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ANTIMICROBIAL POLYPROPYLENE AS MATERIAL FOR SAFE WATER SUPPLY

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Abstract: *As the drinking water supply system is a risk factor for the degradation of the quality of the water intended for human consumption, the opportunity to use pipes made from antimicrobial materials is a solution to eliminate the microbiological risk to which consumers are permanently exposed. This study investigates the antimicrobial potential of polypropylene pipes containing silver nanoparticles, while using Escherichia Coli as the reference microorganism. In order to better emphasize the advantages of using antimicrobial pipes, the trials were conducted in comparison with polypropylene random (the most commonly used material for the construction of drinking water supply systems).*

Key words: *polypropylene, pipes, silver nanoparticles, Escherichia Coli.*

1. Introduction

Waterborne epidemics are a long standing problem worldwide, far from being solved even in the developed countries [2], [7]. A recent example in this respect is the waterborne epidemic in France, Le Havre, where shigellosis affected over 1000 inhabitants. As a result of the excessive use of disinfectants in recent times, germs have become increasingly resistant, which is the reason why it is expected that water microbiological security will be a serious problem of the 21st century [1], especially as the waterborne diarrhoeal disease is the second most frequent cause of death worldwide after cardiovascular diseases [4]. In these circumstances, prevention and control are mandatory actions for maintaining the health of people, an issue also highlighted in the Drinking Water Directive 98/83/EC which aims to "obtaining safe water by applying scientific knowledge". Obtaining and supplying safe water to the consumer involves good risk management [2]. The preliminary stage of risk management consists in identifying critical control points by applying *The hazard analysis and critical control point* (HACCP) system. A critical control point is the drinking water supply system, an issue discussed by the World Health Organization [13] in congresses and conferences in order to find optimal solutions that lead to safe water [14].

Prevention and control are currently ensured by the drinking water producers by disinfection and monitoring, control and audit plans, established under Law no. 458/2002 on drinking water quality (republished) - amended by Law no. 311/2004, however these actions entail certain side effects. Today, the most widely used method of disinfection

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worldwide is chlorination. This method of disinfection has some inconveniences: it dehydrates the skin and causes ageing, leads to toxic derived agents, carcinogens and mutagens (trihalomethans, chloroform, dibromo-chloromethane etc.) [9]. In these circumstances, the reduction of chlorine level, by using supply systems made from antimicrobial materials, would be a solution for to eliminate the above mentioned inconveniences, especially since antimicrobial materials are currently proving to be effective in an increasing number of fields of activity [5]. There are also studies that prove the efficiency of antimicrobial materials against the formation of biofilms, an issue that must be considered when discussing about drinking water supply systems [8], [11].

2. Materials and Methods

In view of the fact that the pathogenic bacteria that may be present in the drinking water are of the kinds *Escherichia, Shigella, Salmonella* and given the fact that the bacteria most often responsible for the waterborne diarrhoeal disease is *Escherichia Coli* [3], for the microbiological determinations in this study the reference microorganism used was *Escherichia Coli*, in the forms described below:

• *Escherichia Coli* ATCC 35218 strain, certified reference material, of known concentration, lot 495 64 3, coming from Microbiologics USA;

• *Escherichia Coli* ATCC 25922 strain, as a reference material selected by KWIK STIK, lot 335-100-4, coming from Microbiologics USA;

• *Escherichia Coli* culture, coming from Compania Apa Braşov, Drinking Water Laboratory;

• Sterile distilled water;

• Pipe made from polypropylene random PP-R, coming from Valrom Industrie Bucharest;

• Polypropylene pipe containing silver nanoparticles, coming from Valrom Industrie Bucharest.

Due to the fact that the field of antimicrobial materials is recent, the microbiological methods applied to determine their antimicrobial efficacy are still being continuously improved, very few being standardized [6]. In the awareness that results must be reproducible and that they must have a high degree of confidence, in order to determine with certainty the anti-microbial nature of the pipes with Ag nanoparticles, 5 determination methods were tested (4 quantitative and 1 qualitative methods) as follows:

