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THERMAL PROPERTIES OF PVA/TEOS/AgNps THIN HYBRID FILMS AND THEIR FUNGICIDAL ACTIVITY TOWARDS REPRESENTATIVES OF GENUS CANDIDA

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ABSTRACT

The role of Candida infections in patients with weakened immune systems or hospitalized and catheterized patients is a worldwide problem with increasing importance. Due to the high fungal resistance to different type antifungals drugs, nowadays, the attention is paid towards novel hybrid coatings possessing strong antibacterial and fungicidal properties. The present article deals with the thermal behavior and fungicidal properties of novel hybrid materials based on polyvinyl alcohol (PVA)/tetraethyl orthosilicate (TEOS) films with included silver nanoparticles (AgNps) synthesized by sol-gel method. Thermal properties of the hybrid materials were investigated using TGA and DSC analysis. The fungicidal activity of the synthesized hybrid materials with included silver nanoparticles were tested onto control and clinical strains of genus Candida resistant to different antifungals. The antifungal activity of the synthesized materials was determined by Disk Diffusion Method (DDM) were the zone of inhibition of hybrid materials against control and clinical strains was monitoring.

Keywords: hybrid materials, silver nanoparticles, thermal properties, antifungal activity, Candida sp.

INTRODUCTION

There has recently been a growing interest in the development of antibacterial medical polymer materials. The reason for this attempt is the effort to reduce health complications caused by bacteria commonly found on various types of medical equipment. Since the most types of the commonly applied polymers do not have antibacterial activity, they have to be modified in order to obtain polymer materials with the desired properties. In this regard, hybrid organic-inorganic materials gain significant interest due to their extraordinary properties with improved physical, mechanical and thermal properties, compared with pure polymeric or inorganic materials [1]. Moreover, the introducing of silver nanoparticles into their matrix can lead to desired antibacterial properties of the obtained materials [2 - 4]. Due to their high surface to-bulk ratio, nanoparticles significantly affect the matrix leading to some new properties which are not present in either of the pure materials [5].

Among the agents causing various nosocomial infections are representatives of genus *Candida*. The literature data show that *Candida sp*. is the main etiologic agent causing urinary tract infections, vulvovaginitis as well systemic infections [6]. *Candida* bloodstream infections are associated with high morbidity and mortality in both neutropenic and non-neutropenic critically ill patients. Risk factors are diverse and include exposure to broad spectrum antimicrobial agents, mucosal colonization, indwelling vascular catheters, surgery and cancer chemotherapy [7]. Nearly 150 nonsporogenous yeast species are presently classified in the genus *Candida*. Among them, *C. albicans, C. tropicalis* and *C. glabrata* include more than 80 % of isolated clinical *Candida* strains, while others such as *C. krusei, C. paraplilosis, C. guilermondii* and *C. kefyr* are isolated sporadically and they are less virulent [8]. Recent data indicate that more than 30 % of nosocomial candida infections are result of species other than *C. albicans* [9]. Antifungal therapy alone is insufficient for cure. Affected devices generally need to be removed and replaced. Removal of these devices has serious implications in the case of infected heart valves, joint prostheses, and central nervous system shunts [6]. Powerful antifungal drugs can decrease or eliminate this necessity in future but it is important to take preventive steps by using devices coated with polymer films containing silver nanoparticles that possess high fungicidal activity. The offensive invasion of hybrid materials into everyday life, practice and medicine [10 - 11] is the main reason to study the thermal and the antifungal activity of novel hybrid materials based on polyvinyl alcohol (PVA)/tetraethyl orthosilicate (TEOS) with included silver nanoparticles (AgNps).

EXPERIMENTAL

Materials

Polyvinyl alcohol (PVA) (Sigma – Aldrich; 87 - 88 % hydrolyzed, Mw =13000 - 23000 mol^{-1}); HNO_3 (Riedel de Haën, Standard solution 2 mol/L); Silver nitrate (AcrosOrganics) and Tetraethyl orthosilicate (TEOS) (Fluka) were used as received without further purification. Standard disks from chromatographic paper FN7 "Munktell" with 0.32 thickness and diameter of the disk (d=6mm) were used (Bul Bio-NCIPD).

