Antimicrobial Polymer Films Functionalized with Cyclodextrins

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The present paper suggests the applying of some support activation methods (dry rubbing, cold plasma or alkaline treatment) for the improvement of the subsequent chemical treatment effects for the polyethylene terephthalate (PET) and polypropylene (PP) films for food package materials. Polyvinyl alcohol (PVA) coating grafted with monochlorotriazinyl-β-cyclodextrin (MCT) provide hosting cavities that can include a large variety of chemicals for specific antimicrobial coating. Some substances (ferulic acid, allantoin) have been included in the cavities of MCT grafted on coated layer. The inclusion compounds have been characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), Fourier Transform Infrared Attenuated Total Reflexion (FT-IR ATR) and Ultraviolet (UV) techniques. The antimicrobial properties of the samples were confirmed by microbiological tests. The antimicrobial activity against four different strains of microorganisms, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans, was evaluated by measuring the growing inhibition zone. Our results have shown the included components are efficiently hosted in the cyclodextrin (CD) nanocavities and the antimicrobial properties of the films are significant after the proposed chemical treatment.

Keywords: chemical treatment, cold plasma treatment, polymeric films, monochlorotriazinyl-β-cyclodextrin

Active packaging is a new concept of packaging that changes the condition inside the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food [1]. Among many applications such as oxygen-scavenging packaging and moisture-control packaging, antimicrobial packaging is one of the most promising innovations of active packaging technologies [2]. Antimicrobial packaging is a system that can kill or inhibit the growth of micro-organisms and thus extend the shelf life of perishable products and enhance the safety of packaged products [3]. It can be constructed by using antimicrobial packaging materials and/or antimicrobial agents inside the package space or inside foods [4]. Antimicrobial package materials may be classified into two types: those containing antimicrobial agents that migrate to the surface of the package material and thus can contact the food, and those that are effective against food surface microbiological growth without migration of the active agent(s) to the food [5]. Incorporation of antimicrobials into 'plastic' or 'bio' polymer films has been studied using a variety of antimicrobials and polymer materials. Antimicrobials tested include silver ions, triclosan, chlorine dioxide, peptides (such as lactoferrin), bacteriocins (primarily nisin), organic acids, enzymes, chelating agents, essential oils, seed extracts, plant skin extracts, plant pigments, and other plant extracts [6, 7].

Monochlorotriazinyl-β-cyclodextrin (MCT) is a reactive derivative of β-cyclodextrin , a cyclic oligosaccharide that provides hosting cavities able to include a large variety of substances for specific antimicrobial effects [8-11].

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a ubiquitous phenolic acid in the plant kingdom; it is found in the leaves and seeds of most plants, and especially in high concentration in the brans of cereals such as rice, wheat and oats. Ferulic acid has been reported to have many physiological functions, including antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis and anti-cancer activities. Due to its properties and low toxicity, ferulic acid is at present widely used in the food and cosmetic industries [12]. Allantoin (5-ureidohydantoin), present in botanical extracts of the comfrey plant (*Symphytum officinale*), is largely used in cosmetic products due to its moisturizing, keratolytic, anti-inflammatory and anti-irritant properties [13].

Cold vacuum plasma systems are of special interest for surface treatments because they often produce 'activated' states of matter, able to improve plasma–surface chemical reactions and physical processing, which cannot be achieved under thermodynamic equilibrium regime [14]. As a result, micro/nano-structures can be obtained by plasma processing, different from any other known surface functionalization method.

Advanced food packaging materials have a multilayer structure of different polymers, because only a combination of several polymers can provide efficient barrier properties toward many aggressive gases and microbes [15]. In order to realize barrier properties comparable with those provided by multilayer polymer food packaging materials, an additional coating has to be applied to homo-polymeric food packaging [16, 17], to attain an extended shelf life by product preservation [18].

