Antimicrobial activity of silver composites obtained from crosslinked polystyrene with polyHIPE structures

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Abstract

The literature reports several potential applications of polymers prepared with high internal phase emulsions (HIPEs). However, the evaluation of these materials as supports for antimicrobial agents has not been explored. In this work, silver composites based on polyHIPEs were prepared. Initial studies indicated that these materials can be efficient to prevent biofilm formation. The silver composites were prepared in three steps. First, HIPEs based on styrene-divinylbenzene were polymerized by aqueous suspension polymerization. These particles showed surface areas of 18 and 48 m^2/g. These polyHIPEs were sulfonated with concentrated sulfuric acid or acetyl sulfate and showed cation exchange capacities of 4.03 and 5.07 meq/g respectively. The sulfonated material was impregnated with silver ions, followed by reduction of the ions to prepare silver composites. These composites showed inhibition halos against *E. coli* and *P. aeruginosa*. and did not present adhesion of bacterial cells of *K. variicola* and *S. aureus* on their surface.

Keywords: *high internal phase emulsions, polyHIPEs, silver composites, styrene-divinylbenzene copolymers, biocidal polymers.*

1. Introduction

The literature indicates that high internal phase emulsions (HIPEs) (containing more than 74 vol% of internal phase) can be employed as templates for porous polymers, generating polyHIPE structures[1]. Despite a large number of publications and patents involving the preparation of polyHIPE structures, only a few of these works involve polymerization of these HIPEs by aqueous suspension polymerization (Hainey et al.[2]; Cameron et al.[3]; Desforges et al.[4]; Stefanec and Krajnc[5,6]; Yang et al.[7]; Mert and Hildirim[8]; Cui et al.[9]; Torquato et al.[10]) and US patents 5,583,162[11], 6,048,908[12], 6,100,306[13] and 6,218,440[14]). The polymerization of HIPEs by employing aqueous suspension polymerization, also referred as water-in-oil-in-water polymerization (W/O/W), can generate spherical particles with an open porous morphology with pores connecting larger cavities (windows)[10]. This type of morphology can favor access of reactants and products through the internal structure of the polymer[15,16], contributing to reduction of mass transfer limitations, especially intraparticle diffusion, and consequently allow the development of more

efficient materials. The application of these polyHIPEs is widespread in many areas[15-18], such as supports for catalysts[15], CO_2 capture[16], stationary phase for preconcentration of PAHs in environmental water samples[17], and development of enzymatic reactors based on immobilization of enzymes on these polymers[18].

Crosslinked porous copolymers have been used as supports for several antimicrobial groups such as ammonium and phosphonium quaternary groups, charge transfer complexes involving iodine and quaternary ammonium groups, N-halamines, sulfo-derivatives and metal particles[19]. Antimicrobial polymers based on silver composites and nanocomposites have potential applications for the inhibition of biofilm formation[20]. Among the studies involving the preparation of silver composites from crosslinked copolymers can be cited the works of Gangadharan et al.[21] Santa Maria et al.[22], Mthombeni et al.[23] and Mandu et al.[20]. In these studies, the antimicrobial activity of the final products was evaluated by different strategies. Gangadharan et al.[21] developed a silver nanocomposite through impregnation

of silver nanoparticles in microspheres of methacrylic acid and divinylbenzene, prepared by aqueous suspension polymerization. The antimicrobial activity of this material was evaluated against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* employing the plate method and in test tubes. Santa Maria et al.[22], Mthombeni et al.[23] and Mandu et al.[20] prepared silver composites from commercial sulfonic resins (Lewatit VPOC1800, Amberlyst 15WET, Amberlite IR-120, respectively). The method of impregnation of silver nanoparticles in these resins was based on exchange of H+ ions by Ag+ ions followed by chemical reduction of these ions. Santa Maria et al.[22] evaluated the antimicrobial activity of these materials against *E. coli* in tests employing micro syringes. In turn, Mthombeni et al.[23] evaluated the antimicrobial activity of these silver nanocomposites against *E. coli* in macroscale column experiments. Mandu et al.[20] evaluated the antimicrobial activity of silver composites against *E. coli*, *P. aeruginosa* and *S. aureus* through plate, batch, and column experiments. From the column experiments, also realized on a macro scale, they determined the breakthrough point and working biocidal activity. However, in these works, no tests were performed to simulate the ability of the final products to inhibit biofilm formation.

Literature data show that polyHIPEs have not been investigated as supports for antimicrobial groups. Thus, here we report a strategy to prepare silver composites from polyHIPEs of styrene-divinylbenzene (Sty-DVB) containing sulfonic groups. The antimicrobial activity of these silver composites was evaluated by employing inhibition halo tests against two Gram-negative bacteria of medical importance (*Escherichia coli* and *Pseudomonas aeruginosa*). The silver composites were also evaluated regarding bacterial adsorption and adhesion employing the bacteria *Klebsiella variicola* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) to simulate inhibition on biofilm formation.

