Evaluation of the Biocidal Capacity of Hypercrosslinked Resins Containing Dithiocarbamate Groups

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Summary: In this study we report both the development of dithiocarbamate resins from the commercial hypercrosslinked resin MN-250 and the evaluation of the biocidal capacity of this material against *E. coli* ATCC25922 suspensions. The preparation of dithiocarbamate resins followed a synthetic pathway based on nitration of resins, reduction of nitro groups and reaction with CS_2 in an alkaline medium. The biocidal capacity was evaluated by means of elution of *E. coli* suspensions (10^3–10^7 cells/mL) through columns containing the resin and plating on LB nutrient medium solidified with Bacto agar. We can conclude that hypercrosslinked resins with dithiocarbamate groups have potential biocidal action.

Keywords: biological applications of polymers; dithiocarbamate resins; functionalization of polymers; hypercrosslinked resins

Introduction

Waterborne diseases remain the leading cause of death in many developing nations. Approximately 30% of all cases of death in Latin America are associated with ingestion of contaminated water. In Brazil, many hospital admissions are attributable to the lack of sanitation in communities.[1,2] Waterborne diseases can be caused by viruses, bacteria, fungi and other microbes.

Various types of biocidal polymers have been prepared and studied for water disinfection. Disinfection using biocidal polymers has several advantages over ozone and many soluble disinfectants. The main advantage is the possibility of inactivating or destroying microorganisms present in contaminated water without leaving behind residues that can yield halomethane analogues.[3–6] Biocides polymers can act on microorganisms in different ways: (i) inhibiting protein synthesis which causes metabolic lesions that impede cell growth (bacteriostatic action), an effect that is reversible with the agent's removal; or (ii) provoking the death of the cells by interfering with one or more essential metabolic process or by injury of some physical structure of the microorganism (biocidal action), an effect that is irreversible.[7,8]

The preparation of biocidal polymers usually consists of incorporating a bactericidal group into a previously synthesized polymer matrix. It has been established that the efficiency of a biocidal polymer is determined by the nature of its bactericidal group and also by the type of porous structure and swelling properties of the polymer support. These characteristics influence the process of reagent diffusion and consequently the extent of modification

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reactions by introducing the biocidal groups in this matrix, as well as the accessibility to those introduced groups by bacteria and the diffusion of the bactericidal agent in the medium.[6–8]

Styrene-divinylbenzene (Sty-DVB) copolymers have been extensively used as supports for biocidal groups.[7–16] These materials are mainly prepared by free-radical crosslinking copolymerization of styrene (Sty) and divinylbenzene (DVB) in the presence of an initiator and a diluent. The porosity characteristics of these copolymers are determined by diluent nature, dilution degree of the monomers and crosslinker concentration.

Styrene-divinylbenzene copolymers (Sty-DVB) containing dithiocarbamate groups have been shown to be effective in removing several heavy metals.[17–22] However, the literature has not yet addressed the biocidal capacity of these materials. The development of polymeric resins with biocidal capacity and ability to remove heavy metals is very interesting from both economic and ecological standpoints.

Hypercrosslinked resins are special materials of low density, with nanopore structures and high internal surface area.[23] These materials have been utilized for removal of phenyl compounds,[24–26] aniline,[27] pesticides [28] and toxic metal ions.[29] Their porosity is introduced not during the polymerization process, but during an extensive post-cross-linking of polymer chains in the highly swollen state via the Friedel–Crafts reaction. In comparison with traditional Sty-DVB copolymers, hypercrosslinked materials have the ability to swell only in thermodynamically good solvents for the networks, but also in solvents that do not normally solvate polymer chains, such as water, despite their large surface area and microporosity. A high degree of functionalization is expected in this type of material because of the swelling and high surface area. As commented before, the accessibility of the biocidal groups by microorganisms in contact disinfectants or the diffusion of the biocidal agent in the aqueous medium in demand-release disinfectants depend on the support characteristics. Therefore, since hypercrosslinked resins have high surface area and swelling capacity in water, it is likely that these materials have strong biocidal action. However, the biocidal capacity of hypercrosslinked resins containing biocidal groups has not been sufficiently studied.[23–33]

In order to fill this gap, we evaluated the antibacterial properties of the commercial hypercrosslinked resin MN-250 (Purolite), functionalized with dithiocarbamate groups, towards *E. coli* ATCC 25922 cells.