Control strains *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Candida tropicalis* B 6030413, *Candida glabrata ATCC* 90050 and five clinical isolates of the same genus fungi as *C. krusei 8-112; C. krusei 8-126; C. glabrata 8-122; C. albicans 8-127* and *C. albicans 8-137* were involved in the investigation. The sensitivity of some of them was tested with the trade kit ATB Fungus 3 "Bio Merieux" with five included antimycotics: Flucytosin (5FC), Amphotericin B (AMB), Fluconazol (FCA), Itraconazol (ITR), Voriconazol (VRC). The antimycotics Flucytosin (5FC), Amphotericin B (AMB), Fluconazol (FCA), Itraconazol (ITR), Voriconazol (VRC) are included in the kit at the following concentration: 5FC: 1=4 mg/L; 2=16 mg/L; AMB: 1=0.5 mg/L; 2=2 mg/L; 3=4 mg/L; 4=8 mg/L; 5=16 mg/L; 6=32 mg/l; 7= 64 mg/L; 8=128 mg/L; FCA: 1=1 mg/L; 2=2 mg/L; 3=4 mg/L; 4=8 mg/L; 5=16 mg/L; 6=32 mg/L; 7=64 mg/L; 8=128 mg/L; ITR: 1=0.125 mg/L; 2=0.25 mg/L; 3=0.5 mg/L; 4=1 mg/L; 5=2 mg/L; 6=4 mg/L; VRC: 1=0.06 mg/L; 2=0.125mg/L; 3=0.25 mg/l; 4=0.5 mg/L; 5=1 mg/L; 6=2 mg/L; 7=4 mg/L; 8=8 mg/L.

The most of the clinical strains of *Candida sp.*, which demonstrated clear resistance toward various antimycotics, were obtained from the reference microbiological laboratory "Mycosis" to National Centre of Infectious and Parasitic Diseases (NCIPD), Bulgaria.

Methods

Synthesis of PVA/TEOS/AgNps hybrid materials via thermal annealing of the films

The synthesis of PVA/TEOS/AgNps hybrid films was reported in details in [2]. Briefly, 5 g of polyvinyl alcohol (PVA) was dissolved in 95 mL deionized water while heating for 20 min at 80°C. Different amount of silver nitrate (0.025 g, 0.05 g, 0.1 g, 0.25 g and 0.5 g dissolved in 0.5 mL (water) was added to the 25 mL PVA (5 %) with concentrations of silver nitrate in the solution equal to: 0.98 mg/mL, 1.96 mg/mL, 3.92 mg/mL, 9.8 mg/mL and 19.6 mg/mL. The silica sol was produced by hydrolyzing tetraethyl orthosilicate (TEOS) (0.93 mL) in acidified water (0.93 mL) using HNO_3 as a catalyst to yield TEOS/H_2O/HNO_3 volume ration equal to 1/1/0.1. The mixture was stirred, until a clear solution was obtained and subsequently added drop-wise to the PVA/$AgNO_3$ solution thus achieving final concentration of silver nitrate in the solution equal to: 0.9 mg/mL, 1.8 mg/mL, 3.6 mg/mL, 9.0 mg/mL and 18 mg/mL. The final mixture was stirred for 80 min and then cast into a film. The films were dried for 3 days at room temperature in dark place. Further thermal annealing was performed for 1 hour at 100°C leading to the formation of silver nanoparticles (AgNps) in the PVA/TEOS matrix. The color of the annealed films depending on the Ag concentration varied from dark yellow to brown. The PVA/TEOS samples were prepared in the same manner without the addition of silver precursor into the PVA solution.

Synthesis of PVA/TEOS/AgNps hybrid materials via preliminary preparation of silver nanoparticles

The synthesis of PVA/TEOS/AgNps hybrid films via preliminary preparation of silver nanoparticles was reported in details in [2] as follow: different amount of silver nitrate (0.025 g, 0.05g, 0.1g and 0.25 g and 0.5 g dissolved in 0.5 mL water is added to three flasks containing 25 mL PVA (5 %). The mixtures were stirred and heated for 1 h at 100°C. The color of the solutions changed during the heating and at the end of the reaction the yellow to dark yellow/brown colors were obtained depending on the Ag concentration used. Further, to these solutions, partially prehydrolyzed TEOS was added drop-wise (hydrolysis of TEOS was achieved by using acidified water at volume ratio TEOS/H_2O/HNO_3 - 1/1/0.1). The obtained mixtures were stirred for 80 min followed by film casting. The films were dried for 3 days at room temperature. The PVA/TEOS samples were prepared in the same manner without the addition of silver precursor into the PVA solution.

