Effect of natamycin on the physicochemical properties of corn starch based films and their effect on *Penicillium spp* activity

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Abstract

The incorporation of antimicrobials in foods by means of the use of films where they are entrapped collaborates to decrease their diffusion rate. In this work, the physicochemical properties of starch-based films loaded with 1 % wt. natamycin were analyzed, and the antifungal activity of these films was evaluated against *Penicillium spp*.

Variations in the properties of films with 1% natamycin were minimal, leading to the conclusion that this material could be applied to avoid mold development on the surface semi-hard cheeses. Corn starch-based films containing natamycin at 1 % w/w inhibited the *Penicillium spp*. growth in a solid matrix.

Keywords: **Starch based films, Natamycin, material properties, Penicillium.**

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Introduction

Controlling microbial growth can be achieved by applying antimicrobial agents to food surfaces by dipping, spraying, or brushing. However, these direct application techniques are laborious and have limited benefits [1], and the compound generally exhibits a rapid loss of activity due to a reduction of its active concentration resulting from interaction or reaction with food components; therefore the benefits of incorporating these components into polymer matrices that allow controlled release and dosing [2]. When the additive diffuses to the bulk of the food, a phenomenon of dilution also acts against its effectiveness. This is the reason why the incorporation of antimicrobials in foods by means of the use of edible films where they are entrapped collaborates to decrease their diffusion rate, thus assisting in the maintenance of high concentrations of the active ingredient where it is required [3].

In the particular case of cheese coating, the incorporation of antimicrobials by spray or dipping without a matrix that controls the amount of active agent could modify the desirable process during ripening [4]. A more serious aspect of microbial contamination is the possibility of risks to human health due to the presence of possible pathogenic microorganisms and/or their toxic metabolites leading to outbreaks of food poisoning. Recognized and foodborne pathogens include multicellular parasites, protozoa, fungi, bacteria, and viruses [5].

The use of natural antimicrobial compounds from a wide variety of natural sources is being explored as a mean to improve the safety and stability of several foods while maintaining a natural, high quality and healthy product [6]. Natamycin, is a polyene macrolide produced by *Streptomyces natalensis* and it is widely used in the food industry to prevent yeasts and molds contamination of cheese and other non-sterile foods [7,8]. It has been approved as a food additive in over 40 countries and has been considered as a GRAS (generally recognized as safe) product by the FDA [9] and also designed as a natural preservative by the European Union

(EEC No. 235). Natamycin acts by binding the membrane sterols, primarily ergosterol, the principal sterol in fungal membrane, distorting the selectivity of the membrane permeability, and therefore, it is active against fungi but not against bacteria [10,11].

Molds and yeasts on the surface of cheese could be a great threat for all cheese varieties, but especially for hard and semi-hard cheeses that need long ripening periods [12]. Certain molds can produce mycotoxins at temperatures as low as -2 to 10 °C; many of these molds belong to the genus *Penicillium* [13].

To the best of our knowledge, scarce data exist pertaining avoiding mold development during ripening of cheese. Various attempts have been made to control the growth of molds on the surface of cheeses regarding shelf life, for example by impregnation of the wrapper or materials with fungicidal or fungistatic chemicals [12,14,15].

According to bibliography, the addition of antimicrobials like potassium sorbate or nisin to polysaccharide matrices increases the solubility, tensile strain and water vapor permeability of edible films [16,17], stating the importance of evaluating the changes in the material properties. Therefore, the purpose of this study was to analyze the potential changes caused by natamycin on the physical and chemical properties of composite films of starch, poly (vinyl alcohol) and polyurethane; and their antifungal effect on *Penicillium spp* activity.

2. Materials and methods

2.1. Materials

Commercial corn starch (Maizena Duryea®, Unilever Argentina S.A.) was used, containing 0.11 g water, 0.006 g lipids, 0.003 g ash, 0.003 g proteins/g starch, and an amylose/amylopectin ratio of 25/75 (dry basis) [18]. Poly (vinyl alcohol) (PVA) was purchased in the form of powder, 98 % hydrolyzed and with a range of molecular weight (M_w) from 13,000 to 23,000 (Sigma Aldrich, USA). The polyurethane (PU) was prepared following a prepolymer process from

dicyclohexylmethane-4,4´-diisocyanate (H_{12}MDI, Desmodur W, Bayer) and polypropylene glycol, M_n 2,000 (PPG 2000, Voranol 2120, Dow). Ethylene diamine (EDA, Sigma Aldrich, USA), dibutyltindilaurate (DBTDL, Sigma Aldrich, USA) and 2-hydroxy ethylmethacrylate (HEMA, Sigma Aldrich, USA) were used as received and were of analytical grade. Triethylamine (TEA) was provided by ADELFA S.A. and it was also used as received. Dimethylol propionic acid (DMPA, Sigma Aldrich, USA) was dried in an oven at 100 °C for 2 h. Commercial natamycin (Delvo®Cid Salt) containing 50 % NaCl was provided by Harmony Group, Food Specialties division.

2.2. Processing

2.2.1. Film preparation

Starch-gelatinized dispersion was prepared by heating starch at 3 wt % in water at 90 °C for 1 hour with magnetic stirring. PVA was dissolved in water at 90°C with magnetic stirring for 24 hours. The polyurethane dispersion was synthetized according to González-Forte et al. [19].

Mixtures of starch, PVA, PU with or without natamycin were prepared. In the case of natamycin loaded dispersions, 6 ml of water were replaced with a solution of natamycin at 1 % prepared at 40 ºC in absence of light.

The composite films were prepared by casting on a Teflon® surface. The ratios of starch, PVA and PU were chosen fixing the starch composition to 70 wt %. The remaining 30 wt % was completed with PVA and PU. The selected ratios were starch/PVA/PU 70:30:0, 70:25:05; 70:20:10 and 70:15:15.

