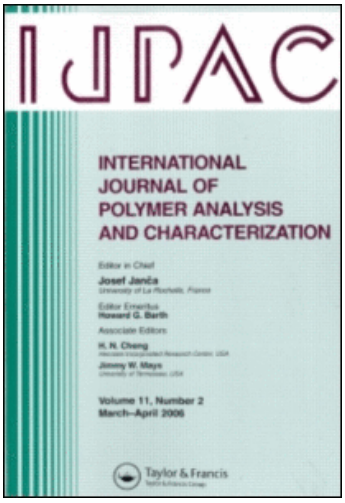

International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

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Online publication date: 27 October 2010

To cite this Article Necula, Adina Maria , Dunca, Simona , Stoica, Iuliana , Olaru, Niculae , Olaru, Liliana and Ioan, Silvia(2010) 'Morphological Properties and Antibacterial Activity of Nano-Silver-Containing Cellulose Acetate Phthalate Films', International Journal of Polymer Analysis and Characterization, 15: 6, 341 — 350
To link to this Article: DOI: 10.1080/1023666X.2010.500524
URL: http://dx.doi.org/10.1080/1023666X.2010.500524

International Journal of Polymer Anal. Charact., 15: 341–350, 2010

ISSN: 1023-666X print
DOI: 10.1080/1023666X.2010.500524

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MORPHOLOGICAL PROPERTIES AND ANTIBACTERIAL ACTIVITY OF NANO-SILVER-CONTAINING CELLULOSE ACETATE PHTHALATE FILMS

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Modification of the rheological properties of cellulose acetate phthalate in 2-methoxyethanol/acetone/water, at different compositions of solvent mixtures, allowed the identification of optimal composition of solvent mixtures for obtaining fibers with controlled diameters. Changing the solvent content in the casting solutions favors modification of the morphological aspects of cellulose acetate phthalate (CAP) films, as observed from atomic force microscopy images. Silver nitrate was incorporated into CAP, as a dispersion medium, and the silver-containing polymer (Ag-CAP) films thus obtained were studied for obtaining information on antimicrobial activity, using* Escherichia coli *ATCC 10536 and* Staphylococcus aureus *ATCCC 6538 microorganisms. The results were compared with the antibacterial activity of nano-silver-containing cellulose acetate (Ag-CA) films. The different inhibiting effects of CA or Ag-CA and of CAP or Ag-CAP on the tested* Escherichia coli *and* Staphylococcus aureus *bacteria are due to a different antimicrobial activity of the polymers and to the antiseptic character of nano-silver.

***Keywords*:** Antimicrobial activity; Atomic force microscopy; Cellulose acetate phthalate; Rheology

INTRODUCTION

The increasing need of materials for new applications requires polymers with diverse architectures, inducing specific properties.[1,2] Among such materials, cellulose acetate (CA) evidences excellent properties, such as biocompatibility and relatively low cost. At the same time, cellulose acetate phthalate (CAP) has been used for several decades as a pharmaceutical excipient; enteric coatings based on CAP are resistant to acidic gastric fluids, but easily soluble in the mildly basic medium of the intestine. The pH sensitive solubility of CAP is mainly determined (as are other properties of this mixed ester) by the degree of substitution (DS), namely the average number of substituent groups bound to an anhydroglucose unit, as well as by the molar ratio (acetyl and phthaloyl groups). These two structural characteristics of the polymer are dependent on the method employed for its synthesis.

Submitted 17 February 2010; revised 15 April 2010; accepted 19 May 2010.

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Recently, its potential to inhibit infections by human immunodeficiency type 1 virus, several herpes viruses in vitro, and other sexually transmitted disease pathogens has been investigated.[3,4] Also, CAP has been used for enteric film coating of tablets and capsules, which confirms its well-established safety for human use.

In addition, intense research efforts have been focused on the design and fabrication of metal nanoparticles in polymers, because of their novel electronic, optical, magnetic, and chemical properties.[5,6] These methods, exploiting the polymer matrix, have been reported to assure relatively better control over nano-metal cluster formation.[7–9] In this context, literature makes special mention of the importance of silver nano-metals in different applications.[8–10] Silver has an oligodynamic effect, that is, it is capable of causing bacteriostatic (growth inhibition) or bactericidal (antibacterial) impact.[11,12]

In some previous articles, the synthesis of cellulose acetate[13] and cellulose acetate phthalate,[14] as well as the some properties of cellulose acetates,[15–18] were reported.

