Preparation and Bactericidal Effect of Composites Based on Crosslinked Copolymers Containing Silver Nanoparticles

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Abstract: Commercial copolymers based on styrene and divinylbenzene containing sulfonic acid groups were used as support for the incorporation of silver nanoparticles. These nanoparticles were obtained *in situ* by the reduction of Ag^+ by using hydroxylamine in the presence of a protective agent. These materials were characterized with determination of the silver content and their morphological characteristics. The antibacterial activity of the final products against *Escherichia coli* was evaluated and the results show that the synthesized copolymers had antibacterial effect varying from 54 to 100%. The most efficient composite was made with small, well distributed particles.

Keywords: *Nanocomposite, silver, copolymer, resin.*

Introduction

Contamination of water by bacteria can cause many waterborne diseases, such as dysentery and cholera[1]. Important sources of this contamination are urban sewage and industrial effluents. Sewage contamination is usually identified by the presence of fecal coliforms.

Escherichia coli is a bacterium belonging to the coliform group and to the family *enterobacteriaceae*[2]. Typical untreated domestic wastewater usually contains from 10^6 to 10^9 total coliforms per 100 mL, according to the wastewater pollution level[3]. This bacterium has been detected in food and drinking water[4]. To avoid levels harmful to human health and well-being, efficient water purification is necessary. This procedure allows the recycling of water without harming human health and the environment.

Silver nanoparticles have shown high antibacterial effect against several species of harmful bacteria[5]. They have been used not only against environmental problems but also in the textile industry[6] and for medical applications[7]. The qualities of silver in addition to the polymer morphology provide some features that give copolymers containing silver nanoparticles advantages over traditional techniques used to disinfect water, such as chlorination, use of antibiotics and ozonization[8]. The non-formation of byproducts, absence of cross-resistance, material stability and possibility of recycling are some of these features[9].

This paper deals with the synthesis, characterization and evaluation of four different composites containing silver nanoparticles, obtained from the modification of two commercial copolymers, for disinfection of water contaminated by bacteria. Our goal is to study the influence of these composites' characteristics on the capability of reducing the number of viable *Escherichia coli* cells.

Experimental Part

Incorporation of silver in the copolymers

First, the commercial resins were activated by treatment in an acid solution. About 10 g of each commercial copolymer – Lewatit® VPOC1800 (sulfonated copolymer based on styrene and divinylbenzene, from Bayer Co.) and Amberlyst® 15WET (sulfonated copolymer based on styrene and divinylbenzene, from Rohm and Haas Co.) – were placed in 2% v/v aqueous solution of HCl for 48 hours. Then the resins were filtered out and washed thoroughly with distilled and deionized water in order to reach a pH close to neutral. The resins were thoroughly rinsed with portions of 95% ethanol and dried at 60 °C for 24 hours.

The acid resins were immersed in 100 mL of 2% w/v $AgNO_3$ solution and acidified with 2 drops of HNO_3 for 48 hours. The impregnated resins were filtered off and washed with cold distilled and deionized water until the AgCl precipitate disappeared in the NaCl aqueous solution.

Reduction reaction of Ag^+ ions

For each impregnated resin the following procedures were performed: Two solutions of gelatin and 2-hydroxy-ethyl cellulose at 0.6% w/v at proportion of 1:1 were prepared and cooled in an ice bath. The resin was added to one of these solutions and in the other 3 g of hydroxylamine chloride was added. The pH was controlled by adding drops of a 2 $mol.L^{-1}$ solution of NaOH or NH_4OH to keep the pH at approximately 12. The hydroxylamine chloride solution was added to the resin solution during 20 minutes. After that, the solution was left at rest for 10 minutes in an ice bath and 25 minutes at 25 °C. The resins were then washed with distilled and deionized water at 60 °C.

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The two commercial resins and the two different solutions used for pH control (S_{pH}) resulted in four distinct composites at the end of each reaction: VPOC1 (S_{pH} NaOH); VPOC2 (S_{pH} NH_4OH); WET1(S_{pH} NaOH); and WET2 (S_{pH} NH_4OH).

Characterization of the composites

The characterization of the copolymers was determined by nitrogen adsorption data obtained with a Micromeritcs surface area and porosity analyzer (ASAP). The ASAP was employed to compare the surface area before and after the silver incorporation.