Experimental part

Materials and methods

Two types of biaxially oriented films have been used as *supports*: polyethylene terephtalate (PET) with a thickness of 70 μm (3M Visual systems Products, France) and polypropylene (PP) with a thickness of 8μm (Geboplast, Iaşi, Romania). As *host,* chemically modified cyclodextrin (monochlorotriazinyl–β-cyclodextrin-MCT, with a degree of substitution of 0.46 per anhydrous glucose unit, from Wacker Chemie Gmbh, Germany) has been used. As guests, a few substances with different protection

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properties have been used: a cinnamic derivative [ferulic acid FA - trans 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid, Fluka, Switzerland] and an urea derivative (allantoin Al - 5-ureidohydantoin or glyoxyldiureide, Fluka, Switzerland). As a polymer with hydroxyl groups for the inclusion in protective films and functionalization with MCT, polyvinyl alcohol with residual vinyl acetate groups (PVA, from Loba Chemie Ltd., India) has been chosen.

Polymeric film coating

The obtaining of coatings on polymer films has been performed in three stages:

a-the preparation of the coating solutions in the next order:

- the realization of the inclusion compound in a stoichiometrical ratio host:guest of 1:1;
- the realization of the final coating solution with functionalized polymer (PVA) in a mass ratio PVA:MCT of 2:1, so that the substitution degree to be 1.4%;

b-the increasing of the adherence (the increasing of the roughness) for the PET and PP polymer films by 3 methods: *dry rubbing* ***(r)*** *with a polyamide-6 brush*, *cold plasma treatment* ***(pl)*** (pressure 0.9 torr; electric field intensity of 30 kV/cm, discharge power of 100 W; for 10 min; gaseous mixture oxygen:argon 80:20 in volumes) [19] and *alkaline treatment* (cold impregnation for 2 h, 50 g/l NaOH+1g/l wetting agent, rigorous washing at neutral *p*H) of PET films [20];

c-the coating **of films (PET, PP)** with solid polymeric layers by depositing the different solutions with blade deposition. The solvent was removed with a warm air flow (75-80°C, for 5 min) and by drying in the vacuum chamber (75°C, for an hour).

Surface film characterization techniques

Spectral methods

FT-IR ATR analysis was carried out on the Multiple Internal Reflectance Accessory (SPECAC, SUA) with KRS-5 (thallium/bromide – iodide) ATR crystal, with 25 reflections, at an investigation angle of 45 degrees, 250 scans in the 1800 - 600 cm $^{-1}$ range. This accessory was attached to the FTIR-IR Affinity-1 (Shimadzu-Japan) Spectrophotometer.

UV-VIS analysis was realized with a Beckman Coulter - DU 800 UV-VIS Spectrophotometer, in the 200-800 nm range, registration speed of 1200 nm/min, wavelength interval (reading step, $\Delta\lambda$) of 1 nm.

Microscopic methods

The PET and PP samples were evaluated by **SEM analysis**, using a QUANTA 200 microscope. The most important parameters were: magnifying power between 13 and 1,000,000 X in the resolution mode, a scanning speed between 200 ns and 10 ms per pixel, the working pressure is lower than $1x10^{-2}$ Pa, with a potential between 200 V and 30 kV and a 3 nm resolution at 30 kV.

AFM images were performed in air, on a SPM SOLVER Pro-M platform (NT-MDT, Russia), having a NSG10 cantilever with a 10 nm tip radius. The setup was operated in semi-contact mode with 230 kHz cantilever oscillating frequency, over 20 μm ×20 μm scan area, 256×256 scan point size images being thus obtained. The AFM image processing and the calculation of the surface texture parameters were realized by the Nova Software (NT-MDT, Russia).

Microbiological tests

The antimicrobial effect was tested by the Kirby-Bauer diffusimetrical method [21]. The 18 h-bacterial cultures were obtained from a bacterial inoculum, which was standardized according to the McFarland scale, yielding to 10^7-10^8 CFU/mL. The culture medium that has been used (LB) was inoculated with that inoculum and afterwards, polymer film disks, with the diameter of 1 cm, subject to different treatments, have been applied on the surface of the medium. The antimicrobial activity against four strains of microorganisms, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*, was assessed by measuring the inhibition zone after a 24 h-incubation at 37°C.

