Iodine Bactericidal Action Adsorbed in 2-Vinylpyridine Copolymer Networks

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ABSTRACT: In the present work, different kinds of porous copolymer networks based on 2-vinylpyridine (2VP) and divinylbenzene (DVB) containing anchored iodine were prepared and evaluated for inactivating *Escherichia coli* in an aqueous medium. By using bacterial viability assays with *E. coli* (AB 1157, wild-type), the iodine bactericidal effect was investigated at room temperature by elution through short columns filled with copolymer beads. Saline solutions containing bacterial cells at several contents (10^2 to 10^7 cells/mL) were eluted. After each elution, cell content viability was determined by plating these suspensions on nutritive agar plates, incubating them at 37°C for 24 h, and counting as colony-forming units (CFUs). It was verified that the networks containing anchored iodine were completely bactericidal within a few minutes. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 93: 972–976, 2004

Key words: biological applications of polymers; resins; functionalization of polymers

INTRODUCTION

Copolymer networks with controlled porous structure are widely employed as starting materials for ion exchangers, as specific sorbents, as packing for gel permeation chromatography, and as catalyst supports.[1–5] Although the production of controlled porous polymeric network for these applications (mainly for ion exchanger and catalyst supports) has been mentioned for many polymeric systems, there are few investigations in the literature about porous copolymers based on vinylpyridines.[4,5,6–8] The morphological control of the copolymer beads and their porous structures may result in many advantages concerning the removal process of metal ions and other pollutants of wastewater. These characteristics have a strong influence on the removal kinetics and flow properties.[4,9]

Literature has pointed out that poly(vinylpyridine *N*-oxide) would inhibit the pathogenic activity of a microorganism.[10] For applications in aqueous systems (e.g., sterilized distilled water), in biomedicine, and reactions using polymer as support, it is of interest to have a suitable accessibility to the polymer matrix (wetting). In this respect, the use of oxidation reaction of crosslinked copolymer networks based on 2-vinylpyridine (2VP) has not been studied yet. In addition, it is known that iodine strongly forms electron-transferring complexes even with weak donors.[11–13] Hence, the *N*-oxide groups are also able to anchor halogen (e.g., Br_2 and I_2). As is well known, iodine has presented *in vitro* activity against bacteria, viruses, fungi, and protozoans.[14] Although halogens (chlorine and iodine) present low water solubility that restricts their applications for water treatment,[15] their use for water disinfection is useful. This feature increases the potentiality of application as a new type of functional polymer network with the antibacterial activity for sterilized distilled water.[13,15] This article deals with the evaluation of different kinds of porous copolymer networks based on 2-VP containing anchored iodine for use in disinfecting aqueous solution containing *Escherichia coli*.

EXPERIMENTAL

Materials

Commercial divinylbenzene (DVB; grade 45% DVB, with ethylvinylbenzene) and 2VP (both from Nitriflex,

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TABLE I
Synthesis Parameters, Chemical Compositions, and Physical Characteristics of Copolymer Networks

Sample	Monomer in the feed (2VP/DVB, mol %)	Diluents system[a]	N_c (%)[b]	d_a (g/cm^3)[c]
R1	40/60	7Hep/3Tol	6.0	0.35
R2	80/20	Hep	12.0	0.20

[a] Volumetric ratio (Hep = *n*-heptane; Tol = toluene).
[b] N_c = nitrogen content determined by elemental analysis (CHN).
[c] d_a = apparent density.

Rio de Janeiro, Brazil) were used as received. α,α′-Azobisisobutyronitrile (AIBN) and 2-hydroxyethyl-celullose (HEC; Cellosize QP-100MH) were donated by Metacril do Brasil (Camçari, Brazil) and Union Carbide (São Paulo, Brazil), respectively. The other reagents were commercially purchased, namely, gelatin, *n*-butanol, propanone, methanol, sodium chloride, *n*-heptane, hydrogen peroxide (30% w/v), glacial acetic acid, toluene (Vetec, Rio de Janeiro, Brazil), and iodine of proanalysis degree were used as received. Agar, tryptone, and yeast extract of Bacto™ grade for use in microbiological culture media were purchased from Difco. Aqueous solutions were prepared by using distilled deionized water. The uxotrophic *E. coli* AB1157 strain[16] was obtained from P. Howard-Flanders (Yale University, New Haven, CT).

