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PVC silver zeolite composites with antimicrobial properties

Daniela Zampino · Tiziana Ferreri · Concetto Puglisi · Monique Mancuso · Renata Zaccone · Roberto Scaffaro · Danila Bennardo

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Abstract Poly(vinyl chloride) (PVC) composites containing increasing amounts (2–20%, w/w) of silver zeolite (SZ) were prepared by melt mixing and characterized by thermal, mechanical and rheological analyses. The addition of large amount of SZ did not influence the processability and the formability of the composites, if compared to neat plasticized PVC. The antibacterial activity of PVC SZ composites was tested on *Escherichia coli* and *Staphylococcus epidermidis* and resulted promising both in culture broth and on agar plate and also in sterile urine seeded with these strains, for simulation purposes. In sterile urine, composites induced a significant reduction (4–6 log units) of viability of both strains already at 24 h, inhibiting *E. coli* growth up to 20 days, whereas their antimicrobial action against *S. epidermidis* vanished within 5 days. The silver release in sterile urine was determined up to 20 days. It was found that the highest amount of silver ions was released during the first day (0.365 ppm), whilst from days 6 to 20 the silver release decreased, reaching a steady daily mean value of 0.02 ppm.

D. Zampino (✉) · T. Ferreri · C. Puglisi
Institute of Chemistry and Technology of Polymers, CNR,
Via Paolo Gaifami 18, 95126 Catania, Italy
e-mail: zampino@ictmp.ct.cnr.it

M. Mancuso · R. Zaccone
Institute for Coastal Marine Environment, CNR,
98122 Messina, Italy

R. Scaffaro
Department of Chemical, Process and Materials Engineering,
UNIPA, 90128 Palermo, Italy

D. Bennardo
Milena Pharmaceutical Srl, 92100 Agrigento, Italy

Introduction

During the last decades, the growing demand of materials with antimicrobial properties has produced an increasing research interest both in academy and industry to the development of antimicrobial thermoplastics [1–6]. The main polymeric matrices used for the production of antimicrobial materials ranged from polyolefins to polyesters, polyurethanes and silicon polymers [1, 6–21], together with other materials including glass, titania nanofibers, etc. [22, 23].

The large increase in the number and occurrence of antibiotic-resistant bacterial strains has prompted a renewed interest in the use of silver as an antibacterial agent. From this point of view, the incorporation of active metals into polymer matrices still represents an effective method to develop polymers with antimicrobial properties.

Silver is a metal with well-known antimicrobial properties. At low concentrations, it does not cause toxicity and its use can reduce the resistance and sensibilization problems due to resistant bacteria [24–26]. Its activity influences bacteria growth of a broad spectrum of bacterial strains on medical and industrial processes [2, 6, 11, 25], inhibiting the transport functions in the cell wall (respiration) and the cell division (reproduction) and interrupting the cell energy generation (metabolism) [27–29].

Though recently argyria cases and a silver concentration-dependent toxicity have been demonstrated, silver has not been listed amongst the most prevalent hazardous heavy metals to public health [2, 25, 27].

Conventional approaches involving the deposition of metallic silver directly onto the surface of a substrate by vapour deposition, sputtering, ion beam coating and electrochemical deposition from solution have been developed [8, 30–34]. However, these coating techniques generally

suffer from poor adhesion onto surface and coating uniformity and therefore special and time consuming processing conditions or special techniques for the surface preparation are needed [20].

The incorporation of silver into molten polymers is another conventional approach to obtain antimicrobial polymer composites. Consequently, silver ions become available on both inner and outer surfaces of composites that could experience contact with infective agents present in blood or other body fluids, like in the case of indwelling catheters and others medical devices [20].

Silver zeolites are compounds containing elemental silver, ionically bound to biocompatible and bioinert ceramic mineral molecules of aluminosilicates. Silver zeolite crystals result randomly oriented and distributed through the surface of polymers or coatings, thus providing silver ions release independently on the particle orientation in the substrate. The microbiocide effect of zeolite crystal carrier depends on silver ions that are tightly bonded to zeolite and released only by active ion-exchange mechanism in biological environments. In fact, under conditions that support the bacterial growth, the sodium ions, in wet environments, exchange with silver ions at reversible bonding sites on the zeolite, with a consequent microbial growth control [35–37].

Recently, they have been extensively used in various technological fields, such as water treatment, food packaging, plastic handles for food service cutlery, hospital equipments, healthcare furnishings, medical packaging and cosmetics [2, 4, 35, 37–49]. Nevertheless, at the authors' best knowledge, very few information is available in the scientific literature about their use in plasticized poly(vinyl chloride) (PVC).

The PVC is widely used for different industrial applications (building products, cosmetics, medical and paramedical items) for its ease of processing, compatibility with different additives and versatility. In medical and paramedical fields, it is extensively used to produce a lot of products, such as equipments for blood transfusion, medical devices, body fluids collection and enteral feeding products. As a consequence, the availability of antimicrobial plasticized PVC is undoubtedly important for health care applications.

In this study, we prepared and characterized antimicrobial PVC silver zeolite composites to prevent the bacterial colonization on polymeric surfaces of biomedical products.