• Method I

Under the microbiological niche and in an environment with controlled anaerobic microflora, one pellet of *Escherichia Coli* ATCC 35218 certified strain, lot 495 64 3, concentration 5.9×10^3 ufc, coming from Microbiologics USA, ATCC Licensed Derivative was placed in a 1000 ml sterile volumetric flask using tweezers. Sterile distilled water and ~100 mL pH 7 buffer were used for dispersion according to the manufacturer's instructions. Thus 1000 mL suspension of *Escherichia Coli* was obtained, containing 5900 ufc of *Escherichia Coli*. Two Berzelius beakers were used, one containing a piece of common polypropylene, and the other one polypropylene with Ag and 100 mL of well homogenized suspension of *Escherichia Coli* was placed in each beaker using a sterile calibrated dropper. So ~590 ufc of *Escherichia Coli* was placed in each Berzelius beaker. The two thus constituted samples were covered with sterile covers and were incubated for 24 h at a temperature of 37 °C in a thermostatic enclosure equipped with an electronic permanent temperature monitoring system.

After the 24 h of incubation, the suspensions in which the materials to be analysed were placed were analysed using the membrane filtration method according to SR EN ISO 9308-1/2004 *AC:2009 - Water quality. Detection and countering of E. coli*. The principle of this method consists in filtrating the 100 mL suspension to be analysed by means of 0.45 μm pore size membranes. The bacteria in the sample to be analysed thus remain on the surface of the filtering membrane and then the filled membranes are placed on selective culture media using sterile tweezers. A reduction of the number of colony forming units expected indicates the antimicrobial nature of the analysed material.

To ensure the quality of results, Nutri Disks Tergitol TTC were used, containing lactose, triphenyl tetrazolium chloride (TTC) and sodium heptadecyl sulphate (Tergitol). Nutri Disks Tergitol TTC includes single-use, sterile Petri boxes containing ready-made, dehydrated and sterile culture media and are accompanied by the filtering membranes corresponding to the medium type, with the same diameter and individually packed.

The samples obtained from the two material samples were placed over the media that were first hydrated by moistening with sterile water that was filtered using 0.2 μm pore size membranes. The thus prepared samples were incubated for 24 h at a temperature of 36 ± 2 °C.

• Method II

A specific Tryptone Soy Agar (TSA) medium was prepared as follows: 40 g of TSA dehydrated medium, of Scharlau origin was dissolved by heating in 1000 mL sterile distilled water and pH was adjusted to 7.2 ± 0.1 at 25 °C. The thus prepared medium was sterilized at 121 °C for 15 minutes and distributed to plates. After cooling, the plates were kept in the refrigerator at 4-12 °C, in sealed bags. When used, they were first heated at 37 °C for 2 hours. Over the thus prepared plates 1 mL, respectively 1.2 mL of initial suspension of *Escherichia Coli,* concentration 5.9×10^3 ufc was placed using a sterile dropper. In the first Petri box a sample of common polypropylene was fixed and in the second box, a sample of antimicrobial polypropylene was fixed. The thus prepared samples were incubated for 24 h at a temperature of 36 ± 2 °C temperature variations were excluded, according to the schedule indicated by the electronic temperature monitoring system (metrologically very-fied).

• Method III

By means of a sterile stick with cellulose end, soaked in the *Escherichia Coli* suspension with concentration 5.9×10^3 ufc, obtained according to the description in experiment no. I, *Escherichia Coli* germs were transferred to 2 Petri plates in which there is TSA medium, prepared according to the description in experiment no. II. A sample of common polypropylene, respectively a sample of antimicrobial polypropylene was fixed on the thus prepared plates. Samples were incubated for 24 h at a temperature of 36 ± 2 °C, permanently monitored. The purpose of this method is to obtain an inhibition zone (halo) around the sample with antimicrobial nature.

• Method IV

In the polypropylene pipe with silver there were introduced 800 mL of suspension of *Escherichia Coli* with concentration 5.9×10^3 ufc, prepared according to the description in experiment no. 1. The pipe filled with the germ suspension was submitted to stagnation for 24 h at room temperature (23 °C). After the 24 h of stagnation, the concentration of the suspension in the pipe was determined in

duplicate using the membrane filtration method according to SR EN ISO 9308-1/2004 *AC:2009 - Water quality. Detection and countering of E. coli*.

This experiment simulates the real situations found in the supply systems.

