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SILVER-POLYMER COMPOSITE MATERIALS WITH ANTIBACTERIAL PROPERTIES

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A series of new polyurethane acrylates were prepared by *in situ* free-radical polymerization of acrylic acid or 2,2bis[5(methacryloyloxyethylcarbonylaminomethyl-1-carbamoyloxymethyl)-3,3-trimethyl-ciclohexyl] propionic acid (*UMA-1*) into a polyurethane matrix, accompanied by the total/partial ionization of the carboxyl groups from the acrylic component with the amino groups from the macromolecular chain. For a set of samples, silver nitrate was added so that through an *in situ* electron transfer reaction, atomic silver was formed in the metal particles/polymer composites. The characterization of the polyurethane acrylates and hybrid composites was achieved through thermal methods (TGA) and surface analysis (contact angle), whereas the presence of silver nanoparticles was confirmed by energy-dispersive X-ray (EDX) and UV-vis spectroscopy. The mechanical parameters of the polymeric materials were investigated comparatively to the precursors as well as to the silver/polymer composites by tensile stress. Antibacterial properties of such hybrid composites were determined by using an agar diffusion test against both Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*) cultures, the results showing a significant effect of these materials to inhibit the growth of bacteria on surface by silver nanoparticles.

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1. Introduction

The properties and applications of polymeric systems can be varied within large limits by their modifying or copolymerization with other polymers, so that the performances of the resultant materials to meet the predetermined standards [1]. Thus, in recent years, a remarkable effort has been directed to exploit the potential of various copolymers to blend and sometimes to potency the useful properties of each component at the expense of undesirable characteristics [2].

Polyurethanes have found many applications in different areas due to their advantageous properties such as a wide range of flexibility, high abrasion and chemical resistance, excellent stability over time and a low processing temperature, these features making polyurethanes a commonly used class of polymers [3, 4]. Despite the outstanding performances, polyurethanes shows and a number of disadvantages such as high production costs (mainly due isocyanate component) or inconveniences appeared during synthesis owing to the high reactivity of the isocyanate groups towards a number of impurities (e.g. water). Currently, secondary reactions are generally removed, a facile approach to achieve materials with improved properties being to introduce acrylic sequences into the polyurethane matrix [5].

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Traditionally, the polyacrylates are extensively used as adhesives or for the obtaining of coatings since such polymers are characterized by a low glassy transition temperature, which makes them suitable for handling, processing or purification [6]. In addition, the broad variety of acrylic structures available allows the design and selection of desired physical properties, without neglecting its lower cost comparatively with the polyurethanes. On the other hand, the problems associated to the lucrative polyacrylates are related to the macromolecular chain flexibility which determines a limited thermal stability and low mechanical strength.

The most frequently used method is to incorporate the precursor vinyl monomers into polyurethanes and to perform the UV polymerization of acrylic derivatives into the polyurethane templates [7]. Under these conditions, any UV-polymerizable system is normally composed of reactive urethane oligomers, reactive diluents and photoinitiators. The reactive urethane oligomer is often the main component in determining the final properties of UV-treated coatings, which is usually a segmented polyurethane oligomer mixed with acrylic derivatives (like 2-hydroxyethyl acrylate or 2-hydroxyethyl methacrylate). The microphase separation of the urethane segments, governed by the type and length of flexible segments, is the key parameter in controlling the dynamic mechanical properties of these polyurethane-acrylates [8]. Reactive diluents are acrylic monomers added with the purpose to modify the properties of polyurethane precursors. Polyurethane-acrylates ideally combine the high abrasion resistance, hardness, tear resistance and good properties at low temperature of polyurethanes with superior optical properties and durability of the polyacrylates [3, 9]. Generally, monoacrylates lead to a module decrease and a ductility increase, while multifunctional acrylates as tri(propylene glycol) diacrylate or trimethylolpropane triacrylate cause a reverse effect. Moreover, the multifunctional diluents are more sensitive to irradiation.

Recently, a feature intensively investigated is represented by the metal/polymer composites, due to the fact that these materials may have novel and versatile properties originating from the combination of properties of the inorganic component with those of the polymer backbone [10-12]. Metals typically, are added to improve mechanical, optical, electrical or chemical properties of the polymers and confer them increased performance in specific applications [13]. Silver is a commercially available metal and its nanoparticles are superior to other nanosized metal particles for instance, by excellent electrical conductivity, antimicrobial effects, optical and catalytic properties [14].