Copolymers synthesis

The aqueous suspension copolymerizations 2VP/DVB were carried out in a 1-L, three-necked, round-bottomed flask glass reactor fitted with mechanical stirrer, reflux condenser with a silicon oil seal at its top. Aqueous phase (AP) was composed by gelatin and 2-hydroxyethyl-celullose (both at 0.3 wt % in relation to AP) and NaCl (2 wt % in relation to AP). The organic phase (OP) was composed of monomers (total amount = 0.5 mol) and diluents (namely, *n*-heptane and toluene) at 100 v/v % in relation to the monomers and AIBN was used as initiator (1.0 mol % in relation to the monomers). OP was added slowly to AP previously prepared under stirring at room temperature, employing an AP/OP ratio = 4/1. These two phases were maintained under stirring (300 rpm) at room temperature for 10 min. Hence, the suspension copolymerization system was kept at 70°C under stirring for 24 h. The resin beads were thoroughly washed with hot water (eight portions of 500 mL) and with propanone (three portions of 500 mL) and dried at 60°C. The copolymer yields were around 90% and the copolymer beads presented a white color and a narrow particle size distribution (180–250 μm). Table I summarizes the synthesis parameters.

Oxidation of pyridine rings

The oxidation reaction (Fig. 1) of the 2VP-crosslinked copolymers was carried out with swollen beads (2.0 g of dried beads) in *n*-butanol. The oxidation agent (peracid acetic) was produced *in situ*. The swollen beads were heated at 75°C in glacial acetic acid (5.25 mol) with (1 mol) of 30 wt/wt % H_2O_2 solution under mechanical stirring for 24 h.[17] The modified resin was filtered off, thoroughly washed with hot water (five portions of 100 mL) and propanone (three portions of 50 mL) to remove the excess of reagents, and then dried at 60°C for 24 h.

Preparation of resin beads containing anchored iodine

One gram of white resin beads was kept in a saturated aqueous iodine solution for 24 h until maximum iodine adsorption. Then, the brown beads were thoroughly washed with hot water (three portions of 100 mL) and last with propanone (three portions of 20 mL). The brown beads were dried at 70°C for 24 h.

Resin characterization

The resins were characterized by elemental analysis by using CHN Perkin–Elmer 2400 equipment. The

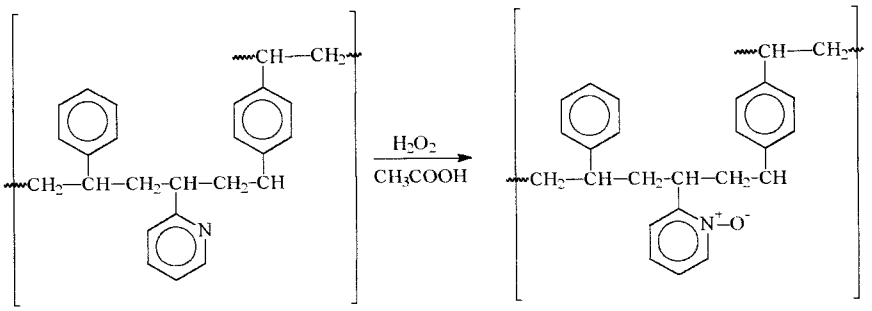
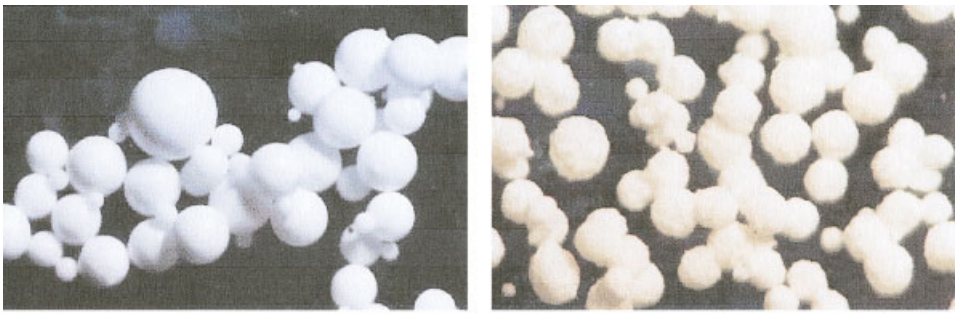


Figure 1 Oxidation reaction on the resin based on crosslinked 2-vinylpyridine.



(a) (b)

Figure 2 Optical micrographs of R1 (a) and R2 (b) resins (magnification, ×70). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