The sulfonation of polyHIPEs has been sparsely studied, by employing concentrated sulfuric acid[24,25] or lauroyl sulfate in cyclohexane[26] as sulfonating agents. Thus, in the present work the sulfonation of the polyHIPE particles was studied by using concentrated sulfuric acid or acetyl sulfate as sulfonating agents (in a comparative study), aiming to learn the effect of the type of sulfonating agent on the morphology of the polyHIPEs and cation exchange capacity of the sulfonated particles.

All steps of this work are shown schematically in Figure 1.

2. Materials and Methods

2.1 Materials

Styrene (Sty) and divinylbenzene (DVB) (donated by Nitriflex Indústria e Comércio S.A.) were used after acid-base extraction by using 5% w/v NaOH aqueous solution. 2,2-azo-bis-isobutyronitrile (AIBN) was acquired from Mig Quimica and used after recrystallization with methanol. Poly(vinyl alcohol) (PVA) 224 (hydrolysis degree of 87-89%) was donated by Kurary Inc. and polyvinylpyrrolidone (PVP) (Mw 1,300.000) was acquired from Fluka. The microorganisms used in this work were *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 15442), *Klebsiella variicola* (ATCC 31488) and *Staphylococcus aureus* (ATCC 25923). The culture media used were Mueller-Hinton II broth (MHII) (BBL), Mueller-Hinton II agar (Kasvi), and plate count agar (PCA) (Neogen). Other reagents and solvents were purchased from Sigma-Aldrich and used as received.

2.2 Synthesis of Sty-DVB polyHIPEs

First, high internal phase emulsions (HIPEs) of Sty-DVB were prepared in a round-bottom three-necked flask with 250 cm³ capacity, fitted with a mechanical stirrer reactor. The organic phase was composed of Sty (0.072 mol), DVB (0.029 mol), AIBN (0.0009 mol) and Span 80 (0.0053 mol). The aqueous phase (AP) was composed of NaCl (0.67 g), potassium persulfate (0.45 g) and distilled water (33 mL). The aqueous phase was dripped through an addition funnel into the organic phase (35 minutes) under mechanical stirring
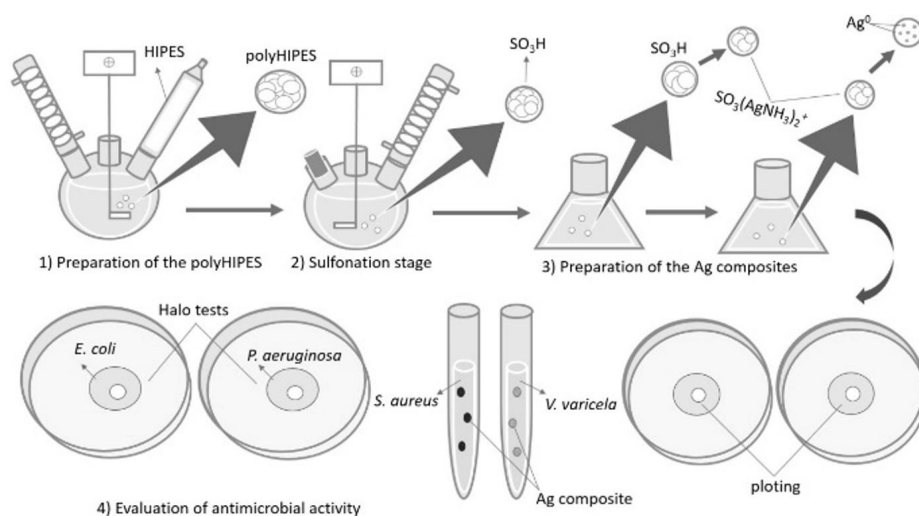
Figure 1. Schematic representation of all stages of the experiment.

at 250 rpm. In the second stage, HIPEs (as viscous white solutions) were polymerized by aqueous suspension, forming polyHIPEs structures. The aqueous phase was prepared using PVP (1.98 g) or PVA (1.98 g) as suspending agent, NaCl (1.49 g) and distilled water (99.4 cm^3), and then transferred to a round-bottom three-necked flask with 500 cm^3 capacity. The HIPE was added to the aqueous phase (pre-heated to 80 ºC) dropwise for 30 minutes under mechanical stirring at 150 rpm. The reaction mixture remained for 24 hours in these conditions at 80 ºC[4-6, 10]. After preparation, the particles were treated with hot water, ethanol and acetone to remove residues. After washing, the polyHIPEs were oven dried for 24 hours at 60 °C.

