 1.8$ mmol/g of resin). Propanone (Vetec, Rio de Janeiro, Brazil), polyvinylpyrrolidone (PVP; molecular weight = 29,000 g/mol; Fluka, St. Louis, MA), nitric acid (Merck, Rio de Janeiro, Brazil), silver nitrate (Merck), hydrazine (Merck), hydroxylamine (Merck), formaldehyde (Vetec), sodium chloride (Herzog, Rio de Janeiro, Brazil), sodium carbonate (Reagen, Rio de Janeiro, Brazil), sodium hydroxide (Vetec), chloride acid (Reagen), and ammonium thiocyanate (Merck), all pro-analysis (P.A.) degree, were purchased and used as received. Aqueous solutions were prepared with distilled and deionized water. Agar, tryptone, and yeast extract of Bacto, used for the microbiological culture media, were purchased from Difco (Detroit, MI). Aqueous bacterial suspensions were prepared with distilled and sterile water. The auxotrophic *E. coli* AB1157 strain was taken from our laboratory stock¹⁸ and obtained from P. Howard-Flanders (University of Yale, New Haven, CT).

Ion exchange and Ag⁺ reduction

Two ion exchanger/chelating commercial resins (i.e., Amberlite GT73 and Amberlite IRC748) were used for anchoring the submicrometer silver particles. These binary materials were prepared as reported in the literature.¹⁷ Typically, the resins were previously treated with 2M HCl for 24 h. The Ag⁺ exchange was carried out with 5 g of resin beads (GT73 or IRC748 resins) immersed in an aqueous solution of silver nitrate (250 mL at 0.25M). Two drops of concentrated nitric acid were added to reduce the pH and consequently to prevent silver salt hydrolysis. The resin was kept in this condition for 24 h. Then, the beads were filtered off and washed thoroughly with distilled and deionized water until no more silver ion could be detected in the washed water by a chloride test, and they were finally washed with propanone. The beads were dried in an oven at 65°C overnight. One gram of each resin containing Ag⁺ ions was used for each treatment with a particular reduction agent. The reduction agents were employed in aqueous solutions containing 4% (w/v) PVP. Twenty milliliters of a 0.1M aqueous solution

of NH_2NH_2 (at pH = 13), NH_2OH (at pH = 13), or HCHO [combined with Na_2CO_3 at 1% (w/v), pH = 12] was slowly added to the aqueous suspension of resin beads dispersed in 10 mL of a PVP aqueous solution. This addition was conducted in an ice bath (temperature $\approx 5^\circ\text{C}$) for 15 min, and the mixture was kept for 30 min more at room temperature. The beads became dark gray after the reduction process. The beads were thoroughly washed with distilled and deionized water, until neutrality, and with propanone. The dark beads were then dried at 65°C . The obtained silver composites were named according to the reductant used; for instance, when hydrazine, hydroxylamine, or formaldehyde was used in the process of anchoring silver onto the originally pure IRC resin, the silver composites were called IRC748HZ, IRC748HY, or IRC748FO, respectively. The silver content was determined by dissolution of the metallic silver with nitric acid (6M) under reflux for 24 h and then titration with an ammonium thiocyanate standard solution.

Morphological characterization

A Philips (Cambridge, UK) XL-30 scanning electron microscope was used for morphological analysis of the microbead surfaces and inner microstructures. The silver and polymer regions were analyzed with a backscattered scanning electron detector (BSE). The presence of metal particles was verified by energy-dispersive X-ray spectroscopy (EDS) with a microprobe. The samples were dispersed on conductive tape and submitted to gold sputtering to make them conductive before the scanning electron microscopy (SEM)/BSE analyses.

Thermogravimetry (TG)

Thermal analyses of the copolymers were conducted with a TA Instruments (Mount Holly, NJ) model 2050 instrument at a heating rate of $20^\circ\text{C}/\text{min}$ under an oxidizing atmosphere (air) with open alumina crucibles at temperatures ranging from the ambient temperature ($\sim 25^\circ\text{C}$) to 1000°C . Approximately 20 mg of each sample was used in each analysis, and the gas flow rate was kept at 90 mL/min.