Experimental

Materials

Pellets of poly(vinyl chloride) (PVC), plasticised with di(isononyl)phtalate (DINP), having melt flow index (MFI) of 0.63 g/10 min and density of 1.22 g/cc, were provided by Milena Pharmaceutical Srl (Agrigento, Italy). The zeolite fine powder was supplied by AgION Technologies Inc. (Wakefield, MA, USA). It was constituted by sodium aluminosilicate crystals, containing 10.4% w/w of ordinary silver ions and having mean particles size of 2–3 μm, surface area of 600 m^2 and bulk density of 0.5 g/cm^3.

Composites preparation

Before compounding, PVC pellets and silver zeolite (SZ) powder were dried at 80 °C under vacuum for 2 h and then cooled to room temperature. PVC composites were obtained by mixing PVC pellets and SZ powder as filler with loadings varying between 2 and 20 wt% in a Brabender mixer at 140 °C, rotor speed 50 rpm and mixing time 3.5 min. The samples were stored under vacuum to prevent moisture adsorption.

Thermogravimetric (TG) analysis

The TG analysis of neat PVC and composites was carried out by a Q500 thermogravimetric analyzer (TA Instruments) under nitrogen flow rate of 50 mL/min. The samples (4 mg) were heated from 30 to 800 °C at a heating rate of 10 °C/min.

Mechanical and rheological testing

To avoid phase segregation or separation during the specimen preparation, the materials were first immersed in liquid nitrogen, finely grounded in a lab homogenizer (IKA A10) at 20,000 rpm and then compression moulded at 150 °C for 10 min at 150 bar and then rapidly cooled to room temperature.

Sheets 0.5 mm thick were used for mechanical analysis whilst cylinder with 25 mm radius and 2.5 mm thick were used for the rheological tests.

Mechanical properties (elastic modulus E, tensile stress TS and elongation at break EB) were measured by using an Instron 3365 dynamometer with crosshead speed of 50 mm/min, on rectangular specimens (90 × 10 mm). The rheological characterization was performed on a parallel plates rheometer RDA II (Rheometrics) in the frequency range of 0.1–500 rad/s at $T = 150$ °C.

Microbiological testing

All microbiological tests were performed on squared film specimens of 10 × 10 mm. Before microbiological analyses, samples of PVC containing 10% (w/w) of silver zeolite (PVC10SZ) and PVC containing 20% (w/w) of silver zeolites (PVC20SZ) were activated by immersing

them in a solution of acetic acid/distilled water 30/70 v/v for 30 min. Then they were washed three times in distilled water, dried at room temperature and sterilized by a UV lamp (wavelength 280–240 nm) through two steps of 3 min. The same procedure was applied to neat PVC samples to evaluate eventual antimicrobial activity that could be caused by the presence of acid residues on the material surface.

The antibacterial effect of composites was examined on *Escherichia coli* and *Staphylococcus epidermidis* in trypticase soy broth (TSB) at an initial concentration of 5×10^3 colony forming units (CFU)/mL and of 9×10^3 CFU/mL, respectively.

The antimicrobial activity towards these strains was determined by immersing films of neat PVC and PVC20SZ in TSB and incubating at 37 °C. Bacterial growth was followed by online measurements of optical density (OD) at 600 nm, at fixed time intervals (0, 30 min, 1, 2, 3, 4 and 24 h) using a Biophotometer (Eppendorf). At each time interval, 0.1 mL of bacterial suspensions were also spread on trypticase soy agar (TSA), in triplicate, to determine microbes concentrations, expressed as CFU/mL.

The direct inhibition of bacteria induced by the composite films (PVC10SZ and PVC20SZ), placed on the surface of TSA plates, seeded with *E. coli* and *S. epidermidis* (both strains at an initial concentration of 10^6 CFU/mL) and incubated at 37 °C, was evaluated by measuring the width in millimeters of the inhibition zone surrounding the polymeric films. The inhibition zone was determined after 6 and 24 h (according to standard methods). The experiment was also prolonged up to 30 days, leaving the films on the surface of the same TSA plates and incubating at 37 °C for all the time period, to evaluate the antibacterial activity of composites over time. After 7, 14 and 30 days the inhibition zone, if present, was measured.

To simulate an urinary tract infection, we also followed the inhibition of bacterial growth by immersing and incubating at 37 °C films of neat PVC and PVC composites in human sterile urine seeded with *E. coli* and *S. epidermidis* at two initial concentration (10^8 and 10^6 CFU/mL). At fixed time intervals (1, 5, 10 and 20 days) bacteria concentrations, expressed as CFU/mL, were determined by spreading 0.1 mL of bacterial suspension from each sample on TSA, in triplicate, and incubating at 37 °C.

Silver ion release

To determine the silver ion release two kinds of experiments were performed. The total amount of free silver ions released from composites (first experiment) was determined by immersing films of PVC20SZ composites on tubes containing human sterile urine and incubating at 37 °C for 20 days. The composite samples were removed from urine medium after 1, 5, 10 and 20 days. For each time interval, at least two replicate specimens were removed and the urine medium containing the silver ions released during the fixed time periods was analysed for the determination of the silver released.

The daily release of silver ions (second experiment) was determined by incubating at 37 °C films of PVC20SZ composite in urine medium for steps of 1 day, after that the films were removed and the solutions were analysed. The same composite samples were washed with distilled water and incubated again in fresh urine medium for another 24 h and then removed again. The same procedure was repeated for 6 days on the same sample so that six different samples, in replicates, of urine containing the daily release of silver ions were obtained.