2.3 Sulfonation of Sty-DVB polyHIPEs

The polyHIPE composed of Sty-DVB prepared with PVP as stabilizer was sulfonated with sulfuric acid or acetyl sulfate to promote silver ion anchorage in the silver impregnation step (Figure 1). The first sulfonation was carried out with sulfuric acid. PolyHIPE (4.0 g) was previously soaked in 1.2 dichloroethane (18.5 cm^3) for 2.5 hours. After this period, the material was sulfonated with sulfuric acid (95-99%) (1.13 mol) in a 500 cm^3 flask coupled to a mechanical stirrer and reflux condenser, and was stirred at a rate of 90 rpm at 90 °C for 24 hours. Previous swelling of the particles by soaking with 1.2 dichloroethane allowed greater access of the reactant sulfuric acid to the internal structure of the polymeric beads, aiming to preserve their spherical morphology[27]. The second sulfonation method was based on employment of acetyl sulfate as sulfonating agent[28]. This reagent was prepared employing acetic anhydride (1.13 mol), anhydrous dichloromethane (0.39 mol) and concentrated sulfuric acid (1.13 mol) under N$_2$ atmosphere. PolyHIPE (4.0 g) was swollen in anhydrous dichloromethane (30 cm^3) for 1 hour under N$_2$ atmosphere in a round-bottom flask. After this period, the sulfonating agent prepared in the previous step was added to the flask. The reaction mixture was kept at a constant temperature of 40 °C for 24 hours under stirring at 90 rpm. The sulfonated polyHIPEs were filtered under reduced pressure, washed with distilled water to neutral pH and oven dried at 60 °C for 24 hours.

2.4 Impregnation of silver in sulfonated Sty-DVB polyHIPEs

The impregnation of silver nanoparticles in polymers was performed according to the descriptions of Mandu et al.[20], Santa Maria et al.[22] and Gangadharan et al.[21], with modifications. Both sulfonated polyHIPEs (1 g) were treated with AgNO$_3$ (aqueous solution of 0.1 mol L^{-1}, 10 mL) for 48 hours in a sealed environment protected from light. After filtration and washing with water, the reduction of Ag$^+$ to Ag^0 ions was performed by applying 4.7 g of hydroxylamine hydrochloride and 2 mol L^{-1} NH$_4$OH solution (to maintain the pH at 12.0) and a solution of distilled water (50 cm^3), gelatin (1.5 g) and hydroxyethyl cellulose (1.5 g) (Figure 1). The mixture of gelatin and HEC was used as colloid protector, aiming to reduce the reaction of Ag$^+$ ions in the polymer matrix in controlled form[29]. The composite produced was washed thoroughly with deionized distilled water (60 °C), then ethanol (50 cm^3), and dried at 60 °C for 24 hours.

2.5 Characterization of the polymers

Sty-DVB polyHIPEs were characterized regarding apparent density (ASTM D1895-69)[30] by optical microscopy (Olympus BX51M) and scanning electron microscopy (SEM) (JEOL-JSM 6460 LV), and the specific area, pore volume and pore diameter were determined through nitrogen physisorption (Micromeritics ASAP 2020 apparatus) following the BET and BJH equations[31], and the particle size distribution was ascertained (Malvern Hydro 2000S model). Sulfonation was accompanied by determination of cation exchange capacity[32]. The FT-IR spectra of the all polymers (KBr pellets) were recorded with a Perkin-Elmer Spectrum One spectrometer (4 scans, 4 cm^{-1} resolution). TG and DTG curves of the copolymers were obtained using a TA Q50 instrument in the temperature range of 50–600 ºC (20 º C min^{-1}) under N$_2$ atmosphere (60 mL min^{-1}).

2.6 Evaluation of antimicrobial activity

Antimicrobial evaluation of silver composites was performed by inhibition halo tests to verify the ability of polymers to inhibit growth of the bacteria *E. coli* and *P. aeruginosa*[33]. Petri dishes (100 mm diameter) were filled with 30 cm^3 of MH II agar. Bacterial suspensions in MHII medium with 1x10^8 CFU/mL were used to inoculate the entire sterile surface of the agar in the Petri dishes using sterile swabs. The polymers were then inserted into 5 mm diameter holes in the inoculated MHII agar. The dishes were incubated in a culture oven at 37 ºC for 24 hours. The test was performed in triplicate. After the incubation period, the halos of the microbial inhibition zones were measured, corresponding to the shortest distance between the outer surface of the well and the beginning of the microbial growth region. They were measured with a millimeter ruler.

Silver composites also were evaluated in relation to bacterial adsorption and adhesion[21] aiming to evaluate potential applications to inhibit biofilm formation. This study was conducted by employing suspensions of *K. variicola* and *S. aureus*. Suspensions of these bacteria (10^6 CFU/mL) were inoculated using nutrient medium in test tubes. Silver composites (0.2 g) were also transferred to these tubes and kept in contact with the bacterial suspensions for 24 h at 45 ºC. After this, the silver composite was filtered by using sterile syringes containing filter paper and washed with sterile deionized water. The silver composite was plated on nutrient agar (PCA medium) and incubated for 48 h at 45 ºC to check bacterial adsorption/adhesion. For statistical analysis, the PAST v2.17 data analysis package was used and the Kruskal-Wallis test for nonparametric data was applied, with p <0.05 being considered statistically significant.

