

ANTIBACTERIAL EFFICACY OF SILVER OR ARSENIC DOPED
POLYMER COMPOSITES AGAINST SEVERAL KINDS OF BACTERIAWitold Brostow^{1, ✉}, Marina Gahutishvili^{1, 2}, Anthony W. Wren³, Timothy J. Keenan³,
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Abstract. Structure and several properties of AgNO₃ and As₂O₃ doped polymer composites have been investigated, including their antibacterial activity against *E. coli*, *S. aureus*, *C. albicans* and *S. epidermidis*. New silver or arsenic doped polymer composites have been characterized by an X-ray diffraction (XRD), a scanning electron microscopy combined with an energy dispersive X-ray spectroscopy (SEM/EDS) and ion release studies. The antibacterial evaluation of each of the composite samples was conducted using *S. aureus* in the liquid broth culture, with 10, 20 and 30 % of liquid extract added to the bacterial culture. Control *S. aureus* stocks were used for comparison at each time period and were recorded at 100 % at each time period. For samples with the PLA plasticizer the bacterial viability was significantly reduced for each composition containing Ag/As and was similar for each dosage concentration.

Keywords: arsenic composites, silver composites, polymer composites, antibacterial behavior.

1. Introduction

Currently there exists a clinical need for materials and surfaces that inhibit the growth and proliferation of pathogenic microbes. Studies have been conducted to incorporate organic compounds such as antibiotics into medical materials and surgical contact surfaces to eliminate opportunistic pathogens such as *S. aureus*. However, overuse of these compounds has resulted in the generation of antibiotic resistant organisms such as

methicillin resistant *S. aureus* (MRSA) and vancomycin resistant *S. aureus* (VRSA). Currently, post-surgical infection rates of orthopedic implants range from 0.3 to 8.8 %. These infections are reported to be caused by a surgical contamination of wounds, hematogenous spread, reoccurrence related to sepsis from a previously infected joint, or spread of infection from a preexisting source. Clearly it is desirable to impart medical materials and contact surfaces with non-antibiotic containing antimicrobial properties which can eliminate opportunistic microbes upon contact. A potential mechanism of achieving this is through the addition of metallic cations such as silver (Ag⁺), copper (Cu²⁺) and zinc (Zn²⁺) – in addition to metallic nanoparticles. It is widely known that Ag⁺ is an effective antibacterial agent as it has a low cytotoxicity in wound dressings and is highly bactericidal – the primary reason for its inclusion in antimicrobial materials and coatings^{1,2}.

Largely the development of antimicrobial polymers has been based on the dispersion of metallic nanoparticles in a medium within a polymeric matrix. In order to achieve the desired properties, Ag⁺ nanoparticles typically have to be smaller than 50 nm in diameter, otherwise they exhibit similar properties to bulk particles³. Polymeric matrix without metallic nanoparticles is investigated for other applications such as improving the mechanical properties. Gold (Au) nanoparticles have previously been created by a laser ablation and placed in polystyrene (PS) matrices⁴. PS is characterized by a high wear, but the addition of Au nanoparticles with concentrations below 1 wt% results in reducing friction and lowering wear rates⁵. Moreover, values of brittleness of PS are much higher than of other engineering polymers^{5,6}. Earlier some of us reported procedures for the separation of natural trivalent oxides of arsenic (As₂O₃) and antimony (Sb₂O₃)⁷, in addition to the synthesis and processing of adamantane-containing hydrazide-hydrazones with Co and Ni. Such materials have been used to prevent biocorrosion of cultural heritage and museum exhibits⁸.

This paper deals with the antibacterial effects of Ag₂O and As₂O₃, the addition of these oxides to polymeric networks, investigation of resulting structures and their

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solubility. One of the primary reasons to conduct the present work is the easy availability of As_2O_3 in the Republic of Georgia^{9, 10} while these oxides are known to exhibit also anti-cancerous properties¹¹⁻¹³. The potential benefit of arsenic as an antibacterial agent was recognized when As_2O_3 was added in small concentrations to poly(vinyl chloride)¹⁴. Sufficient antibacterial activity has been achieved when tested in *E. coli* and *P. syringae* media¹⁴. We explore the antibacterial properties of As_2O_3 when added to a widely used polymer, namely poly(vinyl chloride) (PVC), and comparing it to a known antimicrobial agent, Ag_2O .

2. Experimental

2.1. Material Synthesis

To create samples of silver and arsenic doped polymer composites, the following materials were used: PVC, Zn/Ca salt, arsenic(III) oxide, silver nitrate, and also bio-based plasticizers: Hexamoll DINCH (Hex-C), LPLAS HT3 (Pla-c) and ECOLOBRIUM (Eco-C).

