Styrene-butadiene Block-copolymers Used as Immobilization Support for Drugs with Prolonged Antibiotic Effect

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The paper presents a study about obtaining materials with prolonged biocidal effect based on styrene-butadiene block-copolymers (SBS) grafted with methacrylic acid (ACM) and coupled with antibiotics from the ampicillin group. The IR spectroscopy and the elemental analysis were used to confirm the structure of SBS block-copolymers grafted with methacrylic acid and of the medical ones. The content of grafted methacrylic acid was about 18.8 % in accordance with the 1,2 enchained butadiene content, proving that the methacrylation polymer-analogous reaction takes place at these pendant vinyl groups of the polybutadiene chain. The concentration of the methacrylic acid-ampicillin pendant group is about 38 % and corresponds to the theoretical calculated one. The antibacterial testing of the synthesized polymers showed that the final reaction product has a relatively high biocidal activity on gram-positive and gram-negative microorganisms.

Keywords: styrene-butadiene block-copolymers, methacrylic grafting, polymer with antibiotic effect.

In the recent years literature, many papers were dedicated to the research of medical polymers with prolonged effect and antimicrobial, antifungal and other properties [1, 2].

The research in this pharmaceutical area led to the development of new systems for controlled release of biologically active substances, mainly based on their targeted action [2].

Many of the biologically active polymers exceeded the boundaries of theoretical study and have been implemented in practice [3]. The retention of the biologically active groups on polymeric supports is a relatively new area, giving promising results in the development of new synthesis technologies for drugs deposited on a polymer support. Today, due to biotechnological pharmaceutical development, antibacterial preparations become specific therapeutic agents with high efficiency [4].

It is known that the development of resistant or multiresistant pathogens to a series of antibiotics has become a major problem in the modern medicine. In this context, an important role belongs to the chemistry of polymers with specific functionality for protection against bacteria / fungi and other microorganisms [4]. One of the advantages of using medical polymers is supported by the fact that even their macromolecular structure can provide efficiency over time of the antibacterial activity for a broad spectrum of bacteria in a short contact time, plus the lack of toxicity and chemical stability. The obtaining of polymer materials in order to produce coating films for furnishings in medical clinics and in household uses to give them antibacterial properties presents a special importance [3, 4].

Experimental part

Linear SBS block-copolymer used in this study was obtained by sequential anionic polymerization of monomers in cyclohexane solution, using n-butyl lithium as initiator [5-8]. Molecular characterization of SBS block-copolymer and of the component blocks was performed by gel permeation chromatography (GPC). The polystyrene and vinyl groups content were determined by IR spectroscopy. Physicol-mechanical properties were determined on the polymer film obtained in toluene solution using the centrifugal casting process. The linear styrene-butadiene block copolymer showed the following properties:

Polystyrene content: 31.8 %
Vinyl groups content: 9.5 %
Total molecular weight: 89 000 g/mol
Polystyrene block molecular weight: 14 150 g/mol
Polybutadiene block molecular weight: 60 700 g/mol
Tensile strength: 18 MPa
Elongation at break: 620 %
Hardness: 67 °Sh A

For the purpose of SBS controlled grafting with methacrylic acid (ACM), the purification of both was performed. The purification of methacrylic acid was carried out by distillation under a reduced pressure of 20 mm Hg at a temperature of 116-118 °C. Distillate methacrylic acid was kept at low temperature to avoid its self-polymerization. The purification of the SBS copolymer was achieved by dissolving it in cyclohexane and precipitation with hexane [9].

SBS copolymer grafting with methacrylic acid was carried out in solution, by radical polymerization at a temperature of 80 °C, under nitrogen atmosphere, using azobisisobutyronitrile (AIBN) initiator. The graft polymer purification was performed by dissolving and repeated precipitation with diethyl ether, in order to remove unreacted methacrylic acid [10].

The coupling of SBS-ACM support copolymer was performed according to scheme 1.

