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Preparation and Characterization of the Novels Terpolymers of Poly-{trans-[$RuCl_2(vpy)_4$]-styrene-divinylbenzene} and Styrene-divinylbenzene-vinylpiridine impregnated with Silver Nanoparticles

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Summary

This paper reports the preparation and characterization of poly-{trans-[$RuCl_2(vpy)_4$]-styrene–divinylbenzene} and styrene-divinylbenzene-vinylpiridine filled with nanosilver. Theses materials were synthesized by non aqueous polymerization through a chemical reaction using benzoyl peroxide as the initiator. The nanosilver was obtained from chemical reduction using $NaBH_4$ as reducing agent and sodium citrate as stabilizer. The nanometric dimension of nanosilver was monitored by UV–visible and confirmed through TEM. The morphology was characterized by SEM and the thermal properties were done by TGA and DSC. The antimicrobial action of the polymers impregnated with nanosilver was evaluated using both microorganisms, *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial activity of the poly-{trans-[$RuCl_2(vpy)_4$]-styrene–divinylbenzene} filled with nanosilver was confirmed by the presence of an inhibition halo of the bacterial growth in seeded culture media, but was not confirmed to the styrene-divinylbenzene-vinylpiridine. The present work suggest that trans - [$RuCl_2(vpy)_4$] complex facilitate the release of silver ion from the media.

Keywords:

Ruthenium complex; polymer; silver nanoparticles; antimicrobial

Introduction

Metal nanoparticles have attracted considerable attention because of their novel physical properties and their potential applications in several areas of technology.

Metal nanoparticles with shape control can have structures and properties significantly different from those conventional materials [1].

Network polymers based on styrene and divinilbenzene, prepared by polymerization process in which the reactional medium contains the nanoparticles suspention, retain many advantageous properties of the nanoparticules and the host matrix. The characteristics of network polymers include ease of preparation, controlled surface area, porous structure, good thermal resistance and reasonable chemical inertness [2]. Surface modification of metal nanoparticles, particularly silver, gold and copper with functional polymers are of significant importance and have been the focus of great interest [3]. Silver has long been known to exhibit strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities [4-5]. For this reason silver-based compounds have been investigated for many bactericidal applications [6-7]. Silver nanoparticles also exhibit such bactericidal effect [8-9] and have therefore been seen as candidates for new developments in the field of biomaterials.

In this paper we describe the preparation of an antimicrobial material based on the terpolymer poly-{*trans*-[$RuCl_2(vpy)_4$]-sty–dvb impregnated with silver nanoparticles in aqueous medium.

Studies conducted in our laboratory have shown that ruthenium complexes can be covalently incorporated on the polymer network [10]. The incorporation of these complexes in the polymeric matrix should be accompanied by benefits in the biological systems [11]. since the transition metal complexes of the same class o of complex bring potential therapeutic benefits like acting as antioxidant amongst other.

Preliminary studies Carried through in our group suggest that the ruthenium complex facilitates the release of the silver for the media in view of that polymers that does not contain this complex in the polymeric matrix when are impregnated with silver nanoparticles do not present inhibition of the bacterial growth in the studied of culture media. In this sense, poly-{*trans*-[$RuCl_2(vpy)_4$]-sty–dvb impregnated with silver nanoparticles promise application in biomedical implants as well small devices for clinical applications.

Experimental

Reagents and Synthesis

Commercially available analytical grade reagents were employed throughout this study. $RuCl_3.3H_2O$ (Aldrich), DVB (Fluka), VPY (Aldrich), and STY were used without further purification. The synthesis method used to produce the precursor ruthenium blue solution and the monomer *trans*-[$RuCl_2(vpy)_4$] is described elsewhere [12-13].

Synthesis of poly-{trans-[$RuCl_2(vpy)_4$]-styrene- divinylbenzene}

To a toluene solution of monomer complex *trans*-[$RuCl_2(vpy)_4$], it was added the benzoyl peroxide (Fluka), 1% by mol to the amount of monomer in the toluene media. The synthesis was conducted under reflux in a blank of argon atmosphere, with vigorous stirring at (65 ± 2) °C for 2 hours. To this solution an aqueous solution of Ag nanoparticles was poured. The remaining solution was then evaporated and further was casted on a disk plate. The filled silver nanoparticles were used in a ratio of 2:1 (v/v) of terpolymer to metallic nanoparticles.