• Method V

A Tryptone Bile X-Glucuronide (TBXG) medium, of Scharlau origin was prepared as follows: there were weighted 31.6 g of dehydrated TBXG medium, which was dissolved in sterile distilled water and quantitatively transferred to a 1000 mL volumetric flask. The thus obtained solution, adjusted to the pH 7.2 ± 0.2 at 25 °C, was sterilized at a temperature of 121 °C for 15 minutes. The thus prepared medium was poured in Petri boxes and subsequently seeded with a subculture of *Escherichia Coli* ATCC 25922, obtained according to the scheme in Figure 1.

On 2 plates with TBXG there were inoculated *Escherichia Coli* colonies from the work subculture (3) using a seeding loop.

The common polypropylene sample and the antimicrobial polypropylene sample were fixed on the thus prepared plates. The thus prepared samples were incubated for 2 h at a temperature of 37 °C and then for 22 more hours at a temperature of 44 ± 2 °C.

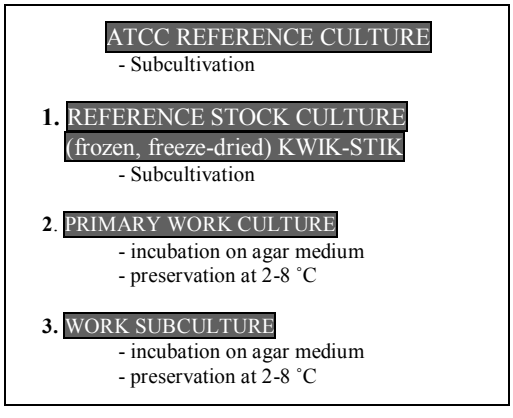


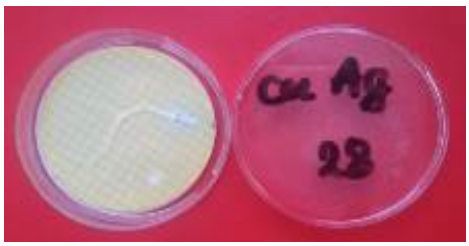
Fig. 1. *Scheme for obtaining the work subculture Escherichia Coli*

3. Results and Discussions

• Results of method I

For the sample with polypropylene PP-R the number of colony forming units was over 200, while for the sample with polypropylene with Ag nanoparticles only one colony unit was obtained, as shown in Figure 2.

a)



b)

Fig. 2. *Comparative results between the two types of propylene: a) without Ag; b) with Ag*

The results achieved above prove the antimicrobial nature of polypropylene containing Ag nanoparticles. Moreover, it may be considered that the efficiency of polypropylene with Ag nanoparticles is remarkable, given the very high initial concentration of the bacterial suspension (590 ufc/ 100 mL).

• Results of method II

By using the volumes of 1.2 and 1 mL suspension of *Escherichia Coli* with concentration 5.9×10^3 ufc and admitting that the materials have no antimicrobial nature, the expected results are 7-8 ufc for the sample with Ag, respectively 6 ufc for

the sample without Ag. 8 ufc were obtained for the sample with polypropylene with silver (Figure 3a) and 11 ufc were obtained for the sample with polypropylene without silver (Figure 3b).

a)

b)

Fig. 3. *Results for polypropylene with Ag (a) and results for polypropylene without Ag (b)*

• **Results of method III**

The result achieved by this method was 0 ufc as shown in Figure 4.

Fig. 4. *Results for polypropylene with Ag*

According to the result achieved, it is considered that polypropylene with Ag has a strong antimicrobial effect.

• **Results of method IV**

The results achieved by this method, which simulates very well the real situation in the supply systems, are remarkable: 0 ufc/100 mL (Figure 5), were values of ~59 ufc/100 mL were expected. The results achieved in duplicate using this method prove once again the efficiency of the antimicrobial material.

Fig. 5. *Result achieved at stagnation in the antimicrobial polypropylene pipe*

• **Results of method V**

The method of seeding on TBXG sought to achieve the emergence of a zone of inhibition at the antimicrobial material as compared to common polypropylene. Upon careful analysis of the images in Figure 6 it can be seen that polypropylene PP-R let the germs develop even on its on edge, while the antimicrobial polypropylene did not allow this phenomenon.