The PVC grade called SE1300N was provided by Shintech Inc, Houston, TX, USA, and was used as the main polymer matrix. It contains vinyl chloride monomer up to 10 ppm by weight and was used without further purification. All materials are commercially available.

For all samples, the amounts of PVC, Zn/Ca stabilizer, and plasticizer were kept constant at 10, 0.1 and 0.25 g, respectively. Antimicrobial functional agents As_2O_3 and $AgNO_3$ were incorporated into polymeric matrices (Table 1).

Since three different plasticizers named above were used to prepare three different series of samples, we thus created nine samples.

PVC was mixed with Zn/Ca stabilized with a strong agitation until a homogeneous dispersion was obtained; a plasticizer was added to this mixture preheated up to 333 K and agitated before use. The purpose of this

step is to homogenize the plasticizer as much as possible in case any crystallization, aggregation or settling occurred at room temperature during storage. The plasticizer was then slowly added and mixed until full plasticizer absorption was achieved (this varies with the plasticizer). 10 mM $AgNO_3$ solution was initially prepared. Arsenic(III) oxide powder was dissolved in water. Finally antibacterial agent arsenic(III) oxide or 10 mM solution of silver nitrate, or both together, were added slowly with the strong agitation. Since our three plasticizers were used to prepare three different series of samples, we created overall twelve samples. Thus:

- control samples HEX-C, PLA-C and ECO-C are free from an antibacterial agent;
- HEX-1, PLA-1 and ECO-1 contain arsenic (III) oxide;
- HEX-2, PLA-2 and ECO-2 contain silver ions;
- HEX-3, PLA-3 and ECO-3 contain both silver and arsenic ones.

All samples were processed in a DAKE compression molding machine, Model 44-250, Grand Haven, MI, USA, at 423 K and at the compressive pressure of 1.33 kPa. Samples of each formulation were prepared as discs 2.0 cm in diameter and 0.5 cm in thickness for antibacterial and structural analysis.

2.2. X-Ray Diffraction

Diffraction patterns were collected using a Siemens D5000 X-ray Diffraction Unit (Bruker AXS Inc., Madison, WI, USA). Polymer plate samples were loaded into standard stainless steel sample holders. A voltage generator of 40 kV and a tube current of 30 mA were employed. Diffractograms were collected in the range of $10^\circ < 2\theta < 70^\circ$, at a scan step size 0.02° and a step time of 10 s. Any crystalline phases present were identified using JCPDS (Joint Committee for Powder Diffraction Studies) standard diffraction patterns.

Table 1. Compositions of Ag/As doped polymer composites

Sample	PVC, g	Zn/Ca stabilizer, g	Plasticizer, g	10 mM $AgNO_3$, mL	As_2O_3 , g
HEX-C	10	0.1	0.25 Hexamoll DINCH	–	–
HEX-1	10	0.1	0.25 Hexamoll DINCH	–	0.2
HEX-2	10	0.1	0.25 Hexamoll DINCH	2.5	–
HEX-3	10	0.1	0.25 Hexamoll DINCH	1.5	0.2
PLA-C	10	0.1	0.25 LPLAS HT3	–	–
PLA-1	10	0.1	0.25 LPLAS HT3	–	0.2
PLA-2	10	0.1	0.25 LPLAS HT3	2.5	–
PLA-3	10	0.1	0.25 LPLAS HT3	1.5	0.2
ECO-C	10	0.1	0.25 ECOLOBRIUM	–	–
ECO-1	10	0.1	0.25 ECOLOBRIUM	–	0.2
ECO-2	10	0.1	0.25 ECOLOBRIUM	2.5	–
ECO-3	10	0.1	0.25 ECOLOBRIUM	1.5	0.2

2.3. Scanning Electron Microscopy and Energy Dispersive X-Ray Analysis (SEM/EDX)

A Quanta 200F Environmental Scanning Electron Microscope (SEM) was used to obtain images of the samples under a vacuum of 0.12 kPa. The electron beam was used at an accelerating voltage of 20 kV and a spot size of 4.0. Energy dispersive X-ray spectroscopy (EDS) was carried out using FEI EDAX system equipped with a silicon-drift detector.