Determinations

Moisture content

The moisture content of the films was determined after drying in an oven at 105 ºC until constant weight. Moisture content was calculated as the weight loss percentage, regarding the original weight [20]. Assays were performed three times for each sample and the results were expressed as percentage (%) using equation 1:

$$Moisture\ \% = \frac{m_3 - m_1}{m_2 - m_1} x\ 100 \qquad (1)$$

where m_3 is the final weight of the capsule with the film, m_1 is the capsule weight and m_2 is the initial weight of the capsule with the film.

Swelling degree

The swelling degree was determined by setting films in an 80 % relative humidity atmosphere. To obtain this atmosphere, a saturated solution of 50 % NaCl and 50 % KCl was prepared. The films were weighted by the hour during the first 8 hours, and then at 24 and 48 h. The assays were performed in triplicate and the results were expressed in percentage using the following equation:

$$Swelling\ degree\ \% = \frac{m_3 - m_1}{m_2 - m_1} x\ 100 \qquad (2)$$

where m_3 is the final weight of the film and the capsule, m_1 is the capsule weight and m_2 is the initial weight of the film and the capsule.

2.3.1. FTIR spectroscopy

The FTIR spectra were measured in transmission mode and attenuated total reflection (ATR) mode using a FTIR Nicolet 380 spectrometer, Thermo Scientific, USA. The samples were free

standing films or, in the case of natamycin, the powder was mixed with KBr to obtain disks by pressing.

The spectra were obtained by recording 64 transmission scans between 4000 and 400 cm^{-1} or between 4000 – 650 cm^{-1} (ATR, ZnSe IRE) with a resolution of 4 cm^{-1}. Spectra processing was performed using the software EZ Omnic (Thermo Electron Corporation, USA).

2.3.3. X-ray diffraction analysis (XRD)

X-ray diffraction analysis was performed using a PANalytical, model X´Pert PRO instrument. Samples were irradiated with Cu Kα radiation 1.5403 Å using 40 mA and 40 kV. The diffractograms were obtained in the range of 3 – 40° / 2θ with a scan speed 0.0044°/s.

2.3.4. Thermogravimetric analyses (TGA)

The thermogravimetric analyses (TGA) were carried out with a Shimadzu TGA-50 instrument (Japan) at a heating rate of 30°C/min in a nitrogen atmosphere at 30 ml/min, from room temperature to 550°C using 4 mg of sample in aluminum pans [21]. A convenient way of expressing thermal stability is using the initial decomposition temperature (IDT) and the temperatures corresponding to a 5 % and to 50 % of weight loss, i.e. the thermal indexes T_5 and T_{50} respectively. The residual water content of films was determined from the first weight loss in the TGA curve. The residual mass at 540 °C was also determined.

Ultraviolet–visible spectrometry

The spectra were obtained using a Nicolet Genesis 10 spectrometer in the wavelength range of 200 to 800 nm, in transmission mode.

Water vapor permeability

Water vapor permeability (WVP) of different film formulations was determined following the guidelines of the ASTM E96-95 [22], using circle samples with a free surface area of 18.85 cm^2. Silica gel was used as desiccant. Briefly, samples were placed in individual test cups in a desiccator cabinet maintained at 10 ºC and 80 % of relative humidity. Samples were weighed every hour to calculate weight loss with time until constant weight. WVP [g.cm/(m^2.day.mmHg)] was determined by duplicate, as follows:

$$WVP = \frac{(G/t) \times L}{A \times P_w \times (RH_1 - RH_2)} \quad (3)$$

where (G/t) is the weight gain of the beaker in stationary state (g/s), L is the film thickness (cm), A is the area of exposed film (m^2), (RH_1 - RH_2) is the difference of HR (0.90) between the two chambers, and P_w is the water vapor partial pressure difference (Pa) across the two sides of film calculated on the basis of relative humidity. The P_w value for 10 ºC was P_w=9.2118 mm Hg.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of the surface and cryo-fractured surfaces of the starch/PVA/PU composites were performed using a FEI-Quanta 200 (The Netherlands) microscope in the low vacuum mode operated at 20 kV acceleration voltage and current of 100 μA. Samples were prepared by breaking them in liquid nitrogen and coated using gold sputtering prior to inspection to prevent sample-charging effects.

Microbiological Assay

Strains and Growth Conditions

Three *Penicillium spp.* strains were isolated from the surface of various cheeses after ripening from "Cátedra de Agroindustrias, Universidad de Ciencias Agrarias y Forestales de la Plata"

and from “Colegio Agrotécnico Don Bosco Uribelarrea de la Provincia de Buenos Aires”. The cheeses were scraped with a sterile swab, and the strains were grown in Sabouraud slanted agar at 28 °C for ten days [23]. After that, they were replicated on glucose-potato agar and Czapek-Dox agar. Macro and micromorphological studies were performed to big colonies and microcultures to complete identification.

Agar Diffusion Method

The agar diffusion test was used to determine the antimicrobial effect of films with and without natamycin al 1 % wt. against *Penicillium spp* 1, 2 and 3. Briefly, 0.5 µL of inoculum containing 1×10^9 CFU/mL of each *Penicillium spp*, was spread on the surface of Petri dishes containing Mueller-Hinton agar. Three film disks of 10 mm diameter loaded with natamycin (starch/PVA/PU 70:30:0, starch/PVA/PU 70:25:5 and starch/PVA/PU 70:15:15), were placed on plates previously inoculated. Control films without natamycin were evaluated as well. The plates were incubated at 28 °C for 96 h. The inhibitory activity was quantified by measuring the total diameter: disk plus inhibition zone. Determinations were made in duplicate.

2.3 Statistical analysis

Data were analyzed through ANOVA (α=0.05) using InfoStat software (Di Rienzo et al., 2018) and Tukey was the post-hoc test applied. Results are reported based on their mean and standard deviation.

Results and discussion

Moisture content, swelling degree and thermogravimetric analysis (TGA)

In Table 1, values for moisture content, swelling degree and different parameters obtained with TGA are presented for films with natamycin at 1 % w/w.

All formulations showed a moisture content around 10 %, with no significate differences among them. Analyzing swelling results, the films containing 1% w/w natamycin exhibited statistically similar values of swelling degree, all around 6 %.