On the basis of these considerations, in the present work we report the first results concerning the generation of silver nanoparticles into polyurethane-acrylate copolymers and its effect on the mechanical characteristics and antibacterial activity. It is believed that the carboxylic groups from the acrylic derivatives favour silver ion increased mobility in the organic matrix, which is considered responsible for the improved antimicrobial surface activity of these hybrid materials.

2. Experimental

2.1. Materials

Poly(tetramethylene oxide) diol (PTMO) (M_n = 1kDa) was obtained from BASF (Germany), whereas poly(butylene adipate) diol (PBA) (M_n = 1kDa), 4,4'-diphenylmethane diisocyanate (MDI), N,N'-β-hydroxyethyl-piperazine (HEP), N-methyldiethanolamine (NMDA), 1,4-butanediol (1,4-BD), acrylic acid (AA), 1,1'-azobis(cyclohexanecarbonitrile), silver nitrate, N,N-dimethylformamide (DMF) were purchased from Sigma Aldrich Chemical Co. and used without further purification.

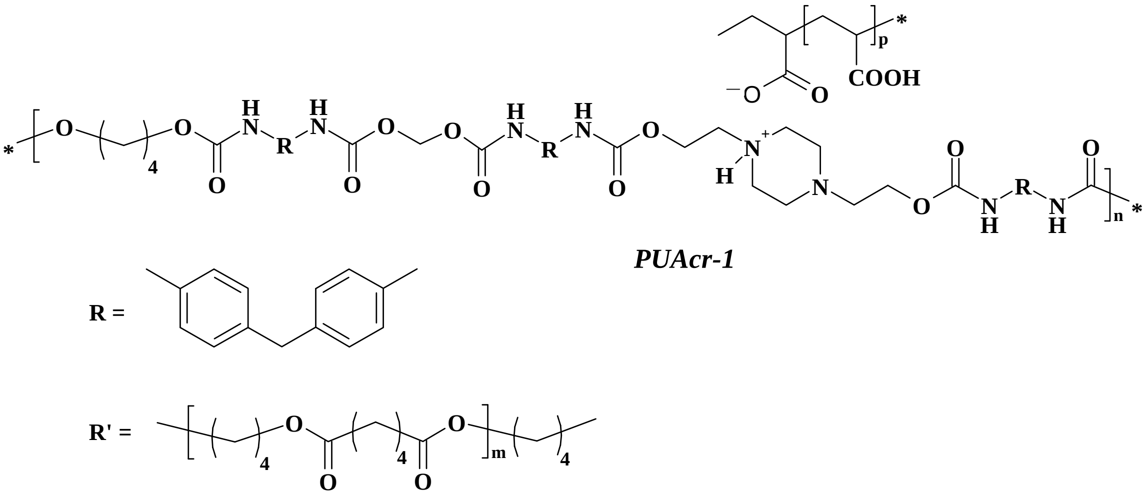
2.2. Polyurethane acrylate synthesis

The synthesis of precursor polyurethanes PU-PR1, PU-PR2 and PU-PR3 was reported previously [15, 16], but the main characteristics of these polymers are included in Table 1.

Table 1. Characteristics of the polyurethane precursors.