In the present work, our primary focus is on the effect of "solvent properties" (2-methoxyethanol/acetone/water) on CAP by rheological and morphological investigation. Moreover, silver nitrate is incorporated into cellulose acetate phthalate. The morphological characteristics of silver-containing polymer films thus obtained are investigated by atomic force microscopy (AFM), and their antibacterial activity is tested. The results are compared with the antibacterial activity of silver nanoparticles contained in cellulose acetate films.

EXPERIMENTAL SECTION

Cellulose acetate with a 1.93 a substitution degree was used for the synthesis of cellulose acetate phthalate. Cellulose acetate was phthaloylated with phthalic anhydride in acid acetic using anhydrous sodium acetate and triethylamine as basic catalysts.[14] The degrees of CA acetylation (DS_{ac}) and of CAP phthaloylation (DS_{ph}) were 1.73 and 0.7, respectively.

Viscometric and oscillatory measurements for cellulose acetate phthalate with a phthaloylation degree of 0.7 in 2-methoxyethanol (2-M)/acetone (Ac)/water (W) were obtained with a Bohlin Instrument, by the cone and plate measuring system; the cone had an angle of 4° and a diameter of 40 mm. The dependencies between viscosity, η, and shear rate, $\dot{\gamma}$, were determined at shear stress, σ, of 30 Pa, at 20°C. Table I shows the compositions of the solvent mixtures used for obtaining a CAP solution with a concentration of 25 g/dL.

Oscillatory measurements were performed over a frequency range of 0.1–150 Hz.

Atomic force microscopy (AFM) images were obtained on a SPM SOLVER Pro-M instrument. A NSG10 "Golden" silicon probe tip with a 10 nm curvature radius and 255 kHz oscillation mean frequency was used to investigate membrane surface morphology. The apparatus was operated in semi-contact mode, over a $20 \times 20\,\mu m^2$ scan area, 256×256 scan point size images being thus obtained.

The membranes used for AFM investigations were prepared from cellulose acetate phthalate solutions of 25 g/dL concentration, in 2-methoxyethanol/acetone/water solvent mixtures, at different composition ranges (Table I).

Table I. Composition of solvent mixtures (% v/v/v) for cellulose acetate phthalate solutions

No.	2-Methoxyethanol	Acetone	Water
1	50	47.5	2.5
2	50	40	10
3	50	30	20
4	50	25	25
5	50	22.5	27.5
6	50	20	30

The polymer solutions were cast on glass plates and initially solidified by slow drying in saturated atmosphere of the used solvent, and finally under vacuum, at 30°C. The CAP membranes thus prepared were subjected to AFM analysis.

For obtaining nano-silver in a cellulose acetate phthalate film, silver-containing CAP (Ag-CAP) solutions with a concentration of 25.4048 g/dL were prepared using 24.9992 g of cellulose acetate phthalate and 0.4056 g of $AgNO_3$ dissolved in 100 mL 2-methoxyethanol. The resulting mixture was boiled gently in a fume hood for 15 min, until reaching a solution concentration of 36.293 g/dL. By boiling, the incorporated silver nanoparticles obtained by $AgNO_3$ thermal decomposition (according to the reaction: $2AgNO_3 \rightarrow 2Ag + 2NO_2 + O_2$) act as cross-linking reagents at higher temperatures. Thus, important interactions appear between metal cations and the oxygen atoms of the hydroxyl, acyl, and phthaloyl groups of CAP. As such interactions might occur in an intermolecular way, the cellulose acetate phthalate molecules are thought to be cross-linked through such interactions or at least through partial coordinations, which result[7,9] in an increased glass transition temperature of CAP. After boiling, the Ag-CAP films were cast on flat glass and gradually oven-dried at different temperatures, to control the solvent evaporation rate. Finally, the obtained film was placed in a vacuum oven for two days at 35°C.

The in vitro antimicrobial activity of a nano-silver-containing CAP film, assessed on two strains of bacteria, namely *Escherichia coli* ATCC 10536 (*E. coli*) and *Staphylococcus aureus* ATCC 6538 (*S. aureus*), by the disk-diffusion method (Kirby-Bauer) certified by the National Committee on Clinical Laboratory Standards (NCCLS), was evidenced by the occurrence of an inhibition zone. The bacteria were pre-incubated for 18 h at 37°C.