Experimental Part

Chemicals

The hypercrosslinked resin MN-250 was donated by Purolite SRL (Victoria, Jud. Brasov., Romania). The resin was used after being washed several times with hot water until neutral pH, and then with ethanol and methanol, after which it was dried at atmospheric pressure for 48 h at 60 °C.

The growth broth Luria Bertani Broth MO80 and plating broth Agar Power Bacteriological were purchased from Himedia Laboratories PVT Limited (Mumbai, India) and prepared according to the manufacturer's instructions. The *E. coli* ATCC 25922 strain was obtained from Laborclin Ltda. (Rio de Janeiro, Brazil). Other reagents and solvents were purchased from Vetec Química Fina Ltda. (Rio de Janeiro, Brazil) and used as received.

All glassware and solutions for bacterial experiments were sterilized in an autoclave at 120 °C for 20 min before the experiments.

Preparation of Dithiocarbamate Resin

The dithiocarbamate resins were prepared by modification of the hypercrosslinked MN-250 resin through of the nitration of benzene rings, reduction of nitro groups with $SnCl_2$ and reaction with carbon disulfide.[17–22]

Nitration: the hypercrosslinked resin MN-250 (18 g) was introduced in a three-

necked, round-bottom flask equipped with a mechanical stirrer and a reflux condenser containing a silicon oil seal at the top. A mixture of sulphuric acid (60.5 mL) and nitric acid (51.3 mL) was added to the flask containing beads. The reaction mixture was stirred continuously (200 rpm) at 60 °C for 2.5 h. The resulting nitro beads were washed repeatedly with deionized water until neutral pH, then further washed in ethanol (300 mL) and methanol (150 mL), and dried in a vacuum at 60 °C for 48 h.

Reduction of the nitro groups: The nitro resin (16 g) and 40 cm^3 of ethanol were added in a three-necked, round-bottom flask equipped with a mechanical stirrer and a reflux condenser containing a silicon oil seal at the top. A mixture of stannous chloride (226.3 g) and concentrated hydrochloric acid (257 mL) was added into to the flask, and nitrogen was bubbled in the reaction mixture. The reaction proceeded under an inert atmosphere for 16 h at 90 °C, under stirring of 200 rpm. The product was filtered, washed with 2 mol L^{-1} NaOH aqueous solution (1 L) to recover the free amino polymer, then washed further with deionized water until neutral pH and then with ethanol (250 mL) and methanol (150 mL), after which it was dried at 60 °C for 48 h.

Reaction with CS_2: The amino resin (16 g) was treated with a solution of NaOH in ethanol 1mol/dm^3 (50 mL) and carbon disulphide (45.6 mL) in a three-necked, round-bottom flask equipped with a mechanical stirrer and a reflux condenser containing a silicon oil seal at the top. The reaction mixture was stirred continuously (200 rpm) at room temperature for 6 days. The resulting product was filtered, washed repeatedly with water until neutral pH, the washed with ethanol (250 mL) and acetone (200 mL) and dried at 60 °C for 48 h.