Natamycin is known as an amphoteric molecule, due to the presence of a carboxylic group in the ring and an amino group in the sugar moiety, with an isoelectric point of about pH 6.5 (pKa 4-4.5, pKb 8.6) [24]. Natamycin also appears as amphiphilic, explained by a very polar long end (carboxylic group and mycosamine) and a macrocyclic ring in which each side has different properties (rigidity and hydrophobicity for the tetraene structure, flexibility and hydrophilicity for the opposite side). Although it has been studied that natamycin does not form self-associated structures in water, unlike high molecular weight polyene antibiotics such as amphotericin or nystatin [9], it exhibits an intermediate behavior and a rigid conformation that should be taken into account when analyzing the results. Particularly the latter, added to its high molecular weight (665.73 g / mol) considerably limits the solubilization of natamycin in water or solvents. The presence of hydroxyl groups and zwitterionic groups (ion-dipole) makes natamycin insoluble or slightly soluble in some solvents such as alcohols, ethers, esters, aromatic or aliphatic hydrocarbons or ketones. On the other hand, the solubility in water is very poor (20-50 ppm), mainly because of tetraene structure, and can be improved by dissolution in acidic or alkaline solutions with natamycin being active against fungi in the range of pH between 4 and 9. Only high solubilization levels have been found for some polar solvents such as methanol, dimethylsulfoxide, glycerol and propylene glycol [24,25].

The solubility and amphoteric nature of natamycin can be understood by looking at this antibiotic as two chains: the chain containing the four double bonds is completely hydrophobic whereas the chain with the two hydroxyl groups has a hydrophilic and a hydrophobic side, causing the chain to be amphiphilic. The polar end of the amphiphilic chain contains the carboxyl and mycosamine groups. The other end is mostly non-polar with only one hydroxyl

group. Natamycin forms a cylindrical structure as the hydroxyl groups of the amphiphilic chain align with each other. The outside of the cylinder is completely non-polar [25].

In previous studies, films without natamycin presented swelling values between 7 and 9 %. By comparing the swelling degree of films with and without natamycin, it can be seen that those containing natamycin absorb less water since the hydrophilic part of the natamycin molecule probably interacts with the hydrophilic part of the starch and PVA molecules, making the film more hydrophobic.

Regarding data obtained with TGA, Table 1 shows the values of residual water (%), initial decomposition temperature (IDT), thermal indexes T5, T50 and residual mass at 540 °C for films added with natamycin at 1% w/w.

For all formulations, the residual water content was about 10 %, unlike films without natamycin, which showed a decrease in this value when 10 or 15 % PU was added to replace PVA [19]. These results are in accordance with the humidity values of the films obtained by drying. In other works, it was found that the effect of the addition of natamycin strongly depends on the nature of the film, finding that the moisture content of pectin films increased when incorporating natamycin, but there were no significant changes in this parameter for alginate films [26].

The residual mass at 540 °C presented a minimum for the Starch/PVA/PU 70:25:5 mixture, unlike what happened with the films without additives where this mixture presented a maximum residue [19]. Bierhalz et al. [27] found that the addition of natamycin as part of the alginate film-forming solution decreased the IDT by 10 °C, unlike what is observed for this case where the IDT did not vary for any formulation with the addition of natamycin.

When PVA was replaced by PU, the T5 index decreased, presenting the minimum value for the films Starch/PVA/PU 70:15:15. The values of T5 for films with PU are within the range of water loss temperature (60-100 ºC), showing that on the one hand the hydrophobic nature of

the PU facilitates the loss of water in the samples but that, on the other hand, the amphiphilic nature of natamycin produces some variations in these values. When there is natamycin present in the formulation, it was observed that the T5 decreased, modifying the behavior of the polymeric structure.

For the T50 thermal index, a differential behavior was observed for the Starch/PVA/PU 70:20:10 mixture with natamycin, showing an increase in stability. For the three mixtures with PU in the presence of natamycin the values of T5 and T50 are statistically equal.

FTIR Spectroscopy. ATR mode

The spectra shown in Figure 1 were obtained by ATR except for natamycin, which was obtained by transmission with KBr.

For the starch/PVA/PU 70:30:0 film on the substrate side containing 1 % natamycin, it is observed in Figure 1 A a widening of the OH band because of the natamycin deformation contribution of the NH_2 at 3277 cm^{-1}. Then at 1715 cm^{-1}, the contribution of the carbonyl stretching of the conjugated esters of the lactone ring of natamycin is observed. In the 1577 cm^{-1} zone, a band can be seen corresponding to the stretching of the carbon-carbon double bond. The band corresponding to the epoxy ring of natamycin is visible at 1266 cm^{-1} [24,28].

In the case of starch/PVA/PU films 70:25:5 and 70:20:10 (Figure 1 B and C), the band at 1715 cm^{-1} can be observed, and also the appearance of a band around 1577 cm^{-1} corresponding to the stretching of the CH=CH double bond.

For the formulation starch/PVA/PU 70:15:15 with 1% natamycin (Figure 1 D), between 3000 and 2800 cm^{-1} the spectrum is modified showing changes that corresponds to the stretching of the CH groups, particularly the stretching of the CH_2 group at 2950 cm^{-1}. An increase in the band intensity is observed at 1715 cm^{-1} corresponding to the stretching of the carbonyls of the conjugated esters of the lactone ring of natamycin as well as for the other formulations. It also

shows changes in the area between 1500 and 1600 cm^{-1}, where the stretching of the double bond C=H for the other formulations was observed. The appearance of two bands at 1110 cm^{-1} and 1266 cm^{-1} are coming from the asymmetric stretching of the C-OH bond and from the epoxy bond between C_4 and C_5 in natamycin, respectively [24,28].

In all cases the occurrence of bands and shifts in the spectrum are detected, indicating interactions between starch, PVA, PU and natamycin [27,29].

UV spectrophotometry

UV-visible spectra for the control films (without natamycin) shows one shoulder corresponding to the PVA around 280 nm [19].