The concentration of silver ions in the urine medium was determined by atomic absorption spectroscopy (Varian Spectra AA) at a wavelength of 328.1 nm, flame flow rate of 1.5 mL/min, spectral bandwidth of 0.5 nm and lamp intensity of 5 mA. The concentration of silver ions was calculated using a calibration curve obtained with solutions at known concentration of silver ions, within the range of 0.1–1.5 ppm.

Results and discussion

Thermal analysis

Generally materials for biomedical applications are not used at temperatures higher than that of the human body, nevertheless during moulding and processing technologies for industrial production of end products may occur degradation processes that could affect their performance.

To evaluate the thermal stability of PVC SZ composites, TGA measurements were performed. Neat PVC pellets and neat processed PVC samples were analysed too. As previously observed [50], neat PVC shows two thermal degradation steps: the first starts at about 200 °C and is mainly due to hydrochloric acid loss ($\sim$60% w/w); the second one starts at about 360 °C and implies the rearrangement of the new formed polyenic chains and their subsequent degradation. The TG data reported in Table 1 indicate that the thermal properties of the composites were not affected by the presence of SZ. No significant differences between neat PVC and composites were found at temperature below 200 °C and on the degradation temperatures (T_{d1} and T_{d2}) of composites with increasing amount of SZ. The residue, observed at 800 °C, increases from 11.5 to 28%, proportionally to the amounts (2–20%, w/w) of SZ loaded into composites. Differently from the plasticized PVC used in this study, the introduction of SZ into other polymeric materials may influence their thermal properties. It was

Table 1 TG data for PVC and PVC silver zeolite composites

	T_{d1}^{a} (°C)	T_{d2}^{b}	$\%R^{c}$
Neat PVC	288	459	8.8
Neat processed PVC	283	457	8.7
PVC SZ 2% (w/w)	287	460	11.5
PVC SZ 4% (w/w)	289	461	13.5
PVC SZ 6% (w/w)	290	458	16.4
PVC SZ 10% (w/w)	291	458	20.3
PVC SZ 20% (w/w)	290	456	28.1

[a] Decomposition maximum temperature of thermal degradation first step

[b] Decomposition maximum temperature of second step

[c] Weight residue (%) at 800 °C

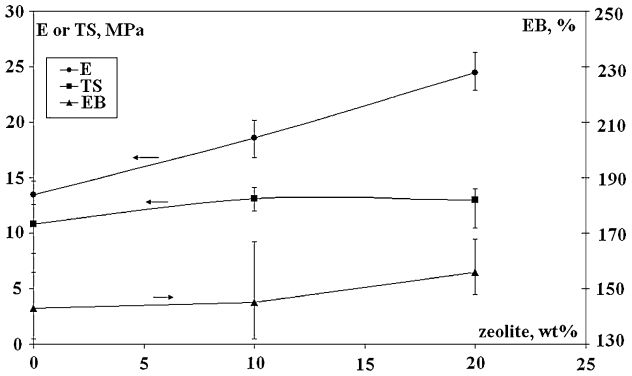

Fig. 1 Mechanical properties of PVC and PVC SZ composites as a function of silver zeolite content: *EB* elongation at break, *TS* tensile stress and *E* elastic modulus.*Y-error bars* standard deviation

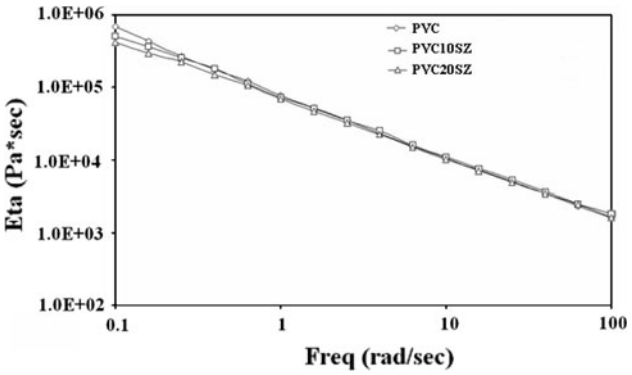

Fig. 2 Viscosity behaviour of PVC with different silver zeolite content as function of system frequency

observed, in fact, that polypropylene (PP) is much more susceptible to thermal decomposition in the presence of silver zeolite than pure PP because the zeolite addition into PP matrix speeds down the decomposition reaction [51]. The heat applied during the preparation of composites of polylactide (PLA) with silver zeolites by melt mixing and compression moulding has a strong annealing effect and influences the crystallization kinetics [52]. Also the incorporation of silver zeolite into polyurethane (PU) leads to changes in the polymeric properties during in vitro ageing, inducing initiation and progress of oxidation in PU with the addition of 2% zeolite [53]. On the contrary, other authors [54] found that zeolites did not cause any deterioration in the thermal properties of zeolite–polyurethane composites. This different behaviour of zeolite composites depends on various parameters, such as polymer matrices, zeolite types, silver content, methodology chosen to process the polymer composites, etc.

Tensile properties and rheological behaviour

To verify the influence of SZ on the processing and performances of PVC composites, mechanical and rheological measurements were carried out. In Fig. 1, the mechanical properties of composites (PVC10SZ and PVC20SZ) are reported. It can be observed that there is a monotonic increase of the elastic modulus (*E*) on increasing the amount of zeolite in the blend. At the highest amount of zeolite (20% by weight) the modulus is almost doubled, evidencing a stiffening of the composite. The *TS* and the *EB* properties are practically unaffected by the presence of the filler.

