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Graphical abstract

Nanostructured polymer composites with potential applications into the storage of blood and hemoderivates

Abstract

AgNPs appears to be a useful alternative to confer antibacterial properties to PVC by its physical inclusion in the polymer phase; however, AgNPs are materials with hemolytic properties and its direct addition for antimicrobial control of blood and hemoderivates is inadequate. The objective of this work was to develop nanostructured PVC and evaluate its application for the improving of bio-security during the storage of blood and hemoderivates. For that, different AgNPs were synthesized by chemical reduction using different stabilizing agents (N-methyl-D-glucamine, cysteamine and cysteine); in addition, poly(allyl chloride) (PACl) was synthesized by radical polymerization of allyl chloride. Later, PACl was functionalized using different polymer:AgNPs ratios in order to produce hybrid nanostructured PACl (PAClne). Composites of PVC and PAClne were

Keywords

Nanoparticle
Bacteriemia
Blood
Poly(vinyl chloride)

obtained by dissolution-evaporation using cyclohexanone as solvent. Products were characterized by different techniques and antimicrobial and hemolytic properties were evaluated. Results suggest that AgNPs can be functionalized and capped with PACl for an adequate exchange of dispersion phases. Besides, for PVCne, viscosity and mechanical properties are not significantly affected respect to PVC (concentration of PAClne < 2.0 wt%). PVCne no showed hemolytic activity evidencing that can be used for applications with direct contact with blood. Antimicrobial properties against *E. coli* were limited suggesting a low migrability of AgNPs in the polymer phase.

Compositos poliméricos nanoestructurados con potenciales aplicaciones en el almacenamiento de sangre y hemoderivados

Resumen

Las nanopartículas de plata (AgNPs) son una alternativa útil para otorgar propiedades antibacteriales al PVC mediante su inclusión en la fase polimérica; sin embargo, AgNPs son materiales con propiedades hemolíticas y no pueden usarse directamente para el control de inmunohematológico de la sangre y hemoderivados. El objetivo de este trabajo fue desarrollar PVC nanoestructurado y evaluar su aplicación para el mejoramiento de la bioseguridad durante el almacenamiento de sangre y hemoderivados. Para eso, diferentes nanopartículas de plata se sintetizaron mediante reducción química usando diferentes agentes estabilizantes (N-metil-D-glucamina, cisteamina y cisteína); Además, poli(cloruro de alilo) (PACl) se sintetizó mediante polimerización radical de cloruro de alilo. Después, PACl se funcionalizó usando diferentes relaciones de polímero:AgNPs con el fin de obtener PACl nanoestructurados híbridos (PAClne). Compositos de PVC y PAClne se obtuvieron mediante disolución-evaporación usando ciclohexanona como solvente. Los productos se caracterizaron por diferentes técnicas y sus propiedades antimicrobianas y hemolíticas se evaluaron. Los resultados sugieren que las AgNPs pueden funcionalizarse y recubrirse con PACl para lograr un intercambio adecuado en la fase dispersa. Además, para el PVCne, la viscosidad y las propiedades mecánicas no se afectaron significativamente respecto al PVC (a concentraciones de PAClne < 2.0 %p/p). PVCne no mostró actividad hemolítica evidenciándose que estas pueden usarse para aplicaciones donde esten en contacto directo con la sangre. Las propiedades antimicrobianas contra *E. coli* se vieron limitadas, sugiriendo una baja migrabilidad de las AgNPs al estar en el interior de la fase polimérica.

Palabras claves

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Nanostructured polymer composites with potential applications into the storage of blood and hemoderivates