The spectra in the UV zone of the starch/PVA/PU 70:30:0, 70:25:5, 70:20:10 and 70:15:15 films with 1 % natamycin and without it are presented in Figure 2. It is known that the maximum absorbance for pure natamycin are detected at 281, 291, 304 and 319 nm [24]. Figure 2 shows the UV spectrum for pure natamycin obtained at 0.03 % in aqueous solution, between 200 and 350 nm in the upper right square.

As it can be seen, when natamycin was added to the films, the characteristic peaks of natamycin were found in all cases, although with slight shifts related to the interactions between the film components and the natamycin.

X- ray diffraction diagram analysis

Through the study of the X-ray diffraction diagrams the structure of the films was studied. In Figure 3 films with and without natamycin at 1 % w/w are shown for each formulation.

In all cases it is observed that by adding natamycin to the film, the internal structure is modified, and signals corresponding to the crystalline form of the starch can be detected (14.8, 17 and 20º). The maximum at 20°, markedly appears for the films Starch/PVA/PU 70:30:0, and as

PVA is replaced by PU its intensity decreases. This signal corresponds to the amylose-lipid complex, and is usually more noticeable in cereal starches, such as corn [30].

The signal at 14.8° was maximized with higher amounts of PU, unlike what was found when studying the crystalline behavior of the non-loaded films.

On the other hand, it became evident that as PU is replaced by PVA, a diffraction diagram with less noise is presented, which would indicate the presence of a less amorphous structure [31].

Water vapor permeability (WVP)

Table 1 also shows the WVP values obtained for films loaded with natamycin.Several authors found that the addition of natamycin to biopolymer films such as alginate or pectin significantly increased water vapor permeability [26] arguing that in these types of films, the poor packaging of the molecules in the film loaded with natamycin increased the free volume of the polymer structure causing an increase in permeability. In some studies, similar WVP values are presented in presence or absence of natamycin as an additive [4,32]. The diversity of the results reinforces the concept that the WVP depends strongly on the composition of the film and on the natamycin solubility.

Scanning electron microscopy (SEM)

The scanning electron microscopy images of cryo-fracture and surface sections of the films loaded with natamycin are shown in Figure 4 (a), (b), (c) and (d).

Smooth surfaces without gaps were observed for all cases, without evidence of phase separation, showing that the incorporation of natamycin at 1% w/w did not modify the good compatibility that previously existed between the formulation components. No natamycin crystals are observed on the surface, indicating that natamycin was fully incorporated into the polymer dispersion. Figure 4 also shows micrographs of the surface of both sides of

Starch/PVA/PU 70:30:0 films with 1% natamycin (d and e) and Starch/PVA/PU 70:15:15 with 1% natamycin (f and g).

In the images of the surfaces it can be seen that the substrate face is smooth, only showing the pattern of the material on which it was supported. In the case of the air face, an irregular surface is observed, with pores, but without the presence of crystals.

Some authors found the presence of natamycin crystals in the cryo-fracture and on the surfaces when analyzing films of starch, cellulose, methylcellulose and alginate/chitosan containing natamycin by SEM, given the low solubility of natamycin [32–34]. In this work, a good compatibility of natamycin with the components of the film evidenced by the absence of crystals.

Agar diffusion method

Table 2 presents the results for measured inhibition halos. Regarding strains, the behavior was similar for all the films, *Penicillium spp 2* and *3* presented similar results between them and statistically higher from *Penicillium spp 1*.

The films containing PU 15 % showed larger inhibition halos for all strains, and a tendency was observed towards more PU replacing PVA, more inhibition. This is an interesting point because all the films were loaded with 1 % natamycin, but apparently the PU content could have a synergetic effect when combined with natamycin.

Hanušová et al. [35] studied the release of natamycin from PVC films, in Petri dishes containing fungi isolated from the surface of a soft cheese (*Penicillium spp. a*nd *Cladosporium spp.*, mainly), finding halos of inhibition for all strains against the active film. De Oliveira et al. [36] tested cellulose-based films of 33 and 95 μm thickness containing natamycin between 0.2 and 4 %, against a strain of *Penicillium roquefortii*, finding that a minimum of 2% natamycin was needed in the films of 33 μm for effective control and that the 95 μm films were not very flexible

and did not adhere to the agar. Pintado et al. [37] studied the effect of nisin, natamycin and malic acid incorporated in whey protein films against *Penicillium roquefortii* strains (among others), finding that the film containing natamycin produced a zone of inhibition of 11.9 mm for these microorganisms.

Conclusions

When adding natamycin to the films it was found that there are specific interactions between the film components and natamycin. The internal structure of the films was modified by the addition of natamycin, being able to detect X-ray diffraction signals corresponding to the crystalline form of the starch; but variations in the global characteristics and properties of films with 1% natamycin are minimal, leading to the conclusion that this material could be applied to avoid mold development on the surface of foods such as semi-hard cheeses.

Corn starch-based films containing natamycin at 1 % w/w inhibited the *Penicillium spp*. growth in a solid matrix, demonstrating its effectiveness as a reservoir of the antimycotic.

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Table 1. Moisture content, swelling degree after 24 h (equilibrium), residual water, initial decomposition temperature (IDT), thermal indexes T_5 and T_{50}, residual mass at 540 °C and water vapor permeability (WVP) for films containing natamycin at 1 % w/w.