The polymer-analogous reaction of SBS-ACM copolymer with ampicillin was done in dimethylformamide at 0°C in presence of ethyl chloroformate, for 3 h. The reaction product was purified by precipitation twice in diethyl ether [3]. The medical polymer was dried in air and finally under

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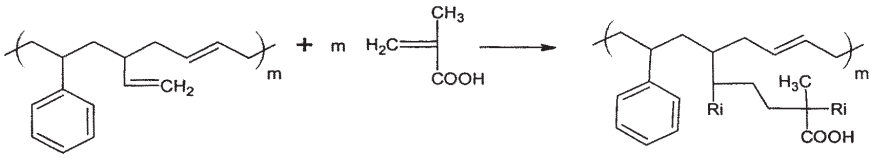
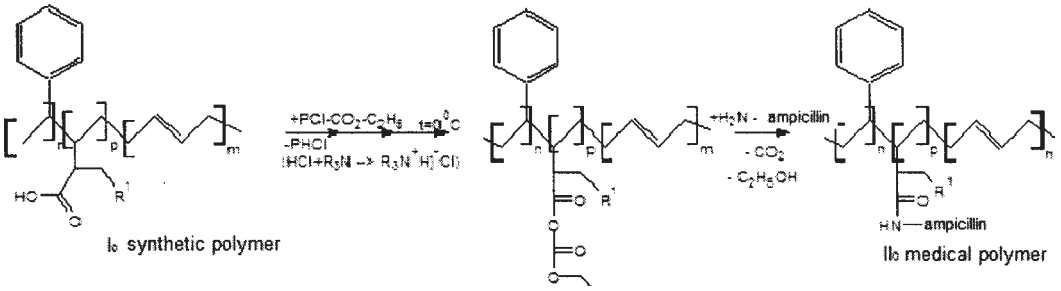


Scheme 1 The coupling reaction of SBS with ACM



Scheme 2 The coupling reaction of SBS-ACM with ampicillin

reduced pressure in a vacuum oven at t=40 ˚C until constant weight. The SBS-ACM (I_0) support polymer and the medical one (II_0) were characterized by IR spectroscopy and elemental analysis that confirm their structure presented in scheme 2.

Results and discussions

The chemical structure of initial copolymer (SBS) and of the grafted SBS-ACM one was investigated using IR spectroscopy. Comparing the IR spectra obtained for the original and grafted SBS block-copolymers presented in figure 1, it can be seen that the absorption band at 1087 cm^{-1} wavelength characteristic for the vinyl double bond (–$CH=CH_2$) disappear in case of the grafted polymer. This disappearance indicates that the grafting reaction of ACM takes place preferential at 1,2 enchained polybutadiene. The determined content of about 18.8 % in methacrylic groups attached to the SBS chain corresponds to the share of 9.8 % in vinyl groups of the polybutadiene block. The higher reactivity of polybutadiene vinyl groups can be found also in other radicalic grafting reactions [11].

In case of the SBS-ACM copolymer, new vibrations characteristic for the functional groups of methacrylic acid appear: λ=3417 cm^{-1} (acid OH groups); λ =2568 cm^{-1} (carboxyl group); λ=1696 cm^{-1} (>C=O carbonyl group); λ =2845 cm^{-1}, 2921 cm^{-1} (methyl and methylene group).

A C content of 63.65 % resulted from the elemental analysis of SBS-ACM copolymer, compared to the theoretical one of 75.4 %, confirm the presence of methacrylic structural units in the polymer chain.

Comparing the IR spectra (fig. 2) of SBS-ACM (I) and SBS – ACM - ampicillin(III) compounds it can be observed the appearance of new vibrations, like: λ_1= 3330-3270 cm^{-1} (-CO-NH- bound); λ_2=2606, 2534 and 2498 cm^{-1} (characteristic for S-H group); λ_3=1650-1630 cm^{-1} (for the phenyl radical); λ_4=1774 cm^{-1} (for the >C=O group from ampicillin). These data confirm the ampicillin coupling at SBS copolymer grafted with methacrylic acid.