The resin's appearance and morphology as well as the silver particles obtained were evaluated with a Philips model XL-30 scanning electron microscope (SEM) equipped with a backscattered electron detector (BSE). The acceleration voltage was 20 kV. An energy dispersive X ray spectrometry (EDS) microprobe from EDAX Co. was used to confirm the silver presence.

The silver content of the composites was measured by a Shimadzu EDX-800HS energy dispersive X ray fluorescence spectrometer (EDXRF) equipped with a liquid nitrogen cooled Si (Li) detector.

Antibacterial analyses

The purpose of the antibacterial analyses was to evaluate the synthesized copolymers' ability to reduce the number of viable cells. The test was conducted in triplicate with a suspension of *E. coli* (ATCC® 25922™) using the viable plate count method described by Jandrey et al.[10]. The *E. coli* strains were cultured in lauryl sulphate tryptose broth for 18 hours at 37 °C beforehand. Successive dilution of the *E. coli* solution was performed with sterile NaCl aqueous solution at 0.9% w/v in order to obtain concentrations from 10^3 to 10^7 cells.mL^{-1}. All solutions from 10^4 to 10^7 were diluted with saline to reach concentrations equal to 10^3 thus facilitating the counting of plates after elution. The different *E. coli* solutions were conducted through columns containing 0.2 mL of each copolymer. The original copolymers without silver added were also tested. All copolymers were neutralized with 2% w/v of $NaHCO_3$ solution to prevent the possible influence of acid groups. After passing through the column, the solutions were placed in eosin/methylene blue Levine agar plates and incubated at 37 °C for 48 hours to count the colonies. As control, the same procedure was performed with the same *E. coli* solutions without passing them through the column. A CP600 Phoenix 617 colony counter was used to measure the colony forming unit (CFU) in each plate. The percentage reduction in bacterial count was calculated by the Equation 1.

Equation 1: The percentage reduction in bacterial count[11].

$$R_p = \left[\frac{(C_b - C_a)}{C_b}\right] \times 100\% \quad (1)$$

where:
R_p = percentage reduction;
C_b = viable count before column;
C_a = viable count after column.
The statistical analysis was performed using Student's *t*-test[12].

Results and Discussion

The ASAP results of the copolymers are listed in Table 1.

Although both resins used have the same polymer base and functional group, the morphology of each has distinct characteristics. The Lewatit® VPOC1800 is a gel-like resin[13] while the Amberlyst® 15WET is a macroreticular resin with high porosity.

The values of surface area were lower for all the composites synthesized compared to the respective polymer matrix. This behavior can be attributed to presence and agglomeration of silver particles within the pores and channels, resulting in a decrease of open spaces and its consequent non-measuring[14]. One of the factors that determine the size of the silver particles is the pore size of the resin, since this space is a physical barrier that prevents agglomeration of particles, serving as template. Macroporous resins showed an increase in pore diameter with addition of silver. This result can be explained by a possible strain of the pores wall generated by the presence of the particle in their interior[15]. However, the resin gel had the opposite trend, possibly in this case the particles were not able to extend the pore of the polymer matrix diminishing the pore size because of its partial filling.

The SEM images showed a significant difference among the four composites. Non-uniform coarse precipitation was observed in VPOC1 (Figure 1a) while the composite VPOC2 (Figure 1b) had narrow size distribution of the silver particles. Thus, the agglomeration of the particles was considerably greater in the VPOC1 in comparison with the VPOC2 composite. The composite WET1 (Figure 1c) had a more homogeneous distribution but larger particle sizes than the WET2 composite (Figure 1d).

The EDS confirmed the formation of elemental silver in all composites (Figure 1a, 1b, 1c and 1d). However, the silver content varied greatly. This result was confirmed by EDXRF, which technique the total amount of silver is calculated as the proportion of silver oxides found per mm^2 of sample. The following percentages were found: VPOC1 = 7.9%; VPOC2 = 9.6%; WET1 = 44.9%; and WET2 = 23.1%.

The NH_4OH solution used for pH control optimized the formation of smaller silver particles than in those controlled with NaOH solution. It can be explained by a possibly stronger reaction control through the formation of an $[Ag(NH_3)_2]^+$ complex by the NH_4OH while the NaOH solution favors the formation of silver clusters in the polymer surface[16]. The Figure 2 shows two possible nanosilver formations.

Antibacterial analyses

The efficacy of the copolymers containing silver nanoparticles against *E. coli* was measured by the viable plate count method. The results are presented as average values over all dilutions in Table 2.