Bacterial test using columns of resin beads

Columns containing different types of resin beads were prepared with sterilized 1.0-mL syringes for samples of about 80–120 mg. A sterile glass bead was used to retain the resin bed within the syringe. Through all columns, 1.5 mL of a sterile NaCl aqueous solution was eluted at 0.9% (w/v; saline solution). Equivalent volumes of saline solutions containing *E. coli* at different concentrations (10^3 , 10^4 , 10^5 , 10^6 , and 10^7 cells/mL) were consecutively eluted

through the bead column at room temperature, and each elution lasted 1–2 min. Triplicates were prepared for each experimental condition. The bacterial suspensions were previously prepared by the dilution, in a sterile saline solution, of a culture ($1\text{--}3 \times 10^9$ cells/mL) grown overnight in a lysogenic broth medium¹⁹ at 37°C . After elution through the bead bed, the bacterial suspensions were appropriately diluted in a saline solution to obtain a suspension containing about 3×10^3 cells/mL to determine the bactericidal activity. Aliquots (100 μL) of these suspensions were plated on a lysogenic broth medium solidified with 1.5% agar. The colony-forming units were counted after 24 h of incubation at 37°C , and the bacterial inactivation was calculated as described elsewhere.^{7,20}

RESULTS AND DISCUSSION

The mechanism proposed here for the formation of silver particles involves the exchange of Ag^+ ions and their subsequent reduction around and/or within the beads, as depicted in Figure 1.

Table I shows the silver content found on the produced composites as determined by titration. All employed reductants were able to reduce Ag^+ under the investigated reaction conditions. The hydrazine was the most efficient reductant, possibly because of its stronger reducing power, in accordance with literature data.^{21–23} In addition, the composites based on the resin containing thiol groups (GT73) presented the highest silver contents.

The silver contents of the GT73 composites were around the nominal resin exchange value (1.5 mmol/g of resin), whereas the silver contents of the IRC748 composites were lower than the nominal

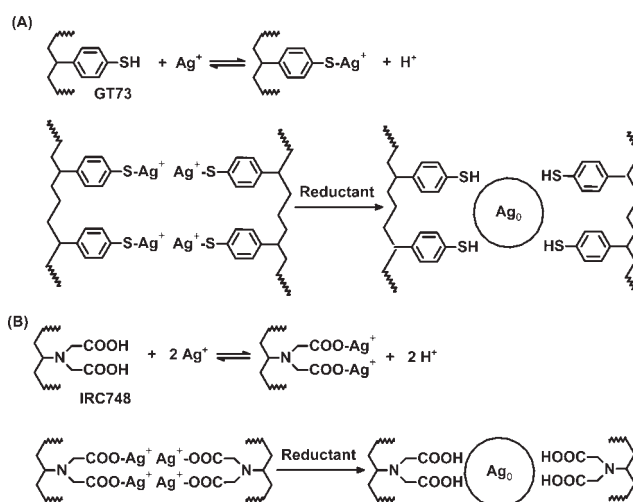


Figure 1 Proposed model for the formation of silver particles around and/or within the bead macrostructure: (A) GT73 and (B) IRC748.

TABLE I
Silver Content in the Composites

Composite	Reductant	Silver content [wt % (mmol/g of resin)] ^a
IRC748HZ	Hydrazine	7 (0.65)
IRC748HY	Hydroxylamine	6 (0.55)
IRC748FO	Formaldehyde	5 (0.46)
GT73HZ	Hydrazine	18 (1.7)
GT73HY	Hydroxylamine	14 (1.3)
GT73FO	Formaldehyde	10 (0.93)

^a Determined by titration (error = 0.1%).

exchange value (1.8 mmol/g of resin). This feature could be explained by Pearson's hard-soft acid-base principle involved in chelating processes: soft acids (e.g., the Ag^+ ion) form more stable coordinate complexes when binding to soft bases (e.g., the $-\text{S}^-$ groups of the GT73 resin).^{24,25} That is, sulfur is a stronger chelator to Ag^+ than the oxygen and nitrogen found in the $-\text{N}(\text{CH}_2\text{COO}^-)_2$ groups of the IRC748 resin.