Thermogravimetric analysis (TGA)

Thermal stability of PVA/TEOS/AgNps films was performed using TA instrument, TGA Q500, under nitrogen atmosphere in the temperature range from 25°C to 600°C at heating rate 20°C/min.

Differential scanning calorimetry (DSC)

DSC analysis was performed using Q100 from TA instruments under nitrogen atmosphere in the temperature range from 25°C to 250°C at heating rate 10°C/min.

Testing the antifungal activity of the films by Disk Diffusion Method

The antifungal activity of PVA/TEOS/AgNps films towards yeasts was performed using disk diffusion method (DDM), described in the document of Clinical and Laboratory Standards Institute CLSI M44-A. The disks from chromatographic paper were impregnated with 5 µL of the solutions prepared as mentioned above and allowing them to dry for 3 days at room temperature. The non-thermal disks were used as prepared. The thermally treated disks were obtained after annealing at 100ºC for 1 h and further used.

Among the many agar media available, the subcommittee on Antifungal Susceptibility Tests considers supplemented Mueller-Hinton agar (MHA) as a good choice for routine susceptibility testing of yeasts. The base medium was supplemented for postproduction to maintain the final concentration of 2 % glucose and 0.5 µg/mL methylene blue dye. Two petri dishes, MHA with 2 % glucose and 0.5 µg/mL methylene blue (GMB) and, impregnated disks for each microorganism were prepared. A control sample of impregnated disks without silver nanoparticles under the same conditions was also prepared. Each plate was examined after 20 to 24 hours of incubation. Then, monitoring of the inhibition zones was performed and determined. When the growth was insufficient after 24-hour incubation, the results were collected after 48 hours according to [12].

RESULTS AND DISCUSSION

Thermal characterization of PVA/TEOS/AgNps hybrid materials

Recently we reported on the preparation of novel hybrid materials based on PVA and TEOS with included silver nanoparticles which have been synthesized via sol-gel method. Two different strategies for the synthesis of silver nanoparticles in PVA/TEOS matrix were applied based on reduction of silver ions by thermal annealing of the films or by preliminary preparation of silver nanoparticles using PVA as a stabilizer and reduction agent [2].

Thus prepared thin thermally and non-thermally hybrid films were characterized and the successful formation of well-defined silver nanoparticles with size between 5 - 10 nm homogeneously distributed into organic-inorganic matrix was confirmed by TEM-EDX analysis and UV-Vis with appearing of peak at 420 cm^{-1} [2]. However, to apply such hybrid films as coatings, it was important to investigate their thermal properties. For that purpose, TGA analysis for the both types of films was applied in the range from 30^{o}C to 700^{o}C in an inert atmosphere. The thermally and non-thermally PVA/TEOS/AgNps hybrid films were tested and comparison between pure PVA, PVA/TEOS and PVA/TEOS hybrids with embedded silver nanoparticles with three different silver concentrations (1.8 mg/mL, 3.6 mg/mL, 9.0 mg/mL) has been made. TGA thermograms showed two main decomposition peaks as for the pure PVA film, the first peak was centered at 315°C which is due to PVA side chain elimination and the second peak was detected at 440°C which was originated from degradation of the main PVA chain. For PVA/TEOS hybrid materials without silver nanoparticles, the decomposition temperatures were shifted to higher temperatures in the range from 453°C to 465°C depending of the concentration of TEOS (Figs. 1, 2). When the concentration of TEOS was increased twice into hybrid materials the thermal stability increased up to 465°C. The improved thermal stability of PVA/TEOS films in comparison to pure PVA is a result of crosslinking reactions between PVA chains and hydrolyzed TEOS during the polycondensation and annealing process. These results indicated that TEOS