An agar plate with pH 7.2–7.4 at room temperature was uniformly inoculated with the test microorganism using a sterile cotton swab, and disinfected steel discs were placed on the agar surface. Circular films, 9 mm in diameter and 100 μm thick, from the nano-silver-containing cellulose acetate phthalate films and from a control CAP film were introduced in the disks. The plates were incubated at 37°C for 24 h. The diameter of the inhibition zone is a function of the polymer present in the disk and of microorganism susceptibility. The results were compared with those obtained for the nano-silver-containing cellulose acetate films previously discussed.[18]

RESULTS AND DISCUSSION

Rheological Properties and Morphological Aspects of Cellulose Acetate Phthalate

Some studies have reported that the chain shape of a polymer in solution could affect the morphology of the polymer in bulk.[19,20] Also, the solvent or the solvent mixtures influence the spinning process of polymer solutions, through modification of solution properties. In this context, the cellulose acetate phthalate solutions used in rheological investigation were prepared in 2-methoxyethanol/acetone/water solvent mixtures. The solvent systems were selected as a function of polymer solubility, at constant concentration of 25 g/dL and constant composition of 2-methoxyethanol in solvent mixtures, 50 vol.%. Figure 1 plots the modification of dynamic viscosity, η, versus shear rate, $\dot{\gamma}$, and water content. Increasing the water content leads to decrease in dynamic viscosity, concomitantly with increasing the Newtonian plateau and flexibility. The small plot from Figure 1 shows that, at constant values of the shear rate, dynamic viscosity varies insignificantly until an approximately 25 vol.% water content, while, for a 27.5 vol.% composition, dynamic viscosity increases, attaining approximately the same values for different shear rates. At higher water composition, decreasing of dynamic viscosity signifies the rearrangement of macromolecules in solution.

Also, the values of transition frequency from viscous to elastic domain and values at which storage, G′, and loss, G″, moduli are equal slightly decrease with increasing water content, according to Figure 2; a sharp increase up to a water content exceeding 25 vol.% also being seen.

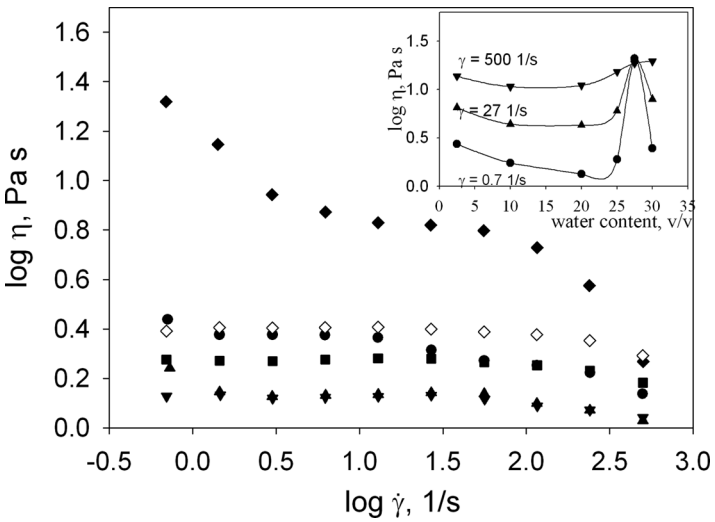

Figure 1. Logarithmic plot of dynamic viscosity as a function of shear rate for CAP in 2-M/Ac/W: 50/47.5/2.5 v/v/v (●); 50/40/10 v/v/v (▲); 50/30/20 v/v/v (▼); 50/25/25 v/v/v (■); 50/22.5/27.5 v/v/v (◆); 50/20/30 v/v/v (◇). Inset plot represents the dynamic viscosity as a function of water content for different shear rates, where the curves were detached with 0, 0.5, and 1 from bottom to top, for good visualization.