Results and disscussions

Spectral analysis

FT-IR spectroscopy

Solid samples (powders, support films with/without polymer coatings) have been registered FT-IR ATR by the direct contact with the KRS-5 ATR crystal. FT-IR ATR spectra of the solid coatings corresponding to the polymer solutions have been obtained by deposition on the ATR crystal and drying.

In figure 1 the absorption bands characteristic for PVA-MCT-FA (spectrum A, 1607, 1425, 1375, 1318, 1245, 1027, 946 and 846 cm^{-1}) are also present in the PVA-MCT-FA coated PETr films (spectrum C, 1609, 1432, 1374, 1317, 1240, 1035, 947 and 843 cm^{-1}). In figure 2 the absorption bands characteristic for PVA-MCT-AL (spectrum A, 1535, 1479, 1422, 1371, 1314, 1025, 945, 810 and 763 cm^{-1}) are

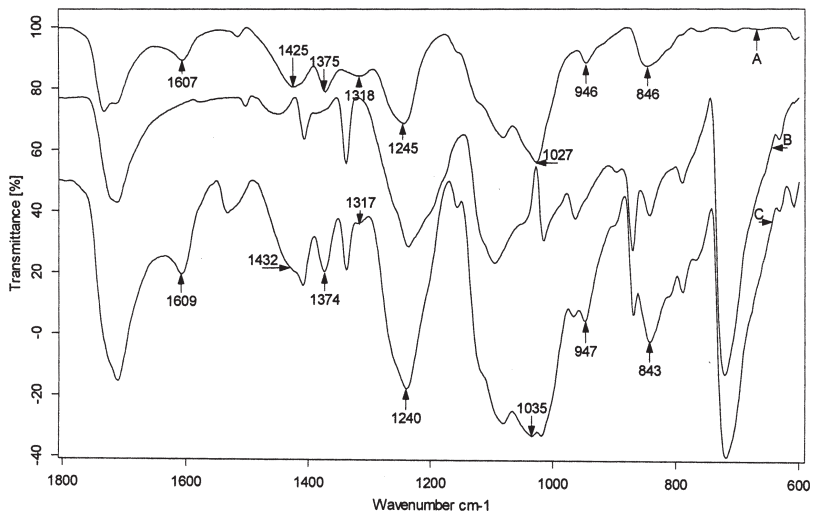

Fig. 1 FT-IR ATR spectra for: A=PVA-MCT-FA; B=PETr; C=PETr-PVA-MCT-FA

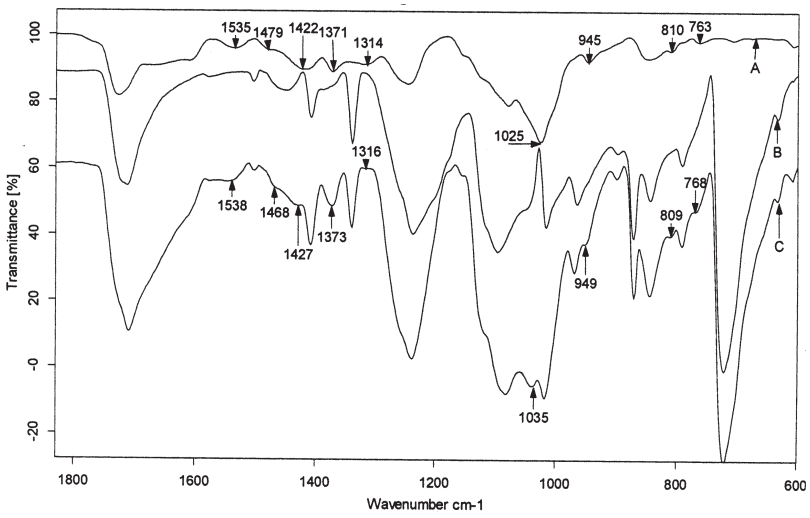