2.4. Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES)

Each sample (5 mm×5 mm×0.5 mm) was immersed in a sterile de-ionized H₂O for 30 days and rotated on an oscillating platform at 310 K. The ion release profile of each glass was measured using the Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) on a Perkin-Elmer Optima 5300UV (Perkin Elmer, Waltham, MA, USA). ICP-AES calibration standards for As and Ag ions were prepared from a stock solution on a gravimetric basis. Three target calibration standards were prepared for each ion and de-ionized water was used as a control.

2.5. Antibacterial Analysis of Composites using *S. aureus*

The antibacterial properties of the Ag and As-doped samples were tested in comparison to the control samples free of Ag or As with UAMS-1 *S. aureus* (tripticase soy agar and broth). Prior to testing, each microbe was initially grown aerobically in a liquid broth at 310 K for 24 h and spread plated on agar plates with a sterile swab dipped in a 1/50 dilution of the appropriate 24-hour culture of bacteria. One colony of the appropriate 24-hour plated culture of bacteria was grown aerobically in the liquid broth at 310 K for 24 h. The 1/50 dilution of the bacteria was performed and 150 μL was used to inoculate the culture for testing; 1 mL bacterial broth was used per sample. Samples were placed in 24-well plates and these plates incubated for 24 h at 310 K. After incubation, 100 μL of broth was taken from each well and pipetted into a 96-well plate ($n = 3/\text{sample}$, total $n = 9$). To determine bacteria concentration within the broth, the extracts absorbance was analyzed with a Bio-Tek μQuant plate reader at a wavelength of 490 nm. Samples were initially tested at concentrations of 10, 20 and 30 % to determine the effect of aliquot concentration. Further testing was conducted using 10% aliquot concentration so

that the viability of *S. aureus* in the liquid broth was evaluated at 6, 12, 24 and 48 h ($n = 3$) with the Bio-Tek μQuant plate reader where total aliquots of 100 μL were used at each of the respective time intervals.

3. Results and Discussion

Testing results. Initially characterization of the materials was conducted to determine differences in the structure, morphology and solubility of the processed composites. The characterization included X-ray diffraction to determine if the addition of Ag₂O or As₂O₃ affected the structures. The results are presented in Figs. 1, 2 and 3 for the HEX, PLA and ECO series of composites, respectively. HEX-C did not contain any Ag₂O or As₂O₃ as expected, while organic components of the polymer phase were evident: C₁₂H₇NO₂ and C₂H₃Cl. Amorphous humps are present within the 15–30° 2 theta range. HEX-1 and HEX-3 are found to contain crystalline peaks indicative of As₂O₃ while the incorporation of Ag₂O did not induce any crystallinity in HEX-2 and HEX-3. X-ray diffraction patterns of PLA (Fig. 2) and ECO (Fig. 3) follow a similar trend to HEX where crystalline As₂O₃ was present in PLA-1, PLA-3, ECO-1 and ECO-3 while crystalline Ag₂O was not evident in any of the samples tested.

Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis were used to further image the composite samples to verify processing consistency and determine the elemental composition. Representative SEM images are presented in Fig. 4 for ECO, PLA and HEX samples. ECO-2 and 3 samples are presented in Fig. 4a and 4b. The higher contrast in ECO-2 is likely a result of the higher Ag₂O concentration in this composite. ECO-3 shows a higher degree of adhesion between the constituents when compared to ECO-2. PLA-1 and PLA-2 are presented in Fig. 4c and 4d; the higher contrast in PLA-2 than in PLA-1 is likely due to the presence of Ag₂O. HEX-1 and HEX-3 are presented in Fig. 4e and 4f. The scattered patches seen for ECO-3, PLA-1, HEX-1 and HEX-3 are likely clusters of As₂O₃ since these clusters are only evident in the As₂O₃ containing composites. The corresponding EDX is presented in Fig. 5 for ECO, PLA and HEX. EDX of the ECO series of composites is shown in Fig. 5a. Ag and As are absent in ECO-C - as expected. As is present in ECO-1 and ECO-3 and Ag is present in ECO-2 but absent in ECO-3. Possibly the concentrations of Ag are below the detection limit of EDX. Also, Zn is observed in EDX for each sample since it is incorporated during processing in a plasticizer. Both the PLA and the HEX series of composites (Fig. 5b and 5c) show a similar trend while Ag and As are absent in PLA/HEX-C, As is present in

PLA/HEX-1 and PLA/HEX-3 and Ag is present in PLA/HEX-2 and PLA/HEX-3, as expected.