After the Ag^+ reduction process with different reductants to produce colloidal silver particles onto/within the resin pearls, the silver formation was confirmed by EDS. The silver particles were detected on the internal and external areas of all prepared composites. As depicted in Figure 2, the presence of

silver was confirmed by the EDS spectra of some typical composites. The EDS spectra provide evidence for the presence of silver particles on the surface (external area) and inside fissures or pores of the beads (inner regions) in all the composites. Figure 2 shows typical EDS spectra of a typical silver composite (IRC748FO) based on the IRC748 resin with Ag^+ ions reduced to Ag^0 by formaldehyde [Fig. 2(a,b) for the internal and external surfaces of the composite, respectively] and a GT73HZ composite based on the GT73 resin with Ag^+ ions reduced to Ag^0 by hydrazine [Fig. 2(c,d) for the internal and external surfaces of the composite, respectively].

The anchorage of silver particles onto both external and internal surfaces of the resin beads may be attributed to their porosity, which allowed the diffusion of the Ag^+ ion and the reductant to their inner regions, reaching their functional groups. Although the composites based on the GT73 resin presented the highest silver contents (Table I), a sulfur signal was still found in their EDS spectra. Figure 2(c,d) indicates that the silver did not fully covered the GT73HZ bead surface, evidencing heterogeneity of the silver particle distribution.

Figure 3 shows typical micrographs of the prepared composites. The presence of silver particles on/within these composites was confirmed by the SEM micrographs. Figure 3(a-c) illustrates the