Synthesis of terpolymer styrene- divinylbenzene- vinylpiridine

Benzoil peroxide (1% in of monomers) is dissolved in toluene. Further that, the divinylbenzene is added, followed by vinylpyridine and finally styrene. The system was maintained under reflux, with vigorous mechanical stirring, at a temperature of 90 °C for 20 minutes. To this solution an aqueous solution of Ag nanoparticles was poured. The filled silver nanoparticles were used in a ratio of 2:1 (v/v) of terpolymer to metallic nanoparticles.

Synthesis of silver nanoparticles

An equimolar solution of $AgNO_3$ (Riedel-de Haën) and sodium citrate (Nuclear) 0,01 mol.L^{-1} of $NaBH_4$ (Nuclear) in ultra pure water was kept under vigorous stirring for 30 minutes. The solution acquired a yellow-gray turbid aspect after the addition of $NaBH_4$ and a colloidal suspension was formed [14-15].

Microbiological study

Microbiological studies were performed to investigate the antimicrobial activity of this new material. Both microorganism, the *Escherichia coli* (ATCC-25922) and *Staphylococcus aureus* (ATCC-25923) were used for this study as both are reference strains used for antimicrobial susceptibility testing. Also, *E. coli* and *S. aureus* are microorganisms that are commonly involved in hospital-acquired infections [16].

Equipments

Spectroscopy UV-visible

The silver nanoparticles preparation was monitoring by a UV-visible HP model 8452A. All the measurements were carried out in quartz cuvettes with an optical path of 1.00 cm.

Raman spectroscopy

Raman spectra were obtained with a Renishaw Raman System 3000 equipped with a CCD, using either the 632.8 nm line of an He-Ne laser (Spectra-Physics model 127) or the 514.5 nm line of an Argon ion laser (Omnichrome), for the terpolymers 1 to 4. The laser was focused onto the sample surface by means of a microscope (Olympus BH2T) and the laser power was kept at 3 mW. The powdered samples were pressed onto a microscope slide. The spectral resolution employed was 4 cm^{-1}.

Thermal analysis

Thermal stability and thermal transitions of the terpolymer with and without silver were evaluated by thermogravimetry and differential scanning calorimetry. TG/DTG curves were obtained with a thermobalance model TGA 50 (Shimadzu) in the temperature range of 25 to 700 °C, using Pt crucibles with ~5 mg of samples, under dynamic N_2 atmosphere (50 mL.min^{-1}) and heating rate of 10°C.min^{-1}. DSC curves were obtained in a DSC-50 cell (Shimadzu), under dynamic N_2 atmosphere (50 mL.min^{-1}), heating rate of 10 °C.min^{-1} in the temperature range from 25 to 600 °C and 25 to 200 °C (three successive heating cycles), using Al crucibles with, respectively, 2 mg and 6 mg of samples.

Transmission Electron Microscopy (TEM) and Scanning Electron Microscope (SEM)

TEM micrographs were taken on a Philips CM200 microscope operating at an accelerating voltage of 200kV. Samples for TEM observations were prepared by placing a drop of an aqueous solution containing the silver nanoparticles in a coated carbon grid.

Microscopic analysis of the terpolymers was carried out using a scanning electron microscope (SEM) (Philips X30).

Results and discussion

Spectroscopy UV-visible

The UV-vis. absorption spectrum of silver nanoparticles in aqueous medium is displayed in Figure 1. A strong absorption peak at approximately 400 nm originates from the surface plasmon resonance absorption of nanosized silver particles. The good symmetric absorption peak implies that the size distribution of the nanoparticles is narrow. The absorption spectrum of an aqueous solution of the nanoparticles can be visualized on Fig. 1 which reveals a single band with maximum absorption at 388 nm that correspond to the surface plasmon ressonance band of spherical silver particles [17-21]. Fig. 1 also reveals a tailing effect in the SPR band that can indicates the existence of a broad distribution in size for these silver nanoparticles [19].

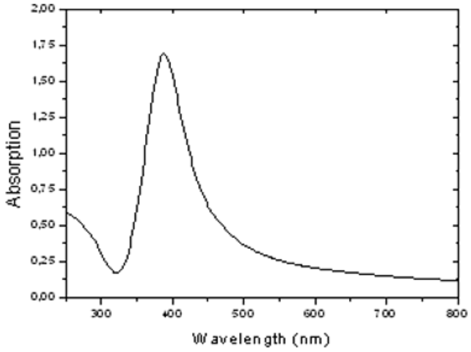

Fig. 1. UV-vis. spectrum of the nanoparticles of silver in aqueous media.

