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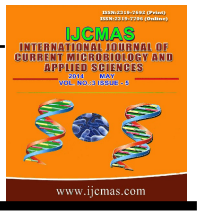

Original Research Article

Synthesis and Evaluation of Toxicity of Polymer Base of Acrolein

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Keywords

Polyacrolein, Poly (acrolein-co-styrene), Cytotoxicity and Genotoxicity Tests

A B S T R A C T

The present study was obtained from acrolein homopolymers and copolymers of styrene and acrolein via ionic polymerization initiated by tert-butyllithium or sec-butyllithium, at - 90°C to 1:00h of reaction. The synthesis products were evaluated for solubility in water and the contents of aldehyde grouping in the microstructure. It was found that copolymers of styrene and acrolein present in excess of the solubility of acrolein homopolymers H2O. The samples were also evaluated for cytotoxicity and genotoxicity by means of indirect diffusion in agarose gel. It was found that both the homopolymers of acrolein as copolymers of styrene and acrolein had no effect on DNA and cell membrane of the bacteria *Staphylococcus aureus* strain.

Introduction

Functional polymers have been studied continuously because of its great versatility in the production of new materials since they can undergo further modifications by changing their physical-chemical properties. Several classes of polymers have been used with the aim of producing new materials such as polyamides, polyamines, polyacid, polyalcohols among others (Marques, *et al.*, 2009) The interest in polymers that exhibit linearity in jail, water solubility and biocompatibility has grown considerably to meet the biomedical,

pharmaceutical and biotech areas. Lynn *et al.,* (2001) proposed the synthesis of poly (β - amino ester) to determine the solubility of these polymers in water and even samples studied that formed complexes with DNA molecules. Hartmann *et al.,* (2006)developed a linear synthetic route to poly (amidoamine) monodisperse with a defined sequence of monomers. However, little has been studied about the cytotoxic and genotoxic behavior of these molecules across the microorganisms. Anvisa, 2003 recommends the toxicological knowledge of each ingredient used in the manufacture of medicines, food or cosmetology as well as its features. The adoption of these measures avoids problems during development of the final product and even after their placing on the market.

Materials and Methods

The synthesis of acrolein homopolymer and copolymer of styrene and acrolein following the methodology described in the literature (Marques, *et al.,* 2009). In a 120mL reactor, flamed and equipped with magnetic stir bar, 60 mL of THF were collected previously dried and distilled in the presence of sodium. After bath reaches below -90 ° C, styrene and / or acrolein were added. When the system reached -90 ° C was added tert-butyllithium initiator or sec-butyllithium. At the end of 1 hour of reaction 0.5mL of ethanol were added to finish the polymerization. The crude product was recovered by precipitation in hexane and dried in a vacuum oven to constant weight. The molar ratios used to obtain the samples described in Table 1.

The groups were derivatized to hydrazones aldehydes and quantified by ultraviolet absorption spectrometry (Table. 1) after the reaction with 2,4- dinitro phenylhydrazine (DNFH). It was considered the molar absorptivity value equal to 22000 l / mol.cm in accordance with the literature data (Lohman, 1958; Ledissz, *et al*., 1966). The quantification was performed in duplicate, obtaining as a final result the average of the same.

The solubility tests (Table.1) were performed by solubilizing a mass of about 0.0010 g of each sample of the polymers in 1.00 mL of distilled water for 48 hours and centrifuged for 10min at a speed of 8000 rpm. The supernatant was removed and the residual precipitate was dried at 70° C for approximately 24 hours, until constant weight. The quantification was done in triplicate, yielding a final result of the same medium. From the results of solubility test (Table 1) the more soluble samples were chosen to perform cytotoxicity and genotoxicity tests. The groups were separated for each system of primers for both the homopolymer to copolymer as being: PA01, PAS02 (sec-BuLi) and PA04 and PAS04 (tert- BuLi). Bacterial activities of colonies of *S. aureus* were not affected by the presence of PA04 samples and PAS02 and therefore these samples were discarded.

The cytotoxic and genotoxic potential of PAS04 and PA01 samples were evaluated by indirect diffusion in agarose gel on the bacterium *Staphylococcus aureus* strain ATCC 8096. For this protocol Anvisa (1990) was adapted used. The controls used for the cytotoxicity tests were saline and antibiotics were respectively, amoxicillin, chloramphenicol, and gentamicin ampicilin. The antibiotics amoxicillin (50mg/mL), chloramphenicol (30mg/mL), gentamicin (10 mg/ mL) and ampicilin (30mg/mL) were dissolved in 10 mL of distilled water according to the recommended clinical dose for each manufacturer. For genotoxicity tests saline and mutagens tin II chloride ($SnCl_2$) and

hydrogen peroxide (H_2O_2) were used. The concentration of homopolymer and copolymer samples were 0.7 mg/ mL employing the volumes of 8 µL, 25 µL and 12.5 µL for tests.