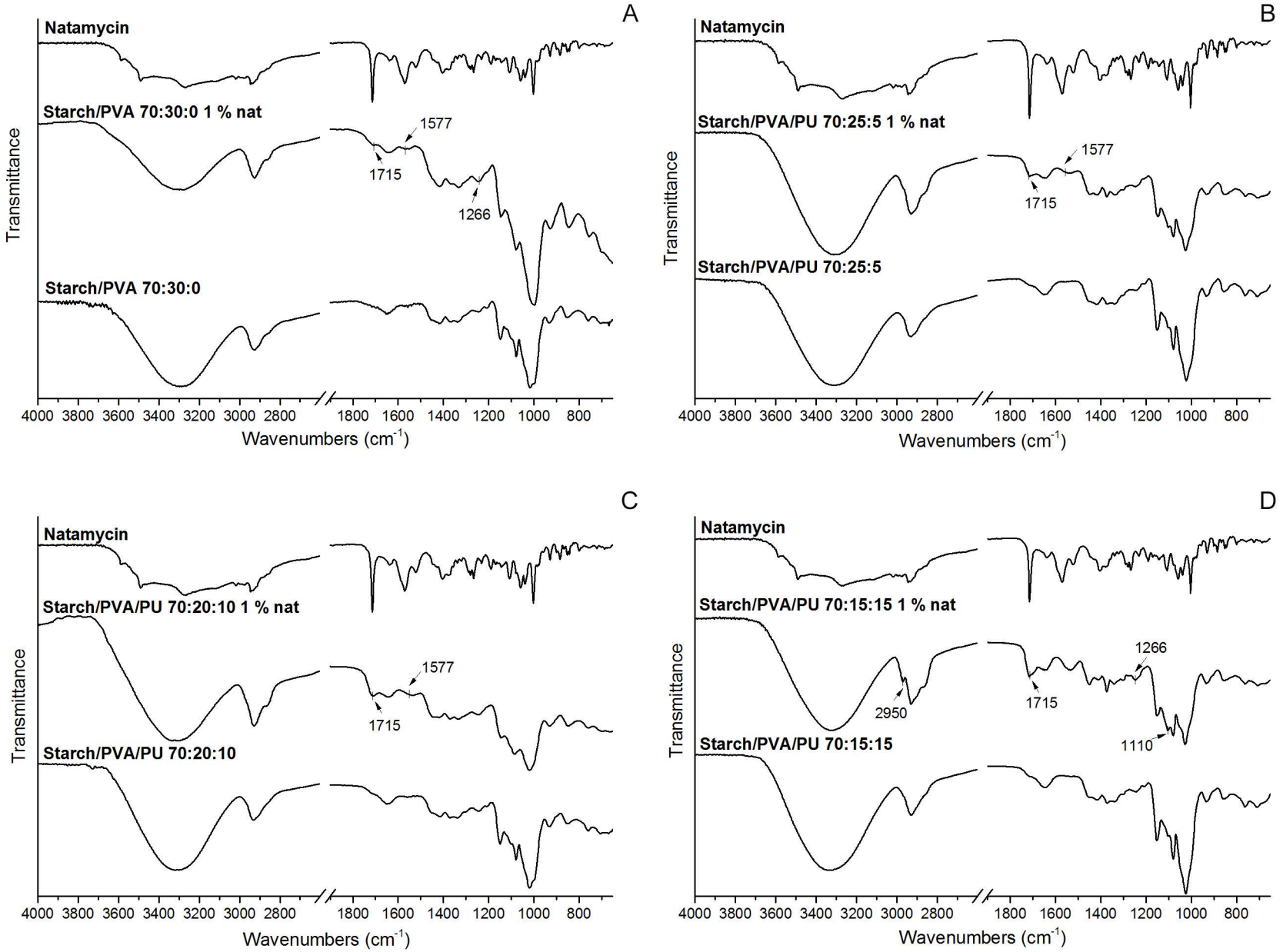
Formulation	Moisture content (%)	Swelling degree (%)	Residual water (%p/p)	IDT (ºC)*	T_5 (°C)	T_{50} (°C)	Residue# (%p/p)	WVP x 10^{11} ($g.s^{-1}.m^{-1}.Pa^{-1}$)
Starch/PVA/PU 70:30:0 1 % nat.	9.9[a]	6.23[a]	10.0[a]	240.0[a]	105.8[b]	344.8[a]	14.7[a]	0,069[a]
Starch/PVA/PU 70:25:5 1 % nat.	10.4[a]	6.71[a]	10.5[a]	240.0[a]	96.73[ab]	347.0[ab]	10.3[b]	0,076[a]
Starch/PVA/PU 70:20:10 1 % nat.	9.8[a]	6.13[a]	10.2[a]	233.5[a]	96.0[ab]	361.9[b]	13.8[a]	0,062[a]
Starch/PVA/PU 70:15:15 1 % nat.	10.2[a]	5.30[a]	9.8[a]	234.0[a]	82.1[c]	350.0[ab]	14.6[a]	0,094[a]