The solubility of each of the composite materials was evaluated using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), where Ag and As levels were recorded specifically after 24 h incubation in sterile DI water. The results of the ICP-OES study are presented in Table 2. For each of the control materials tested, *i.e.* HEX-C, PLA-C and ECO-C) no Ag or As release was found – again as expected. As release was recorded for HEX-1 (13.6 mg/L), PLA-1 (8.6 mg/L) and ECO-1 (19.3 mg/L). Ag release was found to be < 1 mg/L for all composite samples tested. In the composite series containing Ag and As, As release was found to be slightly higher than in the As alone series. As level for HEX-3 was 12.2 mg/L, for PLA-3 11.2 mg/L, and for ECO-3 21.5 mg/L.

Antibacterial evaluation of each of the composite samples was conducted using *S. aureus* in the liquid broth culture, with 10, 20 and 30 % of liquid extract added to the bacterial culture. Control *S. aureus* stocks were used for comparison at each time period and were recorded at 100 % at each time period. Regarding PLA, the results are presented in Fig. 6a; the bacterial viability was significantly reduced for each composition containing Ag/As and was similar for each dosage concentration. For 10 % addition, PLA-1, PLA-2, PLA-3 were found to be 58, 45 and 57 %, respectively. Similarly for the 20% dosage, bacterial viability was evident at 44, 36 and 56 % for PLA-1, PLA-2 and PLA-3, respectively. The 30% dosage exhibited a slightly higher bacterial viability at 47, 54 and 62 % for each of the PLA samples, respectively. The Ag/As free PLA composition (PLA-C) did not exhibit any bactericidal properties. The HEX samples did not show a significant reduction at 10% dosage; the results are presented in Fig. 6b. The bacterial viability was found to be 93, 95 and 97 % for HEX-1, HEX-2 and HEX-3, respectively, at 10% dosage. At 20% dosage, the viability improved to 51, 72 and 59 % for the HEX series and at 30% – the bacterial viability was at 69, 74 and 56 % for HEX-1, HEX-2 and HEX-3, respectively. The HEX-C samples did not present any antibacterial properties under each condition. Results for the ECO samples are presented in Fig. 6c; bacterial viabilities are 75, 99 and 88 % for ECO-1, ECO-2 and ECO-3, respectively, at a dosage of 10 %. At 20 % the bacterial cell viability was reduced to 63, 59 and 49 % for each composition respectively, while the 30% dosage increased the bacterial viability slightly to 74, 77 and 73 % for ECO-1, ECO-2 and ECO-3. The Ag/As ECO-C free sample does not show any bactericidal effect under any condition.