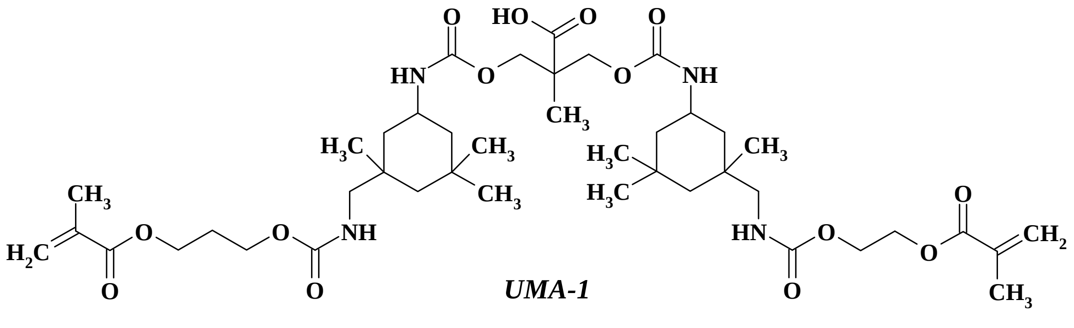
Sample	Weight of raw materials (mol)						M_w (GPC) (g/mol)	η_{red} (dL g^{-1})
	PBA	PTMO	MDI	HEP	NMDA	1,4-BD		
PU-PR1	1	-	3	1	-	1	34500	0.32
PU-PR2	1	-	3	-	1	1	28000	0.29
PU-PR3	-	1	3	1	-	1	23700	0.2

The polyurethane acrylates were obtaining by *in situ* polymerization of acrylic component into the polyurethane matrix in tandem with the ionization of carboxyl groups in the presence of amino moieties as depicted in Scheme 1. Thus, 4 g of polyurethane precursor PU-PR1 were dissolved in 20 mL DMF and 1 mL acrylic acid were added, together with 10 mg 1,1'-azobis(cyclohexanecarbonitrile) as initiator (1 %), in order to prepare polyurethane acrylate structures containing 20 % wt. acrylic component. The reactions were performed under purified nitrogen at 60 °C for 24 h. Subsequently, the resulting mixture was cast on glass surfaces and kept in a desiccator for controlled evaporation of the solvent. Homogenous films were obtained and then dried at 40 °C under vacuum for 2 days.



Scheme 1. Structure of the polyurethane acrylate PUAcr-1

Another way for the preparation of polyurethane acrylate blends consist in the mixing of polyurethane precursors with a urethane acrylate monomer synthesized in our laboratory (illustrated in Scheme 2), whose synthesis was previously reported [17].



Scheme 2. Structure of the urethane methacrylate UMA-1

Hereby, to a solution of 4 g PU-PR1 in DMF was added 1 g urethane acrylate monomer for a gravimetric ratio of 80/20 wt. % polyurethane/polyacrylate, and the ulterior processing was similar to that previously described.

2.3. Silver composites preparation

The composite resin formulation was typically the following: to a DMF solution of the polyurethane precursors (80 wt. %) and AA or UMA-1 (20 wt. %), silver nitrate (1 wt. %) was incorporated by stirring the mixture for 2 h in the dark at room temperature. Then, the radical initiator 1,1'-azobis(cyclohexanecarbonitrile) (1 wt. %) was added and the mixture was heated to 60 ^{o}C for 24 h, so the crosslinking polymerization of the acrylic sequences and the *in situ* electron transfer reaction to be carried out. After that, the mixtures were poured onto glass slides to obtain polymer composite films by drying at 40 °C under vacuum for 2 days.