3. Results and Discussions

3.1 Synthesis of polyHIPEs

HIPEs based on styrene-divinylbenzene (Sty-DVB) were suspended in an aqueous suspension phase containing as stabilizer poly(vinyl alcohol) (PVA) or polyvinylpyrrolidone (PVP) to prepare polyHIPEs. Optical microscopic images of these polyHIPEs (Figure 2) showed the presence of spherical particles and undefined shapes formed by using both stabilizers.

The formation of agglomerates by using both stabilizers was also observed, even after intense washing of the particles with hot water. The polyHIPE N3, prepared by using PVA as stabilizer, showed a larger distribution of particles sizes (Figure 3c) than the polyHIPE N2, synthesized by using PVP as stabilizer. The optical microscopic images of the polyHIPE N3 revealed the presence of smaller particles and agglomerations, and also a small group of larger particles, some spherical and others stick-shaped (Figure 2d and Figure S1). However, the particle size curve for this material (Figure 3c) did not show a bimodal size distribution for these particles, not corroborating the optical microscopic data. According to Budhlall et al.[34] PVA and PVP adopted varied conformations on the aqueous phase, related to the mechanism of solvating the polymeric chains, which is dependent on molecular mass and hydrolysis degree of these stabilizers. These variations cause significant modifications in particle nucleation mechanisms during the polymerization process. Considering this, it is also possible to suppose that both the process of dispersion up and coalescing in the early stage of the polymerization, and the agglomeration process during formation of the macroscopics structures (generated by w/o/w polymerization) were influenced by the conformations of the stabilizers in the aqueous phase. From a simpler point of view, it is possible to suppose that higher viscosity of the aqueous solution prepared using PVP

as a stabilizer (Mw 1,300.000) generated more compact droplets during early stages of the polymerization, in turn generating more compact particles.

SEM micrographs of these materials (Figure 2) indicated that the polymer prepared with the stabilizer PVP had a more porous structure. This observation was confirmed by data of surface area and pore volume of these two materials (Table 1). Probably more voids between polymeric domains were formed when PVP was used as the stabilizer, generating more porous structures[35]. The influence of the type of stabilizer on the morphology of the polyHIPEs prepared by aqueous suspension has not yet been explored in literature. Thus, more studies are necessary to confirm these data.

The polyHIPE particles showed type IV isotherms (Figure 3)[31,36]. It was visually clear that hysteresis was low between the adsorption and desorption processes, associated with capillary condensation on mesopores structures. The initial portion of this isotherm is related with monolayer-multilayer adsorption[31]. The low content of N_2 adsorbed at low relative pressure indicates that this material did not contain micropores in its structure[36]. The hysteresis loop profiles can be classified as H3 type (or B type) (considered intermediate between hysteresis type H1 and H4). This type of hysteresis loop is characteristic of aggregates of plate-like particles, giving rise to slit-shaped pores[31].
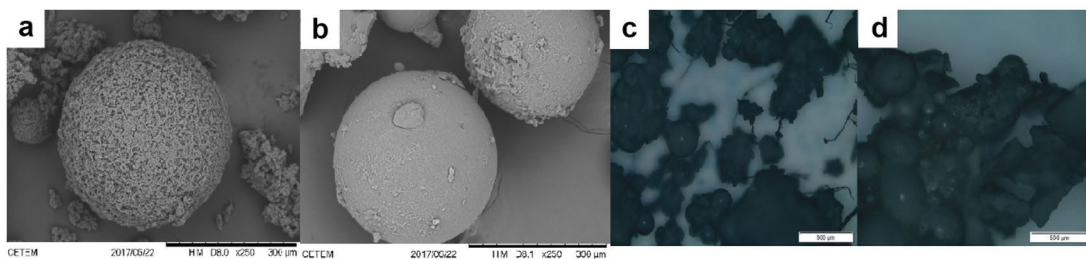
Figure 2. (a, b) SEM micrographs of the polyHIPEs N2 and N3, respectively. Magnifications of 250x; (c, d) Optical microscopic images of the polyHIPEs N2 and N3, respectively. Magnifications of 50x.
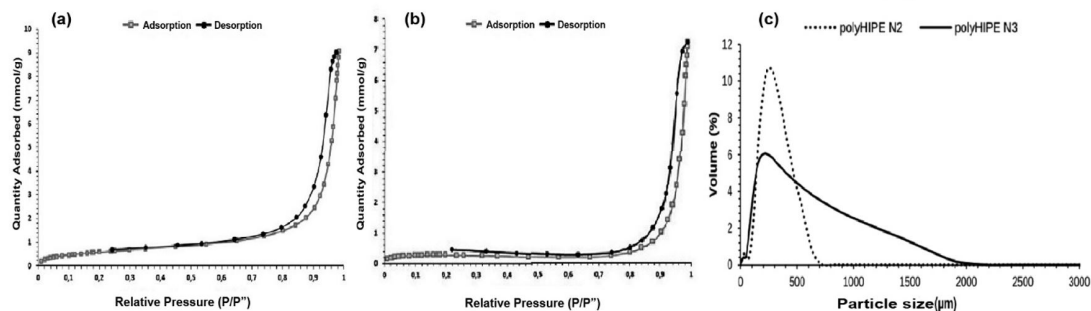
Figure 3. (a) N_2 adsorption-desorption isotherms of polyHIPE N2; (b) N_2 adsorption-desorption isotherms of polyHIPE N3; (c) Particle size distribution of polyHIPE N2 and N3.