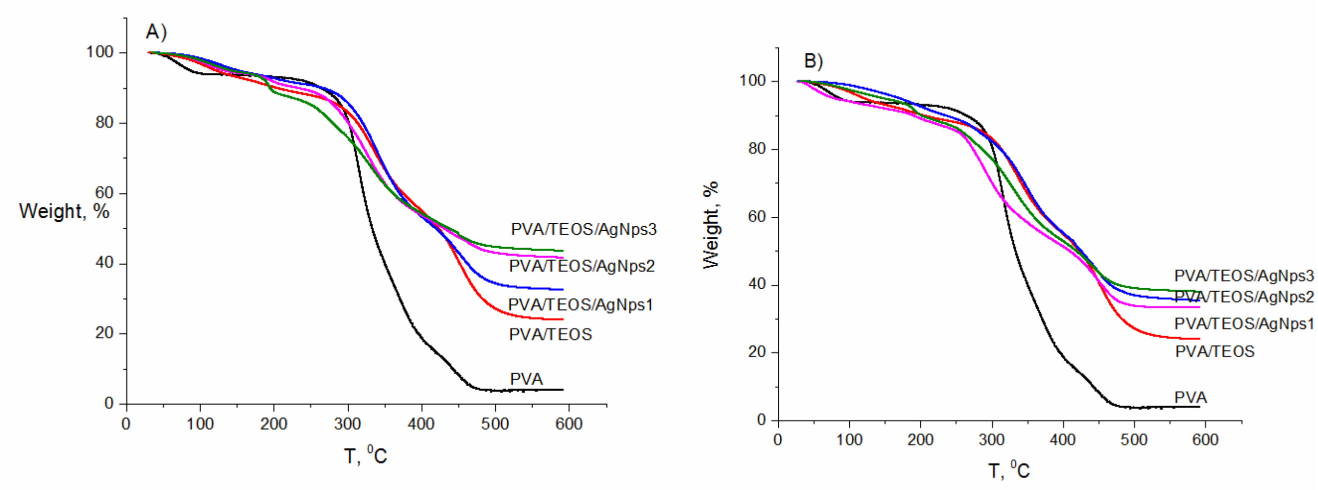

Fig. 1. a) TGA thermograms of PVA/TEOS/AgNps with preliminary preparation of AgNps; b) TGA thermograms of PVA/TEOS/AgNps with in situ preparation of AgNps (AgNps concentrations: AgNps1 - 1.8 mg/mL, AgNps2 - 3.6 mg/mL, AgNps3 - 9.0 mg/mL, TEOS/H_2O/HNO_3 - 1/1/0.1).