Characterization of the Resins

The commercial hypercrosslinked resin MN-250 was characterized by determining its apparent density by the ASTM D1895 method.[34] Its surface area and pore volume distribution were determined by nitrogen adsorption measurements, according to the BET and BJH methods, respectively (Micromeritcs, ASAP 2010 apparatus). Finally, its degree of swelling (*DS*) in water was determined using a graduated cylinder (10.0 ± 0.1 mL), which was filled with about 3.0 mL of dry polymer. The solvent was added up to the 10.0 mL level and 24 h later the final volume was read.[4] The *DS* was calculated according to the following equation:

$$DS(\%) = \frac{(Vf - Vi)}{Vi} \quad (1)$$

The shape and surface texture of the resins were monitored with a FEI Quanta 400 scanning electron microscope (SEM) operating at 20 keV, magnification: 10 000 x and 20 000 x. For SEM, the samples were spread on a conductive tape and sputtered with gold. The sulfur and polymer regions were analyzed by a backscattered electron detector (BSE). The presence of metal particles was confirmed by energy dispersive X-ray spectrometry using an EDAX microprobe.

The modification reactions of the hypercrosslinked resin were monitored by elemental analysis and FTIR spectrometry. The elemental analysis was performed in a Perkin-Elmer CHNS/O analyzer (2400 Series II), applying dynamic flash combustion for sample analysis. FTIR spectra of polymers in the form of KBr pellets were obtained using a Perkin-Elmer (Spectrum One) spectrometer (4 scans and 4 cm^{-1} resolution).

Antibacterial Testing

The antibacterial activity of the polymers was determined against *E. coli* cells (ATCC25922), as previously described.[4,10,11] Columns containing the polymers were prepared by using 1.0 mL sterilized syringes with about 200 mg of the sample. 1500 μL of a sterile NaCl aqueous solution (0.9% w/v) was eluted through all columns. The same volume of saline solutions containing *E. coli* at varying concentrations (10^3, 10^4, 10^5, 10^6 and 10^7 cells mL^{-1}) was successively eluted through the columns. After elution through

the bead bed, the bacterial suspensions were appropriately diluted in saline to obtain a suspension containing about 2×10^3 cells mL^{-1}. Suitable 100 μL aliquots of these suspensions were plated on Luria Bertani nutrient medium solidified with 1.5% Bacto agar. The colonies formed (*CFU*) were counted after incubation for 24 h at 37 °C. The bactericidal activity of the polymers was estimated by calculating the decrease in the number of bacteria, according to Equation 2.[10,11] Triplicates of independent experiments were carried out.

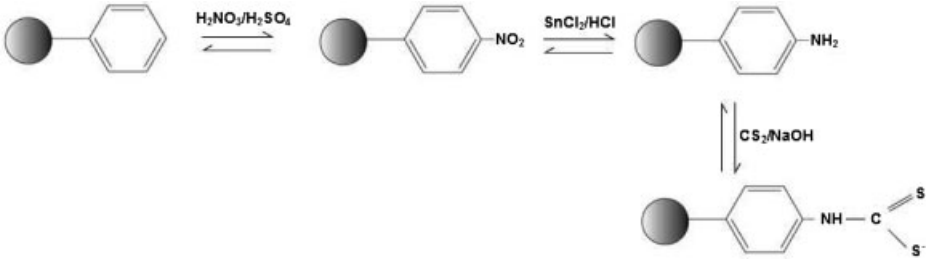
$$\%CFUs = 100x\frac{(CFUi - CFUf)}{CFUi} \quad (2)$$

where *CFUi* and *CFUf* correspond to the *CFUs* of each cell's suspensions before and after elution through the beads, respectively.

Results and Discussion

The introduction of dithiocarbamate groups into the hypercrosslinked resins was based on nitration of the resins, followed by reduction of nitro groups and finally reaction with CS_2 in alkaline medium, according to Scheme 1.

Figure 1 shows the FTIR spectrum of the commercial resin MN-250. The low resolution of the spectrum is due to the difficulty



Scheme 1.
Introduction of the dithiocarbamate groups into the hypercrosslinked resin MN-250.