As regard the analysis of the rheological behaviour (Fig. 2) it is worth noting that the viscosity of both PVC10SZ and PVC20SZ composites did not change significantly if compared to neat PVC, except a slight increase of viscosity in the low frequency range. The results suggest that even at the highest level, the SZ does not significantly change the rheological behaviour of the composites, especially in the high frequency range (50–200 s^{-1}), typical shear rate region of the most common processing operation. As a consequence, the addition of SZ does not influence the processability and the formability of the material.

Antimicrobial activity

As above mentioned, the antimicrobial action of silver zeolite depends on ionic exchange between the silver ions reversible bonded to zeolite and the cations present in wet environments. It is reported in the literature that the activity of the SZ is influenced by several factors, amongst which temperature and pH. A more marked activity of SZ was found at a higher temperature in the range of 0–42 °C and at a higher pH in the range of 6.5–8.5. SZ antimicrobial action is also inhibited by the addition of L-cysteine, L-methionine, L-histidine, L-tryptophan, bovine serum albumin, yeast extract and sodium chloride (at 100 mM) and strongly enhances by the addition of o-phenanthroline

and 2,2′-dipyridyl [36]. Also, in the manufacture of a silver-containing zeolite in PBT, injection moulding followed by an abrasive technique to remove a thin layer of lower silver zeolite resulted in higher antimicrobial efficacy, whereas a fourfold reduction in antimicrobial efficacy occurred when the surface was not abraded after injection moulding [55].

In this study, the antibacterial activity of the PVC SZ composites was strongly improved after a composite activation with diluted acetic acid. Most likely the weak acid is able to remove the idrophobic layer due to plasticizer and/or additives used in PVC formulation, allowing a facile release of silver ions from zeolites.

Inhibition in TSB

The antibacterial effect of composites on *E. coli* and *S. epidermidis* was examined in culture broth (TSB) by measurements of OD at 600 nm, at fixed time intervals. Bacteria concentrations, expressed as CFU/mL, were determined spreading 0.1 mL of bacterial suspensions on TSA and incubating at 37 °C. At time 0 the concentrations of *E. coli* and *S. epidermidis* were 5.0×10^3 CFU/mL (OD = 0.003) and 9.0×10^3 CFU/mL (OD = 0.006), respectively.

The OD values reported in Fig. 3 show that the PVC20SZ composite exhibits a good inhibition of both bacteria growth with respect to neat PVC. The differences between OD values of control and composite samples were not significant up to 4 h for both strains.

After 24 h, the TSB sample seeded with *E. coli* and containing PVC20SZ showed an OD value of 0.005 (corresponding to a bacteria concentration of $4.0 \pm 1.2 \times 10^3$ CFU/mL, mean ± SD) very similar to that initial, whilst the OD value of the control sample containing neat PVC increased (OD = 0.87, corresponding to a bacteria concentration $>10^8$ CFU/mL).

The inhibition trend observed against *S. epidermidis* growth was similar (Fig. 3), showing at 24 h an OD value of 0.002, corresponding to a bacteria concentration of $3.2 \pm 0.9 \times 10^3$ CFU/mL.

To determine the antibacterial activity of composites over time, the control and the composite samples were left in the original culture broth up to 7 days. After 7 days, the TSB sample seeded with *E. coli* and containing neat PVC showed an OD value of 1.32 (bacteria concentration $>10^9$ CFU/mL), whilst the sample containing the PVC20SZ composite showed an OD value of 0.006, corresponding to an *E. coli* concentration of $7.2 \pm 2.5 \times 10^3$ CFU/mL. These results indicate that the antibacterial activity of PVC20SZ composite against *E. coli* growth continued up to 7 days (Fig. 4).

No inhibition of *S. epidermidis* growth was observed after 7 days (OD = 1.194, bacteria concentration $>10^9$ CFU/mL).

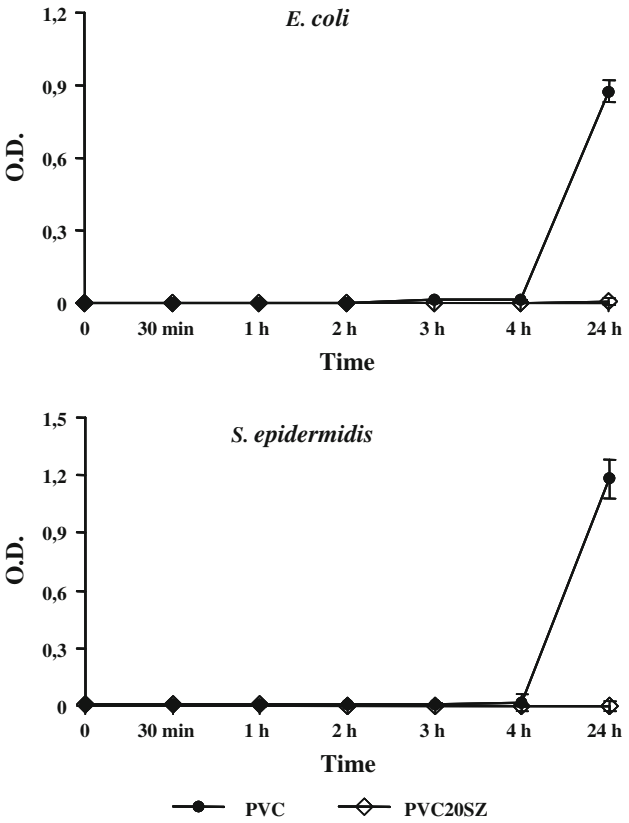

Fig. 3 OD values of TSB samples supplemented with *E. coli* and *S. epidermidis* in the presence of neat PVC and PVC20SZ composite as a function of incubation time. *Y-error bars* standard deviation