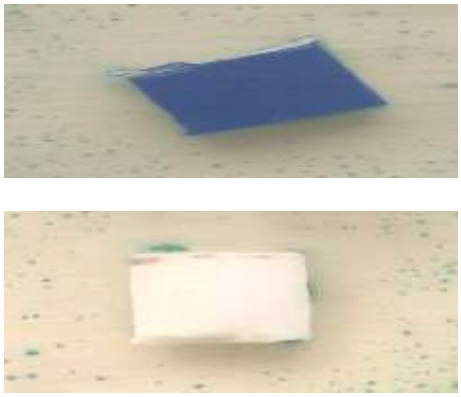


Fig. 6. *Results achieved on TBXG medium*

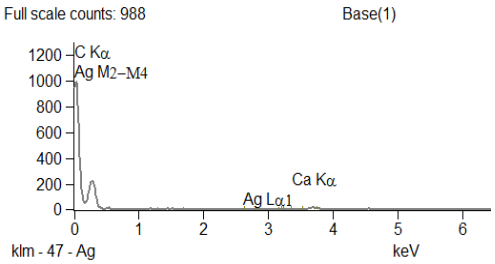
Taking into account that pipes are intended for drinking water and not for water with high bacterial concentrations, as it was done in the experiment, the silver concentration in PP is satisfactory.

4. Material Characterisation

Moreover, studies conducted on plant embryos showed that a too high concentration of Ag ions may have adverse effects on living organisms.

In order to determine the concentration of biocidal agent, polypropylene with Ag nanoparticles was analysed by EDX (Figure 7). The data recorded shows a low concentration of silver, as shown in Table 1.

The material characterisation was also performed by morphological observations using the SEM analysis (Figure 8).



Elem. Line	*Net Counts*	*Weight %*	*Atom %*
C K	1952	90.63	*97.32*
Ca K	130	7.72	2.48
Ag L	24	1.65	0.20
Total		100.00	100.00

Fig. 7. *EDX results*

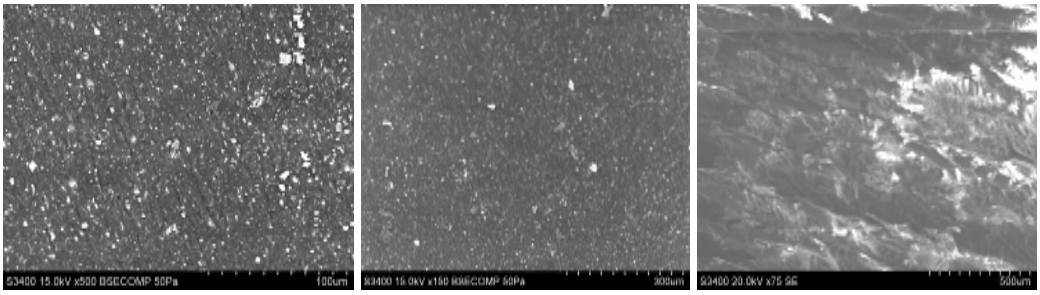


Fig. 8. *SEM images of antimicrobial polypropylene*

5. Conclusions

By cumulating the results achieved using the six test methods it may be concluded that polypropylene with Ag nanoparticles has an antimicrobial nature.

The most representative way to determine the antimicrobial nature of the pipes is provided by method IV, by reproducing the real situations in the supply systems and also by the certainty of the results achieved.

Given the fact that germs became resistant to conventional antibiotics, the use of pipes made from antimicrobial polypropylene for drinking water supply is an ideal solution for the microbiological safety of water. Moreover, by using antimicrobial pipes, the chemical safety of water is also increased by reducing or eliminating chlorine-based conventional disinfectants. The use of pipes with Ag provides constant disinfection, a process that is not provided by the chlorine, due to its interaction with other chemical elements, which leads to the lower concentration of free chlorine. Another advantage is the antimicrobial efficiency of silver in very low concentrations, which means reasonable manufacturing costs and also the elimination of the toxicity risk to the consumer. While chlorine exerts its toxicity by multiple ways: oral ingestion, inhalation and contact with the skin, higher concentration being very harmful, silver in contact with water leads to a very low

concentration of Ag^+ ions into the water. These concentrations are rarely of toxicological significance in view of strong tendency of Ag^+ to form inert precipitates with proteins and inorganic anions. Macrophages perform a central function in mopping up silver precipitates in wound sites and perineural, connective and interstitial tissues [10]. Even some nanoparticles could be accidentally released from the PP pipes, their concentration in water will be very low, due to the initial very low content of Ag into the pipes (1.65%). It was also determined that Ag nanoparticles concentration in water is not toxic up to 25 μg/mL [12]. This concentration is impossible to be obtained by simple contact of the pipe surface with water. Durability of the PP pipes containing Ag nanoparticles, in terms of preserving of antimicrobial activity, will be an issue for future studies.