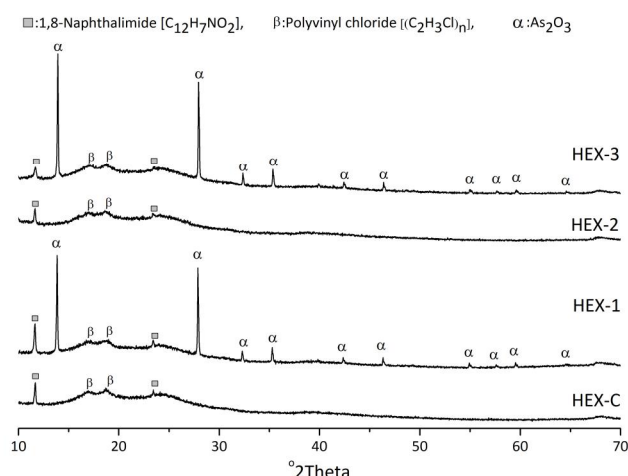


Fig. 1. X-ray diffraction of HEX series of Ag and As doped polymers

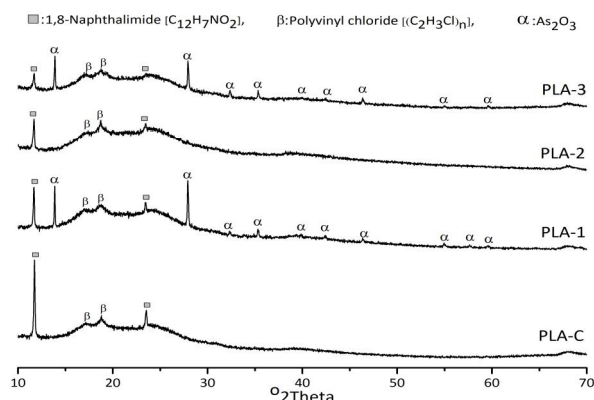


Fig. 2. X-ray diffraction of PLA series of Ag and As doped polymers

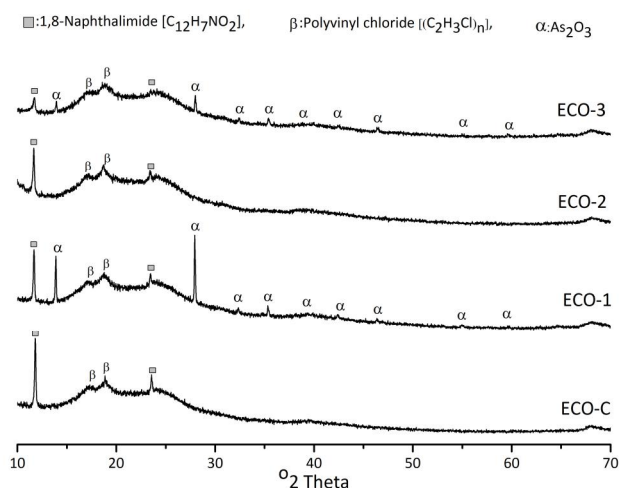


Fig. 3. X-ray diffraction of ECO series of Ag and As doped polymers

For each of the samples tested, ion release profiles were determined, specifically analyzing Ag and As release after 30 days incubation in deionized (DI) water. Each of the control materials, PLA-C, HEX-C and ECO-C did not exhibit any Ag or As release – as expected; the data are presented in Table 2. The HEX-1, PLA-1 and ECO-1

samples showed As release of 14, 9 and 19 mg/L, respectively. HEX-2, PLA-2 and ECO-2 each had very low Ag release rates < 1 mg/L. We recall that HEX-3, PLA-3 and ECO-3 samples contained both As and Ag. For each sample Ag was found to be < 1 mg/L, while the As levels were 12, 11 and 21 mg/L, respectively.

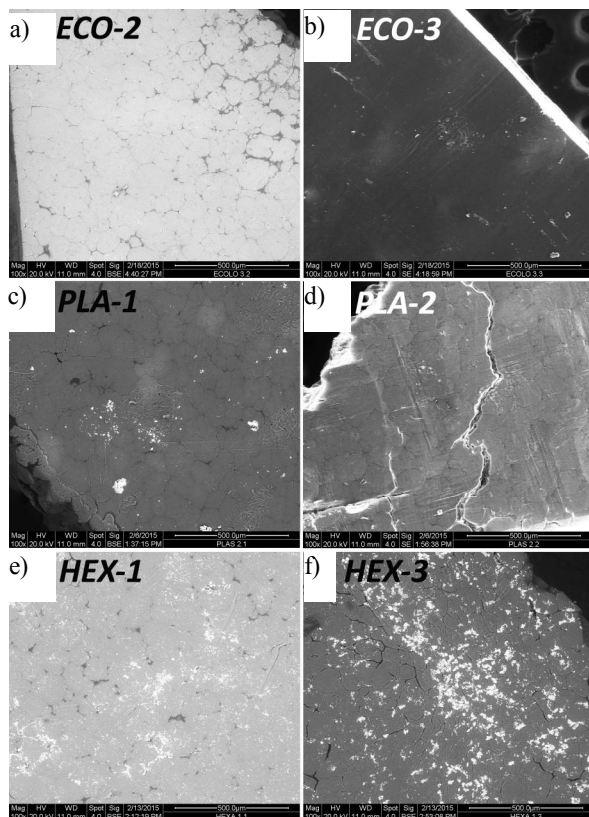


Fig. 4. SEM images of select sample surfaces from ECO (a, b), PLA (c, d) and HEX (e, f)