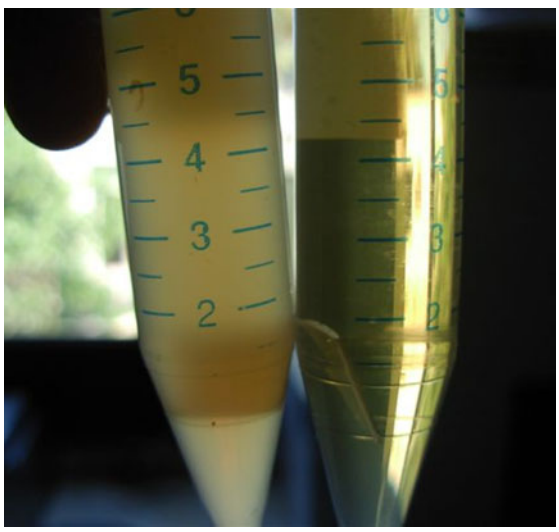

Fig. 4 TSB samples seeded with *E. coli* in the presence of neat PVC and PVC20SZ composite, after 7 days of incubation at 37 °C. The sample containing neat PVC (on the *left*) is turbid due to the microbial growth, whilst the sample containing the composite (on the *right*) is clear, indicating that the antibacterial activity of composite continued up to 7 days

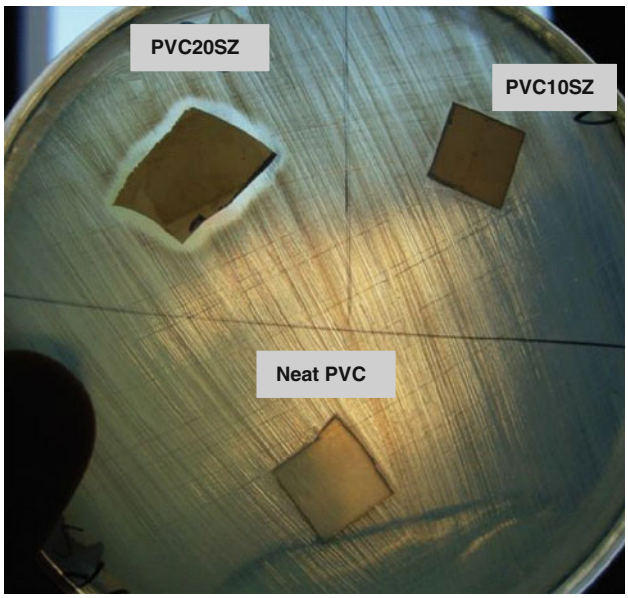

Fig. 5 Direct inhibition of bacterial growth induced by films of neat PVC and PVC SZ composites on TSA spread with *S. epidermidis* after 24 h of incubation

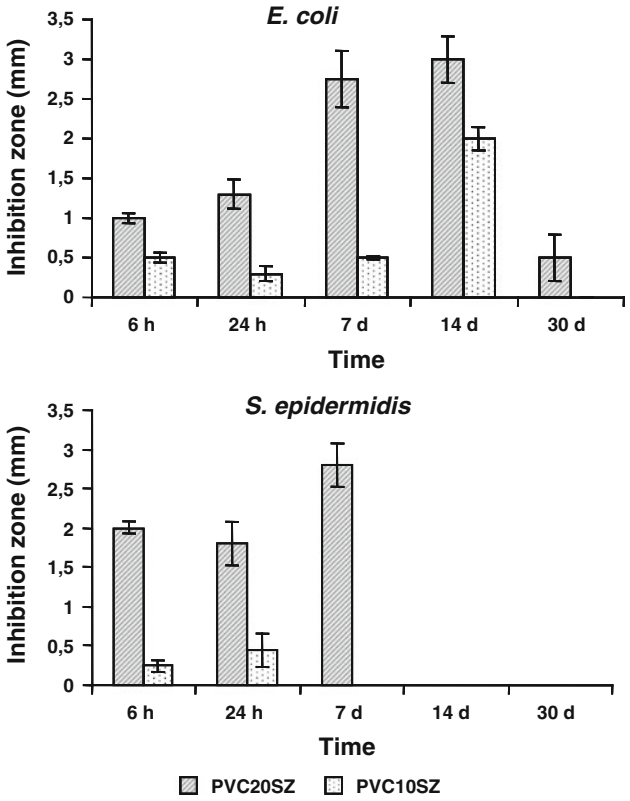

Fig. 6 Width (mm) of the inhibition zones induced by PVC SZ composites on TSA plates spread with *E. coli* and *S. epidermidis* as a function of time. *Y-error bars* standard deviation

These data show a promising antimicrobial activity of composites against both *E. coli* and *S. epidermidis*, in fact, after 24 h, it was found a significant reduction (ANOVA, $F = 56.5$ and 615.2, respectively, $P < 0.001$) on a logarithmic scale basis of bacteria growth in the presence of PVC20SZ composite (10^3 CFU/mL) if compared to neat PVC (10^9 CFU/mL).