Table 1. Data on apparent density, surface area, pore volume and pore diameter of polyHIPEs of Sty-DVB N2 and N3.

PolyHIPEs	Stabilizer	dap (cm³/g)	S (m²/g)	Vp (cm³/g)	D (Å)
N2	PVP	0.36	48.4	0.32	214.9
N3	PVA	0.27	18.3	0.25	272.5

dap: apparent density; S: specific surface area; Vp: pore volume; D: pore diameter; PVA: polyvinyl alcohol; PVP: polyvinyl pyrrolidone.

The morphology of the polyHIPEs prepared by polymerization of HIPEs via aqueous suspension has not been sufficiently studied by N_2 adsorption. Comparison between the results found in this work and data reported by Cui et al.[9] shows similarity between the N_2 adsorption-desorption isotherms. However, the microspheres prepared by Cui et al.[9] had specific surface areas varying between 3 and 4 m^2/g whereas the microspheres synthesized in this study had surfaces areas of 18 and 48 m^2/g. Probably this difference was related to variations in monomeric composition. PolyHIPEs obtained in this work were composed of Sty and DVB while the polyHIPEs prepared by Cui et al.[9] were composed of Sty, DVB, stearyl methacrylate (SMA) and n-butyl acrylate (BA). It is plausible to suppose that alterations of monomer polarity will generate modifications in the phase separation mechanism and the phase separation stage, generating beads with lower porosity[35].

3.2 Sulfonation of polyHIPEs

PolyHIPE N2 prepared with PVP as stabilizer was chosen for sulfonation reactions due to its higher specific surface area and pore volume. It was submitted to sulfonation with concentrated sulfuric acid or acetyl sulfate in order to introduce SO_3H groups in the polymeric matrix. After sulfonation reaction the particles showed color ranging from dark brown to black. Oliveira et al.[28] reported that acetyl sulfate is a better sulfonating agent than sulfuric acid because it contributes to maintaining the spherical morphology of the particles. In this work, alterations of morphology of the particles related to the type of sulfonating agent were not clearly observed by optical microscopy, since both stabilizers generated undefined particles in addition to spherical particles and agglomerates. We observed that part of the polyHIPEs kept their spherical morphology even after sulfonation with sulfuric acid (Figure S2).

FT-IR analysis of polyHIPEs after sulfonation with both sulfonating agents (Figure 4) revealed the presence of new bands at 1216 cm^{-1}, attributed to asymmetric stretching vibration of the S=O bonds in the sulfonate group ($H_3O^+SO_3^-$ group)[28,37], besides a pronounced increase of bands at 3400 cm^{-1} due to hydrogen bonding between sulfonic groups (polar) and water molecules (moist)[37], confirming the occurrence of both sulfonation reactions. These new bands were more accentuated in the FT-IR spectrum of polyHIPE sulfonated with sulfuric acid, indicating that this sulfonation method was more thorough.

After sulfonation with both sulfonating agents, the polyHIPEs showed three stages of decomposition (Table 2), related to water loss, decomposition of sulfonic groups and degradation of the entire carbon chain[38]. The content of residue was higher in polyHIPE sulfonated with sulfuric acid, also indicating that this sulfonation was more efficient. PolyHIPE sulfonated with sulfuric acid showed higher cation exchange capacity (CEC) than polyHIPE sulfonated with acetyl sulfate (Table 3). This result agrees with those obtained by FT-IR and thermogravimetry and indicates that
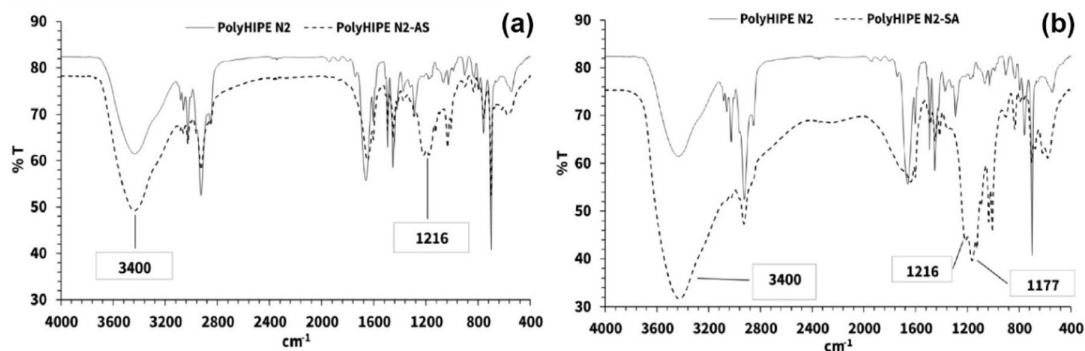
Figure 4. FT-IR spectra of unmodified polyHIPE (polyHIPE N2), polyHIPE sulfonated with acetyl sulfate (polyHIPE N2-AS (a)) and polyHIPE sulfonated with sulfuric acid (polyHIPE N2-SA (b)).