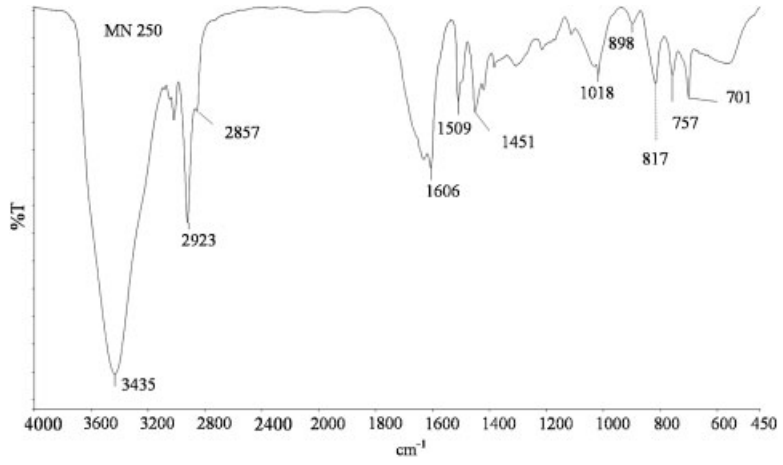


Figure 1.
FTIR spectrum of hypercrosslinked resin MN-250. (3435 cm^{-1} water absorption band; 2923 and 2857 cm^{-1} aliphatic C—H stretching; 1606, 1509 and 1451 cm^{-1} aromatic C=C stretching; 1018 and 898 cm^{-1} aromatic C—H in-plane bending (for p-substitute benzene); 817, 757 and 701 cm^{-1} aromatic out-plane bending (for p-substitute benzene).[35]

of pastilling of the pearls hyperreticulated with KBr pellets.

The porous characteristics of the hypercrosslinked resin MN-250, the nitrogen and sulfur concentrations determined by elemental analysis and the degree of swelling in water are presented in Table 1. In addition to the significant values of surface area and pore volume determined by nitrogen adsorption, the lower apparent density of the resin and the significant values of surface area and pore volume determined by nitrogen adsorption confirm the high porosity of the resin.

Some authors have reported that hypercrosslinked resins contain carbonyl and hydroxyl groups. The presence these groups is attributed to side reaction of oxidation of the chloromethyl groups by oxygen in air during post crosslinking reaction.[6,9] This information was confirmed by the analysis of FTIR spectrum and the swelling degree in water of the commercial resin MN-250. The spectrometry showed the presence of a band at 3435 cm^{-1} due OH stretching associated with the intermolecular hydrogen bond, not present in typical Sty-DVB copolymers, and a broad band at 1800–1600 cm^{-1} that can be due to the association between C=C ring stretching and C=O stretching.[35] The resin showed swelling capacity in water, a characteristic unlike that of typical Sty-DVB copolymers. The test of swelling degree in water is not applicable to Sty-DVB copolymers due to their hydrophobicity.

Modification reactions (nitration, reduction and reaction with CS_2, were evidenced by FTIR spectroscopy (Figure 2) and elemental analysis.

The spectrum of the MN-250 resin after nitration reaction (Figure 2 a) showed two strong bands at 1527 cm^{-1} and 1348 cm^{-1}

Table 1.
Porous characteristics of MN-250 resin.

Resin	d_{ap} (g/cm^3)[a]	S (m^2/g)[b]	V_p (cm^3/g)[c]	SD_w (%)[d]	N_c (mmol g^{-1})[e]	S_c (mmol g^{-1})[f]
MN-250	0.15	987	0.50	6	*nd*	*nd*

[a]d_{ap} = apparent density; [b]S = specific surface area determined by BET method; [c]V_p = pore volume determined by BJH method; [d]SD_w = swelling degree in water (relative standard deviation = 2.4%); [e]N_c = nitrogen content determined by elemental analysis; [f]S_c = sulfur content determined by elemental analysis.