Inhibition on TSA

The direct inhibition of bacteria growth induced by composite films (PVC10SZ and PVC20SZ) was evaluated using the agar diffusion method. The inhibition zone (mm) surrounding the composite films, on TSA plates seeded with inocula of *E. coli* and *S. epidermidis* at an initial concentration of 10^6 CFU/mL, was measured at fixed times.

In Fig. 5, squared films of neat PVC, PVC10SZ and PVC20SZ composites on TSA, spread with *S. epidermidis* and incubated for 24 h, are shown. It can be observed a clearly-defined bacteria free zone around the PVC composite sample containing 20% (w/w) of SZ, confirming the growth inhibition effect induced by the silver zeolite.

In Fig. 6, it is reported the width (average of three replicates) of the inhibition zones induced by PVC10SZ and PVC20SZ composites on TSA spread with both strains. As expected, the PVC20SZ composite showed an inhibition effect greater than PVC10SZ, most likely due to the higher amount of silver ions released during the experiment.

After 24 h, on TSA plates spread with *E. coli*, an inhibition zone of 1.3 ± 0.2 mm (mean $\pm$ SD) surrounding PVC20SZ film was observed. To evaluate the antibacterial activity of composites over time the experiment was prolonged up to 30 days, leaving the films on the surface of the same TSA plates and incubating at 37 °C for all the set time period. The inhibition zone, if present, was measured at 7, 14 and 30 days. The maximum inhibitory effect was found after 14 days for both PVC10SZ (2 ± 0.1 mm) and PVC20SZ (3 ± 0.3 mm) composites.

In the sample of TSA seeded with *S. epidermidis*, an inhibition zone of 1.8 ± 0.3 mm surrounding the PVC20SZ composite was observed at 24 h. The maximum value of inhibition (2.8 ± 0.3 mm) occurred after 7 days, whilst the inhibition activity of the PVC10SZ composite was observed only up to 24 h (inhibition zone of 0.45 ± 0.2 mm). These results allowed to observe that the composite films continued to elute with time, maintaining the inhibition zone up to 30 days for *E. coli* and up to 7 days for *S. epidermidis* and confirming the different duration of growth inhibition of both strains, observed in TSB tests too. This is probably due to a different sensitivity of bacteria to the antimicrobial action of zeolites.

Antimicrobial activity in sterile urine

To simulate an urinary tract infection, films of neat PVC and PVC SZ composites were immersed and incubated at

Table 2 Bacterial concentrations (average of triplicates) after different incubation periods of control (neat PVC) and PVC SZ composite films in sterile urine containing two different inocula (10^8 and 10^6) of *E. coli*

Samples	Days	*E. coli* (10^8 CFU/mL) CFU/mL ± SD	*E. coli* (10^6 CFU/mL) CFU/mL ± SD
Neat PVC	1	$>10^9$	$>10^9$
PVC10SZ	1	$4.3 \pm 1.1 \times 10^3$	$4.2 \pm 1.5 \times 10^3$
PVC20SZ	1	$4.6 \pm 0.9 \times 10^2$	$1.4 \pm 0.6 \times 10^2$
Neat PVC	5	$>10^9$	$>10^9$
PVC10SZ	5	$1.5 \pm 0.8 \times 10^2$	$0.7 \pm 0.3 \times 10^2$
PVC20SZ	5	$0.2 \pm 0.09 \times 10^2$	$0.1 \pm 0.06 \times 10^2$
Neat PVC	10	$>10^9$	$>10^9$
PVC10SZ	10	$>10^9$	$>10^9$
PVC20SZ	10	$1.6 \pm 0.7 \times 10^2$	$0.3 \pm 0.1 \times 10^2$
Neat PVC	20	$>10^9$	$>10^9$
PVC10SZ	20	$>10^9$	$>10^9$
PVC20SZ	20	$6.3 \pm 2.2 \times 10^2$	$6.0 \pm 1.7 \times 10^2$

Bacteria concentration is expressed as colony forming units (CFU)/mL ± standard deviation (SD)

37 °C in sterile urine implemented with *E. coli* and *S. epidermidis*. Two different inocula (10^8 and 10^6 CFU/mL) for both strains were used to evaluate the influence of the initial bacterial concentration on the antimicrobial activity of PVCSZ composites. The bacteria concentration, expressed as CFU/mL, was determined at fixed time intervals, as previously described.

Data reported in Table 2 show that the antimicrobial activity of both PVC10SZ and PVC20SZ composites against *E. coli* was similar in the first 5 days, in fact a strong reduction (4–6 log units) of bacteria concentration was observed already at 24 h. The antimicrobial activity of PVC20SZ composite was expressed for all the period (20 days), inhibiting bacteria growth and thus keeping low the concentration of viable bacteria, whereas PVC10SZ composite inhibited *E. coli* growth only up to 5 days. These results clearly indicate that only the amount of silver ions released from PVC20SZ composite is sufficient to determine the inhibition of *E. coli* growth up to 20 days. No relevant differences of bacterial growth from the two different starting concentrations (10^6 and 10^8 CFU/mL) of *E. coli* were found, indicating that the initial bacterial concentration did not influence the antimicrobial activity of both composites against this strain.