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Resumen

AgNPs appears to be a useful alternative to confer antibacterial properties to PVC by its physical inclusion in the polymer phase; however, AgNPs are materials with hemolytic properties and its direct addition for antimicrobial control of blood and hemoderivates is inadequate. The objective of this work was to develop nanostructured PVC and evaluate its application for the improving of bio-security during the storage of blood and hemoderivates. For that, different AgNPs were synthesized by chemical reduction using different stabilizing agents (N-methyl-D-glucamine, cysteamine and cysteine); in addition, poly(allyl chloride) (PACl) was synthesized by radical polymerization of allyl chloride. Later, PACl was functionalized using different polymer:AgNPs ratios in order to produce hybrid nanostructured PACl (PAClne). Composites of PVC and PAClne were obtained by dissolution-evaporation using cyclohexanone as solvent. Products were characterized by different techniques and antimicrobial and hemolytic properties were evaluated. Results suggest that AgNPs can be functionalized and capped with PACl for an adequate exchange of dispersion phases. Besides, for PVCne, viscosity and mechanical properties are not significantly affected respect to PVC (concentration of PAClne < 2.0 wt%). PVCne no showed hemolytic activity evidencing that can be used for applications with direct contact with blood. Antimicrobial properties against *E. coli* were limited suggesting a low migrability of AgNPs in the polymer phase.

Palabras Claves

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1. Introduction

Polymers have many medical and biomedical applications as a result of low toxicity, adaptability of their properties, biocompatibility and chemical stability. Thus, biopolymers as chitosan, collagen, poly(lactic acid) and poly(L-lysine) have been used for drug controlled release and tissue engineering

[1-2]. On the other hand, vinyl polymers have been the focus of many studies and applications as fabrication of surfaces for hospitals and specialized equipments, surgical instruments, medical textile and container for residual elements, medical materials and fluid storage [3].

In the case of storage of blood and hemoderivates, different stages related with extraction, transfusion

and conservation of these fluids are usual processes with great importance during many medical procedures. However, bacterial contamination can occur during these procedures affecting unavoidably the patient health [4-7]. The prevalence and impact of severe episodes of transfusion-associated bacterial contamination has not been clearly established, however, some authors suggest that can be 1:50000 per platelet unit and 1:500000 per red cell unit transfused [4].

At the present, genotoxic photosensitizing agents are used in order to minimize the effects caused by septic infections transmitted by bacteria. Some photosensitizers as amotosalen-HCl, riboflavin, combination of amotosalen-HCl with glutathione and methylene blue, are used with different light source to produce their activation [8-11]. However, the performing of toxicological studies is difficult and these cannot be efficient to identify genotoxicity events since in many situations can only be detected during long-time observation. In addition, all systems for bacterial elimination of blood products are under intensive development and are not yet routinely available [11].

Here, we proposed the development of nanostructured polymer materials with inherent antibacterial properties for the elimination of bacterial pathogen agents in order to improve bio-security controls in the storage of blood and hemoderivates. Thus, at the present, blood bags are manufacturer using mainly poly(vinylchloride) (PVC) which is plasticized with DOP (dioctylphthalate) to improve their plastic properties. However, though the PVC has been identified to be an excellent material for manufacture of blood bags, is completely innocuous against bacteria.

On the other hand, silver nanoparticles (AgNPs) are nanostructured materials with antimicrobial properties against Gram-positive and Gram-negative bacteria [12-15]; many reports about antibacterial capacity of AgNPs have been recently published [12-15]. Thus, AgNPs appears to be an alternative to give antibacterial properties to PVC by their inclusion in the polymer phase. However, a problem technique is the adequate compatibilizing of nanoparticle phase (which uses commonly hydrophilic stabilizing agent) and polymer phases (which is highly inert, with hydrophobic feature and low solubility in many typical solvents).

The objective of this work was to develop nanostructured polymer materials and evaluate their

application for the improving of bio-security control during the storage of blood and hemoderivates.

2. Experimental section

2.1 Reagents and materials

Silver nitrate (AgNO_3 , 98 %, Aldrich), sodium citrate (99 %, Aldrich), sodium borohydride (Aldrich) and N-methyl-D-glucamine (NMDG, 99 %, Aldrich) were used as reducing and stabilizing agents. In addition, cysteine (cys, Aldrich) and cysteamine (cysa, 98 %, Aldrich) were used as functionalizing agents. Allyl chloride (ACl, Aldrich) and benzoyl peroxide (Aldrich) were used in the synthesis of poly(allyl chloride) (PACl). Commercial PVC (0.22 % of DOP) was used as polymer phase for its nanostructuration. Acetone (Aldrich) and cyclohexanone (Merck) were used as solvent of polymers.