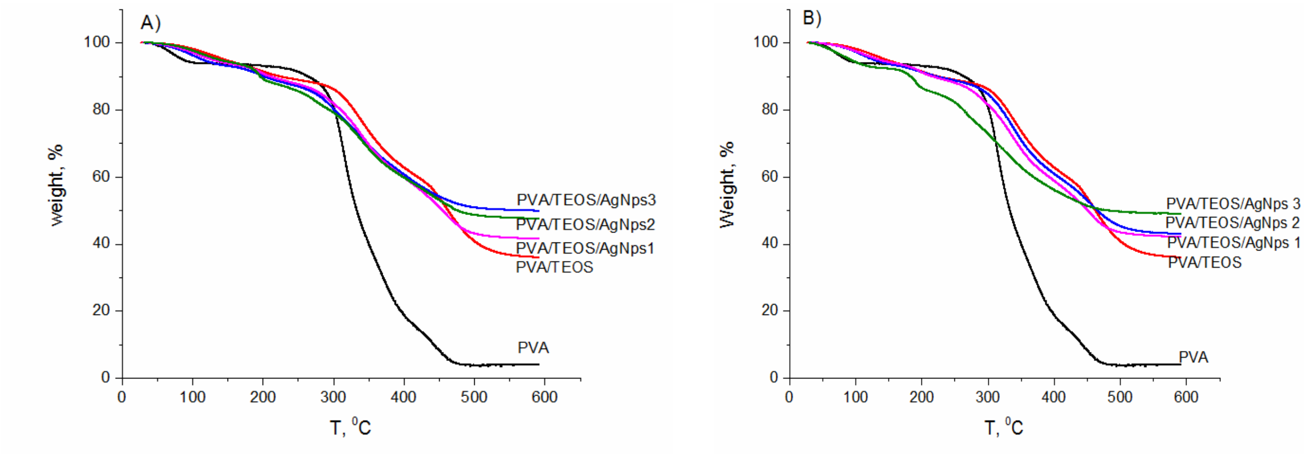

Fig. 2. a) TGA thermograms of PVA/TEOS/AgNps with preliminary preparation of AgNps; b) TGA thermograms of PVA/TEOS/AgNps with in situ preparation of AgNps (AgNps concentrations: AgNps1 - 1.8 mg/mL, AgNps 2 - 3.6 mg/mL, AgNps3 - 9.0 mg/mL, TEOS/H_2O/HNO_3 - 2/1/0.1).

significantly influence the thermal stability of materials.

For PVA/TEOS hybrid materials with embedded silver nanoparticles, the decomposition temperatures were shifted even to higher temperatures (Figs. 1, 2). These observations were more pronounced for the PVA/TEOS/AgNps materials obtained by preliminary preparation of silver nanoparticles using PVA as a stabilizer and reduction agent as the decomposition temperature was increased up to 468°C (Fig. 1). This is a result of occurring interactions between AgNps and hydroxyl (-OH) groups arising from PVA chains which reduced the mobility of PVA chains thus retarding the degradation process and improve the thermal stability of the hybrid materials. Similar observation has been reported in the literature for polymer/Ag nanoparticles nanocomposites [13].

This result indicates that the way of thermal decomposition of the pure PVA and the PVA/TEOS/AgNps hybrid materials proceeded in different manner.

The residue of the hybrid PVA/TEOS/AgNps materials also increased in comparison to the pure PVA. The residue for pure PVA was negligible (3 wt. %) and increased up to 25 wt. % for PVA/TEOS matrix and from 30 wt. % to 42 wt. % for PVA/TEOS/AgNps hybrid materials depending on the content of the embedded silver nanoparticles in the PVA/TEOS matrix (Figs. 1, 2).

Further DSC analysis was also applied in order to investigate the material properties of the prepared PVA/TEOS/AgNps films such as glass transition temperature as well as their thermal stability. The results of the performed DSC analysis showed that glass transition temperature (Tg) of PVA/TEOS/AgNps hybrid materials is also affected by the introducing of TEOS and content of silver nanoparticles into the matrix which is determined by the level of interaction between polymer chains and AgNps. It was established that the Tg of the pure PVA was 66.7°C and increased up to 79.3°C for PVA/TEOS hybrid materials depending of the TEOS content. The increasing of the Tg is a result of the occurring crosslinking and branching reaction. For comparison, the Tg of PVA/TEOS/AgNps hybrid materials has been influenced as from TEOS content as by the silver nanoparticles content and enhance with increased silver nanoparticle concentration up to 80.8°C at highest AgNps content. Obviously at higher silver nanoparticles content polymer chains were closer packed leading to decreasing of the free volume which determines the higher Tg values.

Antifungal activity of PVA/TEOS/AgNps hybrid materials

Previously, the PVA/TEOS/AgNps hybrid materials were tested for sporicidal and antibacterial activity showing very good sporicidic and antibacterial properties [2, 3]. Therefore, it was interesting to investigate their antifungal activity against control and clinical *Candida* strains which showed resistance to a large number of antifungal agents. The antifungal effects by silver are attributable to the disruption of the structure of the fungal cell membrane due to the destruction of membrane integrity, resulting in leakage of intracellular ions and other materials and to the inhibition of the normal budding process by affecting the cell cycle at the G2/M phase [14].