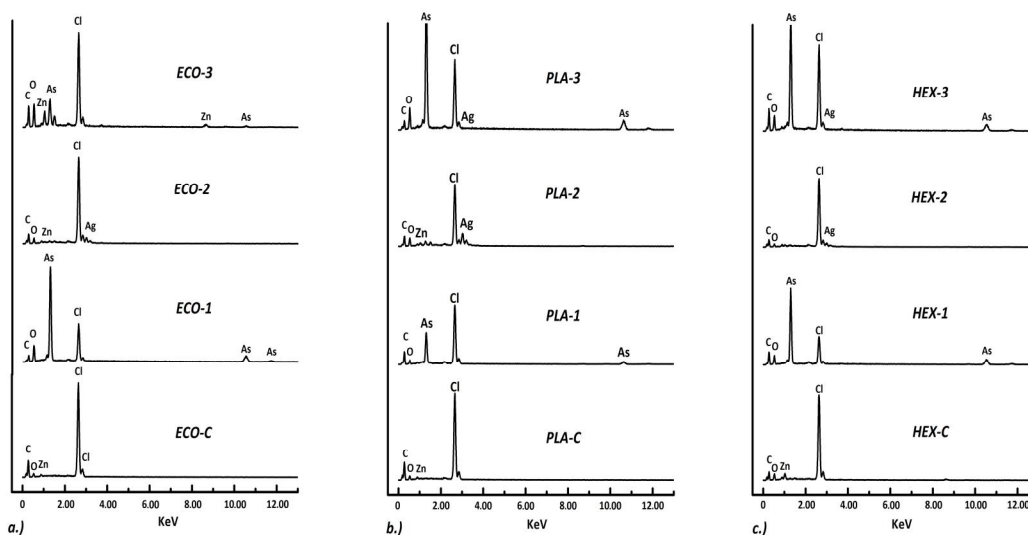


Fig. 5. EDX spectra of ECO (a), PLA (b) and HEX (c)

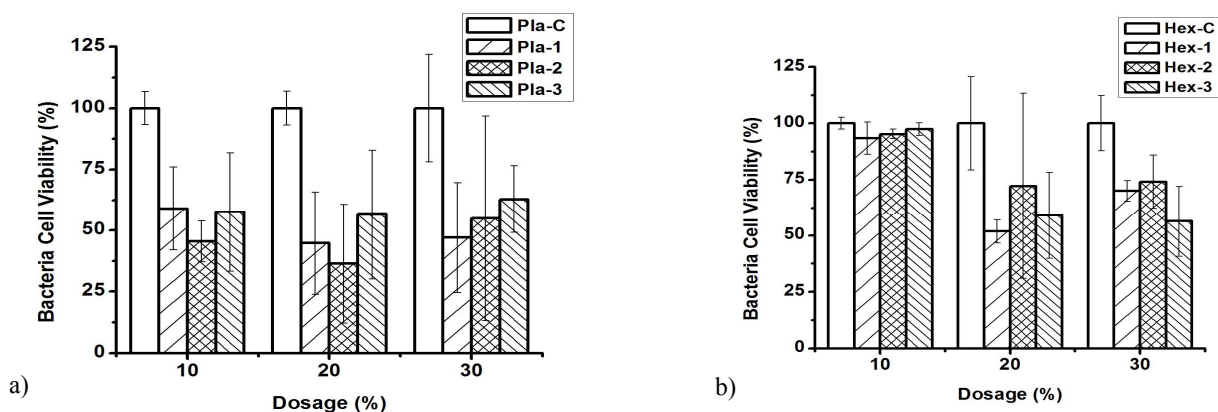
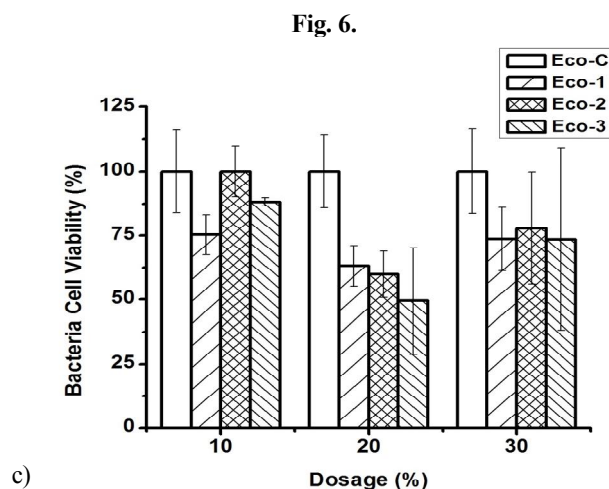


Fig. 6. Antibacterial evaluation of liquid extracts in *S. aureus* at 10, 20 and 30% dosage with respect to PLA (a), HEX (b) and ECO (c)



Further antibacterial testing was conducted using *S. aureus* considering the effect of each of the incubation solutions with respect to time, *i.e.* at 6, 12, 24 and 48 h (Fig. 7).

SEM structures. These results are presented in Fig. 4 and were already discussed above in connection with other kinds of results.

EDX results. These results are presented in Fig. 5 and were also already discussed above in connection with other kinds of results.

Needless to say, there exist also other approaches to imparting antibacterial properties to polymers. Skiba and her colleagues¹⁵ used a non-equilibrium low-temperature plasma technique to synthesize silver nanoparticles (AgNPs). They used poly(vinyl pyrrolidone) (PVP) as a capping agent. Silver nanoparticles (AgNPs)-alginate composite beads with different PVP concentration were synthesized as materials for water purification. Then Skiba

and coworkers¹⁶ have obtained Ag nanoparticles by a simple and eco-friendly atmospheric pressure plasma method, also using a capping agent. Effects of that agent concentration on the formation efficiency of the Ag nanoparticles, their average size and stability have been studied. Significant antibacterial activity on two strains of Gram bacteria has been found.