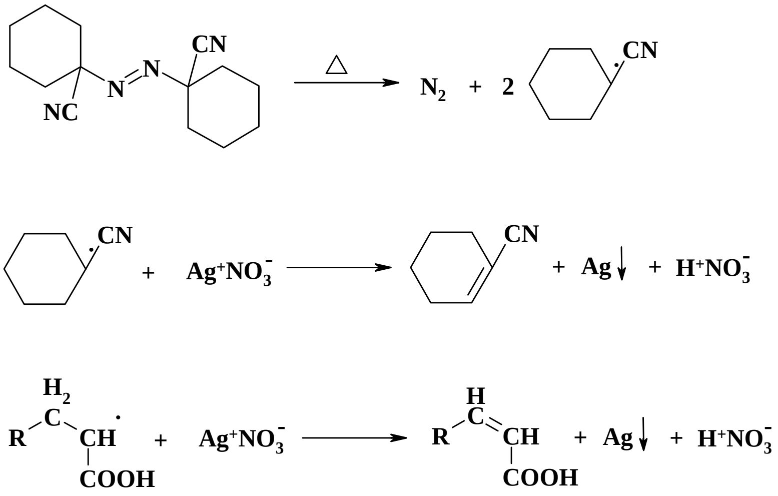
2.4. Instrumentation and measurements

The thermal stability of the polymers was analyzed through thermogravimetry using a MOM Budapest derivatograph. TG and TGA curves were recorded between 20 and 700 °C with a heating rate of 10 $°C\cdot min^{-1}$ in air. Water contact angle measurements were made on homogeneous surfaces using goniometer KSV Cam 200. Ion-exchanged water droplets of 2 μL in volume were dropped onto the composite films, and the average contact angle was calculated starting from at least ten separate measurements. Microscopic investigations were performed on an environmental scanning electron microscope QUANTA200 coupled with an energy dispersive X-ray spectroscope (ESEM/EDX).The dried particle samples were examined in low vacuum mode operating at 30KV using an LFD detector. UV spectra of silver composite films on glass slides were recorded on a Specord 200 Analytik Jena spectrophotometer. The stress/strain curves were recorded on a Shimadzu AGS-J deformation apparatus at ambient temperature and at a rate of deformation of 10 mm/min with a load cell capable of measuring forces up to 1 kN and a sample film of 25 mm × 10 mm × 1 mm. For each data point, five samples were tested and the average value was taken. The antimicrobial properties of silver composites in the film state were determined by the agar disc diffusion method [18]. The bacteria (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 200603) were subcultured to nutrient agar and incubated overnight at 37 °C. Then, the cells were dispersed in the same medium to reach 10^{-4} CFU/ml. The agar plates were streaked with a sterile swab moistened with the bacterial suspension. The silver composite films (5 x 5 mm, thickness: 0.15 mm) previously sterilized by irradiation, also were placed over the surface of the agar plates and then incubated. All the test plates were incubated overnight at 37 °C. Bacteriostatic activity was determined by the ability of the material to inhibit bacterial growth on the agar surface in contact with it. The reactions of the microorganisms with the silver composite films were investigated by measuring the size of the inhibitory zone.

3. Results and discussion

3.1. Design and characterization of the polyurethane-acrylates and silver composite films

Because the coatings achieved by the employing of polyurethane-acrylates can be successfully used in a great variety of applications, we choose to prepare new materials by the in situ radical polymerization of two acrylic derivatives (acrylic acid and 2,2 bis[5(methacryloyloxyethylcarbonylaminomethyl-1-carbamoyloxymethyl)-3,3-trimethyl-ciclo-hexyl] propionic acid – *UMA-1*) into a polyurethane matrix, where the partial/total ionization of the carboxyl groups from the acrylic component with the amino groups from the piperazinium rings occurs (Scheme 2). Moreover, in order to obtain flexible and transparent coatings with antibacterial properties, we have incorporated into the polymeric mixture inorganic particles of silver nitrate, which in the presence of the radical initiator is transformed in silver nanoparticles, without an additional reduction process, according to Scheme 3 [19].



Scheme 3. The proposed mechanism for the reduction of silver cations by an electron transfer reaction

The composition of the resulting polyurethane-acrylates and silver composites is given in Table 2.

Table 2. Characteristics of the polyurethane acrylate derivatives and hybrid composites.

Sample	Initial composition percentage (wt. %)						Contact angle (°)
	PU-PR1	PU-PR2	PU-PR3	AA	UMA-1	Ag	
PUAcr-1	80	-	-	20	-	-	79.5
PUAcr-2	80	-	-	-	20	-	96.7
PUAcr-3	-	80	-	20	-	-	61.1
PUAcr-4	-	80	-	-	20	-	76.1
PUAcr-5	-	-	80	20	-	-	57.8
PUAcr-6	-	-	80	-	20	-	73.6
PUAcr-7	80	-	-	20	-	1	54.9
PUAcr-8	80	-	-	-	20	1	65.1
PUAcr-9	-	80	-	-	20	1	57.9
PUAcr-10	-	-	80	-	20	1	52.8

Wettability of the polyurethane-acrylates and silver hybrid composite films were determined by contact angle measurements, the values of contact angle (°) being summarized in Table 2. Generally, the hydrophilic/hydrophobic character of such hybrid materials was considered to establish the influence of silver nanoparticles on the system interaction with the surrounding medium, thus affecting their antibacterial activity. On the other hand, a certain hydrophobic character improves the polymer-bacteria interaction, disrupting membrane functionality [20]. According to the contact angle data, the polyurethane acrylates containing acrylic acid sequences are more hydrophilic than those based on UMA-1 derivative, and the differences are ascribed to the increased number of hydrophilic carboxyl units introduced by the low molecular weight acrylic acid. Moreover, it can be observed that the inclusion of silver particles into polymeric matrix determined an enhancement of the hydrophilic nature, the values measured for the contact angle in the case of silver composites being lower than those of the corresponding polymers.