Transmission Electron Microscopy (TEM)

TEM image of the colloidal silver nanoparticles revealed the presence of nearly spherical particles with diameters ranging from 5 to 45 nm and corroborates with data show on recent publication (Fig. 2) [22]. From the analysis of TEM micrographs it was selected the diameter of 600 randomly selected particles in enlarged TEM images with resulted in the particle size distribution histogram shown in Fig. 2. The size distribution was found to be well described by a lognormal distribution function from which we obtained the media particles diameter of 19.7 nm and the distribution width of 0.48. A lognormal distribution is widely used for describing the polydispersity of very small particles, where the lower metallic particle size is usually more difficult to measure and shows some aggregation and corroborates with data show on recent publication [22-24].

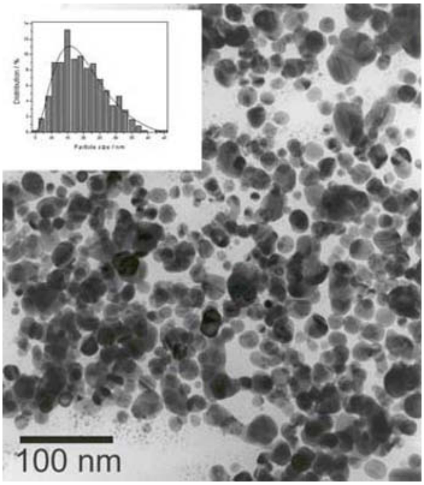

Fig. 2. Transmission electron microscopy image and particle size histogram showing a lognormal size distribution fit (solid line) of silver nanoparticles.

Scanning Electron Microscopy (SEM)

The surface morphology of terpolymer poly-{trans-[RuCl2(vpy)4]-styrene-divinylbenzene} was characterized by SEM showing a film with smooth surface and an increased roughness in the polymer impregnated with silver nanoparticles (Fig. 3). Figure 4 shows a SEM micrograph of the terpolymer poly-{styrene-divinylbenzene-vinylpyridine}. The observed morphyology is powder like polymer with and without silver in the polymer matrix. It is clear from the observation that the presence of trans-[RuCl2(vpy)4] play a significant role in give to the material a smooth property.

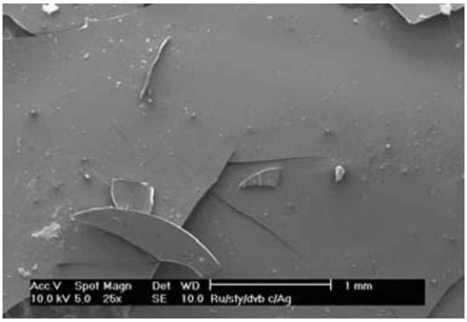

Fig. 3. SEM micrograph of terpolymer poly-{*trans*-[RuCl2(vpy)4]-styrene-divinilbenzene} impregnated with silver nanoparticles.

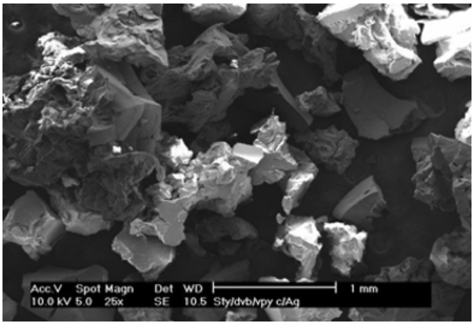

Fig. 4. SEM micrograph of terpolymer {styrene-divinilbenzene-vinylpiridine} impregnated with silver nanoparticles.

Raman spectroscopy

Fig. 5 shows the Raman spectra of terpolymers poly-{*trans*-[RuCl2(vpy)4]-styrene-divinylbenzene} with and without Ag (samples 1 and 2, respectively) along with the spectra of monomers complex *trans*-[$RuCl_2(vpy)_4$], polystyrene and poly-{*trans*-[$RuCl_2(vpy)_4$]} obtained by electrochemical polymerization [13] for the sake of comparison.