Results and Discussion

The results for the solubility test are shown in Table 1. It was found that the homopolymers of acrolein (PA01, PA06) showed lower solubility profile when compared to copolymers of styrene and acrolein (PAS01, PAS06). It was expected that the inclusion of styrene monomer to polymer microstructure decrease the solubility of the polymer chains in water. However, copolymers of styrene and acrolein were more soluble in water compared to homopolymers of acrolein. It is believed that the inclusion of a more rigid monomer to the chain promoted the opening interstitial spaces favoring the entry of the solvent into the random ball and facilitating the solubilization in water. As the chains of homopolymers was expected that the increase in the content of aldehyde groups to increase the solubility of the polymer in water. However, it may have been an effect of association of intra and inter molecular aldehyde groups was that the predominant effect of solvation.

With respect to cytotoxicity and genotoxicity testing samples PA01 and PAS04 showed no inhibition zones for growing colony of bacteria in the culture medium employed. This means that the polymers had no quote or genotoxic effects in the studied concentrations. The PA01 homopolymer also had the result of cytotoxicity, a small potentiation of the bactericidal effect of antibiotics Ampicilin and Gentamicin. For genotoxicity tests the PA01 sample showed inhibition of oxidative effect of hydrogen peroxide (H_2O_2) showing the antioxidant effect of this sample. The copolymer of styrene and acrolein (PAS04) introduced as a result of cytotoxicity, a significant enhancement of the bactericidal effect of the antibiotic Gentamicin when associated therewith. The results of genotoxicity showed that this sample significantly potentiated the effect of the oxidant hydrogen peroxide (H_2O_2) while still associated with $SnCl_2$. The percentage of the average area ratios (area of inhibition of growth of colonies of bacteria) in the sample acrolein homopolymer PA01 are described in the chart shown in Figure 1 for Ampicillin and Gentamicin controls. It was found that the sample of acrolein homopolymer PA01 showed no cytotoxicity. Furthermore, when associated with Ampicillin and Gentamicin controls the efficiency of these antibiotics potentiated, increasing about 4% effectiveness. We also observed a reduction of 4 % of the oxidizing effect of hydrogen peroxide (H_2O_2) in the DNA of the bacteria.

The percentage of the average area ratios (area of inhibition of growth of colonies of bacteria) in the sample of a copolymer of styrene and acrolein are described in PAS04 graph shown in Figure 2. This graph shows the effects of the antibiotic Gentamicin PAS04 sample and oxidizing agent hydrogen peroxide, showing a significant enhancement of 12% in the bactericidal effect of Gentamicin, and a 6% increase in the effect of hydrogen peroxide is, however, associated with the agent mutagenic tin II chloride ($SnCl_2$). We can also observe that the copolymer showed no toxicity. The sample of the copolymer showed greater potentiation of the effect of the antibiotic Gentamicin about 12% when compared to the homopolymer sample potentiated about 4%. Figure 3 shows a comparison of these effects.

Table.1 Molar ratios and aldehyde groups for samples of homo and copolymers of acrolein