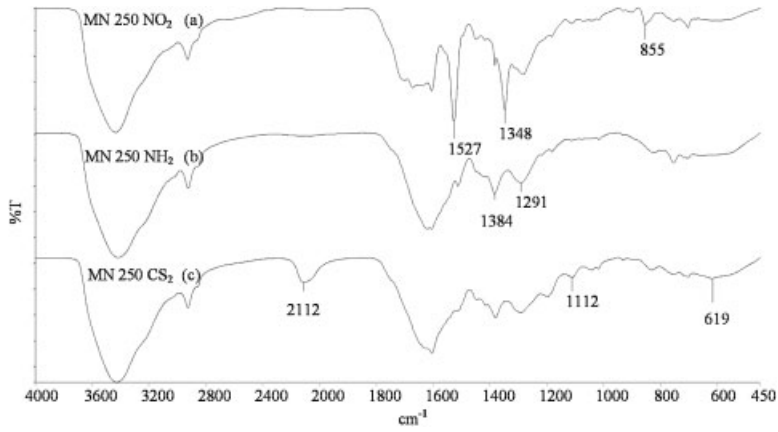


Figure 2.
FTIR spectra of the commercial resin MN-250 following reactions of nitration (a), reduction (b) and reaction with CS_2 (c), used to introduce dithiocarbamate groups.

due to asymmetric and symmetric stretching vibrations of the nitro groups and a new band at 855 cm^{-1} due to the bending vibration of the C–N bond.[35] The content of nitro groups determined by elemental analysis was 3.30 mmol g^{-1}. The spectrum of the resin after the reduction reaction (Figure 2 b) showed the C–N stretching vibration is always mixed with other bands and is usually assigned in the region of 1384–1291 cm^{-1}.[35] The conversion of amino groups into dithiocarbamate groups after reaction with CS_2 (Figure 2 c) was confirmed by new bands at 2112 cm^{-1} due to the C=S bond, 1112 cm^{-1} due to NCS_2, and 619 cm^{-1} due to the stretching vibration of the C–S bond. The sulfur content determined by elemental analysis was 1.51 mmol g^{-1}. The reaction with CS_2 is typically a difficult reaction, thus leading to a low conversion degree.[4,17,20,36]

The SEM micrographic (Figure 3 (a) and (b)) show the external part of the hypercrosslinked resin MN-250. The micrograph

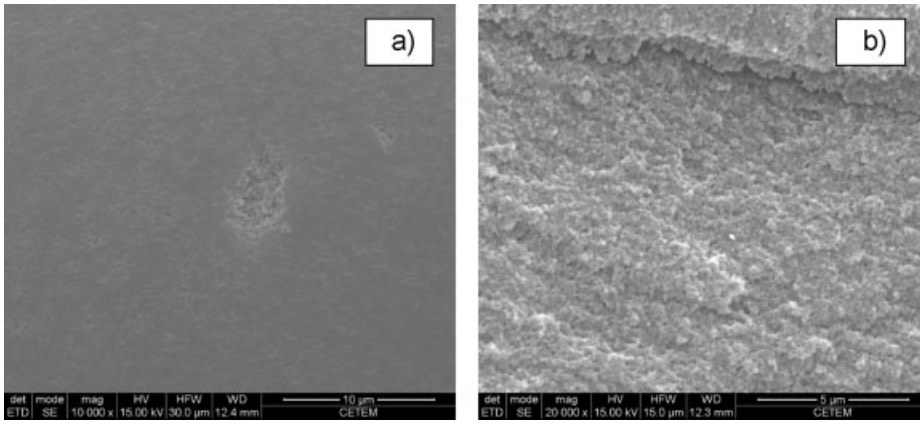


Figure 3.
SEM micrographs of commercial resin beads (external part): 10 000 x (a) and 20 000 x (b), after reaction with CS_2, used to introduce dithiocarbamate groups.