Data reported in Table 3 indicate that the antimicrobial activity of composites against *S. epidermidis* was expressed only for 5 days, showing a different trend with respect to the initial bacterial concentration. At an initial concentration of 10^6 CFU/mL, there was a good antimicrobial action of both composites for the first 5 days with a reduction of *S. epidermidis* viability up to 10^3–10^2 CFU/mL. After 5 days no antimicrobial activity was detected for both composites.

At the initial concentration of 10^8 CFU/mL, PVC20SZ composites induced a strong reduction (from 10^8 to 10^3 CFU/mL) of *S. epidermidis* growth already at 24 h of incubation. The antimicrobial action continued up to 5 days with a reduction of 6 log units in the viability of *S. epidermidis*. These data suggest that the amount of silver ions released from PVC20SZ composites was sufficient to induce a strong short-time bactericidal action, differently from PVC10SZ composite, which expressed only a bacteriostatic action against *S. epidermidis*.

These data confirm the different sensitivity of both strains to the action of silver ions released from PVC SZ composites. This different sensitivity, previously reported [28, 29, 48, 56], is related to differences in the cell wall structure. Gram-positive bacteria have more peptidoglycan than gram-negative bacteria because of their thicker cell walls. Also, since peptidoglycan is negatively charged and

Table 3 Bacterial concentrations (average of triplicates) after different incubation periods of control (neat PVC) and PVC SZ composite films in sterile urine containing two different inocula (10^8 and 10^6) of *S. epidermidis*

Samples	Days	*S. epidermidis* (10^8 CFU/mL) CFU/mL ± SD	*S. epidermidis* (10^6 CFU/mL) CFU/mL ± SD
Neat PVC	1	$>10^9$	$>10^9$
PVC10SZ	1	$7.0 \pm 1.3 \times 10^8$	$2.0 \pm 0.9 \times 10^2$
PVC20SZ	1	$3.2 \pm 0.9 \times 10^3$	$0.2 \pm 0.09 \times 10^2$
Neat PVC	5	$>10^9$	$>10^9$
PVC10SZ	5	$5.0 \pm 1.0 \times 10^8$	$1.5 \pm 0.6 \times 10^3$
PVC20SZ	5	$0.5 \pm 0.14 \times 10^2$	$0.1 \pm 0.07 \times 10^2$
Neat PVC	10	$>10^9$	$>10^9$
PVC10SZ	10	$>10^9$	$>10^9$
PVC20SZ	10	$>10^9$	$>10^9$
Neat PVC	20	$>10^9$	$>10^9$
PVC10SZ	20	$>10^9$	$>10^9$
PVC20SZ	20	$>10^9$	$>10^9$

Bacteria concentration is expressed as colony forming units (CFU)/mL ± standard deviation (SD)

silver ions are positively charged, more silver may get trapped by peptidoglycan in gram-positive bacteria than in gram-negative bacteria. Thus, gram-positive bacteria may allow less silver ions to reach the cytoplasmic membrane than gram-negative bacteria allow and may therefore be less susceptible [48]. The cells of *E. coli* after silver ion treatment appeared to be seriously damaged, showing either localized or complete separation of the cell membrane from the cell wall [56]. Also, many small electron-dense granules either surrounding the cell wall or depositing inside the cell and a remarkable electron-light region in the centre of the cell, containing condensed DNA molecules were observed. It was suggested that the free state of DNA changed to a condensed form, losing its replication ability, as a reaction against the denaturation effects of silver ions, whereas the interaction of silver ions with thiol groups in protein induced the inactivation of the bacterial proteins [29].

Silver release

Silver ions are released from SZ by ionic exchange with other positive ions (often sodium) and their release is dependent on the concentration of cations in the solution [48]. Usually, 45% of Ag^+ rapidly binds to protein resulting in partial inactivation. Also, Ag^+ complexes with free chloride, phosphate and sulphate ions present in the solution [27], whilst a variety of substances can affect the release and activity of silver ions, as above mentioned [36].

In this study, to correlate the long term antimicrobial activity of composites to the amount of silver ions released, PVC20SZ samples were immersed in sterile urine without adding bacteria. In the presence of bacteria, in fact, the amount of free silver ions detectable may be lower because most of them is bound to bacteria. The total amount of free silver ions released from composites was determined by immersing films of PVC20SZ on tubes containing sterile urine and incubating at 37 °C. The composite samples, in replicates, were removed after 1, 5, 10 and 20 days and the urine medium was analysed for the determination of the total amount of silver released (see 'Experimental').

The amount (ppm) of silver ions detected over the time period of 20 days is reported in Fig. 7. The amount of silver ions released after 1, 5, 10 and 20 days of incubation showed a linear increase during the examined time period. The amount of silver ions released during the first day was 0.365 ppm, which decreased to 0.07 ppm (daily mean value calculated) from days 2 to 5 and to 0.02 ppm (daily mean value calculated) from days 6 to 10 and from days 11 to 20. These data indicate that the rate of silver ions released from PVC20SZ composite reached a steady state value after 6 days of incubation.