Barium sulfate (Merck), sulfuric acid (Merck) and agar Mueller-Hinton (Aldrich) were used in the antimicrobial test against *E. coli*. DPBS (Dulbecco's Phosphate Buffered Saline), KCl (Aldrich), KH_2PO_4 (Aldrich), NaCl (Aldrich), KCN (Aldrich), $\text{K}_3\text{Fe}(\text{CN})_6$ (Aldrich) and NaHCO_3 (Aldrich) were used for hemolytic test. Bidistilled water was used in all cases.

2.2 Synthesis and functionalization of AgNPs

AgNPs were synthesized by two methodologies based on chemical reduction of silver atoms which have been previously described [16-17]. First one, 100 mL of 1.0 mmolL^{-1} AgNO_3 solution was heated to boiling under reflux and constant stirring, after, slowly 1.0 mL of $1.0 \text{ mg}/100 \text{ mL}$ of sodium citrate solution and 1.0 mL of 400 mmolL^{-1} of NMDG solutions were added drop to drop and in that order; reaction time was one hour.

On the other hand, for the second methodology, 250 mL of 1.0 mmolL^{-1} of NaBH_4 solution was iced using an ice bath for 15 minutes. Later, 88.33 mL of AgNO_3 solution (1.0 mmolL^{-1}) was added drop to drop (reaction time was one hour). For functionalization of AgNPs, 1.0 mL of AgNPs was mixed separately with 10.0 mmolL^{-1} cys and cysa solutions (1:1 NP/ligand ratio). Mixtures were continuously stirred for 30 minutes. AgNPs were characterized by dynamic light scattering (DLS, Zetasizer Nano ZS90) and ultraviolet-visible

spectroscopy (UV-vis, Shimadzu UV-1700, PharmaSpec).

By procedure described above, three types of functionalized AgNPs were obtained: AgNPs-glu, AgNPs-cys and AgNPs-cysa which correspond to NPs functionalized with NMDG, cys and cysa, respectively. AgNPs stabilized with citrate are denoted AgNP-cit.

2.3 Synthesis of poly(allyl chloride) (PACl)

10.0 mL of ACI and benzoyl peroxide (2 % molar with respect to moles of monomer) were mixed under continuous stirring. Reaction was activated using ultrasound for 30 minutes under N_2 atmosphere and after heated at 40 °C for 24 hours using an ethylene glycol bath. Unreacted monomer was eliminated by heating at 60 °C and polymer was washed with acetone. Finally, PACl was characterized by UV-vis and Fourier-transformed infrared spectroscopy (FT-IR, Shimadzu, FT-IR 8400).

2.4 Nanostructuration of PACl

PACl was dissolved in acetone (2.6 molL⁻¹) and mixed with different volumes of AgNP dispersion (1:1, 1:4 and 1:6 of PACl:AgNPs volumetric ratio). Nanostructured PACl (PAClne) was characterized by elemental analysis, FT-IR and UV-vis. It was selected the 1:6 ratio to permit the highest incorporation of AgNPs.

2.5 Obtaining of nanostructured PVC (PVCne)

PVCne was obtained by mixed of PVC and low amounts of PAClne. For that, 3.6 g of PVC in acetone was separately mixed with 0.168, 0.336 and 0.672 g of PAClne previously dissolved in acetone (i.e., 0.5, 1 and 2 wt% of PAClne). Mixtures were placed in aluminum containers and solvent was eliminated by heating at 45 °C. PVCne was characterized by IR-FT, tensile test (using a universal machine Tinius Olsen model H50KS according to ASTM D638-14 [18]) and by capillary viscosimetry in ciclohexanone solutions.

2.6 Antibacterial test

In order to determine the antibacterial property of material, 5 mm x 5 mm of PVCne was evaluated against *E. coli* using turbidimetric method [19].

Tryptic soy was used as liquid culture medium. Absorbance was measured at 625 nm and turbidity standards were prepared from barium chloride solution (0.048 molL⁻¹) and sulfuric acid (0.18 molL⁻¹). Thus, by McFarland scale, bacterial inoculums were specified to be 0.5 CFU/mL (CFU is colony-forming unit). 10.0 μ L of bacterial dispersion was added to 7.0 mL of culture medium previously sterilized and contacted with the PVCne sample. Finally, system was incubated at 37 °C for 24 hours. Analyzed samples were AgNPs (positive control), PVC (negative control) and PVCne (samples). All experiments were performed by triplicate.