The main problem during testing the hybrid films with biological activity is the choice of testing method. The literature data showed that the availability of inhibition zones is indicative for the presence of such activity. The use of easy for performance and standardized method as Disk diffusion method (DDM) has some advantages over the other methods, therefore it has been applied. The thermally and non-thermally PVA/TEOS/AgNps based hybrid materials with different silver concentrations (from 0.9 mg/mL to 18 mg/mL) were tested for antifungal activity using DDM in which the inhibition zone was monitored. Initially, their antifungal activity was tested against different control strains as the microorganisms involved in the experiment were etalon strains of *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Candida tropicalis* B 6030413 and *Candida glabrata* ATCC 90050. The fungicidal activity of PVA/TEOS/AgNps hybrid films

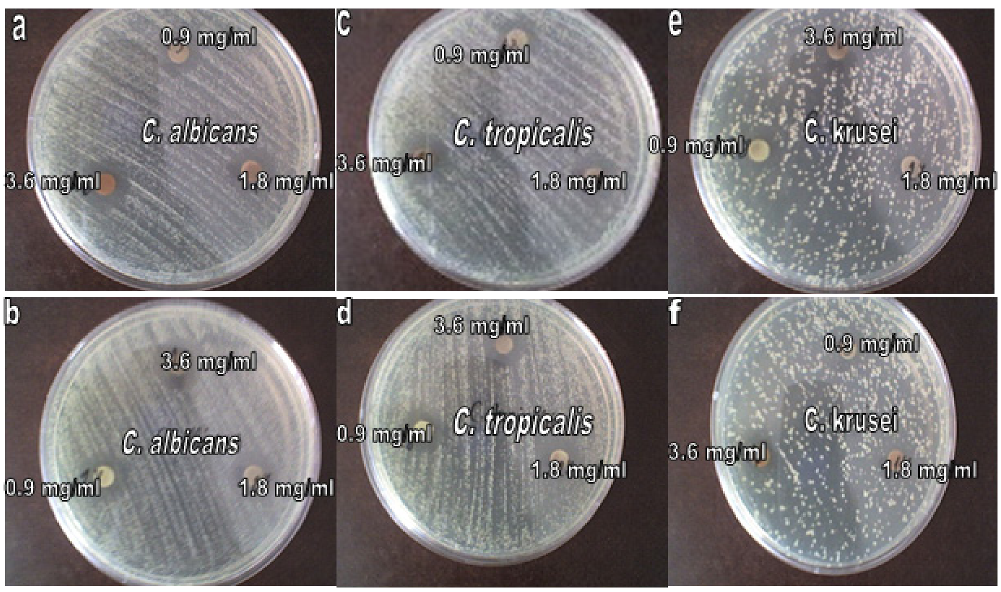

Fig. 3. Antifungal activity of thermally and non-thermally PVA/TEOS/AgNps films at different silver concentration against: (a-b) C. *albicans*; (c-d) C. *tropicalis*; (e-f) C. *krusei.*

Table 1. Fungicidal activity of thermally and non-thermally PVA/TEOS/AgNps hybrid films at 0.5 MF after 48h incubation.

Strain / zone (mm)	Ag precursor 1.8 mg/mL	Ag precursor 3.6 mg/mL	Ag precursor 9 mg/mL	Ag precursor 18 mg/mL
	zone (mm)	*zone (mm)*	*zone (mm)*	*zone (mm)*
C. albicans ATCC 10231	7.5	8.5	9.5	10.5
C. krusei ATCC 6258	7.5	8.5	9.5	10
C. tropicalis B 6030413	8.5	8.5	9.5	9.5
C. glabrata ATCC 90050	9.5	9.5	10.5	11.5

Table 2. Fungicidal activity of thermally and non-thermally PVA/TEOS/AgNps hybrid films at 3 MF after 48h incubation.

Strain / zone (mm)	Ag precursor 1.8 mg/mL	Ag precursor 3.6 mg/mL	Ag precursor 9 mg/mL	Ag precursor 18 mg/mL
	zone (mm)	*zone (mm)*	*zone (mm)*	*zone (mm)*
C. albicans ATCC 10231	7	7	7.5	8
C. krusei ATCC 6258	7.5	8	8.5	8.5
C. tropicalis B 6030413	7.5	8.5	9.5	9.5
C. glabrata ATCC 90050	8	8	9	9

was tested first at 0.5 MacFarland (MF) turbidity as inhibition zones and accounting of brightening around the impregnated disks were monitoring. The results were collected after 48 h of incubation since double zones of inhibition were observed after 24 h. It was found that both thermally and non-thermally treated PVA/TEOS/AgNps films with Ag concentration from 0.9 mg/mL to 3.6 mg/mL exhibit fungistatic activity against *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Candida tropicalis* B 6030413 by appearance of an inhibition zone with a weak pronounced growth of the control strains (Figure 3).