Sample	Initiator system	[M]/[I] ratio	[Acro]/[Sty] ratio	λ_{max}	Abs	Concentration of aldehyde [CHO]/gPol×10^{-2}	Average [CHO]/gPol×10^{-2}	Solubility mg/mL
PA01	*sec*-BuLi	8	-	336.2 335.4	0.0874 0.0733	1.3 1.1	4.7 ± 0.8	0.973
PA02	*sec*-BuLi	20	-	335.7 335.7	0.4265 0.4571	6.6 6.4	5.8 ± 1.3	0.363
PA03	*sec*-BuLi	30	-	335.9 335.7	0.3306 0.3524	4.3 3.8	3.6 ± 0.8	0.097
PA04	*terc*-BuLi	8	-	336.5 336.0	0.2941 0.2437	3.8 2.6	3.2 ± 0.8	0.077
PA05	*terc*-BuLi	20	-	335.7 335.7	0.2392 0.2775	4.1 3.2	3.7 ± 0.5	0.377
PA06	*terc*-BuLi	30	-	335.4 336.5	0.1031 0.1139	1.7 1.8	1.7 ± 0.1	0.283
PAS01	*sec*-BuLi	10	5:1	- -	- -	- -	-	0.307
PAS02	*sec*-BuLi	10	9:1	337.3 338.2	0.1427 0.1260	1.3 1.2	1.3 ± 0.1	0.733
PAS03	*sec*-BuLi	10	49:1	338.7 338.7	0.1141 0.1125	0.8 0.7	0.8 ± 0.1	0.707
PAS04	*terc*-BuLi	10	4:1	334.6 334.8	0.3042 0.3498	4.0 4.0	4.0 ± 0.0	0.977
PAS05	*terc*-BuLi	10	5:1	335.4 335.4	0.3992 0.4487	6.0 6.5	6.5 ± 0.5	0.903
PAS06	*terc*-BuLi	10	7:1	335.1 335.4	0.4905 0.4791	5.1 5.9	5.5 ± 0.6	0.863

The PAS01 sample was not soluble in the solvent analysis (DMSO).

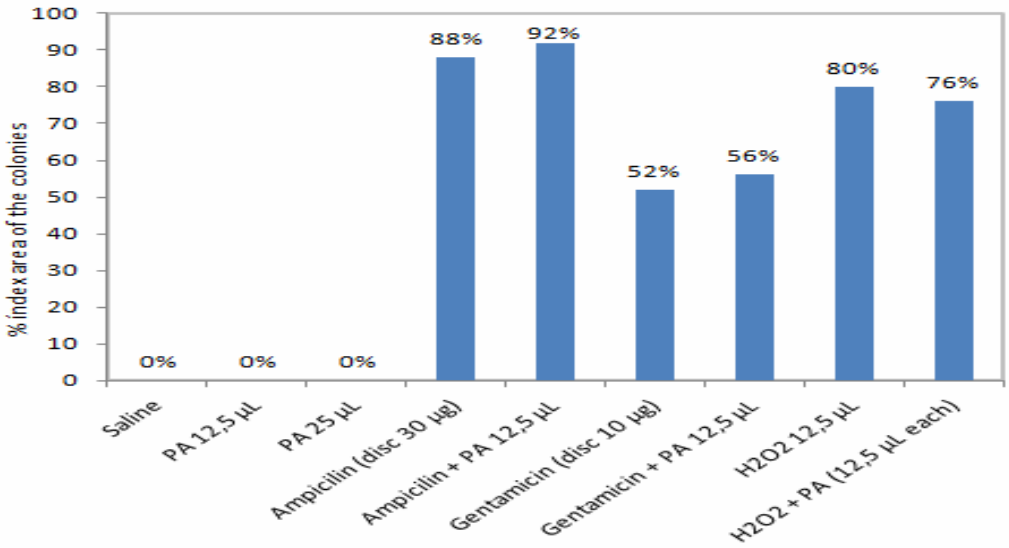

Figure.1 Effects of cyto-and genotoxicity of the sample PA01.