2.7 Hemolytic activity test

Hemolytic test was carried out by cyanmethemoglobin method according ASTM F756-13 and Dobrovolskaia et al., (2008) [20-21]. Thus, 3.5 mL of blood sample was dissolved in 7.0 mL of 10 mgmL⁻¹ of DBPS solution and contacted with PVCne samples (0.5 mm x 0.5 mm). Mixture was incubated at 37 °C for 4 hours. Later, samples were spin-dried at 150 gravities for 15 minutes. Supernatant was mixed with Drabkin's reagent and hemoglobin was quantified by UV-vis at 540 nm. Calibration curve was performed in the range of 0.1 and 10 mgmL⁻¹ of hemoglobin standard at the same experimental conditions. Sorbitol and acetic acid were used as negative and positive controls.

3. Results and discussion

3.1 Synthesis of AgNPs

In the Figure 1 are shown surface plasmon bands and size distributions for functionalized AgNPs. It can be seen from UV-vis spectra, at ~420 nm, the absorption band of surface plasmon associated with AgNPs. These results suggest that AgNPs were satisfactorily synthesized, and in addition, it is concluded that the different functionalization procedures no affected the "nanosize" features of particle dispersion (see Figure 1a-1d). Surface plasmon resonance can be explained by resonant oscillation of conduction electrons as a result of incident radiation. For AgNPs maximal absorbance is displaced to the right with the increase of size (small AgNPs with a size around ~10 nm have a maximal absorption at $\lambda \sim 450$ nm and this is

increased with the increase of size, being more wide as higher is the size distribution) [22].

On the other hand, from results of DLS can be seen that the size of AgNPs-cit and AgNPs-glu were 14-459 nm and 4-615 nm, respectively (see Figure 1e-h). In addition, bimodal size distributions were obtained with maximal frequencies of 30 and 190 nm for AgNPs-cit and 8 and 142 nm for AgNPs-glu. For AgNPs functionalized with cys and cysa, particle sizes were in the range between 78 and 458 nm (with monomodal size distribution for AgNPs-

cysa); whereas for AgNPs-cys were 190 and 6400 nm (bimodal size distribution with maximum values of 200 and 2500 nm). Bimodal distribution can be explained by the presence of two species of silver atoms (ions Ag^+ and complexes of citrate-Ag by interaction of carboxylic acid groups with silver atoms) [23].

In general terms, mean size of AgNPs showed the following order: AgNPs-cit < AgNPs-glu < AgNPs-cysa and AgNPs-cys.

Figure 1. Plasmon bands and size distribution for functionalized AgNPs

Figure 2. UV-vis absorption of PACI

3.2 Synthesis and nanostructuration of PACI

For synthesis of PACI was obtained a low reaction yields (10 %w/w). Low reaction yield can be explained by chain transfer reactions that produce macroradicals stabilized by resonance. The formation of these highly stable macroradicals limits the propagation stage and decreases the polymerization grade [24]. These macroradicals can be evidenced by the UV-vis absorption related with the presence of conjugated double bonds of monomer (see Figure 2).

It has been reported that chain transfer reactions promote the fragmentation of large chains producing smaller PACI chains, in consequence, additional experiments of polymerization in solution using dioxane as solvent, and polymerization in bulk, at different reaction times,

Figure 3. FT-IR spectra of PACl and ACI

Figure 4. FT-IR spectra of PAClne with AgNPs-glu (a), AgNPs-cys (b) and AgNPs-cysa (c)

were performed (2, 7 and 24 hours), however, reaction yields were very small and are here not discussed (yields < 7 %).

In the Figure 3 can be seen the FT-IR spectra of PACl and ACI. Vibrations at 2940 and 890 cm^{-1} are associated with C-H and C-Cl bonds of PACl and ACI, respectively. In addition, vibrations of PCIA at 1757 cm^{-1} are associated with conjugated system C=C, this vibration is congruent with absorption band identified by UV-vis.