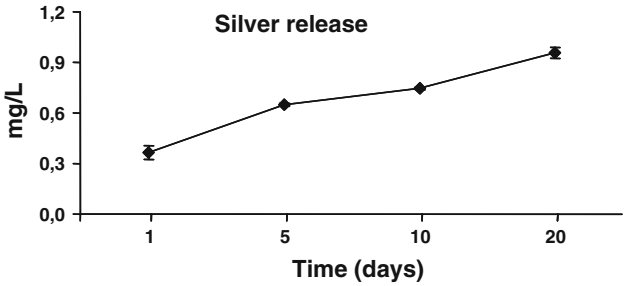

Fig. 7 Amount of silver ions released from PVC20SZ composite in sterile urine as a function of time. *Y-error bars* standard deviation

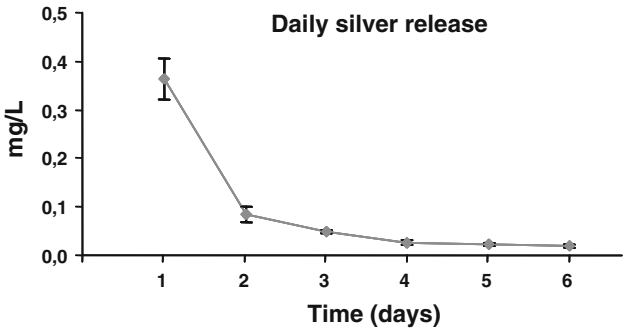

Fig. 8 Daily release of silver ions from PVC20SZ composite in sterile urine as a function of time. *Y-error bars* standard deviation

In another experiment, a sample of PVC20SZ composite was incubated at 37 °C in urine medium for 1 day, then it was removed, rinsed with distilled water and incubated again in fresh urine medium for another 24 h. The same procedure was repeated for 6 days on the same sample so that six different samples of urine containing the daily release of silver ions were obtained and analysed. Figure 8 shows the concentrations of silver ions daily released by the PVC20SZ composite. The largest amount of silver ions was released during the first day, confirming the strong antimicrobial effect induced by composites after 24 h of incubation (Tables 2, 3). The release rate of silver ions is very similar to that of the first experiment, in fact the amount released during the first day (0.365 ppm) decreased to 0.084 ppm on the second day, halving from the second to the fourth day, whilst on the fifth and on the sixth days the release of silver ions was quite constant (21 ppb).

Taking into account that the silver ion release from SZ varies according to the different parameters involved (silver content, polymer matrices, composition and ionic strength of medium, etc.) [2, 20, 52], it is difficult to compare our results with those from literature.

It was reported that a concentration of 10 ppm of $AgNO_3$ caused many changes in bacterial cell structures, affecting DNA replication and inducing inactivation of bacterial proteins [29]. For bacteria, MICs of silver zeolite

containing 38% (wt/wt) Ag ranged from 3.9 to 31.2 ppm (corresponding to 2–12 ppm of Ag), in particular for *E. coli* MIC ranged from 1.9 to 3.9 ppm [57]. Kawahara et al. [48] found that SZ containing 1.9% (wt/wt) Ag showed MICs ranging from 4.8 to 38.4 ppm of silver ions. They reported that in water SZ released no detectable amounts of Ag^+, whilst in phosphate buffered saline (PBS) medium it released 0.53 ppm (0.21%) of its silver ions after 24 h of incubation.

We found that the PVC20SZ composite released 0.365 ppm of silver ions after 24 h, corresponding to 38% of the total amount released during the test period. Also, the silver released during the activation procedure of PVC composite films was quantified too, finding a value of 0.463 ppm. The silver release was not constant over time and proceeded in two phases: a phase of relatively fast decreasing release during the first 4 or 5 days and a phase of slow and steady release during the next 15 days. The amount of silver ions released from PVC20SZ composite was sufficient to exert the antimicrobial effect up to 20 days of incubation in urine samples supplemented with *E. coli*, whereas the inhibition of *S. epidermidis* vanished after 5 days.

Conclusion

In this study, a silver zeolite was loaded, at different amounts, as antimicrobial agent into PVC matrix. The incorporation of SZ did not affect the thermal, rheological and mechanical behaviour of PVC composites and consequently it did not influence the processability and the formability of the material even at high SZ content (20% wt). Since flexible PVC formulations are widely used for biomedical applications (blood or urine bags, transfusion tubing, catheters, etc.), we simulated an urinary tract infection using sterile urine seeded with *E. coli* and *S. epidermidis* at two initial concentration (10^8 and 10^6 CFU/mL), in addition to conventional microbiological tests. The antibacterial effect of the PVC SZ composites was strongly improved after sample activation with diluted acetic acid.

It was found that composites induced, in sterile urine, a significant reduction in the viability (4–6 log units) of both bacteria, already at 24 h, inhibiting *E. coli* growth up to 20 days, whilst their antimicrobial action against *S. epidermidis* vanished within 5 days. Amongst the composites examined in this study, PVC containing 20% (w/w) of SZ showed the highest antimicrobial activity in all culture media used. The highest amount of silver ions was released during the first day of incubation (0.365 ppm). This amount decreased to 0.07 ppm (daily mean value) from days 2 to 5, reaching a steady state value of 0.02 ppm (daily mean value) from days 6 to 20.

It is difficult to compare our results with those from literature due to the different parameters involved in the antimicrobial activity of SZ composites, such as the polymer matrices, silver content of zeolites, composition and ionic strength of medium, etc.

The strong inhibition of microbes proliferation showed by PVC SZ composites represents a valuable result that could be useful for the development of PVC-based antimicrobial medical devices. In fact the availability of silver ions on both the outer and the inner surfaces of the device is of crucial importance for medical purposes. Further studies to assess biocompatibility and toxicity of these composites and to evaluate the antimicrobial activity of silver zeolites as a function of different types of plasticizers used in PVC are in progress.

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