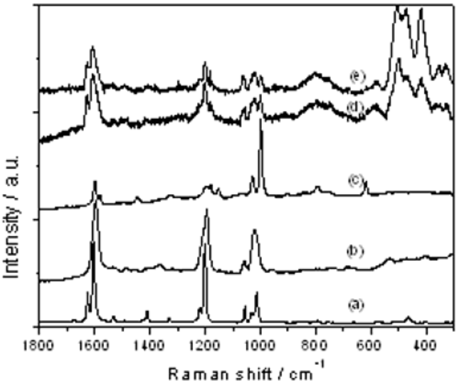

Fig. 5. Raman spectra of **(a)** *trans*-[$RuCl_2(vpy)_4$]; **(b)** poly-{*trans* [$RuCl_2(vpy)_4$]} **(c)** poly-styrene); **(d)** terpolymer 2 (without Ag); **(e)** terpolymer 1 (with Ag), in the region 300-1800 cm^{1}.

The typical vibration frequencies of *trans*-[$RuCl_2(vpy)_4$] (monomer complex) polystyrene, poly-{*trans* [$RuCl_2(vpy)_4$]}, terpolymers 1 and 2 are presented in Table 1. To facilitate this discussion, Figure 6 shows a close zoom of the same spectra as in Fig. 5 with emphasis on the 850-1180 cm^{-1} range. The terpolymers 1 and 2 show bands at 997, 1023, 1062, 1200, 1602 and 1627 cm^{-1}.The band at 1627 cm^{-1} is assigned to the *trans*-[$RuCl_2(vpy)_4$] (monomer), since it is present only in this compound. This band, assigned to ν(C=C) mode, present in the terpolymers 1 and 2, indicate that the polymerization was incomplete. The band at 1062 cm^{-1} is assigned to the *trans*-[$RuCl_2(vpy)_4$] monomer, although the polymer also has a band with frequency close to the value observed by the terpolymers, but with low intensity. The band at 1023 cm^{-1} is assigned to the poly-{*trans*-[$RuCl_2(vpy)_4$]}, which has a band with a very close frequency value and with reasonable intensity. The bands at 1200 and 1602 cm^{-1} can be assigned to the *trans*-[$RuCl_2(vpy)_4$] monomer and polymer, because they are present in both compounds with high intensity. On the other hand, the terpolymer band at 997 cm^{-1} can be safely assigned to the polystyrene, because it is not present in the complex monomer or polymer.

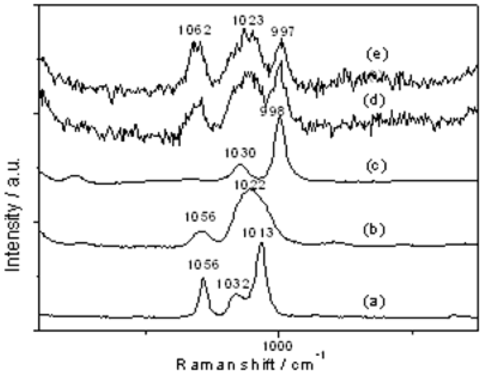

Fig. 6. Close zoom at 850-1180 cm^{-1} range of Raman spectra from figure 5 of: **(a)** *trans*-[$RuCl_2(vpy)_4$]; **(b)** poly-{*trans* [$RuCl_2(vpy)_4$]} **(c)** polystyrene; **(d)** terpolymer 2 (without Ag); **(e)** terpolymer 1 (with Ag).

With a detailed inspection of the bands of the terpolymers 1 and 2, at 997, 1023 and 1062 cm^{-1}, in Fig. 6, it can be observed that in the terpolymer 1 the band at at 1023 cm^{-1} (from poly-{*trans* [$RuCl_2(vpy)_4$]} is slightly enhanced as on compare to the band at 997 cm^{-1} (from polystyrene) in comparison to the same bands in the spectrum of terpolymer 2. This fact indicates that the addition of Ag in terpolymer 1 contributes to the intensification of the bands of the complex. Although not shown in detail in Figure 7, the bands at 1200 and 1602 cm^{-1}, assigned to the *trans*-[$RuCl_2(vpy)_4$] monomer and polymer, were also enhanced (in comparison to the band at 997 cm^{-1}, from polystyrene) in the spectra of the terpolymer 1 (with Ag), so corroborating the hypothesis of the enhancement effect carried out by the presence of Ag in the terpolymer 1.

Another fact worth of mention is the intensification of bands in the low frequency region (around 300-600 cm^{-1}) in the terpolymer 1. These bands are tentatively assigned to ruthenium oxide bands or some ruthenium-oxygen bands of oxygen containing ruthenium compound [25]. The presence of bands due to ruthenium oxide in the terpolymer could be explained by the oxidation of the complex by the initiator used in the polymerization process. In a previous paper reporting the electrochemical polymerization of the trans-[$RuCl_2(vpy)_4$] [13], bands in the same region were observed, being assigned to ruthenium oxide. The enhancement of the bands of the complex and the bands of the ruthenium oxide was due the presence of Ag atoms in the terpolymer.