In these cases, where inhibition zones with incomplete strains suppressing are observed, it can be concluded that both the types of PVA/AgNps/TEOS films showed fungistatic activity. When the silver nanoparticle concentration was increased from 9 mg/mL to 18 mg/mL into PVA/TEOS/AgNps films, the presence of fungicidal activity was detected.

The benefit of this system is that the representatives of *C. glabrata* showed higher sensitivity and expressed fungicidal activity towards PVA/TEOS/AgNps hybrid materials (thermally and non-thermally) with Ag concentration from 0.9 mg/mL to 3.6 mg/mL in comparison to the other fungi involved in the experiment (Fig. 4). It is known that C. *glabrata* infections are second or third in frequency after C. *albicans* infections, they are difficult to treat, since are often resistant to many azole antifungal agents, especially fluconazole and are associated with a high mortality rate in compromised, at-risk hospitalized patients [15].

In all cases, the performed experiments showed an equivalent applicability of the synthesized PVA/AgNps/TEOS films with respect to their fungicidal activity regardless of the method used for synthesis with the appearance of an inhibition zone in the range from 7.5 to 11.5 mm depending on the silver concentration (Table 1).

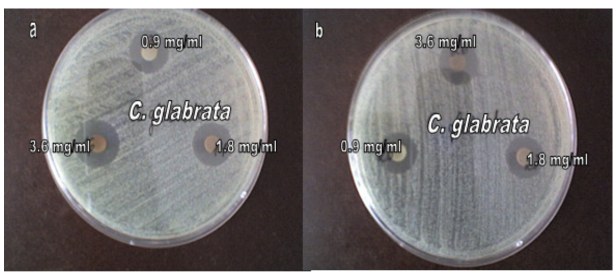

Fig. 4. Antifungal activity of (a) thermally and (b) non-thermally PVA/TEOS/AgNps films at different silver concentrations against C. *glabrata*.

Table 3. The antimycotic resistance of different type clinical isolates of fungi.

Candida sp.	5FC		AMB						FCA								ITR						VRC							
	1.	2.	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.	7.	8.	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.	7.	8.
C.krusei 8-126	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-
C.glabrata 8-122	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-
C.albicans 8-127	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C.albicans 8-137	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

** antimycotics: Flucytosin (5FC), Amphotericin B (AMB), Fluconazol (FCA), Itraconazol (ITR), Voriconazol (VRC) ** The results from the investigation are presented as "+" (presence of growth) and "-" (lack of growth).*

Table 4. Interpretation of the results obtained by the testing of clinical isolates with different type antimycotics.

Candida sp.	Sensible	Intermediate	Resistant
C.krusei 8-126	AMB, VRC	5FC, FCA	ITR
C.glabrata 8-122	5FC, AMB, VRC		FCA, ITR
C.albicans 8-127	5FC, AMB		FCA, ITR, VRC
C.albicans 8-137	5FC, AMB		FCA, ITR, VRC

**antimycotics: Flucytosin (5FC), Amphotericin B (AMB), Fluconazol (FCA), Itraconazol (ITR), Voriconazol (VRC)*

Further, suspensions of control strains were tested at higher turbidity (3MF). At this high turbidity, the presence of fungicidal activity was detected with appearance of inhibition zones in the range from 7 to 9.5 mm. Pronounce fungicidal activity against *C. glabrata* was again clearly demonstrated (Table 2).

The complete inhibition of the tested strains at turbidity of the suspensions 3MF, which is approximately ten time higher than that suggested in CLSI standard gave a clear indication of the pronounced fungicidal properties of the synthesized PVA/TEOS/AgNps films.

In the same manner, the control tests with PVA/TEOS films without AgNps against the tested Candida strains have been performed, where no inhibition zone is observed. These results demonstrated that the fungicidal activity is a result of the formation of silver nanoparticles in the hybrid materials.