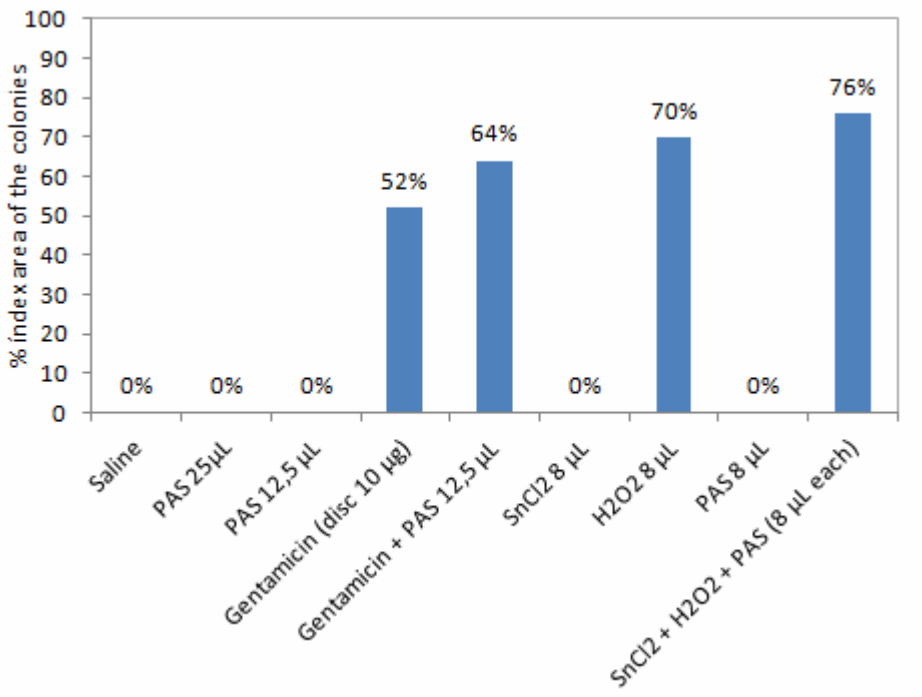

Figure.2 Effects of cyto-and genotoxicity of the sample PAS04.

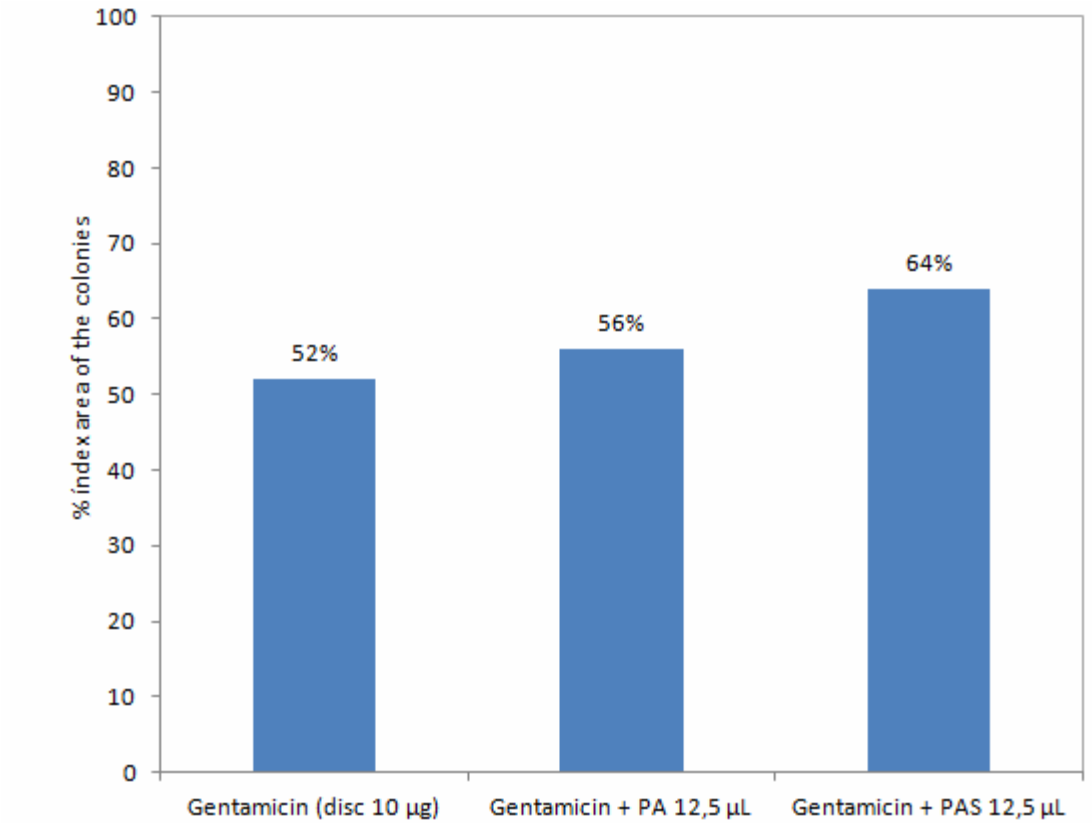

Figure.3 Effects of cytotoxicity of the sample PA01 and PAS04 Gentamicin control.

The homopolymer sample (PA01) and the copolymer of styrene and acrolein (PAS04) showed good solubility in water and showed no cytotoxic effect, nor genotoxic even for high concentrations (25 µL) of the samples. The PAS04 sample more potentiated the effect of the antibiotic Gentamicin that the PA01 sample. Studies have shown that the homopolymer synthesized using a molar ratio [M] / [I] equal to 8, initiated by sec-butyl lithium and styrene and acrolein copolymer synthesized in molar ratio [M] / [I] = 10 to comonomer ratio [acro] / [sty] equal to 4:1, beginning with tert-butyllithium have the potential to be used as biomaterials.

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