In addition, FT-IR spectra of PAClne with AgNPs-glu, AgNPs-cys and AgNPs-cysa are shown in the Figure 4. Absorption at 3288 cm^{-1} was associated with vibrations of -OH groups on NMDG. In addition, for all NPs, vibrations at ~893 and ~1755 cm^{-1} were associated with C-Cl bonds and conjugated C=C double bonds, respectively.

PAClne was indirectly verified by disappearing of plasmon band in remaining solution after to ligand exchange reaction between AgNPs-cys or AgNPs-cysa with PACl in solution (see Figure 5). Besides, it was seen the formation of precipitate with different coloring.

On the other hand, elemental composition of different PAClne evidences the inclusion of AgNPs (see Table 1). For PAClne nanostructured with AgNPs-glu can be seen an increase of nitrogen percent as AgNPs amount was increased. Nitrogen percent is directly related with the presence of high hydrophilic units of NMDG in the polymer phase (hydrophobic phase). For PAClne with AgNPs-cysa and AgNPs-cys can be seen an increase of nitrogen and sulfur percentages as AgNPs amount was increased; these nitrogen and sulfur atoms are directly related with cysa and cys molecules on AgNPs.

Figure 5. Disappearing of plasmon band in remaining solution after to ligand exchange reaction: (a) PACl and AgNPs-cysa and (b) PACl and AgNPs-cys; in addition, (i) before and (ii) after of addition of PACl

Table 1. Elemental analysis of PACI and PCIAne with AgNPs-cys, AgNPs-cysa and AgNPs-glu

Sample composition	PACI:AgNPs	% N	% C	% H	% S
PACI	1:0	0.00	66.44	4.55	0.00
PCIA + AgNPs-glu	1:1	0.10	61.01	4.43	0.00
	1:4	0.56	53.96	5.14	0.00
	1:6	0.55	58.30	4.66	0.00
PACI + AgNPs-cysa	1:1	0.42	62.25	4.46	0.31
	1:4	1.12	85.35	0.72	0.97
	1:6	1.96	50.26	4.75	2.45
PACI + AgNPs-cys	1:1	0.39	62.36	4.55	0.43
	1:4	0.67	60.67	4.41	1.01
	1:6	1.71	55.38	4.63	2.00

Table 2. Intrinsic viscosities (η) for PVC and PVCne at different concentrations

NPs	PACIne (%)	PVCne (g/mL)			R_h (nm)
		0.001	0.010	0.020	
PVC without AgNPs	0.0	25.87 $\pm$ 6.31	45.54 $\pm$ 0.41	48.48 $\pm$ 0.44	3.7
AgNPs-NMDG	0.5	47.37 $\pm$ 22.41	49.38 $\pm$ 1.13	46.62 $\pm$ 0.54	5.8
	1.0	57.81 $\pm$ 9.70	49.34 $\pm$ 0.35	47.90 $\pm$ 0.13	7.0
	2.0	47.89 $\pm$ 5.89	48.33 $\pm$ 0.43	49.69 $\pm$ 0.11	6.0
AgNPs-cys	0.5	31.29 $\pm$ 12.35	48.00 $\pm$ 0.72	45.96 $\pm$ 0.13	1.9
	1.0	39.61 $\pm$ 11.37	49.10 $\pm$ 1.06	47.03 $\pm$ 0.27	5.1
	2.0	45.37 $\pm$ 8.77	47.63 $\pm$ 0.44	47.52 $\pm$ 0.19	5.8
AgNPs-cysa	0.5	49.24 $\pm$ 16.04	52.00 $\pm$ 0.40	50.05 $\pm$ 0.22	6.1
	1.0	62.40 $\pm$ 9.11	55.09 $\pm$ 0.91	49.36 $\pm$ 0.23	7.4
	2.0	85.72 $\pm$ 6.71	53.67 $\pm$ 1.28	49.68 $\pm$ 0.20	9.0

3.3 Nanostructuration of PVCne

PVCne was obtained by mixture of PVC and PACIne by dissolution-evaporation method using ciclohexanone as solvent. Intrinsic viscosities (η) for PVC and PVCne at different concentrations are shown in the Table 2. It can be seen that intrinsic viscosity of PVCne was increased with respect to PVC in all cases and with the increase of PVCne concentration. However, the increase in function of concentration was lower at highest concentration of PVCne (*i.e.*, at 0.020 g/mL⁻¹ of PVCne).