Further, it was interesting to test PVA/TEOS/AgNps films for fungicidal activity toward clinical strains isolated from patients with urinary tract infections. The isolates used were from genus *Candida* (*C. krusei 8-126; C. glabrata 8-122; C. albicans 8-127* and *C. albicans* 8-137) and their resistance to different commercial antifungal agents was tested. The clinical isolates involved in the investigation showed resistance to different antifungal agents such as Fluconazol (FCA), Itraconazol (ITR) and Voriconazol (VRC) using commercial kit ATB Fungus 3 "Bio Merieux" as shown in Tables 3, 4.

The tested clinical isolates with already confirmed resistance to different types of antifungal agents were further studied for their sensitivity to silver nanoparticles embedded into PVA/TEOS hybrid matrices using DDM. This was conducted using disks impregnated with PVA/TEOS/AgNps containing different concentrations of the silver precursor in the range from 0,9 mg/mL to 18 mg/mL (Fig. 5).

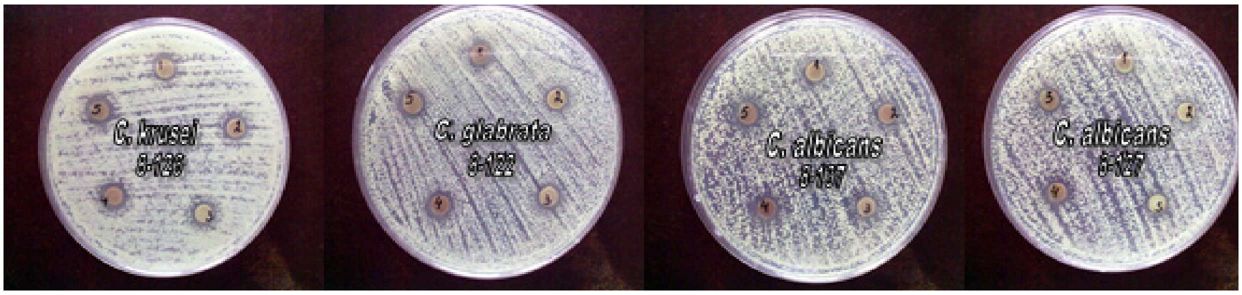

Fig. 5. Fungicidal activity of PVA/TEOS/AgNps disks with different silver concentrations: (1) 0.9 mg/mL (2) 1.8 mg/mL (3) 3.6 mg/mL (4) 9 mg/mL (5) 18 mg/mL against the tested *Candida* strains.

The results showed the presence of fungicidal activity with zones of inhibition similar to those of the control strains in the range from 7 to 11 mm. The most expressed zones of inhibition were observed after 48 h of incubation using disks with silver precursor concentration in the range from 3.6 mg/mL to 18 mg/mL.

The results obtained confirmed that the PVA/TEOS/AgNps thin films can be successfully used as promising antifungal agents even at such clinical candida strains which typically showed proven resistance towards the most used antifungals.

CONCLUSIONS

Novel PVA/TEOS/AgNps hybrid thin films with different silver concentrations were tested for their thermal properties by TGA and DSC analysis. It was established that the introducing of TEOS into polymer matrix improve the thermal stability of the PVA/TEOS hybrid materials. Moreover, the embedded AgNps into hybrid PVA/TEOS matrix significantly increase their thermal resistance. The presence of fungicidal activity was examined and it was demonstrated that PVA/TEOS/AgNps films with silver concentration in the range from 0.9 mg/mL to 3.6 mg/mL PVA/TEOS/AgNps films possessed fungistatic activity against *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Candida tropicalis* B 6030413 and fungicidal activity toward *Candida glabrata* ATCC 90050. With increasing the silver concentration up to 18 mg/mL in PVA/AgNps/TEOS films, the activity toward *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258 and *Candida tropicalis* B 6030413 became fungicidal. Pronounce fungicidal activity was detected against four tested clinical Candida strains. These results showed that the using of PVA/TEOS/AgNps films with silver concentration from 1.8 mg/mL to 18 mg/mL would guarantee the fungicidal activity toward yeasts.

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