Since humans wear clothes, anti-bacterial properties of textiles are pertinent. Matyjas-Zgondek and coworkers¹⁷ have studied bacteriostatic efficacy of selected silver particles in the finishing of textiles: nano-Ag, sub-micro-Ag and AgCl. Two strains of bacteria: Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) were studied. Mostly silver compounds were well dispersed on the fabric surface, but in some cases those compounds form agglomerates of single particles. The authors conclude that the antibacterial treatment of textile fabrics “can easily be achieved”.

Table 2. Ion release profiles of HEX, PLA and ECO samples, specifically Ag and As release (mg/L)

	Ag	As		Ag	As		Ag	As
HEX-C	–	–	PLA-C	–	–	ECO-C	–	–
HEX-1	–	13.6 (2.3)	PLA-1	–	8.6 (1.0)	ECO-1	–	19.3 (2.0)
HEX-2	<1.0	–	PLA-2	<1.0	–	ECO-2	<1.0	–
HEX-3	<1.0	12.2 (2.0)	PLA-3	<1.0	11.2 (1.0)	ECO-3	<1.0	21.5 (1.9)

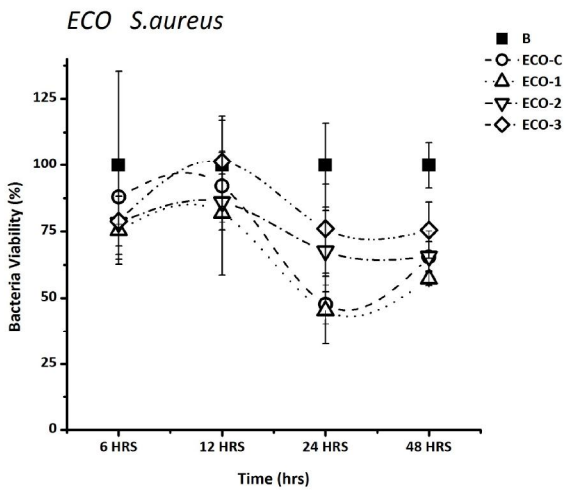


Fig. 7. ECO series tested in *S. aureus* over 6, 12, 24 and 48 h of incubation

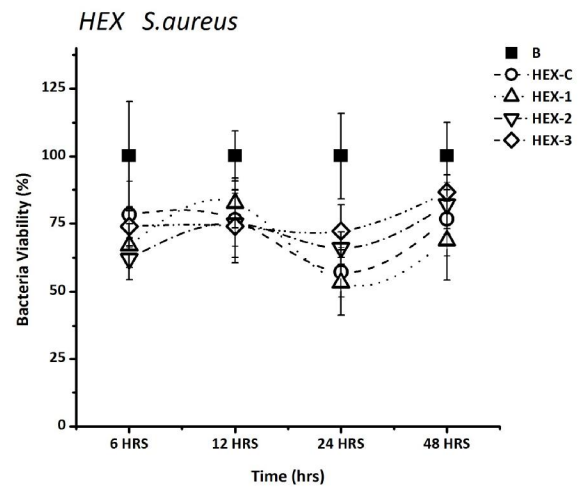


Fig. 8. HEX series tested in *S. aureus* over 6, 12, 24 and 48 h of incubation

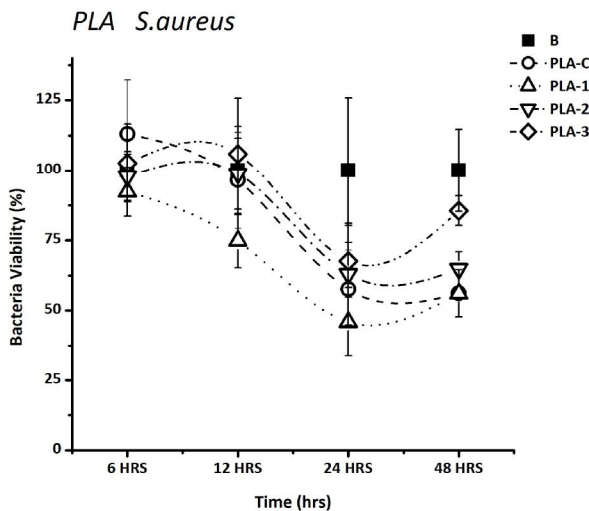


Fig. 9. PLA series tested in *S. aureus* over 6, 12, 24 and 48 h of incubation

Indirect prevention of bacterial activity also deserves to be noted. Ravichand and coworkers in Hyderabad¹⁸ have developed a method of drinking water purification by creation of membranes. Poly(vinylidene fluoride) membranes were hydrophilized by blending with the polyvinyl alcohol and then cross-linking with glutaraldehyde.