Table 2. Thermal characteristics of the polyHIPE N2, polyHIPE N2 sulfonated with sulfuric acid (polyHIPE N2-SA), polyHIPE N2 sulfonated with acetyl sulfate (polyHIPE N2-AS), silver composites derived from these two materials (based on work of Simplicio et al.[38]).

Material	$T_{onset}(1)$ (° C)[a]	$T_{onset}(2)$ (° C)[b]	$T_{onset}(3)$ (° C)[c]	RC[d] (%)	RD[e](%)	ID[f](%)	RD[g](%)
polyHIPE N2	362	-	-	5.9	-	-	-
polyHIPE N2-SA	82	256	351	28.6	22.7	-	-
polyHIPE N2-AS	81	284	360	18.2	12.3	-	-
polyHIPE N2-SA-Ag	74	282	389	40.4	34.5	11.8[j]	41.3[k]
polyHIPE N2-AS-Ag	116	254	393	24.4	18.5	6.2[j]	34.1[k]

[a] T_{onset} related with the first stage of decomposition, [b] T_{onset} related with the second stage of decomposition, [c] T_{onset} related with the third stage of decomposition, [d] RD: residue content after last stage of the decomposition, [e] RD: Residue difference, difference of residue content between modified and unmodified polyHIPE, [f] Inorganic residue: Content of inorganic residue determined by difference between residue of composite and sulfonated materials, [g] Resistance to degradation%: Increase of thermal resistance at 700 °C (comparing sulfonated polyHIPEs and silver composites derived from these materials): resistance %: (residue% composite – residue% sulfonated material)/(residue% sulfonated material) x 100.

the sulfonation reaction with concentrated sulfuric acid generated structures with higher content of accessible sulfonic groups. Considering that the sulfonation with acetyl sulfate was carried out in a milder condition than the sulfonation with sulfuric acid, it is possible to suppose that the degree of sulfonation with acetyl sulfate was lower, i.e., sulfonation with sulfuric acid enabled introducing more sulfonic groups in the innermost layers of the particles. Oliveira et al.[28] also observed that polymers sulfonated with sulfuric acid showed higher CEC than polymers sulfonated with acetyl sulfate.

Table S1 shows CEC values of sulfonic resins synthesized by other authors with commercial sulfonic resins[28,32,39-41]. The CEC value found for polyHIPE sulfonated with acetyl sulfate (PolyHIPE N2-AS) was more than three times higher than the CEC of the Sty-DVB copolymer sulfonated with the same sulfonating agent prepared by Oliveira et al. (RM-10)[28]. The CEC of polyHIPE sulfonated with sulfuric acid (PolyHIPE N2-AS) was higher than that of sulfonic resins prepared by Reis et al.[32], Aguiar et al.[39], Rezende et al.[40] and equivalent to the CEC of the sulfonic resin prepared by Coutinho et al.[37] and several commercial sulfonic resins used in industrial processes, such as Amberlyst 18[39], Amberlyst XN 1010[39], Amberlyst 35[39,41] and Amberlyst 36[32,39,40]. Sulfonation reactions were conducted after swelling of the beads in 1,2-dichloroethane (a good solvent of the polymeric chains) in order to avoid cracking the beads during sulfonation and also to favor diffusion of the sulfonating agents through the structure of the polymers[27]. PolyHIPE structures are characterized by the presence of interconnecting pores and larger cavities formed during polymerization of an external phase containing monomers dispersed in an aqueous phase[10]. Probably this type of structure favored the diffusion of the sulfonating agents through the internal structure of polymers, contributing to the higher CEC values observed.

3.3 Preparation of silver composites

PolyHIPEs sulfonated with acetyl sulfate and sulfuric acid were submitted to impregnation with silver particles in two steps: treatment of sulfonic particles with aqueous solution of $AgNO_3$ and reduction of Ag^+ to Ag^0 employing hydroxylamine hydrochloride and NH_4OH, in the presence of gelatin and hydroxyethyl cellulose (Figure 1). Some researchers have indicated that this method introduces silver

nanoparticles into the polymeric structure[20-23]. EDS spectra of silver composites showed peaks associated with elemental silver located between 2.6 KeV and 3.6 KeV (Figure 5). The presence of these peaks confirmed the reduction of Ag^+ ions present in $[Ag(NH_3)_2]^+$ groups, associated with SO_3^- groups, to Ag^0[21,22].