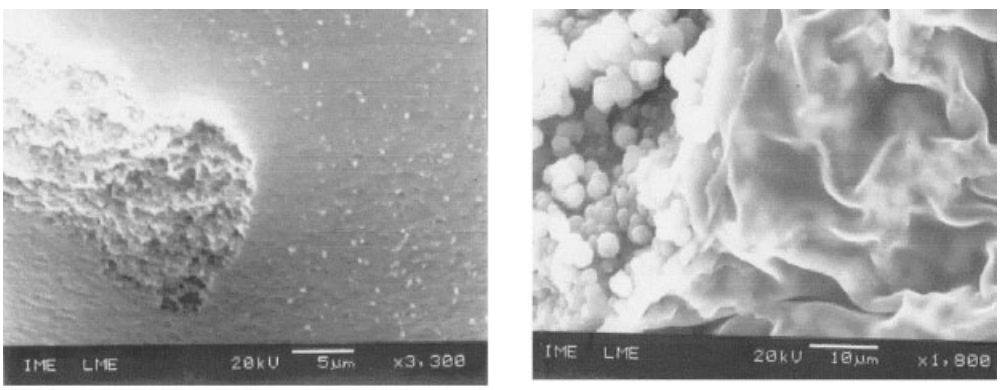
morphological and visual appearance characteristics were observed with a stereo optical microscope (Olympus SZ10). Resins were also characterized by scanning electron microscopy (JEOL, JSM-5800LV). The beads were coated with carbon film and their external and internal parts were observed by using a 20-kV acceleration electron beam.

Bacterial test using column of the resin beads

Columns containing different types of resin beads were prepared by using 1.0-mL sterilized syringes with about 30 to 200 mg of sample. A sterile glass bead was used to retain the resin bed within the syringe. Through all columns, 1500 μL of a sterile NaCl aqueous solution was eluted at 0.9% w/v (saline). The same volume of saline solutions containing *E. coli* at varied contents (namely, 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 cells/mL) was successively eluted through the bead columns at room temperature. Each elution was performed for 1 to 2 min (elution rate $\cong$ 0.75 mL/min). The bacterial suspensions were previously prepared by diluting, in sterile saline, an overnight culture (1–3 $\times$ 10^9cells/mL) grown in nutritive medium[18] at 37°C. After elution through the beads bed, the bacterial suspensions were appropriately diluted in saline to obtain a suspension containing about 2 $\times$ 10^3 cells/mL, to determine bactericidal activity. Suitable 100-μL aliquots of these suspensions were plated on LB nutrient medium solidified with 1.5% Bacto agar. The colonies formed (CFU) were counted after 24 h incubation at 37°C.

RESULTS AND DISCUSSION

After a judicious choice, resin beads of polymeric materials with spherical morphology and controlled porous structure based on 2-VP/DVB crosslinked resins were prepared as reported.[19] Table I shows the chemical composition and physical characteristics of the copolymer networks. The 2VP incorporation was determined by elemental analysis (Table I). It was noted that the R2 resin presented double nitrogen content compared to the other one. This result indicates that both resins totally incorporated the 2VP present in the poly-



(a) (b)

Figure 3 SEM micrographs of the R1 (a) and R2 (b) resins—internal and external parts.

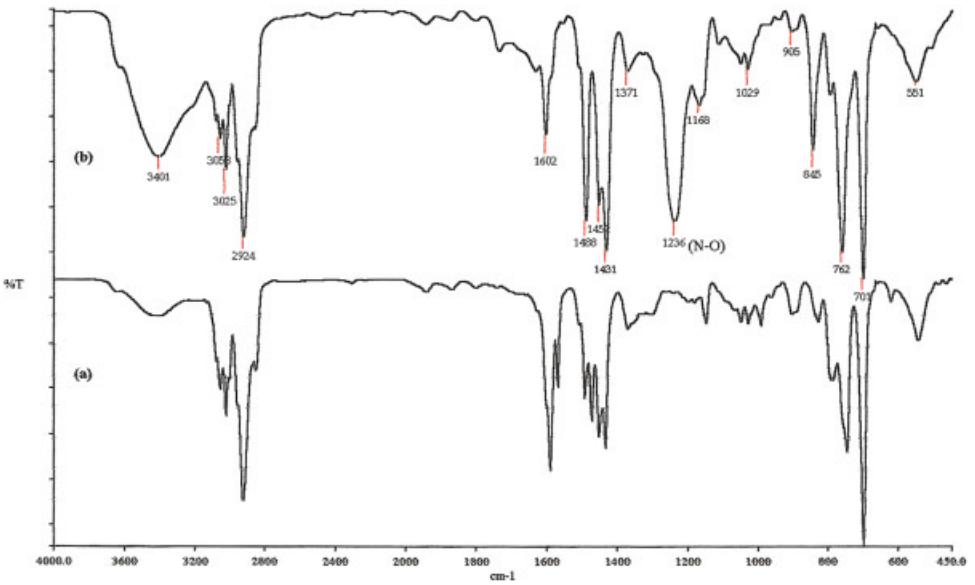


Figure 4 FTIR spectra of unmodified (a) and (b) modified (b) resins.

merization feed. Figures 2 and 3 show optical micrographs and scanning electronic micrographs (SEM) of both resins, respectively. As can be seen in these figures, both resins are porous. However, the external part of the R2 resin [Fig. 3(b)] is rougher than R1 resin [Fig. 3(a)]. These results indicate that the R2 resin presents larger pores than the R1 resin. As a consequence, this porosity produces resins with heterogeneities (presence of pores) that could be easily observed by the material opacity shown on optical micrograph [Fig. 2(b)]. The lower apparent density (d_a) of the R2 resin (Table I) suggests the existence of porous structure. This structure will simplify the transport process when the material will be used as a column filling. The material porosity increases the column efficiency for contacting with other materials by diffusion processes.