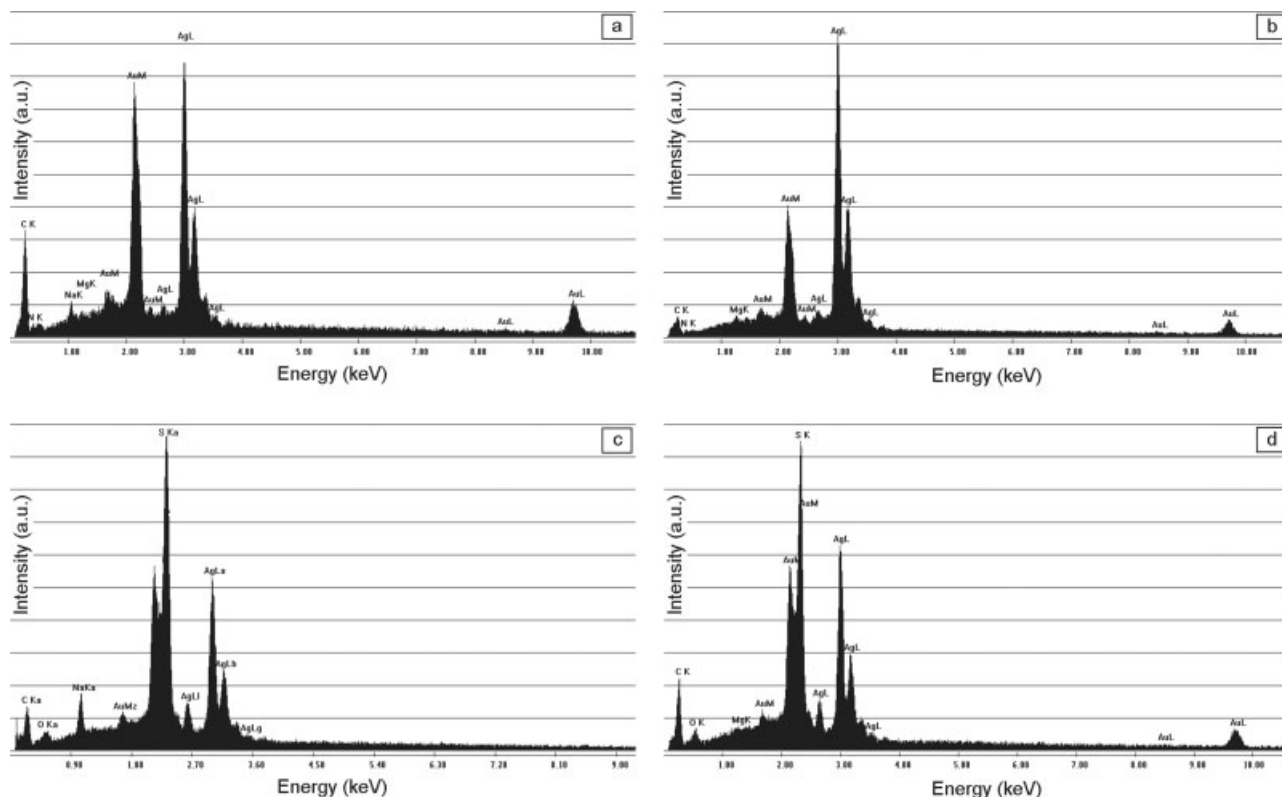


Figure 2 Typical EDS spectra of the IRC748FO composite [(a) internal and (b) external surfaces] and GT73HZ composite [(c) internal and (d) external surfaces].