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References

1. Almagro-Nieves, D., Guisosa-Campos, P., Garrido-Almagro, S., García-Aragón, M.A.: *Epidemic Outbreak of Acute Gastroenteritis Due To Norovirus with Possible Hydric Origin*. In: Enfermedades Infecciosas y Microbiología Clínica **24** (2006) No. 2, p. 93-95.
2. Ashbolt, N.J., Grabow, W.O.K., Snozzi, M.: *Indicators of Microbial Water Quality*. In: Water quality-Guidelines, standards and health: Assessment of risk and risk management for water-related infectious disease. In: Fewtrell, L. & Bartram, J. (Eds.), IWA Publishing, London, 2001, p. 289-315.
3. Batabyal, P., Mookerjee, S., Sur, D., Palit, A.: *Diarrheogenic Escherechia coli in Potable Water Sources of West Bengal, India*. In: Acta Tropica **127** (2013) No. 3, p. 153-157.
4. Bowen, A.B., O'Reilly, C.E., Perez, N.E., Sarisky, J.P., Shepherd, C.A., Miller, M.D., et al.: *A Waterborne Outbreak of Gastroenteritis with Multiple Etiologies among Resort Island Visitors and Residents: Ohio, 2004,*. In: Clinical Infectious Diseas **44** (2007) No. 4, p. 506-512.
5. Damian, L., Patachia, S.: *Antibacterial Materials - A Future Insigt*. In: Bulletin of the *Transilvania* University of Braşov (2014) Vol. **7** (56) No. 1, Series I: Engineering Sciences, p. 25-32.
6. Damian, L.N., Patachia, S.: *Method for Testing the Antimicrobial Character of Materials and Their Fitting to the Scope*. In: Bulletin of the *Transilvania* University of Braşov (2014) Vol. **7** (56) No. 2, Series I: Engineering Sciences, p. 37-44.
7. Guibal, E., Cambe, S., Bayle, S., Taulemesse, J-M., Vincent, T.: *Silver/chitosan/cellulose Fibers Foam Composites: From Synthesis to Antibacterial Properties*. In: Journal of Colloid and Interface Science **393** (2013), p. 411-420.
8. Jooyoung, S., Jyongsik, J.: *Antimicrobial Polymer Nanostructures: Synthetic Route, Mechanism of Action and Perspective*. In: Advances in Colloid and Interface Science **203** (2014), p. 37-50.
9. Mingquan, Y., Mingyang, Li., Xuze, H.: *Behaviour of I/Br/Cl-THMs and Their Projected Toxicities under*

Simulated Cooking Conditions: Effects of Heating, Table Salt and Residual Chlorine. In: Journal of Hazardous Materials **314** (2016), p. 105-112.
10. Naughton, D.P., Nepusz, T., Petroczi, A.: *Metal Ions Affecting the Gastrointestinal System Including the Liver*. In: Met. Ions Life Sci. **8** (2011), p. 107-132.
11. Patrícia S. de A. Mendes, Hugo da C. Ribeiro Jr., Carlos Maurício C. Mendes: *Temporal Trends of Overall Mortality and Hospital Morbidity Due to Diarrheal Disease in Brazilian Children Younger Than 5 Years from 2000 to 2010*. In: Jornal de Pediatria **89** (2013) No. 3, p. 315-325.
12. Takamiya, A.S., Bernabé, D.G., Gorup, L.F., Camargo, E.R., Gomes-Filho, J.E., Oliveira, S.H.P, Barbosa D.B.: *In Vitro and in Vivo Toxicity Evaluation of Colloidal Silver Nanoparticles Used in Endodontic Treatments*. In: Journal of Endodontics, (online 2016), DOI: http://dx.doi.org/10.1016/j.joen.2016.03.014.
13. WHO (World Health Organization): *Guidelines for drinking water quality*. 3rd Ed. Recommendations. Incorporating 1st and 2nd Addenda, vol. 1, Geneva, 2008.
14. http://www.apapura.ro/2010/02/boli-cu-transmitere-hidrica.html. Accessed: 15-01-2015.