Koval and Starchevskyy¹⁹ stress the fact that the water treatment technology has to exclude the use of chemical reagents such as chlorine or ozone - which lead to the formation of toxic compounds. As noted in a textbook of Materials Science and Engineering⁶ "the toxic effects on human health deserve more attention". Koval and Starchevskyy applied the acoustic cavitation *via* ultrasound in the presence of several gases bubbled through the aqueous media: Ar, He, CO₂ and O₂. They studied the viability of *Diplococcus*, *Sarcina lutea*,

Bacillus cereus as well as *Pseudomonas fluorescens* bacteria and also the yeast (*Saccharomyces cerevisiae*) in natural water and in wastewater. The most efficient microorganism destruction has been obtained using the acoustic cavitation via ultrasound in the presence of bubbled Ar.

Consider now review articles written for use in teaching. There are articles on crystallography²⁰, on viscoelasticity²¹, on electronic materials systems for power transmission²², on Materials Science learning experience at a university venue for middle school students²³, on converting traditional Materials laboratories to project-based learning experiences²⁴, on "green" approach with an example of using post-consumer waste²⁵, on polymers in the production of crude oil²⁶⁻²⁹. As noted by Gedde and Hedenqvist³⁰, the same general laws are applied to both synthetic and natural polymers - while relations between

mechanical and tribological properties of polymers exist³¹. Our results reported above seem also to suggest that antibacterial behavior of polymers deserves attention in the instruction as well.

4. Conclusions

Initially characterization of the materials was conducted to determine differences in the structure, morphology and solubility of the processed composites. HEX-C was found to contain no Ag₂O or As₂O₃, while organic components of the polymer phase were evident: C₁₂H₇NO₂ and C₂H₃Cl. Amorphous humps are present within the 15–30° 2 theta range. HEX-1 and HEX-3 were found to contain crystalline peaks indicative of As₂O₃ while the incorporation of Ag₂O did not induce any crystallinity in HEX-2 and HEX-3.

SEM and EDX analyses were used to further image the composite samples to verify processing consistency and determine the elemental composition. As is present in ECO-1 and ECO-3 and Ag is present in ECO-2 but absent in ECO-3.

Antibacterial evaluation of each of the composite against *E. coli*, *S. aureus*, *C. albicans* and *S. epidermidis* was conducted. At 20 % of each of the bacterial agents the bacterial cell viability was reduced significantly.

For each of the samples tested, ion release profiles were determined, specifically analyzing Ag and As release after 30 days incubation in DI water. Some of the compositions showed very low Ag release rates < 1 mg/L.

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АНТИБАКТЕРІАЛЬНА ЕФЕКТИВНІСТЬ ПОЛІМЕРНИХ КОМПОЗИТІВ, ЛЕГОВАНИХ СРІБЛОМ АБО МИШ'ЯКОМ ПРОТИ КІЛЬКОХ ВИДІВ БАКТЕРІЙ

Анотація. Досліджено структуру та ряд властивостей полімерних композитів, легованих $AgNO_3$ та As_2O_3 , зокрема їхню антибактеріальну активність щодо *E. coli*, *S. aureus*, *C. albicans* та *S. epidermidis*. Нові полімерні композити, леговані сріблом або миш'яком, охарактеризовані за допомогою дифракції рентгенівських променів (XRD), скануючої електронної мікроскопії в поєднанні з енергодисперсійною рентгенівською спектроскопією (SEM/EDS) та дослідженнями вивільнення йонів. Проведено антибактеріальну оцінку кожного з композиційних зразків з використанням *S. aureus* у рідкій бульйонній культурі з додаванням до бактеріальної культури 10, 20 та 30 % рідкого екстракту. Контрольні зразки *S. aureus* використовували для порівняння і реєстрували при 100 % в кожен період часу. Встановлено, що для зразків із пластифікатором PLA життєздатність бактерій значно знижується для кожної композиції, що містить Ag/As , і є подібною до кожної дози концентрації.

Ключові слова: миш'якові композити, срібні композити, полімерні композити, антибактеріальна поведінка.