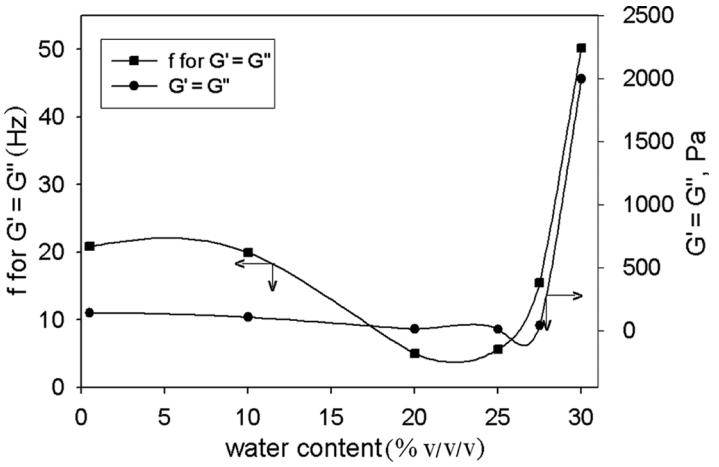

Figure 2. Values of $G' = G''$ and f for $G' = G''$ at different water contents.

Preliminary investigations have shown that the spinning process of CAP in acetone/water solvent mixtures leads to short fibers with small diameter. When 2-methoxyethanol was added to the systems, the diameter of the fibers becomes larger and the irregularity disappears. Fiber diameters were found to vary insignificantly with the water content, yet, at an approximately 25% water content, the fibers possess a smaller diameter when the viscosity of solutions and the boiling point of the solvent mixtures increase.

On the other hand, AFM images (with $20 \times 20\,\mu m^2$ scanning area) from Figure 3 show the influence of casting solutions on film morphology. Table II gives the average values of pore characteristics and surface roughness parameters identified in Figure 3.

According to these images, increasing the water content in the casting solutions determines modification of pore number and their characteristics, so that, at approximately 25 vol.%, the pore number is maximum, while the area, perimeter, and diameter are minimum. This changing trend in morphology is due to the modification in the chain conformation of the polymer, which is influenced by the quality of the mixed solvents.[19,20] Also, it may be assumed that the association phenomena of 2-methoxyethanol, acetone, or water over different composition domains of their mixtures may influence the preferential adsorption of one of the solvents by the macromolecular chain.[18] This phenomenon modifies CAP solubility, determining modification of the rheological properties (Figures 1 and 2) as well as the morphological aspect.

Morphological Aspect of Silver-Containing Cellulose Acetate Phthalate Films and Antimicrobial Activity

The silver-containing polymer films were investigated by atomic force microscopy to identify the silver complexes present in the cellulose acetate phthalate film.

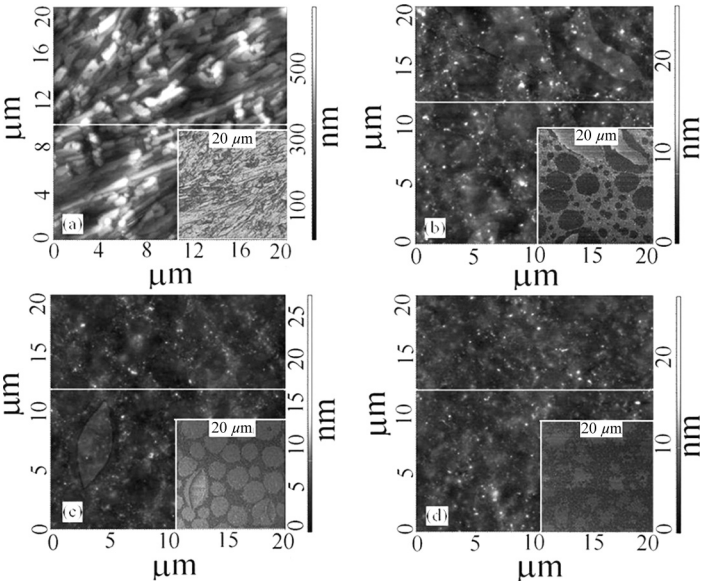

Figure 3. AFM images (2-D and phase image (small inserted image)) of $20 \times 20\,\mu m^2$ scan area for cellulose acetate phthalate films prepared from solutions in: (a) 2-methoxyethanol; (b) 50/47.5/2.5 vol.% 2-methoxyethanol/acetone/water; (c) 50/22.5/27.5 vol.% 2-methoxyethanol/acetone/water; (d) 50/20/30 vol.% 2-methoxyethanol/acetone/water.