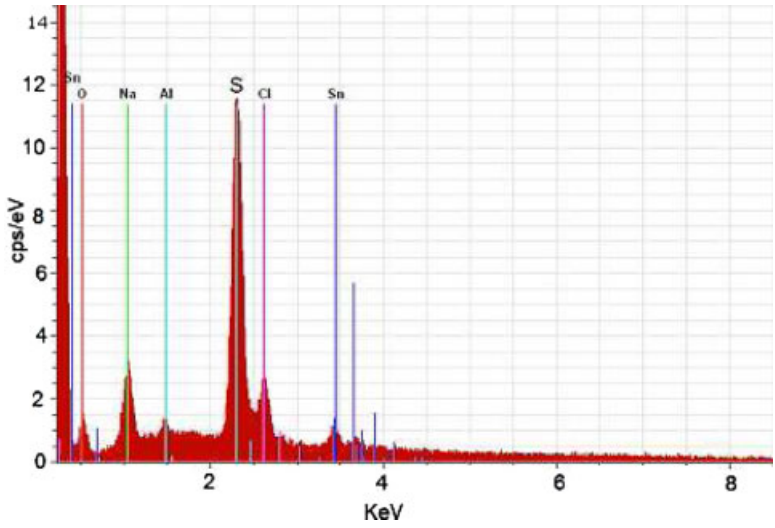


Figure 4.
EDS spectrum of the external surface of the dithiocarbamate resins obtained from MN250 resins.

Table 2.
Preliminary results for the bactericidal action of the dithiocarbamate resin.

Initial Concentration[a] (cells mL^{-1})	Bactericidal Action[b] (%)
2.2×10^3	92
2.6×10^4	72
2.1×10^5	65
2.1×10^6	56
2.4×10^7	47

Relative standard deviation = 10% UFC. Significant bactericidal action > 14%;[a]NaCl aqueous solution at 0.9%, initial concentration of *E.coli* suspension = 2×10^3 cells mL^{-1};[b]mean of triplicates.

with magnification of 20 000 x shows that this resin contains larger pores. It is possible to suppose that this structure will simplify the transport process when the material is be used as column filling. The presence of larger pores favors the contact between the bacteria and biocidal groups located on the inner region of the beads.

Figure 4 shows the spectrum of the external part of the composite obtained with dithiocarbamate resin. The X-ray analysis confirms that sulfur is present in the external part of this dithiocarbamate resin.

The results of preliminary tests to evaluate the bactericidal capacity of the dithiocarbamate resin are presented in Table 2.

Table 2 shows that the bactericidal action of the dithiocarbamate resin was significant for all *E.coli* concentrations. This resin killed 92% of the bacteria at an initial concentration of 10^3 cells mL^{-1}. This indicates that the dithiocarbamate group was effective as an antimicrobial agent. However, the unmodified resin samples and the products of nitration and reduction reactions showed no bactericidal action at any *E. coli* concentration tested.

In general, biocidal activity showed a tendency to decline as the bacterial concentration increased. This may be a result of copolymer saturation, since there was no column exchange of the same resin. Thus, after the elution of *E. coli* at a concentration of 10^7 cells mL^{-1}, over 10^{15} cells can cross the column and form a biofilm around the polymeric pearls, which in turn can hamper the interaction between the biocidal agent and new bacteria.

Conclusion

The introduction of the dithiocarbamate groups into the commercial hypercrosslinked resin MN-250 was possible due to nitration reaction of the resin, reduction of the nitro groups and reaction with CS_2. The modification reactions were confirmed by FTIR by the presence of bands of the nitro groups (1527 cm^{-1}, 1348 cm^{-1}, 855 cm^{-1}), amino groups (1384–1291 cm^{-1}) and dithiocarbamate groups (2112 cm^{-1}, 1112 cm^{-1} and 619 cm^{-1}). The concentrations of the nitro and dithiocarbamate groups estimated by elemental analysis were 3.30 mmol g^{-1} and 1.51 mmol g^{-1}, respectively. EDS microanalysis showed the sulfur particles dispersed in the polymeric matrix. The preliminary tests demonstrated that the commercial resin had no biocidal action. After introduction of dithiocarbamate groups, the resin began to show bactericidal capacity, especially at high *E. Coli* concentrations. At concentration of 10^3 cell mL^{-1}, the biocidal action of the dithiocarbamate resin was higher than 90%.

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