It was possible to observe small variations in the degradation profile of the composites polyHIPE N2-SA-Ag (derived from polyHIPE sulfonated with sulfuric acid) and polyHIPE N2-AS-Ag (derived from polyHIPE sulfonated with acetyl sulfate) (Table 2). For polyHIPE N2-AS-Ag, the first stage of degradation occurred at 116 ºC (1.6% of mass lost) while for polyHIPE N2-SA-Ag, the first step of decomposition occurred at 74 ºC (4.8% of mass lost). This first stage of decomposition can be related with water loss, while the second and third stages are related to the degradation of sulfonic groups and polymeric matrix, respectively[38]. The silver composite polyHIPE N2-SA-Ag had more residue (40.4%) than polyHIPE N2-AS-Ag (24.4%). Resistance to degradation of these composites was calculated considering the difference between residue content of the composites and sulfonated polyHIPEs. The values found were 41.3% (composite polyHIPE N2-SA-Ag) and 34.1% (composite polyHIPE N2-AS-Ag)[38]. These results allow suggesting that the composite derived from polyHIPE sulfonated with sulfuric acid contains more inorganic residue (Ag^0 content) resistant to decomposition.

3.4 Evaluation of antibacterial activity of polymers

According to Table 4, all silver composites produced inhibition halos against *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative bacteria), demonstrating the composites' ability to inhibit bacterial growth around silver particles. Statistical analysis of these data, performed by one-way analysis of variance, demonstrated that the

Table 3. Cation exchange capacity (CEC) of the polyHIPE sulfonated with acetyl sulfate (polyHIPE N2-AS), polyHIPE sulfonated with sulfuric acid (polyHIPE N2-SA).

| Supports	Sulfonating agents	CEC (meq/SO₃H/g)
PolyHIPE N2-AS	acetyl sulfate	4.03
PolyHIPE N2-SA	Sulfuric acid	5.07
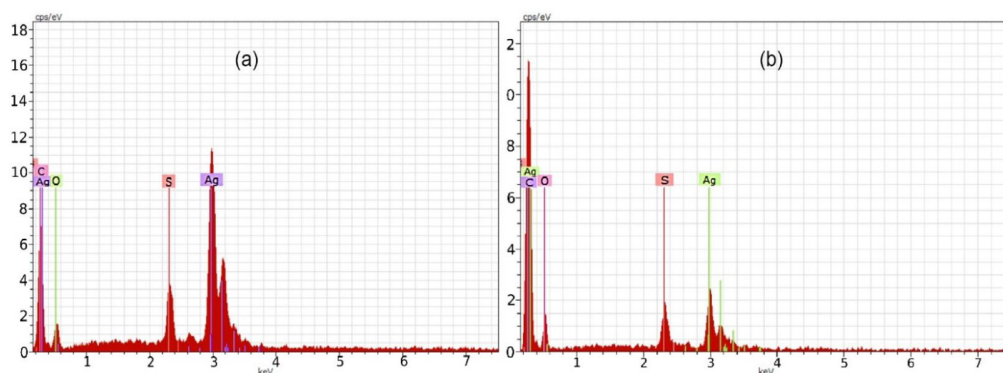
Figure 5. (a) EDS spectra of polyHIPE sulfonated with sulfuric acid; (b) EDS spectra of the polyHIPE.

Table 4. Halo values of polymers tested on Mueller-Hinton agar against *Pseudomonas aeruginosa* and *Escherichia coli* and variable correlations by applying the Kruskal-Wallis test.

	Polymer	Halo values					Variable correlations		
		Inhibition halo (mm)			Mean	Standard deviation	N2*	N2-AS-Ag	N2-SA-Ag
Pseudomonas aeruginosa	N2	0	0	0	0	0.0	-	0.05935	0.04685
	N2-AS-Ag	2	3	3	2.7	0.6	0.178	-	0.1876
	N2-SA-Ag	2	2	2	2.0	0.0	0.1406	0.5629	-
Escherichia coli	N2	0	0	0	0	0.0	-	0.05935	0.04685
	N2-AS-Ag	2	1	2	1.7	0.6	0.178	-	0.505
	N2-SA-Ag	2	2	2	2.0	0.0	0.1406	1	-