The determination of the nitrogen content in the final product was carried out by elemental analysis, resulting in a content of 8.3 % in the SBS - ACM - ampicillin (III) polymer, in comparison with the theoretical value of 9 %. The presence in the final product of the nitrogen contained only by ampicillin confirms once again that the coupling reaction of ampicillin at the support copolymer (SBS-ACM) was successful. The yield of the coupling reaction of about 80-85% of ampicillin onto the polymer support creates premises of an industrial application of these research results.

The bacteriological tests showed a high antimicrobial effect against gram-positive and gram-negative microorganisms and can be recommended for the production of clinical use objects. The antimicrobial tests on the medical copolymer were comparable to the standard N-vinylpyrrolidone copolymer (NVP) with methacrylic acid coupled with ampicillin.

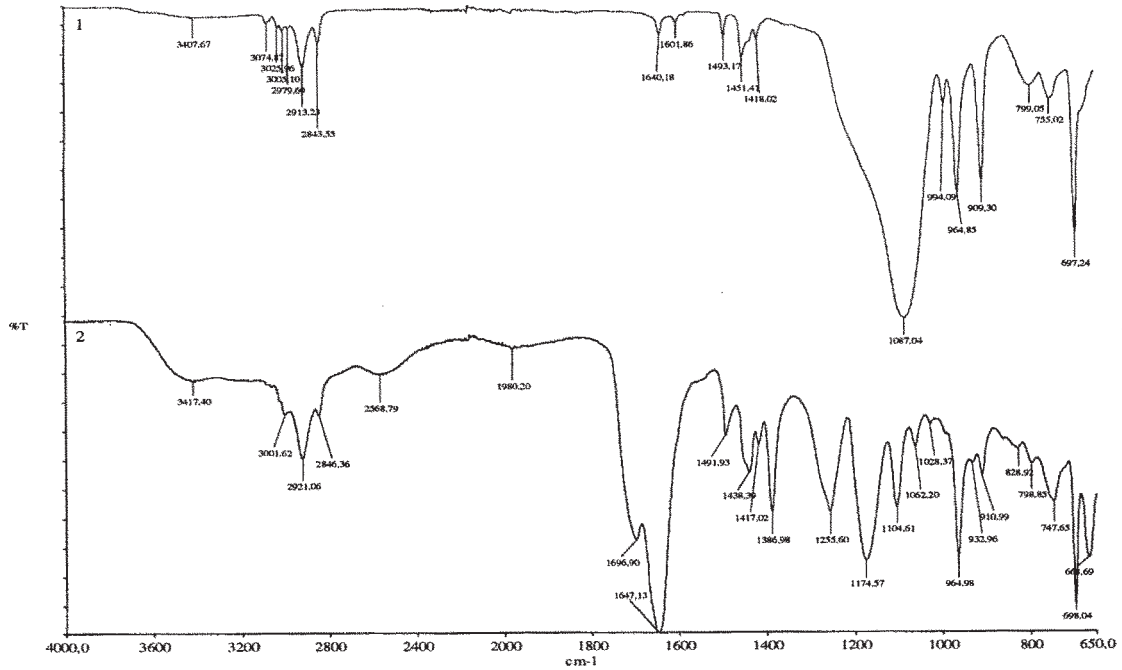


Fig.1 IR spectra of SBS copolymer (number 1) and SBS coupled with methacrylic acid (number 2)