Evidence for the formation of composites based on silver/polyurethane acrylates after inclusion of silver nitrate into the polymeric matrix and occurring *in situ* electron transfer reaction was provided by energy-dispersive X-ray spectroscopy (EDX). In Figure 1 (a), the scanning electron microscopy (SEM) image of the composite PUAcr-8 film shows a homogeneous surface, with uniformly distributed Ag particles in polyurethane acrylates film and no further agglomeration of them.

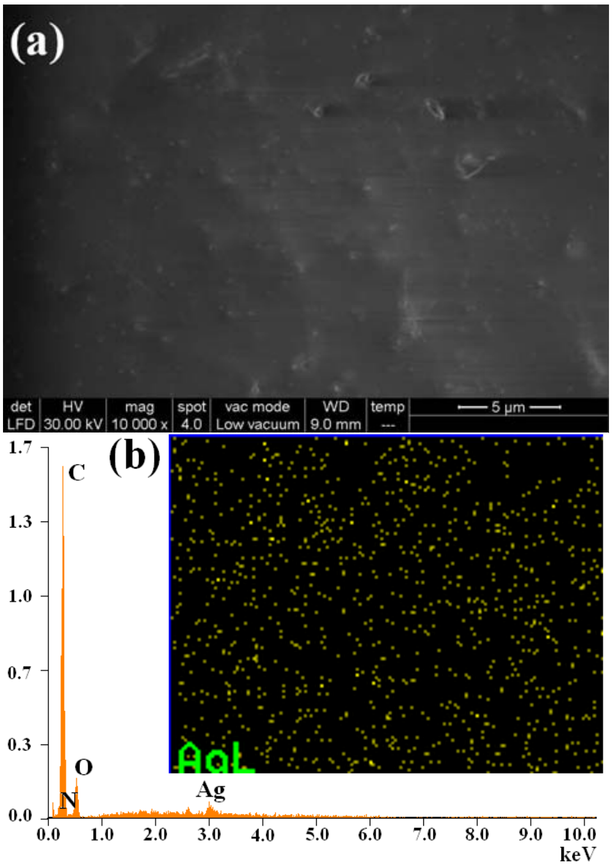


Fig. 1. SEM (a) and EDX(b) image of the polyurethane acrylates - silver film composite PUAcr-8

In the EDX pattern, C, O, and Ag peaks are clearly shown, demonstrating that such polyurethane acrylates – silver composites were successfully prepared by this one step process. In addition, mapping image of silver Lα1 obviously indicates the position of each element as display in Figure 1 (b), where the bright spots indicate the presence of the silver atoms. This result means that silver particles were homogeneously dispersed into the polymeric matrix, without formation of separated silver domain consisting of enlarged or aggregated silver particles.

An alternative technique used to confirm the existence of silver nanoparticles into the polymeric matrix was the UV–vis absorption spectroscopy, and the absorption spectra of the silver composites are presented in Figure 2.

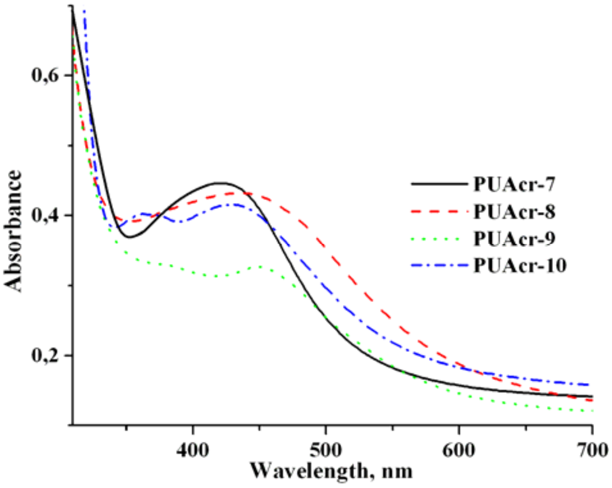


Fig. 2. UV-vis spectra of polyurethane acrylate silver composites PUAcr-7 ÷ PUAcr-10

