THE CHARACTERIZATION OF RECYCLED POLY(STYRENE-CO-DIVINYLBENZENE) FUNCTIONALIZED WITH α-HYDROXYPHOSPHONIC ACIDS FOR NEW STUDY OF ANTIMICROBIAL TESTS

Ileana Nichita[1*], Adriana Popa[2*], Radu Valentin Gros[1], Smaranda Iliescu[2], Marcela Stoia[3], Monica Seres[1], Adriana Morar[1]

[1] „Banat" University of Agricultural Science and Veterinary Medicine, 119 Calea Aradului, 30465,Timisoara, Romani, [*] e-mail: nichita_ileana@yahoo.com
[2] Institute of Chemistry Timisoara of Romanian Academy, 24 Mihai Viteazu Blv., 300223 Timisoara, Romania
[3 c] Politehnica University of Timisoara, Faculty of Industrial Chemistry and Environmental Engineering, 6 Vasile Parvan Blv, 300223, Timisoara, Romania
e-mail: apopa@acad-icht.tm.edu.ro; apopa_ro@yahoo.com

Abstract
Antimicrobials are a wide variety of compounds with the attack for the destruction or inhibition of microorganisms.This article describes the using of recycled α-hydroxyphosphonic acids grafted on styrene-divinylbenzene copolymeras a recycled material for new study of antimicrobial tests.

Introduction
Antimicrobials are a wide variety of compounds with the attack for the destruction or inhibition of microorganisms. The development of the antimicrobial agents have extended with the acceptance germ theory in 1800s, with antibiosis, disinfection, preservation, sterilization and environmental control now an integrated aspect of modern infection control [1-3].
In water treatment, the most usual treatment method to disinfect and sterilize water is to use chlorine and other related chemicals. But, their residues can grow to be concentrated in the food chain and in the environment as well as the possible formation of halomethane analogues that are suspected of being carcinogenic should lead to the prevention of their use [3]. Due to the associated problems result from the use of conventional antimicrobial agents, the idea for using of polymeric antimicrobial agents appeared to be an attractive choice.
Biocide polymer is a polymeric material that has the ability to destroy microorganisms, by acting as a source of sterilizing ions or molecules [4].Polymers can act as matrix for the materials holding the antimicrobial agents [5].
This article describes the using of recycled α-hydroxyphosphonic acids grafted on styrene-divinylbenzene copolymer [1] as a recycled material for new study of antimicrobial tests.

Experimental
Recycled α-hydroxyphosphonic acids grafted on styrene-divinylbenzene copolymers was used as a study material. α-hydroxyphosphonic acids grafted on styrene-divinylbenzene copolymers were synthesized in our laboratory and the antimicrobial effects of the copolymers (S-12%DVB, %Cl= 10.32, G_F = 2.91 mmol Cl/g. copolymer; S-15%DVB, %Cl= 10.21, G_F = 2.88 mmol Cl/g. copolymer) functionalized with α-hydroxyphosphonic groups were tested on two species of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two species of Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus ceresus*) and a species of yeast (*Candida albicans*) [1].

The copolymers using in this work were recovered from previous antibacterial solutions [1] and they were used to new test of the antimicrobial activity. These samples were filtrated, autoclaved at 120 ° C and 1 atm pressure for 30 minutes and subsequent they re-use the antibacterial activity against one specie of Gram-negative bacteria (*Pseudomonas aeruginosa*) and one specie of Gram-positive bacteria (*Staphylococcus aureus*) and a specie of yeast (*Candida albicans*).
These recycled copolymers were characterized by IR, SEM and EDX and they are confirming the presence of active groups' pendant.
The obtained polymeric resins were characterized by Fourier transform infrared spectroscopy with a Jasco FTIR spectrophotometer.
The energy dispersive X-ray analysis (EDX) was performed using an Inspect S scanning electron microscope.
The antibacterial activity of the recycled copolymers were tested on standard strains of bacteria (obtained from MediMark Europe Company, France) as follows: gram-positive bacteria – *S.aureus* (ATCC 25923), gram-negative bacteria –*P.aeruginosa* (ATCC 27853), and on a strain of *C.albicans* (ATCC 10231). For experiments were used active cultures (24 h old for bacteria and, respectively, 48 h for yeasts) using Mueller-Hinton broth (Oxoid) for bacteria and Sabouraud dextrose agar for yeast.Each recycled α-hydroxyphosphonic acids grafted on styrene-divinylbenzene copolymers were mixed with each microbial bacterial culture. New study of antimicrobial tests was according to the method presented in our previous paper [1].

