FOOD PACKAGING WITH ANTIBACTERIAL PROPERTIES

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The incorporation of antimicrobial agents is one of the best routes to limit microbial growth on materials. Synthesis of silver nanoparticles with antimicrobial properties has increased greatly in recent years due to their numerous applications such as food packaging.

The aim of this paper is to study the synthesis of silver nanoparticles by photochemical reduction of $AgNO_3$ in an acrylic monomer (PEG600 diacrylate) using a photo-initiator and characterization of their antibacterial properties against gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacteria. The silver migration into a food simulant (distilled water) was analyzed by ICP-MS in accordance with the conditions specified in Commission Regulation (EU) no. 10/2011 on plastic materials and articles intended to come into contact with food.

Keywords: food packaging, antibacterial activity, silver nanoparticles, ICP-MS

1. Introduction

The package is an "object" for retention, protection, handling, delivery and presentation of products, from raw materials to processed goods, from the producer to the user or consumer. Packages fulfill a number of vital functions in the supply of products from manufacturers to consumers, they wouldn't exist without the products they contain and many products would not exist without packages that provide a method of delivery.

The principal roles of food packaging are to protect food products from outside influences and damage, to contain the food and to provide consumers with ingredient and nutritional information. Food packaging can delay product deterioration, retain the beneficial effects of processing, extend shelf-life, and maintain or increase the quality and safety of food. For this purpose, the packaging provides protection against chemical, biological and physical influences coming from outside [1].

Active packaging is a modern food packaging technique in which the product, packaging and interior environment interact in a positive way in order

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to extend shelf life and improve the sensory properties of food, maintaining the quality and safety of the packaged product.

Antimicrobial packaging is one of the multiple applications of active packaging. It is a packaging system capable of destroying or inhibiting spoilage and pathogenic microorganisms which contaminate food [2]. Antimicrobial packaging is designed especially to control the growth of microorganisms, unlike conventional packaging systems that are used to extend the shelf life and to maintain quality and food safety [3].

In the last years, the use of silver nanoparticles-based antimicrobial films has increased significantly due to the toxicity of silver towards a wide range of microorganisms [4] as well as its stability at high temperatures [5]. Although it is known that silver nanoparticles are an effective antimicrobial agent against a broad spectrum of Gram-negative and Gram-positive bacteria, the exact mechanism of their antibacterial action is not yet fully understood, but possible mechanisms for metallic silver, silver ions and silver nanoparticles have been proposed in accordance with the structural and morphological changes found in microbial cells [6, 7].

The main mechanisms proposed for the antimicrobial action of silver are carried out through: protein inactivating, DNA association, entering the cell and its deterioration, the ionized form of silver [8-13].

The aim of this paper is to test the antibacterial activity of some silver nanoparticles formulations obtained by photochemical synthesis in an acrylic monomer against some pure strains of gram-negative and gram-positive bacteria and correlate the antibacterial activity with the silver amount released into a food simulant which was determined by inductively coupled plasma mass spectrometry (ICP-MS).

2. Experimental part

2.1. Materials and equipments

The silver nanoparticles precursor used in the synthesis was silver nitrate $AgNO_3$ (Aldrich). Polyethylene glycol 600 diacrylate (SR610) monomer and 2-hydroxy-1-[4-(2-hydroxyetoxy)phenyl]-2-methyl-1-propanone photo-initiator (Irgacure 2959) were used as received. The role of the photo-initiator is to reduce silver salt forming nanoparticles in the same time with the initiation of the polymerization reaction of acrylic monomer.

2.2. Methods

4 types of samples with different $AgNO_3$ content (0.2; 0.5; 1 and 2%, % wt) were obtained. In Fig.1 is presented the scheme for sample preparation. The reactive oligomer and photoinitiator (0.5 wt % versus oligomer) were homogenized under stirring and different quantities of silver nitrate (0.2, 0.5, 1 and 2 wt %) were added. Synthesis of nanoparticles was performed by irradiation of the samples which were coated onto a glass substrate in the form of a film with a thickness of ~ 6 microns (Lightning cure L8333 with a Hamamatsu L8253 Xe-Hg 100 W lamp). The synthesis process is the subject of another paper of the authors which is *in press* [14].