Fig. 2 FT-IR ATR spectra for: A= PVA-MCT-AL; B= PETr; C= PETr- PVA-MCT-AL

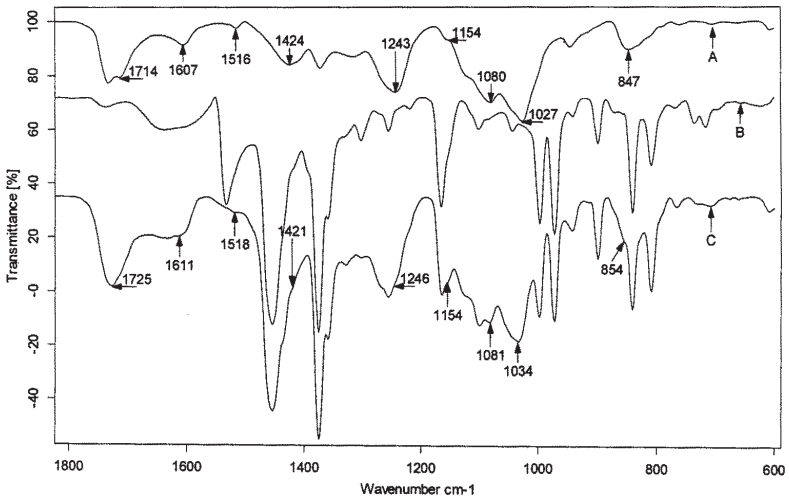

Fig. 3 FT-IR ATR spectra for: A=PVA-MCT-FA; B= PPr; C= PPr - PVA-MCT-FA

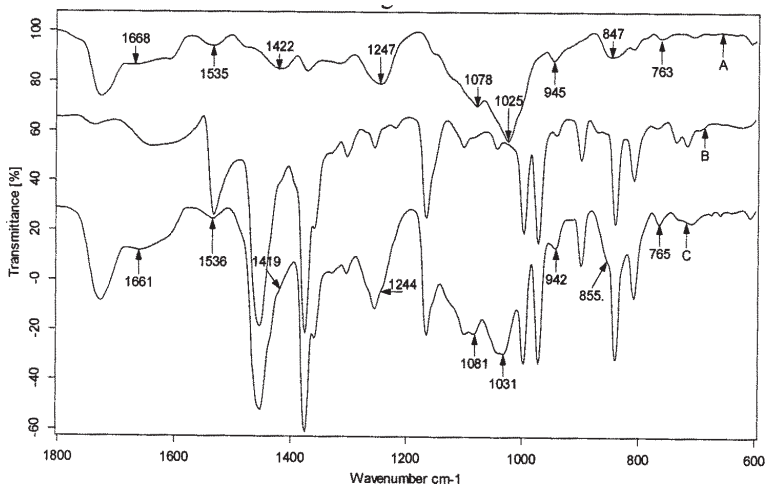

Fig. 4 FT-IR ATR spectra for: A= PVA-MCT-AL; B= PPr; C= PPr -PVA-MCT-AL

also present in the PVA- MCT-AL coated PETr films (spectrum C, 1538, 1468, 1427, 1373, 1316, 1035, 949, 809 and 768 cm^{-1}). In figure 3 the absorption bands characteristic for PVA-MCT-FA (spectrum A, 1714, 1607, 1516, 1424, 1243, 1154, 1080, 1027 and 847 cm^{-1}) are also present in the PVA- MCT-FA coated PPr films (spectrum C, 1725, 1611, 1518, 1421, 1246, 1154, 1081, 1034 and 854 cm^{-1}). In figure 4 the absorption bands characteristic for PVA-MCT-AL (spectrum A, 1668, 1535, 1422, 1247, 1078, 1025, 945, 847 and 763 cm^{-1}) are also present in the PVA-MCT-FA coated PPr films (spectrum C, 1661, 1536, 1419, 1244, 1081, 1031, 942, 855 and 765 cm^{-1}).