In addition, at low concentrations, *i.e.*, 0.001 mg/L⁻¹ of PVCne, the effect of PACIne percent on viscosity was negligible for AgNPs-glu and AgNPs-cys. In the case of AgNPs-cysa a significant change as a function of PACI percent was evidenced.

From molecular simulations and experimental results, it has been concluded that small changes in the particle size of filler particles can to produce changes in the dependence of concentration with the viscosity. However, when particles with a similar

size as the polymer beads are added, particles act as a solvent or a plasticizer and the viscosity decreases, but if added particles are only slightly larger than two times the size of the polymer beads, the viscosity increases with increasing of filler concentration [25].

In our case, mean hydrodynamic radius of AgNPs is larger than hydrodynamic radius of polymer (for PVC used in our experiments hydrodynamic radius was 3.7 nm), therefore, the increase in the viscosity obtained by us is congruent with previous studies. Values of hydrodynamic radius shown the following order PVCne with AgNPs-cysa > PVCne with AgNPs-glu > PVCne with AgNPs-cys > PVCne, in consequence, a major separation of molecules of PVCne in ciclohexanone was evidenced. In addition, these results are congruent with the mean hydrodynamic size determined for AgNPs: AgNPs-cysa > AgNPs-glu > AgNPs-cys. Thus, results suggest that by a rigorous control of nanoparticle size is possible to decrease the changes in the initial polymer matrix.

Table 3. Mechanical properties of PVCne (TS: tensile strength, MS: Maximum strain and YM: Young's modulus)

AgNPs	PAClne (%)	TS (MPa)	MS (%)	YM (MPa)
---	0.0	10.3 ± 1.2	685.04 ± 0.0	1.8 ± 0.1
AgNPs-cys	0.5	8.3 ± 0.8	703.0 ± 36.5	1.7 ± 0.1
	1.0	11.0 ± 0.6	690.1 ± 59.2	2.2 ± 0.1
	2.0	9.2 ± 1.2	649.9 ± 47.9	2.0 ± 0.4
	2.0	9.0 ± 1.1	723.9 ± 36.7	1.8 ± 0.3
AgNPs-cysa	0.5	8.3 ± 0.8	703.0 ± 36.6	1.7 ± 0.2
	1.0	7.2 ± 0.3	697.7 ± 42.6	1.7 ± 0.2
	2.0	8.0 ± 1.0	719.7 ± 55.1	1.8 ± 0.5
AgNPs-NMDG	0.5	7.1 ± 0.4	564.0 ± 54.4	1.7 ± 0.1
	1.0	9.3 ± 1.1	748.0 ± 23.4	2.1 ± 0.5
	2.0			

Another aspect to take account is the dispersion of particles in the phase of PVC, thus, an increase of viscosity of nanocomposites with increasing on dispersion of the filler particles has been reported [25]. In addition, results show that flowability of PVC is affected by nature of PAClne in the following order AgNPs-cysa > AgNPs-glu > AgNPs-cys. This behavior could be associated with the chain size of functionalization agents which increase cys > NMDG > cysa (for NMDG, polyol chain can be adsorbed on AgNP surface whereas cys and cysa are linked to the surface by sulfur of thiol group).

Results of mechanical properties of PVCne are shown in the Table 3. In general, a decrease of tensile strength with respect to PVC was observed; however, differences related with nature of AgNPs were not evidenced. In addition, whereas values of maximum strain no showed a clear tendency, values of Young's modulus showed small changes for the highest PAClne concentration (*i.e.*, 2.0 wt%). It has been reported that at low incorporation ranges (~ 5 wt%) nanocomposites exhibit a significant improvement in tensile strength and elastic modulus [26]. Besides, dispersion of an inorganic nanoparticle filler in PVC is not easily achieved because nanoparticles have a strong tendency to agglomerate and to reduce their surface energy. Thus, grafting polymers, dispersions of sterically stabilized nanoparticles and in situ polymerization have been used in order to avoid the aggregation of nanoparticles in polymer phase evidencing this tendency [27].