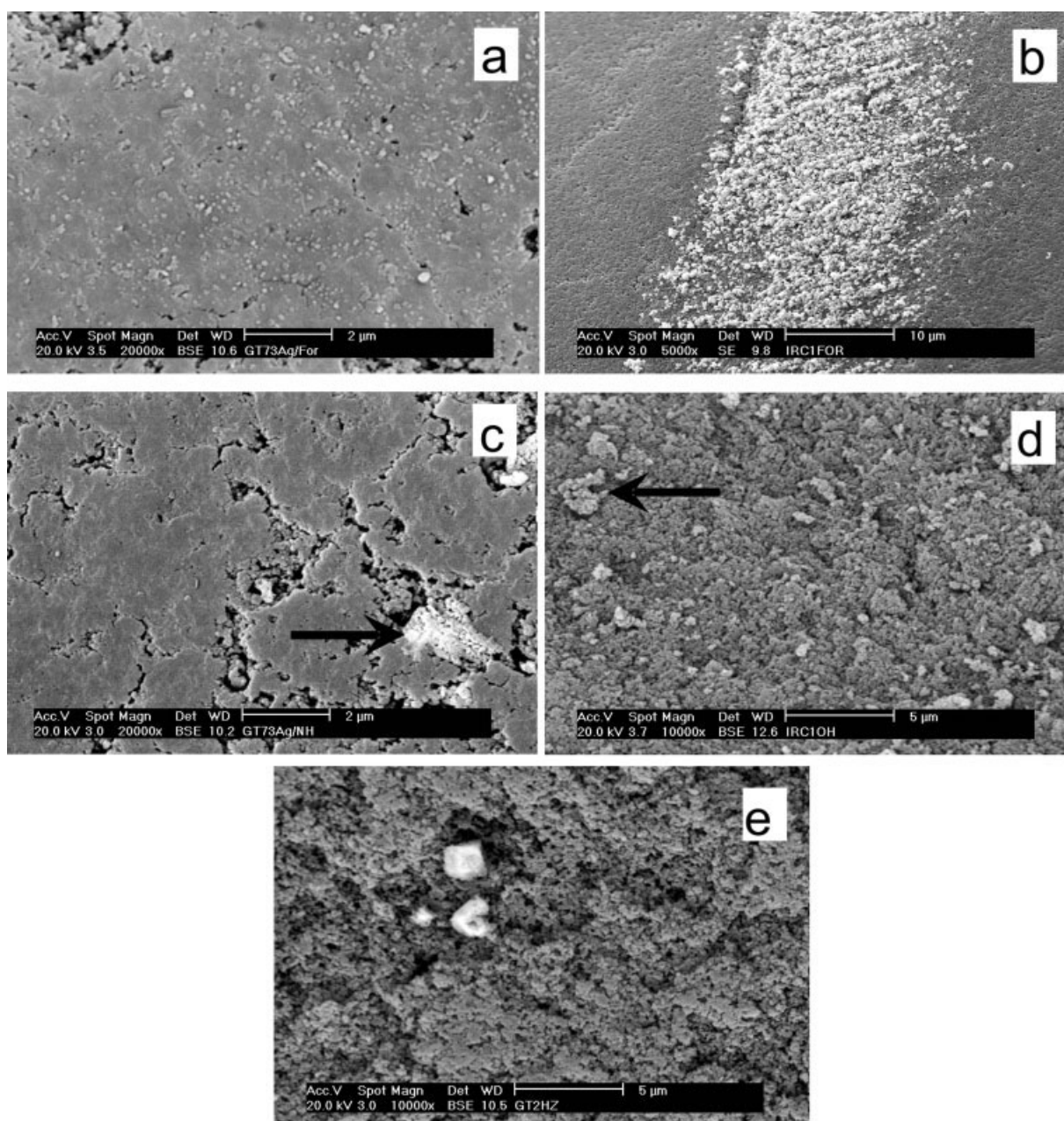


Figure 3 Typical SEM pictures of the external surfaces of three composites [(a) GT73FO, (b) IRC748FO, and (c) GT73HZ] and internal parts of two composites [(d) IRC748HY and (e) GT73HZ].

presence of silver particles on the external surface of three composites: GT73FO, IRC748FO, and GT73HZ, respectively. Figure 3(a) clearly shows that the silver particles were more homogeneously distributed on the external surface of the GT73FO composite in comparison with the IRC748FO micrograph [Fig. 3(b)]. This result could be attributed to the thiol groups, which may strongly chelate the Ag^+ ions (soft acid/soft base), consequently altering the kinetics and equilibrium conditions during the silver particle formation. It was also possible to observe silver particles inside all the composites, as can be seen in Figure 3(d,e), which depicts the internal

surface of the IRC748HY and GT73HZ composites, respectively.

Depending on the colloid preparation conditions, particles 50–500 nm in diameter were obtained according to the SEM micrograph analysis [Fig. 3(a)]. It was shown by a field-emission SEM micrograph that the primary particle size of silver in the colloid was around 50 nm. As a rule, these particles were clustered with a size up to 4 μm . A similar result was reached by Nersisyan et al.²⁶ when they prepared a silver colloid dispersion from Ag^+ reduction with formaldehyde, using sodium dodecyl sulfonate as a surfactant.

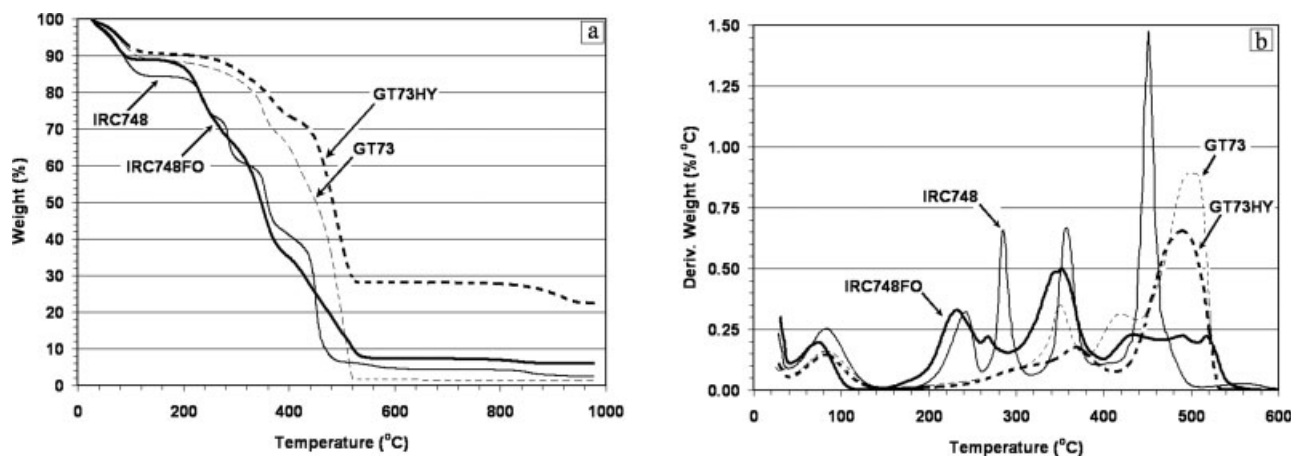


Figure 4 Thermogravimetric analysis of typical polymer/silver composites and pure resins: (a) TG and (b) DTG curves.