UV spectroscopy

The UV spectra which are shown below (fig. 5 and 6) have been realized in transmission mode. For the registration of the UV spectra, aqueous solutions of MCT, AL, MCT-AL (respectively, FA in ethylic alcohol, MCT-FA) have been used, placed in a quartz cell with an optical way of 1 cm; the polymer coatings (PVA, PVA-MCT, PVA-MCT-FA, PVA-MCT-AL), obtained by the water removing from the corresponding solution, are deposited on a quartz plate/ PP film.

In figure 5 the absorption bands characteristic to the polymer PVA grafted with MCT – FA (spectrum C, 218, 290 and 317 nm) are also present in the spectrum B of the PPr film coated with PVA grafted with MCT-FA (218, 290 and 317 nm). In figure 6 the absorption band characteristic to the polymer PVA grafted with MCT – AL (spectrum C, 230 nm) is also present in the spectrum B of the PPr film coated with PVA grafted with MCT- AL (223 nm).

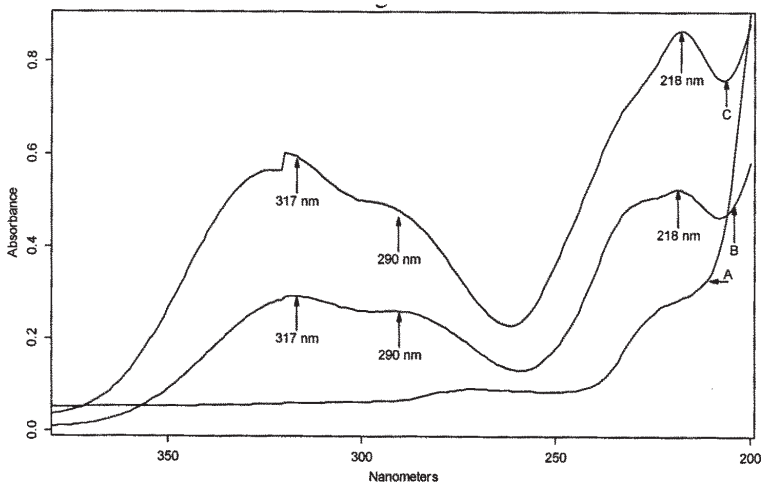

Fig. 5. UV spectra (200-350 nm): A=PPr; B=PPr-PVA-MCT-FA; C=PVA-MCT-FA

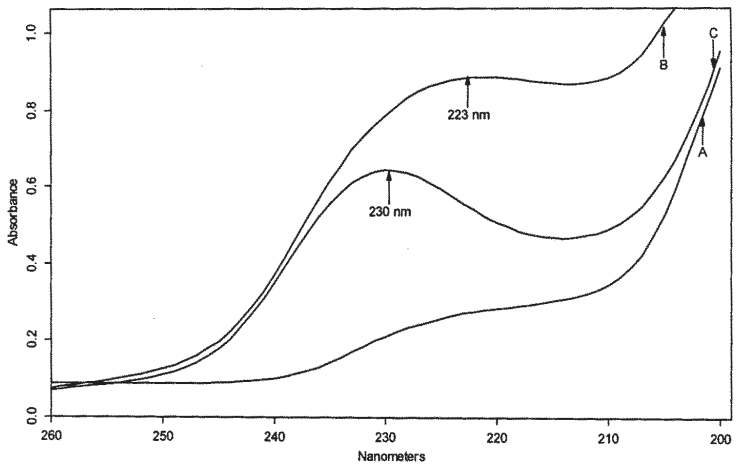