Closer examination of this figure indicated the appearance in the UV spectrum of the any hybrid composite of a surface plasmon resonance band in the region 425-450 nm, which argue the nano-sized character of the silver particles incorporated in our polymer matrix [21]. Moreover, it can be noticed that the surface plasmon generated by the silver nanoparticles embedded in the PUAcr-7 containing poly(acrylic acid) sequences is sharper than that corresponding to the UMA-1 composites, implying a narrow distribution of the sizes and shapes of the silver particles. The broader and red-shifted UV maxima in the case of the PUAcr-8 ÷ PUAcr-10 composites evidenced that the dimensional limits of the silver particles are large, and this suggests a possible aggregation phenomena attributed to the reduced mobility of the formed species due to a higher crosslinking density [22].

3.2. Thermal properties

The thermal stability for the synthesized polyurethane-acrylates and their corresponding silver composites was followed by thermogravimetric analysis. From the TGA curves of the materials were determined the initial decomposition temperatures (T_{onset}), the temperatures where 10 and 50 % weight loss occurs and the residue at 700 ^{o}C (Table 3). The degradation of the polymers started over 160 ^{o}C, conferring a good thermal stability for the resulting materials. The inclusion of silver particles determined an enhancement of the onset degradation temperature with about 10 ^{o}C, being evident that the decomposition temperature of polyurethane-acrylates increased in the presence of Ag nanoparticles. Also, from the data given in Table 3, it can be remarked that the materials containing acrylic acid presented lower decomposition temperatures than those with the UMA-1 derivative but a higher residue content at 700 ^{o}C. Moreover, it might be remarked, that the addition of a small quantity of silver particles to the polymeric matrix results into a proportionally growing residue amount.

Table 3. Thermal behaviour of the polyurethane acrylates and silver composites.

Sample	T_{onset} (°C)	$T_{10\%}$ (°C)	$T_{50\%}$ (°C)	Residue at 700 °C (%)
PUAcr-1	185	295	395	5.6
PUAcr-2	193	280	367	4.1
PUAcr-3	160	275	380	6.6
PUAcr-4	175	300	405	4.8
PUAcr-5	165	277	395	6.4
PUAcr-6	190	282	390	4
PUAcr-7	197	300	410	6.4
PUAcr-8	200	290	395	5.6
PUAcr-9	205	295	415	5.9
PUAcr-10	195	300	395	5.3

3.3. Mechanical characteristics

An important feature of the polymeric coatings assumes achieving good mechanical properties, advantageous for biomedical and food packaging applications. For this reason, the mechanical behaviour of the above polymeric composites was analysed comparatively with that of the pure polyurethane acrylates. Consequently, we have determined the Young modulus, maximum tensile strength and elongation at break for the polyurethanes and hybrid composites, the obtained data being illustrated in Figure 3.

As can be observed from the figure, the polyurethane precursor PU-PR3 having an elongation at break of 500 % proved to be more flexible than the precursors based on PBA (PU-PR1 and PU-PR2 with an elongation at break of 150 % and 300%, respectively), tendency maintained for the polyurethane acrylates and their composites. Thus, the inclusion of acrylic acid sequences into the polyurethane matrix (in the case of PUAcr-1, PUAcr-3 and PUAcr-5) determined a decrease of the tensile strength accompanied by an increase of the elongation at break, while the use of UMA-1 derivative as bifunctional monomer causes an increase in stiffness of the polymeric matrix, reflected into an increase of the tensile strength (for the PUAcr-2, PUAcr-4 and PUAcr-6 samples).