TABLE II
***E. coli* Content in the Initial Suspensions and Decreasing (%CFUs) after Elution in the 0.2-mL Beads Containing Resin**

Initial concentration (cell/mL)[a]	CFU %				
	R1	R2	R3	R4	R5
0[a]	—	—	—	—	—
3.28×10^2	87	70	92	100	100
3.38×10^3	5	70	27	100	100
3.40×10^4	0	70	5	100	100
3.53×10^5	3	44	0	100	100

Standard deviation = 5%CFUs.

E. coli cultures of AB1157 (wild-type) in the mid exponential phase of growth were prepared in Luria–Bertani (LB) medium at 37°C for 24 h; R1 = resin described in Table I (m = 105.1 mg); R2 = resin described in Table I (m = 61.8 mg); R3 = R1 resin modified with *N*-oxide groups (m = 229.2 mg); R4 = R3 resin with iodine (m = 239.5 mg); R5 = R2 with iodine (m = 167.6 mg).

[a] Sterile NaCl aqueous solution at 0.9% wt %.

Typical FTIR spectra of unmodified and modified resins by the oxidation reaction are shown in Figure 4, in which can be noted the presence of band (~ 1230 cm^{-1}) related to the *N*-oxide stretch.[20]

The white color and dry-state morphology of resin beads remained after the oxidation reaction. These results were verified by optical and electronic microscopies, respectively. All resins became brown after treatment with iodine aqueous solution for fixing it into the material macrostructure. This color persists even after heating at 150°C for 12 h. It indicates that the iodine anchorage is effective by stable complex formation by charge transfer.[11]

We have previously investigated the *E. coli* bactericidal action by using the technique diffusion on a disc as a support to one bead fixed on it. The *E. coli* colonies (at 10^7 organisms/mL) were spread (in duplicate) on LB-rich medium solidified with 1.5% Difco agar for all kinds of resin beads. No present halo around the discs was observed. The halo nonappearance could be attributed to the impossibility of iodine molecules diffusion.[21] This result corroborates that the iodine anchorage is effective by stable complex formation.

The sterilization activity of the resins beads was estimated by calculated bacterial decreasing as

$$\%\text{CFUs} = 100 \times (\text{CFU}^i - \text{CFU}^f)/\text{CFU}^i$$

where CFU^i and CFU^f correspond to the CFUs of each cell's suspensions before and after elution through the beads, respectively.

Table II shows the bactericidal results for different

TABLE III
***E. coli* Content in the Initial Suspensions and Decreasing (%CFUs) after Elution in the 0.04-mL Beads Containing Resin**

Initial concentration (cell/mL)[a]	CFU %	
	R4	R5
0[a]	—	—
3.40×10^5	24	99
4.60×10^6	15	79
2.98×10^7	8	3

Standard deviation = 5%CFUs. *E. coli* cultures of AB1157 (wild-type) in the mid exponential phase of growth were prepared in Luria–Bertani (LB) medium at 37°C for 24 h; R4 = R3 resin with iodine (m = 55.5 mg); R5 = R2 with iodine (m = 31.0 mg).

[a] Sterile NaCl aqueous solution at 0.9% wt %.

kinds of resin beads by using the saline solutions with different *E. coli* cells contents.

The original resins modified with *N*-oxide groups have just partially sterilized the saline solutions with low cells contents (up to 10^2 cells/mL), whereas both materials with anchored iodine were able to deactivate *E. coli* cells up to 10^5 cells/mL. At the conditions employed, the results shown in Table II did not differ the R5 resin from the R4 resin. Nevertheless, experiments with larger cell contents and smaller resin amounts were conducted, as can be seen in Table III. It was observed that the R5 resin (R2 resin with anchored iodine) presented a higher deactivation effect than the R4 one. This result could be attributed to higher iodine content of that resin than this one due to higher 2VP content than the other one and also to the presence of large pores into its structure.

CONCLUSION

It was possible to evaluate the iodine bactericidal action anchored on crosslinked resin beads based on 2-VP unmodified or modified with *N*-oxide groups by elution through short columns. Resin beads containing anchored iodine were completely effective at inactivating *E. coli*. Bacterial viability assay with a wild-type of *E. coli* cells present in aqueous solution showed a severe toxicity of iodine to this organism after the passage through a column filled with a bed of resin beads containing anchored iodine. The iodine bactericidal activity was none for static test (disc technique), indicating that iodine is not lost from the resin structure to the medium.

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