Figures 4(a), 4(a′), and 4(a″) show AFM images (2-D image, phase image, and profile) of $2 \times 2\,\mu m^2$ scan areas for silver-containing cellulose acetate phthalate films. Unlike Figure 3(a), in which ordered domains were identified, Figure 4 shows high clusters of the silver nanoparticles with sizes of about 125 nm in the polymer matrix.

In a previous study, silver nanoparticles were obtained in the cellulose acetate matrix (Ag-CA) by the same method, also using 2-methoxyethanol as a solvent.[18] The properties of the cellulose acetate films depend on their chemical structure,

Table II. Pore characteristics including the area, average perimeter, diameter, length, mean width, and surface roughness parameters including average roughness (Ar), root-mean-square roughness (Rms), and nodule height from the height profile (Nhp) of cellulose acetate phthalate films prepared from solutions in 2-methoxyethanol/acetone/water (column 1), with $20 \times 20\,\mu m^2$ scanned areas, corresponding to the 2-D AFM images

	Pore characteristics				Surface roughness		
Solvent mixtures, % v/v/v	Number pores	Area (μm^2)	Perimeter (μm)	Diameter (μm)	Ar (nm)	Rms (nm)	Nhp (nm)
100/0/0	–	–	–	–	83.26	102.81	301
50/47.5/2.5	46	7.28	7.99	2.54	1.22	1.62	5.0
50/40/10	30	3.01	5.97	1.90	1.58	2.18	12.0
50/30/20	20	15.01	12.07	3.89	1.55	2.12	7.6
50/25/25	48	0.76	2.84	0.90	1.65	2.22	12.9
50/22.5/27.5	21	8.53	10.07	3.21	1.16	1.56	4.5
50/20/30	12	6.45	8.88	2.83	1.22	1.65	5.8

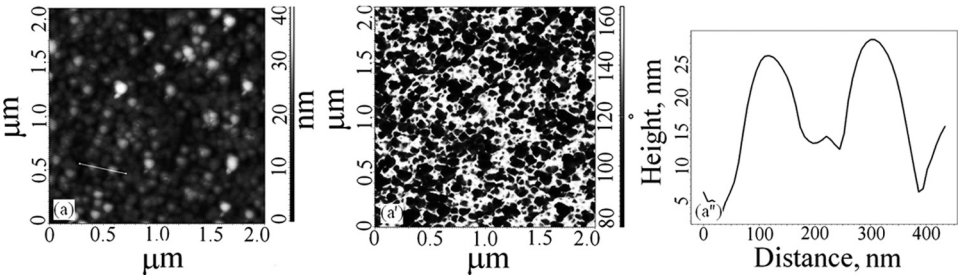

Figure 4. AFM images of $2 \times 2\mu m^2$ scan area for silver-containing cellulose acetate phthalate films prepared from solutions in 2-methoxyethanol: 2-D image (a), phase image (a′), and surface profile taken along a line from 2-D-AFM images (a″).

involving different substitution degrees and positions of the acetyl groups along the chain, on the presence of a metal complex incorporated in the CA film, and on the history of the films prepared from 2-methoxyethanol solutions.

The Ag-CAP and Ag-CA films were used for analyzing the bactericidal effect of the silver nanoparticles in the CA films, in correlation with the substitution degrees of cellulose acetate. The antimicrobial activity of CAP and of nano-silver-containing cellulose acetate phthalate films was investigated against *Escherichia coli* and *Staphylococcus aureus*. Also, the results were compared with those obtained for cellulose acetate with 1.88 and 1.90 substitution degrees ($CA_{1.88}$, $CA_{1.90}$) and for the corresponding nano-silver-containing CA films. As shown in Figure 5 and Table III, the cellulose acetates insignificantly inhibit the growth of microorganisms, in comparison with cellulose acetate phthalate. Inhibition is more intense for nano-silver-containing cellulose acetates and cellulose acetate phthalate.