After 48 hours of incubation, the viable *E. coli* declined by 54 and 100%, according to the composite. There was no reduction in viable counts in the control group and also in the solution that was eluted through the column without silver.

The most efficient antimicrobial copolymer against *E. coli* was VPOC2. This result indicates that the silver content does not seem to be closely related to the bactericide effect since this copolymer did not contain high levels of silver. VPOC2 composite had smaller particles with higher specific surface area and narrower size distribution than the other composites. The combination of these parameters produced 100% antimicrobial efficiency. Despite WET1 presents larger particles than WET2, its distribution in the polymer was homogeneous all through the polymer favoring the interaction between silver and bacteria. These results may explain the similar bactericidal activity of both composites.

Table 1. Characterization of porosity and surface area.

Copolymers	Pore size (Å)	Surface area (m^2.g^{-1})
Lewatit® VPOC1800	21.6	3.0
VPOC1	*	*
VPOC2	*	*
Amberlyst® 15WET	234.9	54.7
WET1	242.7	35.8
WET2	251.9	36.4

*below the detection limit

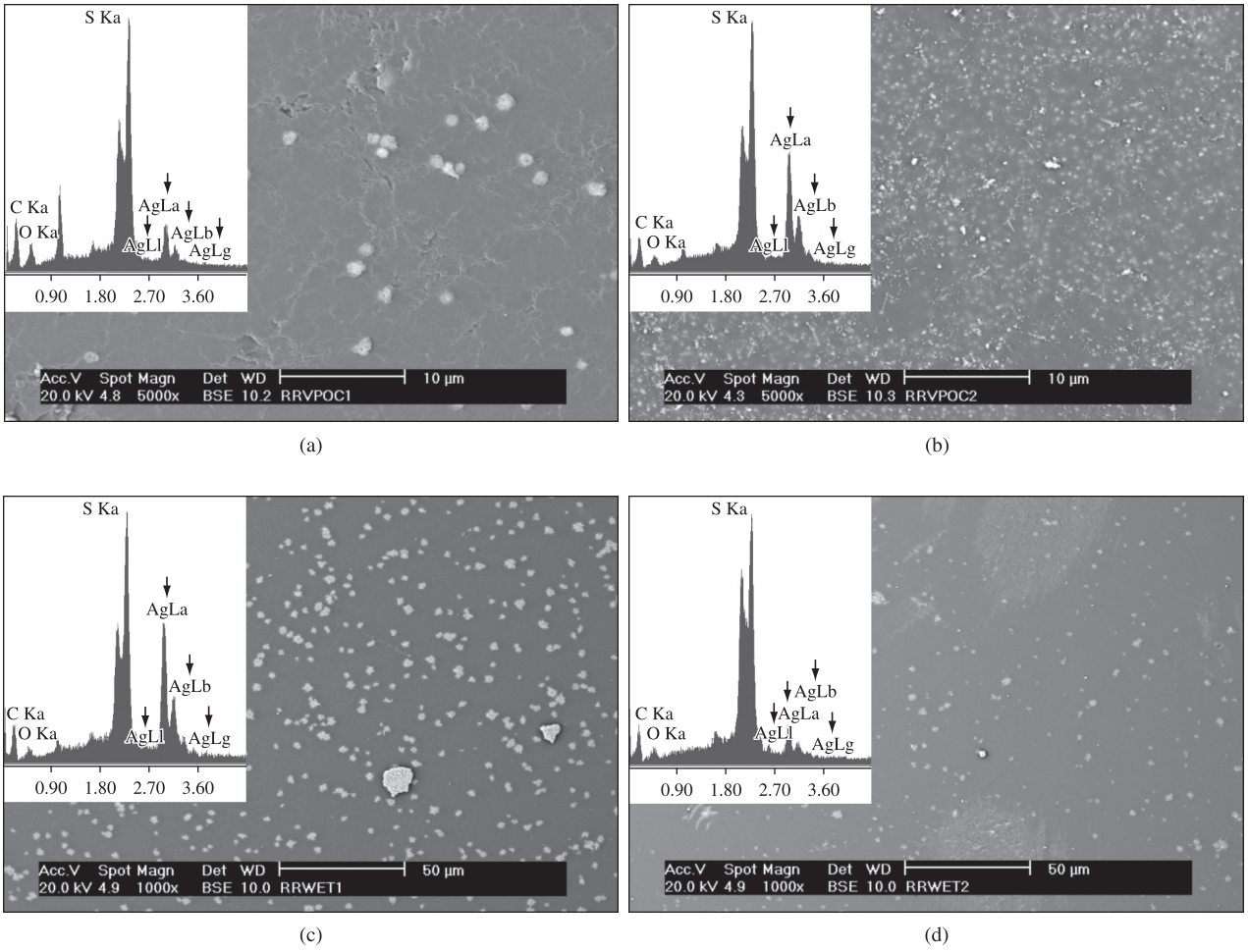