Results and discussion

Samples were analyzed after recovery: it is noted band at 3430 cm^{-1}, which is attributed to the OH group, also the band at ~2930 cm^{-1}, which is assigned to the group P-OH and also identifies the group P=O at ~1250 cm^{-1} [1] (see Figure 1 and 3). The morphology of the polymers grafted with α-hydroxyphosphonic groups can be directly visualized by SEM images (see Figure 2 and 4).The macrospores are presented among the agglomerations of microspheres, micropores being inside the agglomerations of microparticles [1].The phosphorus content was determined (see Table 1) and it has confirmed that the recovered poly(styrene-co-divinylbenzene) containing pendant groups with α-hydroxyl-phosphonic acid.
FTIR spectra, SEM images and phosphorus content confirm the presence of active groups (α-hydoxyphosphonic acid) for new study of antimicrobial tests.

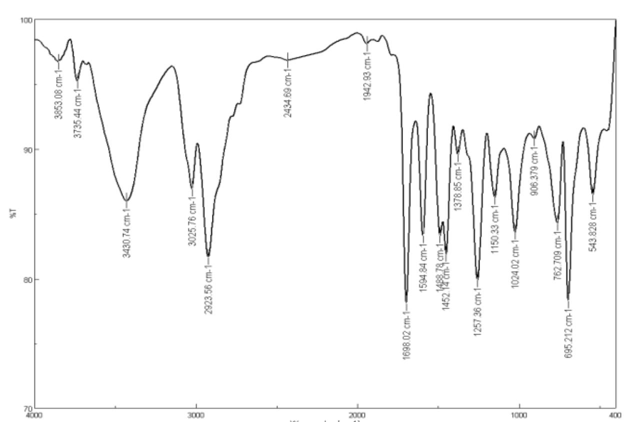

Figure 1. FTIR spectra for S-15%DVB P3 it was obtained after testing with *Pseudomonas aeruginosa*

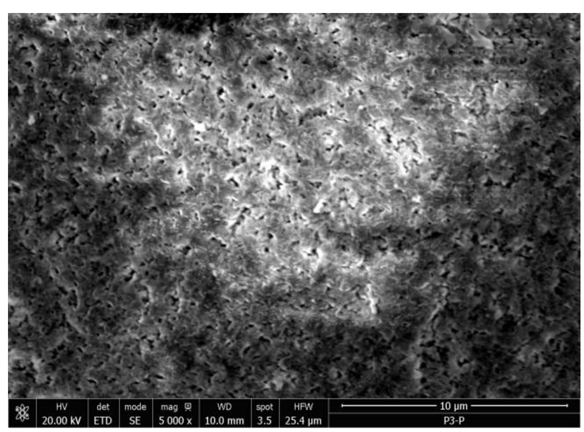

Figure 2. SEM image for S-15%DVB P3 it was obtained after testing with *Pseudomonas aeruginosa*

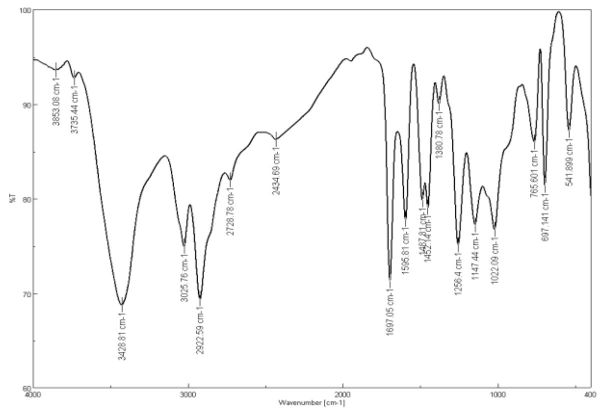

Figure 3. FTIR-spectra for S-12%DVB P2 it was obtained after testing with *Candida albicans*

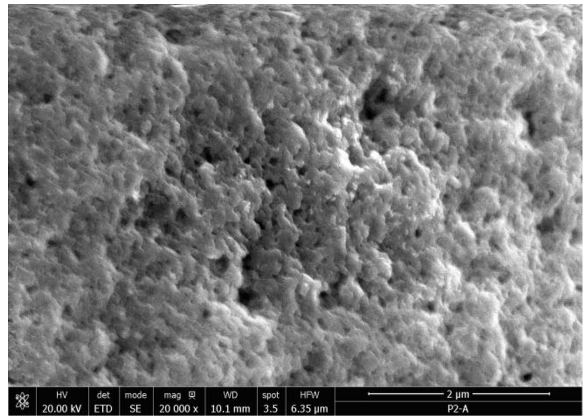

Figure 4. SEM image for S-12%DVB P2 it was obtained after testing with *Candida albicans*

Table 1. The phosphorus content of the recovered poly(styrene-co-divinylbenzene) with α-hydroxyl-phosphonic acid for using in new antimicrobial tests and the comparing with the phosphorus content the S-12%-(15%)DVBPHOS-presented in our previous paper.