Table 1: Raman frequencies (cm^{-1}) of *trans* [$RuCl_2(vpy)_4$], polystyrene, poly-{*trans* [$RuCl_2(vpy)_4$]} and poly-{*trans*-[$RuCl_2(vpy)_4$]-styrene-divinylbenzene} with and without Ag (samples 1 and 2, respectively), in the region above 900 cm^{-1}.

trans-[$RuCl_2(vpy)_4$]	poly-{*trans*-[$RuCl_2(vpy)_4$]}	polystyrene	Terpolymers 1 and 2
		998 (s)	997 (m)
1013 (m)			
1032 (w)	1022 (m)	1030 (w)	1023 (m)
1056 (w)	1056 (w)		1062 (m)
		1153 (w)	
		1180 (w)	
1200 (s)	1194 (s)	1198 (w)	1200 (m)
1204 (sh)	1207 (sh)		1209 (sh)
1220 (w)			
1604 (s)	1597 (s)	1600 (w)	1602 (s)
1625 (m)			1627 (m)

s = strong; m = medium; w = weak; sh = shoulder

Thermal analysis

TG/DTG and DSC curves of the terpolymer poly-{*trans*-[$RuCl_2(vpy)_4$]-sty–dvb} prepared without and with silver nanoparticles are shown in Fig. 7 and 8, respectively. TG/DTG curves of both materials showed similar thermal behaviour with total mass loss of 82.1% (without silver) and 81.6% (with silver) in the interval from 25 to 700 °C. A lower loss of mass of the polymer impregnated with silver is consistent with the presence of the non volatile metallic component in the polymeric matrix. In both materials, an initial loss of mass of approximately 3% was found in the interval from 25 to 200 °C followed by a loss of mass of approximately 17% from 200 to 400 ºC. After reaching 400 °C, a more important loss of mass takes place from 400 to 700 ºC corresponding to 61.9% and 60.5% mass loss for the terpolymer without and with silver nanoparticles, respectively. The DSC curves for the terpolymer with and without silver revealed an exothermic process from 80 to 200 ºC (T_{peak}DSC=140 ºC) that can be attributed to polymerization reactions of the residual unreacted vinyl groups. After three successive heating cycles in the interval from 25 to 200 °C (DSC

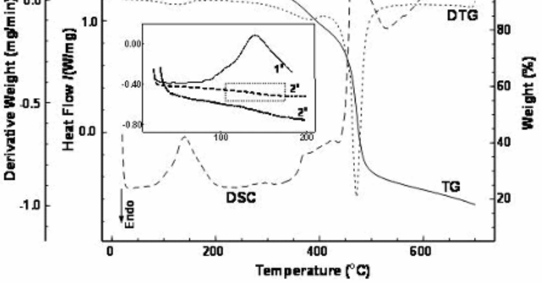

Fig. 7. TG/DTG and DSC obtained in dynamic nitrogen atmosphere (50 mL.min^{-1}) and heating of Ru/STY/DVB (without silver).

curves inserted in the main Figures) it was possible to estimate the glass transition for the terpolymer without silver (145 °C) and for the terpolymer impregnated with silver (155 °C), calculated after second heating. The main exothermic process that corresponds to the terpolymer decomposition occurred between 400 and 500 ºC for both materials. However, the DSC curves indicate an exothermic event at 463 ºC for the terpolymer without silver and at 469 ºC for the terpolymer impregnated with silver. This behavior demonstrates stabilization of the terpolymer due to the presence of silver metal in the polymeric matrix.

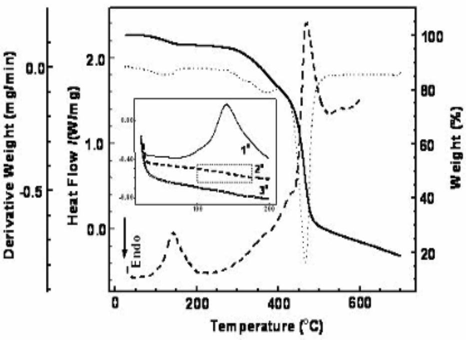

Fig. 8. TG/DTG and DSC obtained in dynamic nitrogen atmosphere (50 mL.min^{-1}) and heating of Ru/STY/DVB (with silver).