Figure 1. SEM and EDS results of the impregnated silver nanoparticles in the different final composites: a) VPOC1; b) VPOC2; c) WET1; d) WET2.

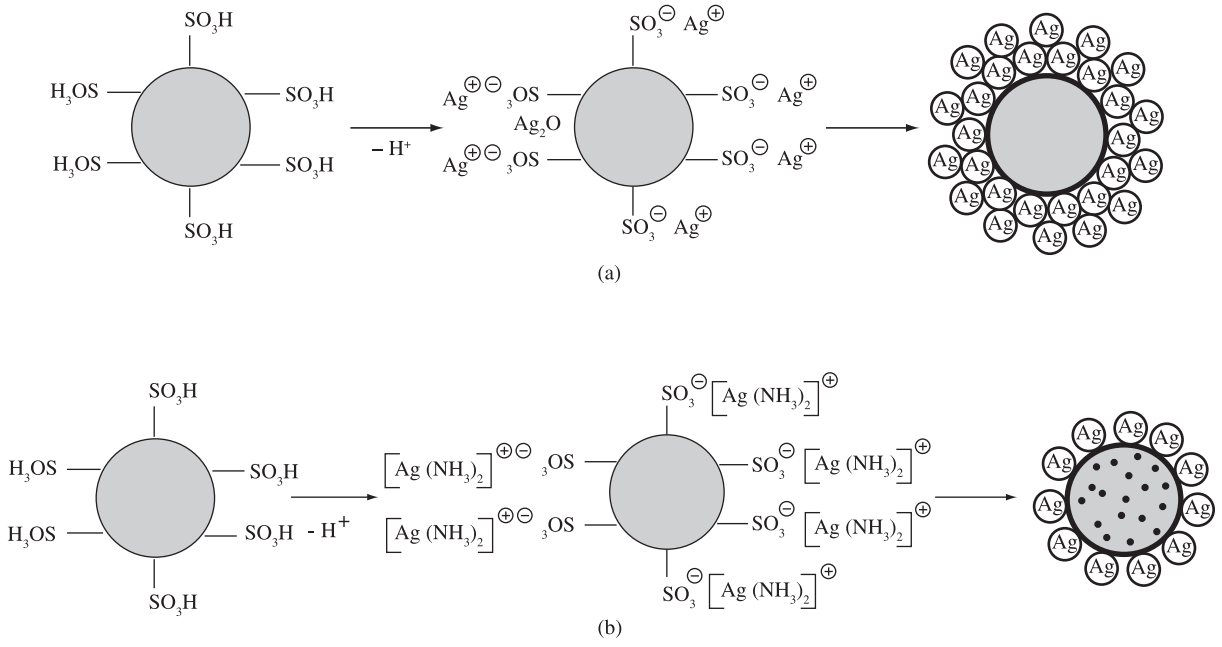

Figure 2. a) Formation of silver nanoparticles in NaOH presence; b) formation of silver nanoparticles in NH_4OH presence.

Table 2. Antimicrobial activity of the copolymers before and after the silver addition.

Copolymers	Antimicrobial activity (%)[a]
Lewatit® VPOC1800	0
VPOC1	54
VPOC2	100
Amberlyst® 15WET	0
WET1	91
WET2	93

Relative standard deviation = 5% UFC; significant bactericidal action > 14%; a) average of concentrations from 10^3 to 10^7.

Conclusion

The different methods used to obtain copolymers containing silver nanoparticles were successful. The NH_4OH presence had an important role on the formation of smaller silver particles due to a possible silver complex formation during the reduction reaction. The bactericide effect is related to the interaction between silver and *E. coli*. Factors such as high surface area and a homogeneous distribution of the particles favor this interface. The absence of reduction in viable counts in the control groups demonstrates that the antibacterial effect is only due to the silver nanoparticles, not the copolymer itself.

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