All obtained composites were prepared by Ag^+ -ion reduction in the presence of a suitable colloid protector, which was necessary to control the growth of the metal colloids through agglomeration. In this study, PVP was employed as the protecting agent. According to Zhang et al.,²⁷ PVP can form a complex with silver ions and also be strongly absorbed onto the silver particles. The reduction of Ag^+ occurs by its interaction with the surrounding matrix (containing chelating groups), including the colloid protector. Chelation of Ag^+ with PVP slows down its chemical interaction with the matrix, allowing the slow growth of silver particles. In the SEM images shown in Figure 3(b) (for the IRC748FO composite), it can be clearly observed that the colloids coalesced into large grains in comparison with the original aspect exhibited in Figure 3(a) (for the GT73FO composite). Once more, this result could be explained by Pearson's hard-soft acid-base principle. Because the iminodiacetic groups of the IRC748 resin are weaker chelators than the thiol groups of the GT73 resin, the Ag^+ -ion release from the IRC748FO composite was more intense than that from the GT73FO one. This result noticeably illustrates one of the undesired characteristics of nanosized particles, the tendency to agglomerate.

With thermogravimetric analysis, samples were investigated under an oxidizing environment (air) to oxidize the organic components and estimate the remaining silver. Figure 4 shows the overlay of TG/differential thermogravimetry (DTG) curves of the samples, revealing that the stability of the composites depended remarkably on their silver content and also on the specific matrix.

As can be seen in Figure 4(a), the IRC748FO composite curve (in air) showed around 6% residue, which was expected be composed of silver oxide and matrix decomposition material, the latter around 2.5%. On the other hand, the GT73HY composite curve showed around 23% residue [Fig. 4(a)]. The

amount of char obtained for the composites based on the GT73 resin was higher than that of the GT73 resin without Ag^0 and also higher than the silver content shown in Table I. In addition, the thermal stability of the composites based on the GT73 resin was higher than that of the pure resin. These results may be an indication that the presence of silver modified the oxidation pattern of the polymeric matrix. Conventional polymer thermal degradation is affected by ubiquitous heterogeneous surface-catalyzed steps, whose real importance was recently reported following studies of the decomposition of several polymers.²⁸ The DTG curves plotted in Figure 4(b) show clearly that the thermal decomposition pathways of the composites are extremely distinct in comparison with those of their pure resins.

Antibacterial activity against *E. coli* was evaluated with the column containing resin beads, and the results are shown in Table II. It can be noted that the pure resins and all their silver composites, except GT73HZ, exhibited strong antibacterial activity because the percentage reduction of colony-forming units was around 100% for up to 10^6 cells/mL suspensions. Possibly the functional groups [$-\text{SH}$ and $-\text{N}(\text{CH}_2\text{COOH})_2$] could have been responsible for a strong decrease of the pH of the bacterial suspensions, causing bacterial death. In fact, the originally neutral ($\text{pH} \approx 7$) saline solution became acidic ($\text{pH} = 3$ for the GT73 and $\text{pH} = 5$ for the IRC748) after contacting the resins, and this indicated an ion-exchange process with Na^+ ions according to the well-known displacement equilibrium.²⁹

The GT73HZ composite, however, exhibited lower antibacterial activity for bacterial suspensions greater than 10^4 cells/mL. Surprisingly, this composite presented the highest content of silver (Table I). This result could be justified by a lower susceptibility to silver release from the metal colloids and/or by more intense particle growth in the internal surface of the

TABLE II
Antibacterial Activity of the Neat Resins and Their Composites Against *E. coli* Suspensions