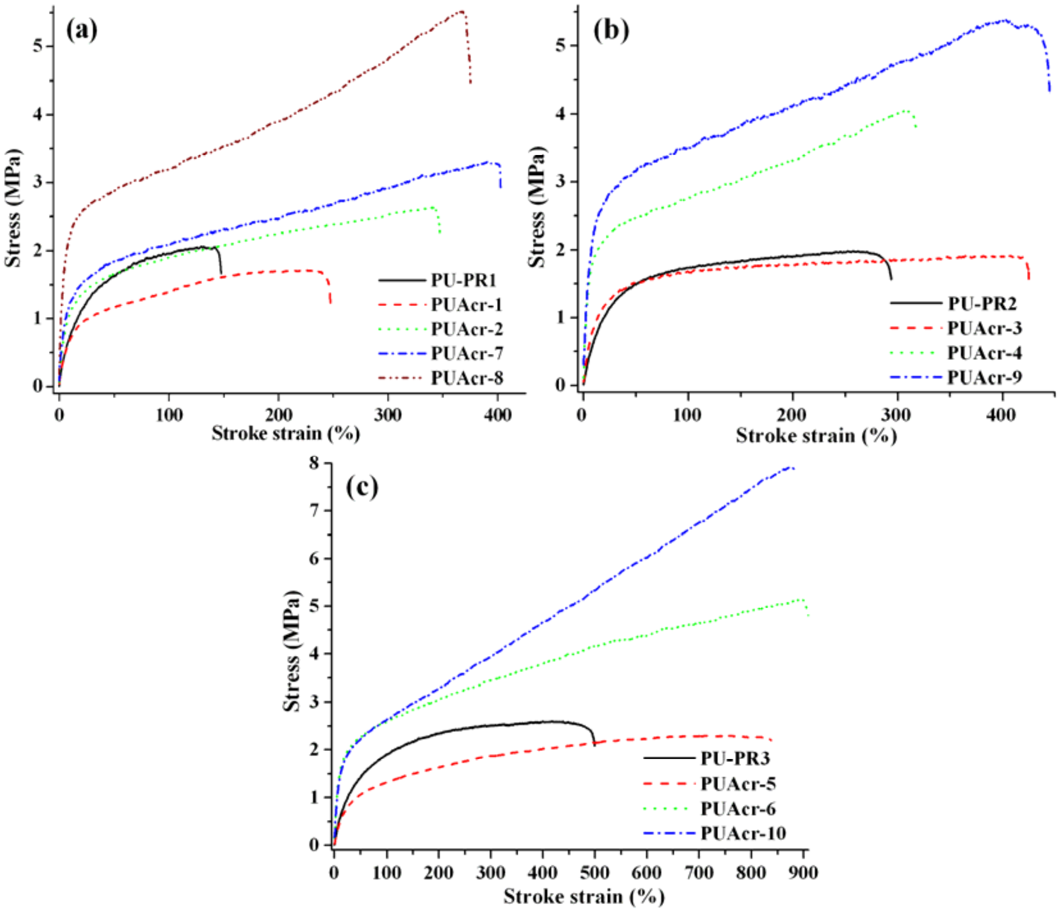


Fig. 3. Stress-strain curves for the polyurethane precursors, polyurethane acrylates and silver composites.

For the hybrid composites, the presence of the silver nanoparticles produced an improvement of mechanical properties as compared to the samples without silver, suggesting that the inorganic component had indeed a reinforcement effect on the polymeric substrate.

3.4. Antibacterial testing

The antimicrobial activity of the silver nanoparticles in polymeric matrix proved to be of great interest for the prevention of adherence and proliferation activities of some bacteria on the materials surface [23, 24]. The antibacterial efficiency of the polyurethane acrylate/Ag composites against Gram-negative or Gram-positive bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were assessed by zone of inhibition testing method. In fact, the hybrid composites exhibited significant efficacy against bacteria of Gram class. The pictures displayed in Figure 4 indicate that after 24 h of incubation, the zones of inhibition for the polyurethane acrylates/silver composite PUAcr-10 versus bacteria are significantly outlined (8.5 – 11 mm).