N2: PolyHIPE of styrene-divinylbenzene, N2-AS-Ag: Composite derived from polyHIPE N2 sulfonated with acetyl sulfate and impregnated with silver, N2-SA-Ag: Composite derived from polyHIPE N2 sulfonated with sulfuric acid and impregnated with silver. * $p < 0.05$: statistically significant.
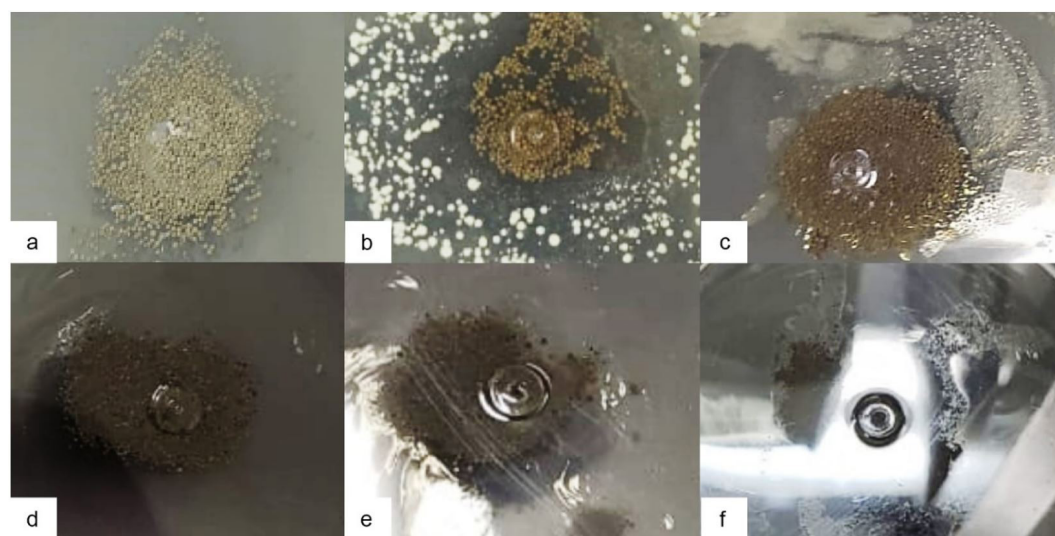
Figure 6. Silver composite derived from the commercial resin Dowex: (a) control; (b) presence of *Staphylococcus aureus;* (c) presence of *Klebsiella variicola* in bacterial adsorption and adhesion tests. Silver composites PoliHIPE N2: (d) control; (e) absence of *Staphylococcus aureus;* (f) absence of *Klebsiella variicola* in bacterial adsorption and adhesion tests.

difference in zone diameter was significant ($p < 0.05$) only in the comparison between the composites derived from polyHIPE sulfonated with sulfuric acid and unmodified polyHIPEs. Tests showed that unmodified polyHIPEs and sulfonated materials did not have any antimicrobial activity, corroborating results previously published by Maria et al.[22]. Silver composites prepared from polyHIPEs sulfonated with sulfuric acid showed equal inhibition halos for the two bacteria (*E. coli* and *P. aeruginosa*), both Gram-negative. Data from thermogravimetry indicated that the composite derived from polyHIPE sulfonated with sulfuric acid presented higher content of silver than the composite derived from polyHIPE sulfonated with acetyl sulfate. The difference in zone diameter was not significant (Table 4).

Silver composites also were evaluated in relation to bacterial adsorption and adhesion by employing *K. variicola* (Gram-negative) and *S. aureus* (Gram-positive) (Figure 6). A silver composite derived from the commercial resin Dowex was used as reference in this test. It was possible to observe bacterial growth in the dishes containing composites derived Dowex. Dishes containing unmodified polyHIPEs and sulfonated materials also showed bacterial growth.

On the other hand, this bacterial growth was not observed in dishes containing silver composites, indicating absence of adsorption/adhesion of bacterial cells on the surface these materials. This test indicated that this material could prevent biofilm formation.

4. Conclusions

Silver composites were prepared by impregnation of silver particles in polyHIPEs of styrene-divinylbenzene. These polyHIPEs, with spherical shape, were prepared by aqueous suspension polymerization of high internal phase emulsions (HIPEs). Both particles produced had structures with macropores and low specific surface areas (18 and 48 m²/g). PolyHIPEs were sulfated with sulfuric acid or acetyl sulfate and the sulfonation was confirmed by EDX, FT-IR and TGA. The cation exchange capacity (CEC) values of polyHIPEs sulfonated with sulfuric acid and acetyl sulfate were 4.03 and 5.07 meq g⁻¹ respectively, indicating that sulfonation with sulfuric acid was more efficient. These sulfonated polymers presented similar CEC values in relation to commercial sulfonic resins

with macroreticular structures. Also, the silver composite prepared from polyHIPE sulfonated with sulfuric acid had higher content of silver than that prepared with polyHIPE sulfonated with acetyl sulfate. Both composites presented similar inhibition halos against the bacteria *E. coli* and *P. aeruginosa* (Gram-negative bacteria). These composites also did not present adsorption/adhesion of bacterial cells of *K. variicola* (Gram-negative) and *S. aureus* (Gram-positive) on their surface, indicating these materials can be efficiently used to prevent biofilm formation.

5. Acknowledgements

6. References

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Supplementary Material

Supplementary material accompanies this paper.

Figure S1. Optical microscopy of polyHIPE N3. Magnification of 50x

Figure S2. Optical microscopies of the Sty-DVB polyHIPE N2 (a) before (b) after sulfonation reaction with sulfuric acid. Magnification of 50x

Table S1. Cation exchange capacity (CEC) of the polyHIPE sulfonated with acetyl sulfate (N2-AS), polyHIPE sulfonated with sulfuric acid (N2-SA) and other sulfonic resins reported in the literature

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