It is clear that an important factor in nanocomposite properties is the dispersion of the AgNPs in the PVC matrix. At lower concentrations, experimental

scatter in large enough to produce only small differences in Young's modulus [28]. Another important factor which affects the Young's modulus is the heterogeneity resulting of aggregates and that is increased with concentration [27-28].

Results suggest that the addition of PAClne with AgNPs-glu, AgNPs-cys and AgNPs-cysa to PVC no produce significant changes in the fluidity properties or in the mechanical properties at low concentrations (< 2.0 wt%). In consequence, it is concluded that composites PVC-PAClne maintain the same processability properties than PVC and an adequate dispersion of AgNPs was achieved.

3.4 Tests of antibacterial and hemolytic activities

Results of antibacterial and hemolytic tests are shown in the Tables 4 and 5, respectively. Thus, antibacterial activity on surrounding was not observed for PVCne. Note that, in this experiment an intrinsic antibacterial activity is not identified, in consequence, PVCne could have antibiofouling properties but it cannot be concluded from our results. However, results indicate that AgNPs have a low migrability and these are completely occluded in the polymer phase. Only a weak AgNPs fraction could be on the surface in direct contact with solution and, in consequence, effective concentration is decreased. In addition, results suggest that functionalization and covering with PACl avoids that ions Ag⁺ can be released.

On the other hand, from results of hemolytic activity is concluded that PVCne is compatible with blood and this could be used for initial application. However, absence of antimicrobial activity could mean in a limitation of application in the storage of

Table 4. Results of antibacterial test (inhibition: +, growth: -). Positive control (26 CFUx10⁸) and negative control (Inhibition 100%)

Sample	AgNPs type	PAClne (%)	CFU (x10 ⁸)	Inhibition (%)	Results
PVCne with	AgNPs-cys	0.5	25	7	+
		1.0	25	4	+
		2.0	26	3	+
	AgNPs-cysa	0.5	26	3	+
		1.0	26	1	+
		2.0	26	0	+
	AgNPs-NMDG	0.5	25	6	+
		1.0	25	5	+
		2.0	24	12	+

Table 5. Results of hemolytic test (absence of hemolysis: -, hemolysis: +)

Sample	AgNPs type	PAClne (%)	Hemoglobin (mgmL ⁻¹)	Hemolysis (%)	Results
Positive control	---	0.0	0.03 ± 0.08	0.5	-
Negative control	---	0.0	7.50 ± 2.20	118.2	+
PVCne with	AgNPs-cysa	0.5	0.02 ± 0.01	0.3	+
		1.0	0.00 ± 0.02	0.0	+
		2.0	0.00 ± 0.05	0.5	+
	AgNPs-cys	0.5	0.12 ± 0.72	1.9	+
		1.0	0.04 ± 0.03	0.6	+
		2.0	0.00 ± 0.01	0.0	+
	AgNPs-NMDG	0.5	0.04 ± 0.01	0.6	+
		1.0	0.01 ± 0.04	0.2	+
		2.0	0.00 ± 0.90	0.2	+

blood, but it is important to take account that this work was limited to low concentration of PAClne, in consequence, effect at high concentration of PAClne in the PVC is not clear from results here showed.

4. Conclusions

AgNPs can be easily synthesized by chemical reduction and functionalized with NMDG, cys and cysa. Mean size of AgNPs showed the following order: AgNPs-cit < AgNPs-glu < AgNPs-cysa and AgNPs-cys. Besides, PACl can be synthesized by free radical polymerization in absence of solvent with a yield near at 10 %. Thus, PAClne can be obtained at room temperature by mixing of AgNPs-glu, AgNPs-cys and AgNPs-cysa; in a second stage, PVCne can be obtained by dissolution-evaporation of PVCne added to PVC solutions using ciclohexanone as solvent. For PVCne, viscosity and mechanical properties are not significantly affected respect to PVC (concentration of PAClne < 2.0

wt%). PVCne no showed hemolytic activity evidencing that can be used for applications related with the direct contact with blood or hemoderivates. Antimicrobial properties against *E. coli* were limited suggesting a low migrability of AgNPs in the polymer phase.

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Interest conflict

No interst conflict

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