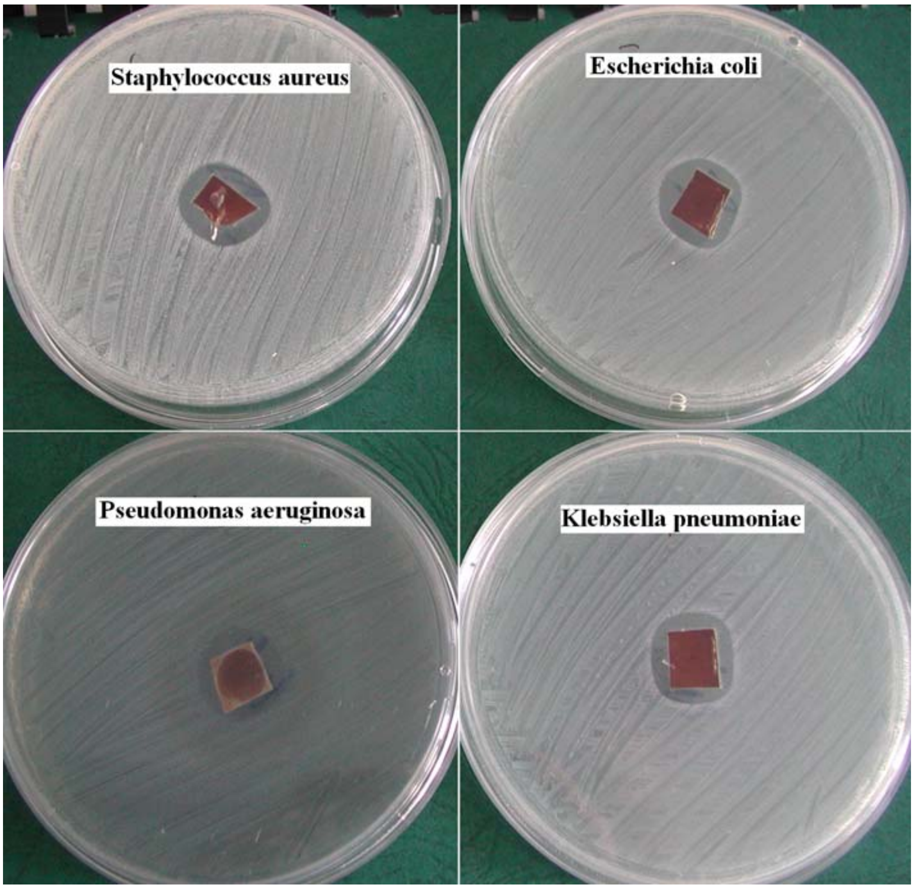


Fig. 4. Antibacterial activity results for silver composite PUAcr-10 films against S. aureus, E. coli, P. aeruginosa, and K. pneumoniae

It can be seen that the lower inhibition zone was obtained in the case of *P. aeruginosa* (about 8.5 mm), whereas the inhibition halos measured for *S. aureus, E. coli* and *K. pneumoniae* are higher signifying that for these situations, the silver composite showed improved bactericidal activities. Therefore, the existence of silver nanoparticles embedded into the polymeric matrix demonstrated to be effective in preventing bacterial growth. Although the exact role of silver nanoparticles in the immediate surrounding of the composite film is not very clear, the literature data explain the huge zones of inhibition produced by other films through the same mechanism of release of silver, which implies a oxidation reaction at the nanoparticle surface and diffusion of the silver ions thus causing structural changes and finally, bacterial death [25]. In agreement with these results, the above hybrid composites prepared by *in situ* polymerization/electron transfer reaction manifest a real potential for antimicrobial coatings in a wide variety of bioapplications.

4. Conclusions

In this study, novel flexible polyurethane-acrylate coatings were prepared by the *in situ* polymerization of some mono(di)acrylates into a polyurethane matrix. These dual materials exhibited remarkable improved mechanical properties, comparatively with the pristine polyurethanes, in particular excellent elongation at break. The coupling of polymeric matrix formation with the *in situ* electron transfer reactions leads to silver hybrid composites, for which the EDX analysis indicates that the silver particles are well dispersed in the polymeric matrix, whereas the UV-vis confirms their nano-sized character. Additionally, the incorporation of silver particles into polyurethane acrylates films improved their thermal stability and elongation properties, whereas the silver particles released from composites present antibacterial activity against *S. aureus, E. coli, P. aeruginosa,* and *K. pneumoniae*. Therefore, these materials have great potential for utilization in the fabrication of flexible antibacterial coatings and textiles.

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