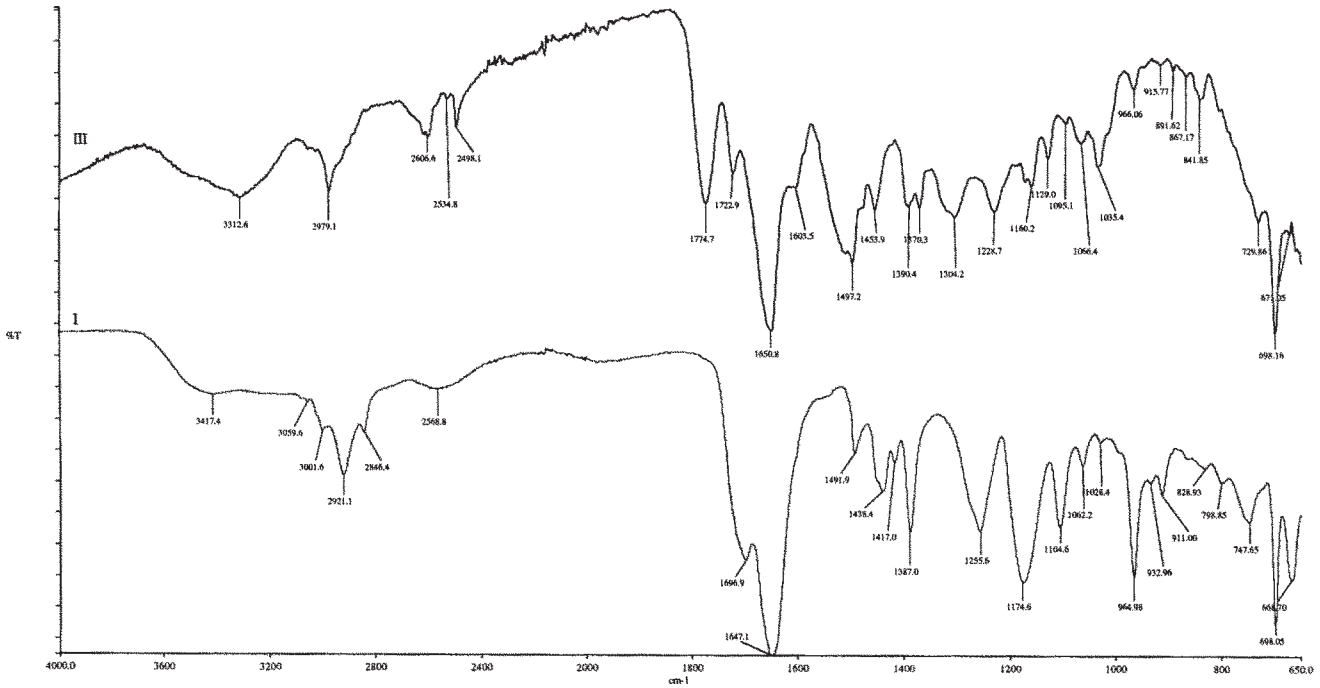


Fig.2 IR spectra of SBS-ACM (I) copolymer and SBS – ACM – ampicillin (III) medical copolymer

The antibacterial activity was tested on reference cultures like: Staphylococcus aureus (209-P strain); Enterococcus faecalis (E. faec.); Escherichia coli (ATCC 25882 strain); Pseudomonas aeruginosa (ATCC 27853 strain); Proteus vulgaris (HX 19222 strain).

The tests consist of research like:

a) "CMI" minimum bacteriostatic activities, which indicates no growth of microorganisms in nutrient medium;

b) "CMB" bactericidal activities, which is determined by the lack of growth of microorganisms within 24 h.

It was established that the SBS-ACM polymer coupled with ampicillin has "CMI" and "CMB" indices similar to those of the NVP-ACM copolymer coupled with ampicillin which was used as standard.

The SBS block-copolymer with a polystyrene content of 30% has a two-phase morphology: a polybutadiene continuous phase in which is dispersed the polystyrene phase as domains, due to the fact that the volume fraction of polystyrene blocks is smaller than 50%. By the grafting polymer-analogous reaction of the ACM phase, the polystyrene-methyl methacrylate plastomer phase becomes the majority of about 68 %, which form in this case the continuous phase that contains the dispersed polybutadiene phase. The new morphology leads to a polymer with film-forming properties, showing also high impact strength, these features allowing its use in the production of antibacterial coating materials.

Conclusions

The AMC and ampicillin grafting on SBS block-copolymer chains was carried out by polymer-analogous reactions that take place preferentially at the 1,2 vinyl double bonds, the mechanism of reaction and the content of methyl - methacrylic - ampicillin groups, being evidenced by IR spectroscopy and elemental analysis.

The antimicrobial tests on the medical polymer showed an activity comparable to that of a standard N-vinylpyrrolidone copolymer with methacrylic acid.

The obtained medical copolymer is recommended for its use in the manufacture of household items and furnishings, parts of clinical utensils, being able to improve the hygienic indices.

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