Sample	P %	Sample	P %
S-12%DVBPHOS [1]	4.65	S-15%DVBPHOS [1]	5.14
S-12%DVB P2[1]	0.83	S-15%DVB P3[1]	1.19
S-12%DVB P2[2]	0.90	S-15%DVB P3[2]	1.07
S-12%DVB P2[3]	0.79	S-15%DVB P3[3]	0.96

Where:[1]it was recovered after testing with *Pseudomonas aeruginosa;* [2]it was recovered after testing with *Candida albicans;*[3]it was recovered after testing with *Staphylococcus aureus*

Antimicrobial activity

The results obtained from the testing of antimicrobial activity of the recycled α-hydroxyphosphonic acids grafted on styrene-divinylbenzene copolymers are presented in table 2.

Table 2. The antimicrobial activity of the recycled α-hydroxyphosphonic acids grafted on styrene-divinylbenzene copolymers

Microbial specie	RecycledS-12%DVB P2			Recycled S-15%DVB P3		
	CFU/mL in the start moment	**CFU/mL after 18 h of contact**	**Percentage of microbial reduction, %**	**CFU/mL in the start moment**	**CFU/mL after 18 h of contact**	**Percentage of microbial reduction, %**
S. aureus	2.05 x 10^8	0.19 x 10^8	90.73	7.06 x 10^8	4.68 x 10^8	33.71
P. aeruginosa	1.50 x 10^4	0.12 x 10^4	92	6.50x 10^4	0.31 x 10^4	95.23
C. albicans	1.84 x 10^8	1.61 x 10^8	12.48	1.29 x 10^8	1.12x 10^8	13.17

Both recycled copolymers still present antibacterial activity. The reduction in the number of bacteria (log CFU/mL) was found to be bigger in case of the recycle copolymer S-12%DVB comparatively with the copolymer S-15%DVB after 18 h of contact. In case of *Staphylococcus aureus* the recycle S-12%DVB had 90.73% reduction and the recycle copolymer S-15%DVB had 48.00% reduction. For the *Pseudomonas aeruginosa* specie were recorded 92 % of reduction in

case of recycle S-12%DVB and 95% reduction for copolymer S-15%DVB. None of two copolymer tested had antimicrobial effect for the yeast specie tested (*Candida albicans*).
The antimicrobial activity of the recicled copolymer comparatively with the first their form is presented in table 3. It coud be observed similar results. The antimicrobial activity of copolimers S-12%DVB was more pronounced than of the copolymer 15%DVBPHOS in case of the bacteria tested.

Table 3. The comparative antimicrobial activity of the α-hydroxyphosphonic acids grafted on styrene-divinylbenzene copolymers before and after recovery

Microbial specie	Percent reduction, %			
	recycled S-12%DVB P2	**S-12%DVBPHOS [1]**	**recycled S-15%DVB P3**	**S-15%DVBPHOS [1]**
S. aureus	90.73	100	33.71	48.00
P. aeruginosa	92	100	95.23	100
C. albicans	12.48	12.3	13.17	13.4

According to what was presented in our previous paper [1] we considered that the antibacterial activity is the result of hydrogen bonding between the organic groups (P=O) from α-hydroxyphosphonic acid groups and the OH groups in the cell walls of the bacteria. For the reason it is considered that the antimicrobial activity of the copolymers happened more quickly for the gram negative bacteria than the gram-positive bacteria. The copolymer S-15% DVB, even its recovery form, is more hydrophobe, comparatively with the copolymer S-12% DVB that is less hydrophobic and its α-hydroxyphosphonic functionalized groups from the interstitial copolymer S-12% DVB are more accessible for bonding through hydrogen bonding with the OH groups of the bacterial membrane.

Conclusion

The recycled copolymers were characterized by IR, SEM and EDX and they are confirming the presence of active groups' pendant.
The recycled α-hydroxyphosphonic acids grafted on styrene-divinylbenzene copolymersstill present antibacterial activity. The recycled S-12% DVB P2 copolymer had a better antimicrobial activity than the recycled S-15%DVB P3copolymer.
The antimicrobial activity of recycled copolymers is similar with those initially tested in our previous paper [1].

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