Fig. 6. UV spectra (200-260 nm): A=PPr; B=PP_r-PVA-MCT-AL; C=PVA-MCT- AL

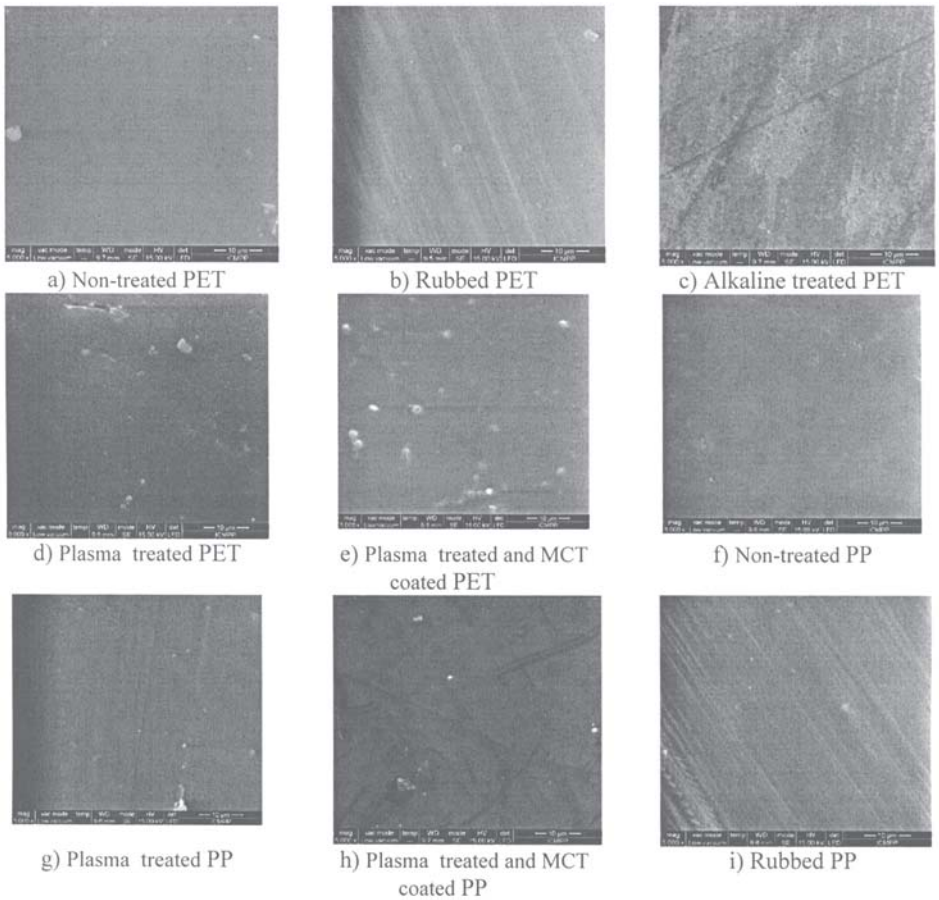

Fig. 7. The SEM images of non-treated and treated PET and PP films surfaces

SEM analysis

The SEM images (investigated area of 55 μm x 55 μm, magnifying power of 5000 X) of untreated and treated PET and PP films surfaces are presented in figure 7.

Comparative SEM analysis of non-treated (a, f), rubbed (b, i), alkaline treated (c) and plasma - treated films (d, g) allows the visualization of rough asperities, superficial microcavities and parallel traces, but for films treated with

Sample	Tested microorganism			
	Staphylococcus aureus(+) ATCC 25923	*Escherichia coli(-)* ATCC 25922	*Pseudomonas aeruginosa(-)* ATCC 27853	*Candida albicans*
Non-treated PET	-	-	-	-
PETr - PVA-MCT-FA	10	8	10	9
PETr - PVA-MCT-AL	9	9	9	9
PETpl - PVA -MCT-FA	9	8	8	9
PETpl - PVA -MCT-AL	9	8	9	8
Non-treated PP	-	-	-	-
PPpl - PVA -MCT-FA	10	8	9	8
PPpl - PVA -MCT-AL	9	9	10	8
PPr - PVA - MCT-FA	9	8	8	8
PPr - PVA - MCT-AL	9	7	8	8