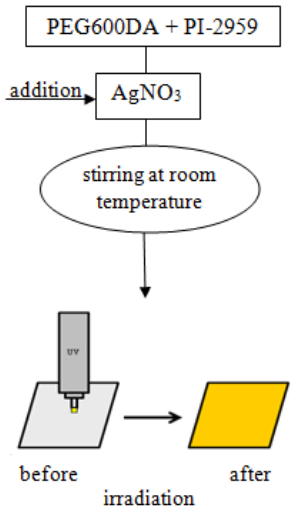

Fig. 1. Scheme showing the steps for sample preparation

Antibacterial activity of obtained silver nanoparticles was determined against pure strains of gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria, using Kirby-Bauer disk diffusion method. The samples were incubated at 37 °C for 24 hours, after which the inhibition zone dimensions were measured.

The released silver amount was determined by ICP-MS, using an equipment NexIon 300q produced by Perkin Elmer. Analysis was performed after immersion of samples into a food simulant (distilled water) at 70 °C for 2 hours, using an extraction ratio sample surface: liquid volume of 1:10. These

conditions can be found in the Commission Regulation (EU) no. 10/2011 on plastic materials and articles intended to come into contact with food.

3. Results and discussions

In Europe, the use of nanomaterials in food packaging is generally regulated under the framework regulation EC 1935/2004, which states that their use in food packaging may not pose a danger to human health (Article 3). In accordance with Article 23 of Regulation EC no. 10/2011, nanoparticles have to undergo case-by-case evaluation before being placed on the market. Nanomaterials have to be authorized even if the equivalent bulk material is already authorized (Directive EEC 89/109). If a non-authorized substance is used, a migration limit of 0.01 mg/kg shall be observed through use of a functional barrier (Regulation EC no. 450/2009, art.14) [15]. For plastic food packaging, currently only three nanomaterials have been authorized, namely carbon black, titanium nitride and silicon dioxide.

In 2011, the European Food Safety Authority (EFSA) published a guidance document "on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain" in which it indicates which physicochemical information is required from the manufacturer. It requests in vitro genotoxicity, absorption, distribution, metabolism and excretion (ADME) tests and a repeated-dose 90-day oral toxicity study. Denmark and France are the only countries currently establishing a registry of products containing nanomaterials [16, 17].

Considering the above specified mentions, it is even more important to determine the amount of silver that can migrate from the packaging material into a food simulant (or food product). Thus, the aim of the present study was to determine the amount of silver migrated from samples applied on glass sheets into a food simulant (distilled water). The results obtained are shown in Table 1.

Table 1

Results of ICP-MS analysis

Sample	Ag amount in the applied sample (mg)	Migrated Ag conc. (μg/L)	Migrated Ag amount (mg)	Migrated Ag (%)
P_2	0,006	46,89	0,0015	25
P_3	0,015	82,53	0,0027	18
P_4	0,030	95,46	0,0031	10,33
P_5	0,059	332,27	0,0108	18,31

The synthesis of silver nanoparticles through a photochemical method was realized and their antimicrobial properties were determined in order to evaluate their possible use in food packaging materials.

For the determination of the antibacterial activity, samples consisting of films with a thickness of 6 microns deposited on glass sheets (with dimensions of 14*12 mm) were placed in Petri dishes containing culture media and bacterial inoculum. After incubation at 37 °C for 24 hours the inhibition zone dimensions were measured. For a better assessment of antibacterial activity the surface of inhibition zone was measured for each sample. Results are presented in Table 2.

Table 2

Antibacterial activity against *E. coli* and *S. aureus*

Sample	Ag amount in the applied sample (mg)	Inhibition zones dimensions (mm)				Surface of glass sheet (mm^2)	Inhibition zone surface (mm^2)	
		E. coli		*S. aureus*				
		L	l	L	l		*E. coli*	*S. aureus*
M	0	14	12	14	12	168	168	168
P_2	0,006	15	12	15	13	168	180	195
P_3	0,015	15	13	16	13	168	195	208
P_4	0,030	15	14	16	14	168	210	224
P_5	0,059	17	14	16	16	168	238	256

The results of antibacterial activity tests show the increase of the inhibition zone surface with the increase of the $AgNO_3$ amount. Samples not containing $AgNO_3$ do not present any antibacterial activity.