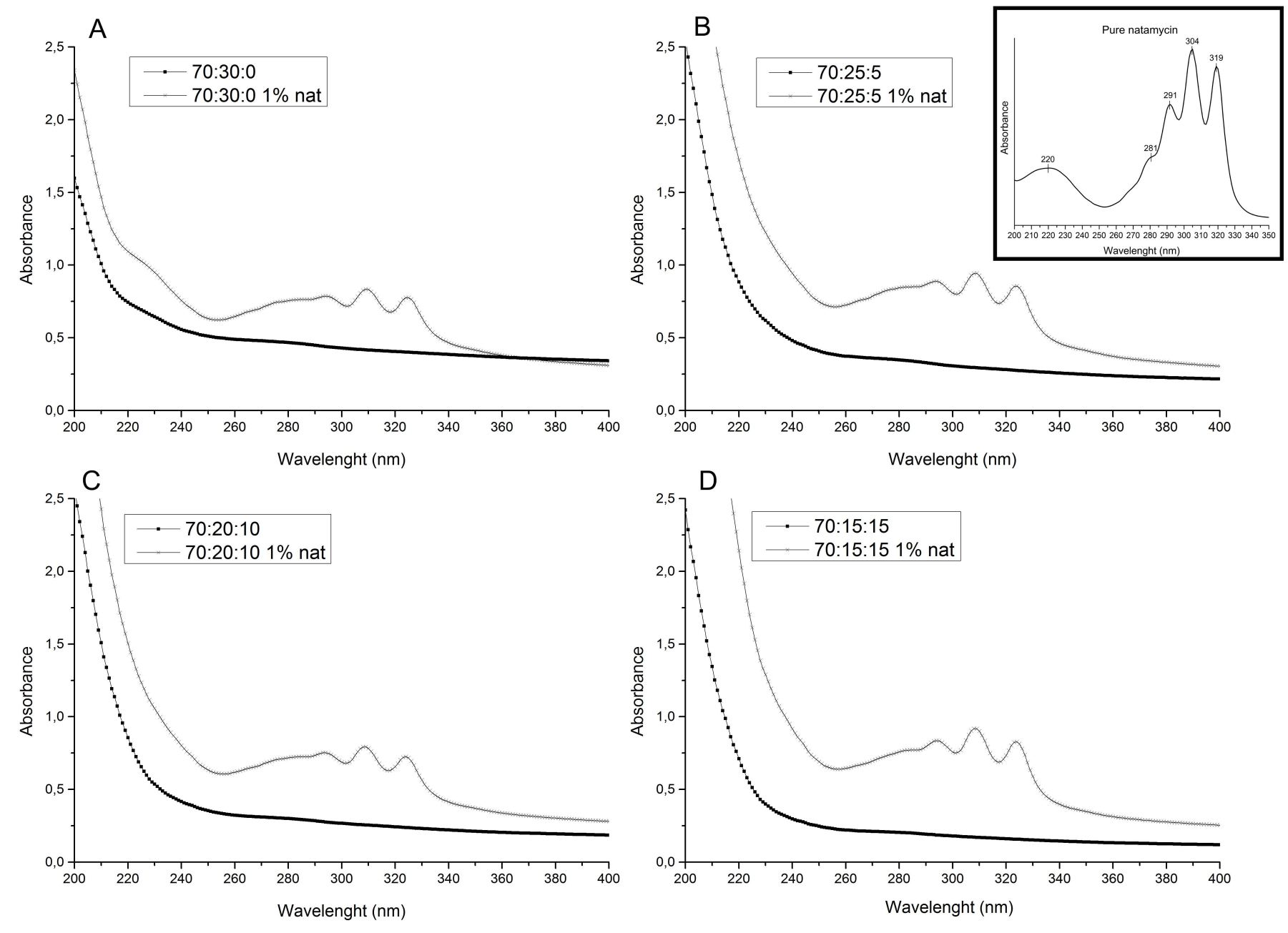
Mean and standard deviation are reported. Different letters in the same column indicate significant differences ($p<0.05$). *IDT: after water loss. # Residual mass at 540 ºC.

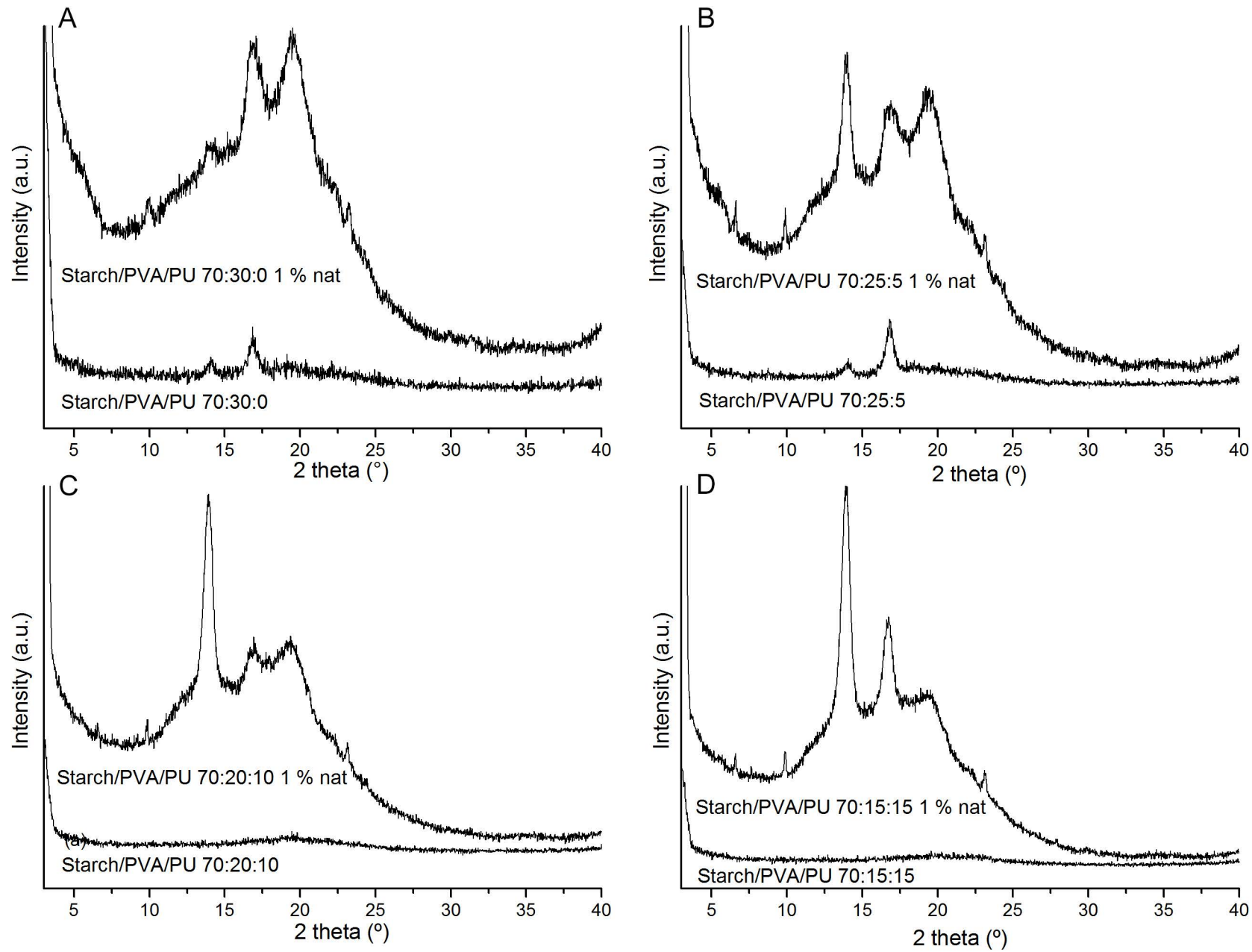
Table 2. Diameters of inhibition halos (mm) for Starch/PVA/PU films 70:30:0, Starch/PVA/PU 70:25:5, Starch/PVA/PU 70:15:15 added with and without 1 % natamycin against *Penicillium spp* 1, 2 and 3.