Table 1
ANTIMICROBIAL ACTIVITY OF POLYMER FILMS - DIAMETER (MM) OF THE INHIBITION ZONE

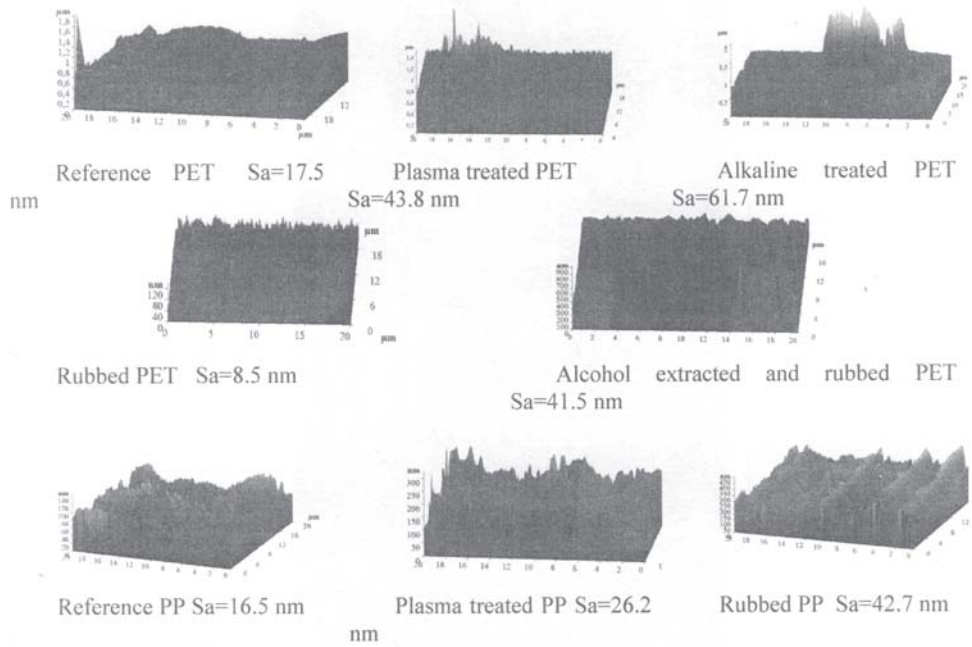

Fig. 8. AFM 3D topography images (20 mm×20 mm scan) and the average roughness values Sa (nm) of the film surfaces with /without treatments

MCT (e, h) microdeposits of reactive product on the surface of modified support can be observed.

AFM

The AFM 3D topography images (20 μm×20 μm scan) and the average roughness values Sa (nm) of the film surfaces with /without treatments are presented in figure 8.

Atomic force microscopy (AFM) measurements indicate the surface of the non-treated films is formed of nanoscaled material asperities. The treatments confer new morphological modifications with broader 3D profiles, which reflect the deterioration of the surface. For these samples the roughness (correlated with the parameter of average roughness in nm) increases both for PP and PET films, leading to an increased adhesion of the surfaces for different polymer coatings deposited from solution.

Microbiological tests

Antimicrobial effects of coated films are presented in table 1.

In table 1 one can notice that both the mechanically stressed by rubbing films (PP and PET) as well as those plasma treated, after being coated with FA or AL containing coatings present antimicrobial effects.

A different sensitivity of the 2 microorganisms (*Staphylococcus aureus (+)*- ATCC 25923, *Escherichia coli(-)* –ATCC 25922) to the coated films could be noticed: the diameters of the inhibition zones varied from 7-10 mm for *E. coli* to 10-13 mm for *S. aureus*.

Differential inhibition of growth and multiplication of the two bacterial species under the action of the coatings deposited on the tested films could be correlated with the different composition and ultrastructure of the cell wall of the two species, which confer a hydrophilic character for the Gram negative bacteria (*E. coli*) and a hydrophobic character for the Gram positive bacteria (*S. aureus*).

Conclusions

FT-IR ATR and UV studies have indeed proved that PVA samples were grafted with the reactive product monochlorotriazinyl-β-CD.

The inclusion of the compounds (ferulic acid, allantoin) in the grafted PVA polymer was confirmed by FT-IR ATR and UV analyses.

The generation of a higher roughness with a better adherence (by dry rubbing, cold plasma or alkaline treatment) of support films for polymer coatings was observed by means of SEM and AFM analyses.

Films coated with polymer composites of PVA, grafted with MCT bearing ferulic acid and allantoin, possess satisfactory antimicrobial activity against four microbial strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*).

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