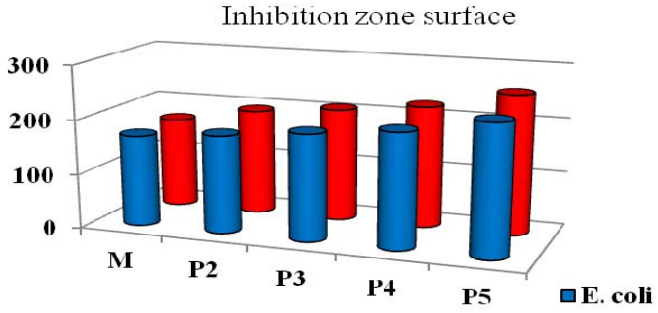

Fig. 2. Inhibition zone surfaces of tested samples

Antibacterial activity is more pronounced against *S. aureus* toward *E. coli* (see Fig. 2). This result is supported by previous reports [18, 19] and can be explained by the different cell wall thickness and structures of these bacteria.

Gram-negative bacteria possess an outer phospholipidic membrane with structural lipopolysaccharide components which is not found in Gram-positive bacteria [20].

In Fig. 3A is shown the correlation between the migrated amount of silver and initial amount of silver in samples P2-P5. It can be noticed that the migrated silver amount is function of initial silver amount but also is determined by the obtained particles shape and size (shape and size of particles are presented in the reference [14]). In Fig. 3B is presented the correlation between inhibition zone diameter and the amount of migrated silver. In this case it can be also observed that antibacterial activity is dependent not only on the amount of silver migrated but also on obtained nanoparticles shape and size.

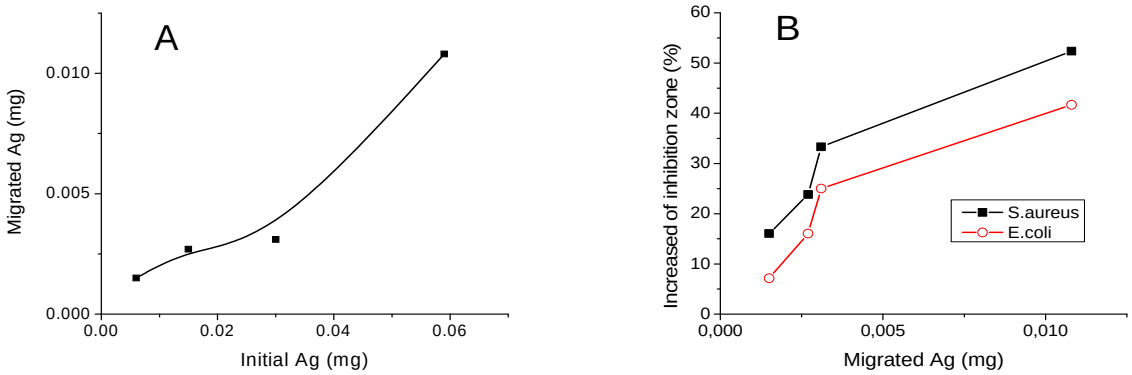

Fig 3. Correlations between initial and migrated amounts of silver (A) and increased of inhibition zone and migrated amounts of silver (B)

4. Conclusions

The use of silver nanoparticles with antimicrobial properties has increased greatly in recent years. Photochemical synthesis of silver nanoparticles in an acrylic monomer by using a photo-initiator capable to reduce silver nitrate at the same time with the initiation of the monomer polymerization is a versatile ”green” method. Antibacterial activity of nanoparticles obtained through this method was more pronounced against *S. aureus* than *E. coli*, probably due to higher thickness and different structure of the cell wall of gram-negative bacteria.

The antibacterial activity is dependent not only on the amount of silver migrated, but also on obtained nanoparticles shape and size. Properties of obtained nanoparticles are very important, influencing both migrated silver amount, and antibacterial activity (measured by growth of inhibition zone diameter).

In our future studies, we will correlate the methods for obtaining silver nanoparticles with the particles characteristics and antibacterial activity, in order to obtain a good activity, but having low amount of migrated silver.

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