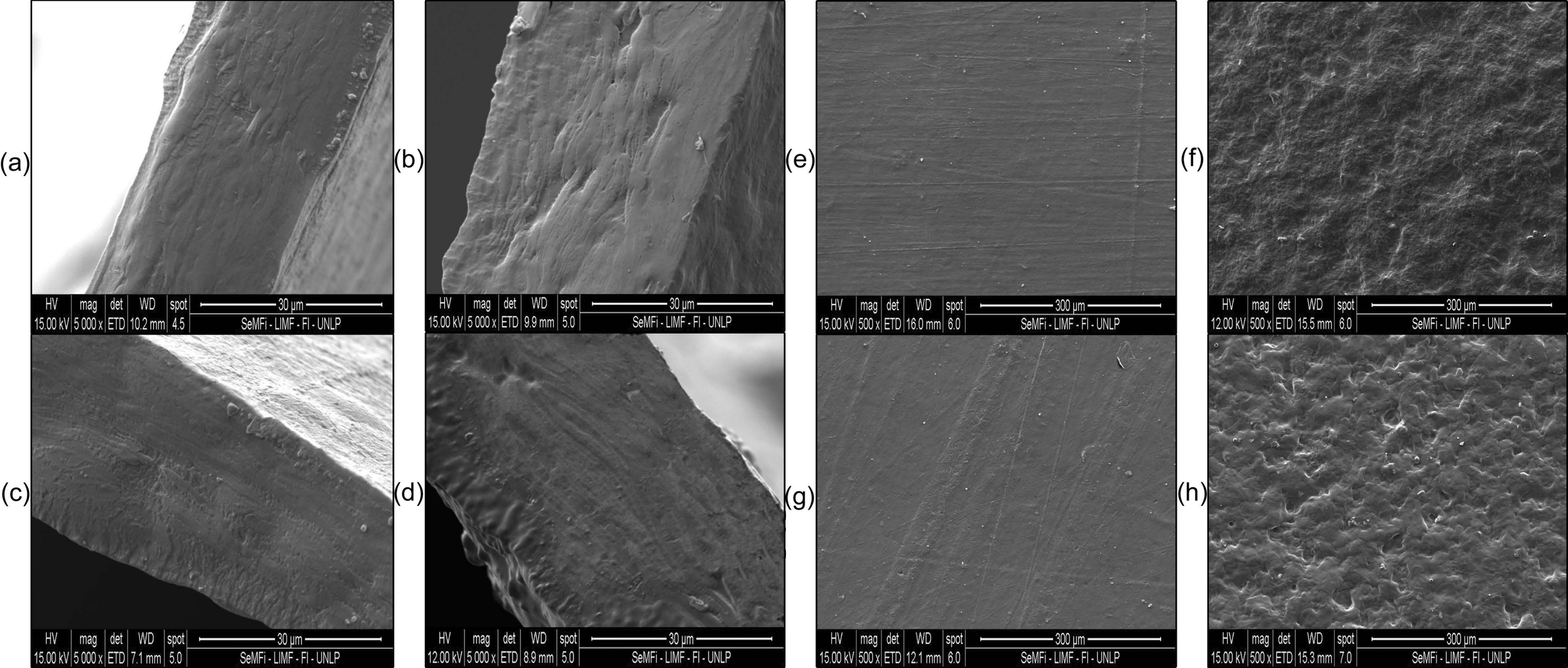
	Inhibition zones (mm)		
Films	***Penicillium spp 1***	***Penicillium spp2***	***Penicillium spp 3***
Starch/PVA/PU 70:30:0	0^{aA}	0^{aA}	0^{aA}
Starch/PVA/PU 70:25:5	0^{aA}	0^{aA}	0^{aA}
Starch/PVA/PU 70:20:10	0^{aA}	0^{aA}	0^{aA}
Starch/PVA/PU 70:15:15	0^{aA}	0^{aA}	0^{aA}
Starch/PVA/PU 70:30:0 1 % Nat	29.75^{bA}	33.00^{bB}	32.90^{bB}
Starch/PVA/PU 70:25:5 1 % Nat	30.05^{bA}	35.15^{cB}	36.15^{cB}
Starch/PVA/PU 70:20:10 1 % Nat	34.95^{dA}	36.30^{dB}	36.10^{cB}
Starch/PVA/PU 70:15:15 1 % Nat	33.40^{cA}	36.95^{dB}	37.25^{dB}