Initial bacteria concentration (cells/mL) ^a	Antibacterial activity (%) ^b							
	IRC748 ^c	IRC748HZ ^d	IRC748HY ^e	IRC748FO ^f	GT73 ^g	GT73HZ ^h	GT73HY ⁱ	GT73FO ^j
3×10^3	100	100	100	100	100	100	100	100
3×10^4	100	100	100	100	100	96	100	100
3×10^5	100	100	100	100	100	52	100	100
3×10^6	99	100	99	100	100	24	100	100
3×10^7	67	100	67	99	99	39	97	100

The standard deviation was 5% colony-forming units. *E. coli* AB1157 cultures in the stationary phase of growth were prepared in LB at 37°C for 24 h.

^a Suspensions in sterile solution.

^b Significant antibacterial activity >14% ($p < 0.05$).

^c Material mass = 94 mg.

^d Material mass = 92 mg.

^e Material mass = 94 mg.

^f Material mass = 87 mg.

^g Material mass = 91 mg.

^h Material mass = 97 mg.

ⁱ Material mass = 112 mg.

^j Material mass = 85 mg.

resin beads (lower exposed surface area) during the reduction step. The large silver load may have caused the clogging of some resin pores, hindering the access of cells to the inner part of the composite. Figure 3(c) confirms that some external pores (arrow) of the GT73HZ composite were partially covered with silver.

To evaluate the bactericidal activity purely due to the silver included in the composites, the neutralization of the neat resins (IRC748 and GT73) and the GT73HY composite was carried out with a Na₂CO₃ aqueous solution (2 wt %) for 24 h, and this was followed by a thorough washing of the beads with deionized water until neutrality. During this treatment with sodium carbonate, gas release (CO₂) was observed. The Ag⁺ release was also evaluated with the chloride test. Table III summarizes the results of the antibacterial activity of the neutralized neat

resins (IRC748 and GT73) and the neutralized GT73HY composite (reduced by hydroxylamine) against *E. coli* suspensions.

It can be clearly observed in Table III that the neutralized pure resins exhibited lower bactericidal activity than the nonneutralized ones (Table II), and the neutralized silver composite showed more intense bactericidal activity than the neutralized GT73 resin for a cell concentration up to 3×10^4 cells/mL. Therefore, the antibacterial activity may be attributed to the combined action of silver and acid-functional groups of the resins.

CONCLUSIONS

The size of silver particles anchored onto the surface (internal or external) of porous copolymer networks

TABLE III
Antibacterial Activity of the Neutralized Pure IRC748 and GT73 Resins and the Neutralized GT73HY Composite Against *E. coli* Suspensions

Initial bacteria concentration (cells/mL) ^a	Antibacterial activity (%) ^b		
	Neutralized IRC748 ^c	Neutralized GT73 ^d	Neutralized GT73HY ^e
3×10^3	22	20	64
3×10^4	—	22	88
3×10^5	21	19	14
3×10^6	31	0	8
3×10^7	39	0	37

The standard deviation was 5% colony-forming units. *E. coli* AB1157 cultures in the stationary phase of growth were prepared in LB at 37°C for 24 h.

^a Suspensions in sterile solution.

^b Significant antibacterial activity >14% ($p < 0.05$).

^c Material mass = 82 mg.

^d Material mass = 86 mg.

^e Material mass = 116 mg.

with different functionalities was clearly evaluated with the SEM/BSE techniques. In addition, the use of BSE images allowed adequate observation of the dispersion of silver particles on the surface of copolymer microspheres.

The silver particle distribution and size depended on the type and amount of functional groups present on the network structure used as the copolymer host. The size-controlled production of composites containing silver submicrometer particles can be fairly simply performed under the reduction conditions employed in this study, that is, by the use of reducing agents hydrazine, hydroxylamine, and formaldehyde in a PVP aqueous solution.

The bactericidal activity of the silver composites based on the Amberlite GT73 and Amberlite IRC748 resins was attributed to the combined action of silver and functional groups of the resins.

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