The studied samples interfere with the bacterial metabolism by electrostatic stacking at the cell surface of the bacteria.[21] Thus, *E. coli* was found be much more

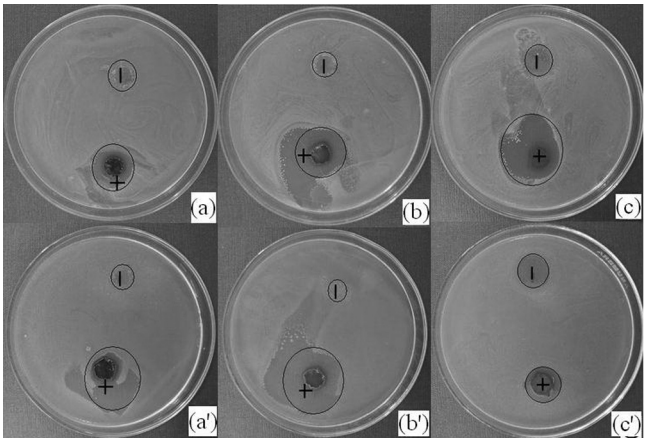

Figure 5. Antimicrobial screening test: (a) Ag-$CA_{1.88}$ against *Staphylococcus aureus*; (a′) Ag-$CA_{1.88}$ against *Escherichia coli*; (b) Ag-$CA_{1.90}$ against *Staphylococcus aureus*; (b′) Ag-$CA_{1.90}$ against *Escherichia coli*; (c) Ag-CAP against *Staphylococcus aureus*; (c′) Ag-CAP against *Escherichia coli*. In each figure, the inhibition area on the top side was recorded for CA and CAP, respectively, as a control sample.

Table III. Antimicrobial activity expressed by the diameter of the inhibition zone (mm) of Ag-$CA_{1.88}$, Ag-$CA_{1.90}$, and Ag-CAP films, and $CA_{1.88}$, $CA_{1.90}$, and CAP used as a control sample against *Escherichia coli* and *Staphylococcus aureus*

Sample	*E. coli*	*S. aureus*
$CA_{1.88}$	12.6	12
Ag-$CA_{1.88}$	33.0	24.0
$CA_{1.90}$	11.4	12.6
Ag-$CA_{1.90}$	33.6	25.8
CAP	17.4	16.2
Ag-CAP	30.0	36.6

sensitive to the Ag-CA film and less sensitive to Ag-CAP than *S. aureus*. On the other hand, the differences in the composition of the cell wall of gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria induce different antimicrobial activity. The component of gram-positive bacteria cell walls is peptidoglycan, while the major constituent of gram-negative bacteria cell walls is peptidoglycan, together with other membranes, such as lipopolysaccharides and proteins. The components of the cell walls assure the hydrophilic character of *E. coli* and the hydrophobic character of *S. aureus*. The different inhibiting effects of CA or Ag-CA and CAP or Ag-CAP on the tested *E. coli* and *S. aureus* bacteria may be due to a different antimicrobial activity of polymers and to the antiseptic character of nano-silver from film compositions. Thus, these aspects indicate that the antimicrobial activity depends not only on the chemical structures of the cellulose derivatives and nano-silver particles, but also on the hydrophilic or hydrophobic character of bacteria, generating different interactions with the bacterial cell membrane; the adhesion of the relatively hydrophilic *E. coli* to the hydrophobic Ag-CA should be higher than the adhesion of hydrophobic *S. aureus* cells, and the adhesion of the hydrophilic *E. coli* to the more hydrophilic Ag-CAP should be lower than that of the hydrophobic *S. aureus* cells. Some deviations from these statements illustrated in Table III recommend taking into account of other types of interactions, such as van der Waals and electrostatic interactions.[22]

CONCLUSIONS

The present study provides information on the optimal composition of 2-methoxyethanol/acetone/water solvent mixtures in cellulose acetate phthalate solutions for obtaining fibers with controlled diameters, as determined by rheological and AFM investigations. Fiber diameters were found to vary insignificantly with water content, but, at approximately 25% water content, the fibers evidence a more reduced diameter, in which case the viscosity of solutions and the boiling point of the solvent mixtures increase. On the other hand, increasing the water content in casting solutions determines modification of pore number and their characteristics in AFM images, so that at approximately 25%, the pore number is maximum, while the area, perimeter, and diameter are minimum. This changing trend in morphology is due to the modification of the chain conformation of polymer in solution.

Moreover, significant antibacterial activities have been demonstrated for nano-silver-containing cellulose acetate and cellulose acetate phthalate films against both the gram-negative *E. coli* and gram-positive *S. aureus* tested bacteria.

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