Microbiological study

The microorganism strains were reactivated and the purity confirmed by the seeding in appropriate culture means with aid of platinum loop for the exhaustion technique. To *E. coli* was utilize culture medium *McConkey* Agar, and to *S. aureus*, *Manitol* Salt Agar. After 24 hours the samples were placed in liquid *Brain Heart Infusion* (BHI) e NaCl solution at 0,9%, respectively and incubated at 37 ºC until approximately 10^6 colony-forming units per mL (CFU/mL). After that, were seeding in *Muller & Hynton* Agar media plate. The polymer samples impregnated with silver nanoparticles were placed in a Petri dish with the *Muller & Hynton* culture medium and incubated at 37 ºC overnight. After this period a bacterial growth inhibition halo was observed

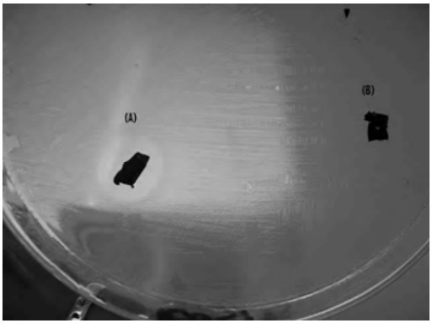

Fig. 9. Microbial culture with terpolymer poly-{*trans*-[RuCl$_2$(vpy)$_4$]-styrene-divinybenzene} with **(A)** and without silver **(B).**

around the polymer poly-{*trans*-[$RuCl_2(vpy)_4$]-sty–dvb} impregnated with silver this not being observed in the polymer without silver (Fig. 9 A and B). To the polymer {styrene-divinylbenzene-vinylpyridine} not being observed bacterial growth inhibition halo in the polymer with and without silver nanoparticles (Fig 10 A and B). The inhibition of the microorganism caused by the presence of silver nanoparticles in the polymer poly-{*trans*-[$RuCl_2(vpy)_4$]-sty–dvb} confers to the material characteristic antiseptic properties (Fig. 9 A).

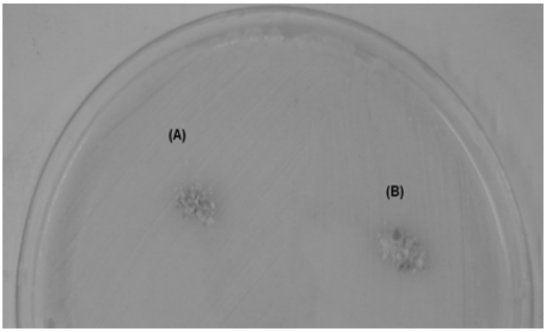

Fig. 10. Microbial culture with terpolymer {styrene-divinybenzene-vinylpyridine} with **(A)** and without silver **(B).**

Silver nanoparticles present oligodynamic activity and such activity can be a valuable alternative as an adverse effect of antibiotics and also disinfecting. The ions silver forms insoluble compounds with sulphydryls groups in the cellular wall of the microorganism, that are composed of responsible essential enzymes for the energy metabolism of *trans* - membranous and electrolytes transport. Additionally the silver ions enters in the cell and link to bacterial DNA and the silver intercalation takes lead a decrease of the stability of the couple helix, inhibiting additional proliferations [26]. The antimicrobial activity of the silver in polymeric matrix can also be important for the prevention of proliferation of adherent bacteria in the surface of the polymeric materials, increasing like this the time of life of this material. Polymeric matrix impregnated with silver nanopartículas present new surface properties that act in the prevention of adherence of microorganisms in the surface and deposits of proteins. This and other issues relating to the understanding of this new material will be subject of future studies in laboratories.

Conclusions

The ternary polymer poly-{*trans*-[$RuCl_2(vpy)_4$]-sty-dvb} filled with silver nanoparticles can be prepared with vigorous mechanical stirring of the aqueous solution with silver nanoparticles. The characterization proved this preparation method to be successfully. The microbiological analysis showed an inhibitory halo of the polymer impregnated with silver nanoparticles in the microculture plates, confirming the antiseptic property of the novel material. The ruthenium complex could be a determinative factor in the release of silver nanoparticles. It was observed that polymer in absence of ruthenium complex in the matrix difficult the release silver ion for the media, not conferring to this material antimicrobial activity.

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