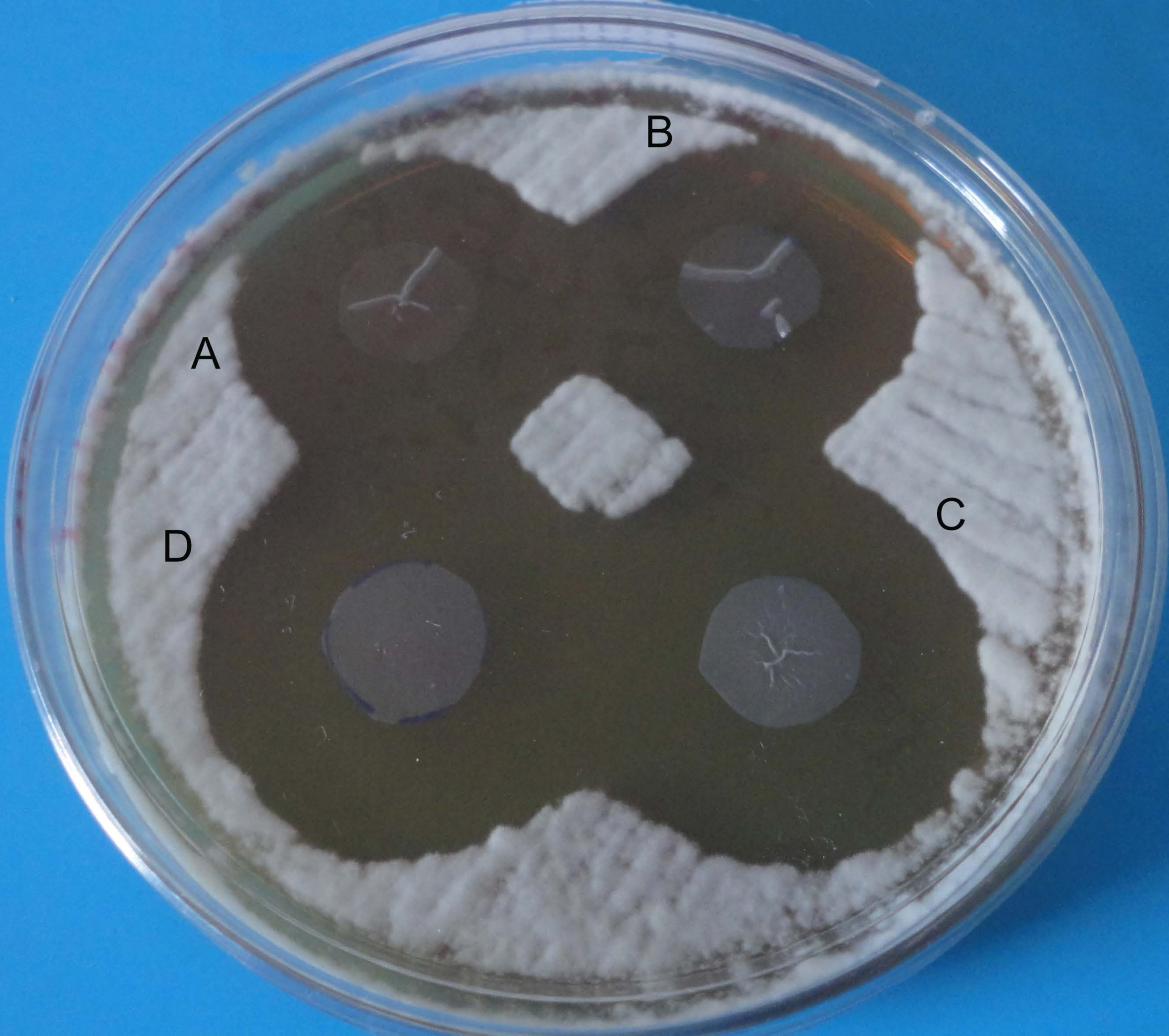
Lower case letters indicate differences among films for a given *Penicillium spp.* strain, and capital letters indicate differences among strains for a given film formulation based on a Tukey test at $P \leq 0.05$.


A
Natamycin
Starch/PVA 70:30:0 1 % nat
Starch/PVA 70:30:0
1577
1715
1266
Transmittance
4000 3800 3600 3400 3200 3000 2800
1800 1600 1400 1200 1000 800
Wavenumbers (cm-1)
B
Natamycin
Starch/PVA/PU 70:25:5 1 % nat
Starch/PVA/PU 70:25:5
1577
1715
Transmittance
Wavenumbers (cm-1)
C
Natamycin
Starch/PVA/PU 70:20:10 1 % nat
Starch/PVA/PU 70:20:10
1577
1715
Transmittance
Wavenumbers (cm-1)
D
Natamycin
Starch/PVA/PU 70:15:15 1 % nat
Starch/PVA/PU 70:15:15
2950
1715
1266
1110
Transmittance
Wavenumbers (cm-1)


A
70:30:0
70:30:0 1% nat
Absorbance
Wavelenght (nm)
B
70:25:5
70:25:5 1% nat
Pure natamycin
220
281
291
304
319
Absorbance
Wavelenght (nm)
C
70:20:10
70:20:10 1% nat
Absorbance
Wavelenght (nm)
D
70:15:15
70:15:15 1% nat
Absorbance
Wavelenght (nm)


A
Intensity (a.u.)
Starch/PVA/PU 70:30:0 1 % nat
Starch/PVA/PU 70:30:0
5 10 15 20 25 30 35 40
2 theta (°)
B
Intensity (a.u.)
Starch/PVA/PU 70:25:5 1 % nat
Starch/PVA/PU 70:25:5
5 10 15 20 25 30 35 40
2 theta (º)
C
Intensity (a.u.)
Starch/PVA/PU 70:20:10 1 % nat
(a)
Starch/PVA/PU 70:20:10
5 10 15 20 25 30 35 40
2 theta (º)
D
Intensity (a.u.)
Starch/PVA/PU 70:15:15 1 % nat
Starch/PVA/PU 70:15:15
5 10 15 20 25 30 35 40
2 theta (º)


(a)
HV 15.00 kV | mag 5 000 x | det ETD | WD 10.2 mm | spot 4.5 | 30 µm | SeMFi - LIMF - FI - UNLP
(b)
HV 15.00 kV | mag 5 000 x | det ETD | WD 9.9 mm | spot 5.0 | 30 µm | SeMFi - LIMF - FI - UNLP
(e)
HV 15.00 kV | mag 500 x | det ETD | WD 16.0 mm | spot 6.0 | 300 µm | SeMFi - LIMF - FI - UNLP
(f)
HV 12.00 kV | mag 500 x | det ETD | WD 15.5 mm | spot 6.0 | 300 µm | SeMFi - LIMF - FI - UNLP
(c)
HV 15.00 kV | mag 5 000 x | det ETD | WD 7.1 mm | spot 5.0 | 30 µm | SeMFi - LIMF - FI - UNLP
(d)
HV 12.00 kV | mag 5 000 x | det ETD | WD 8.9 mm | spot 5.0 | 30 µm | SeMFi - LIMF - FI - UNLP
(g)
HV 15.00 kV | mag 500 x | det ETD | WD 12.1 mm | spot 6.0 | 300 µm | SeMFi - LIMF - FI - UNLP
(h)
HV 15.00 kV | mag 500 x | det ETD | WD 15.3 mm | spot 7.0 | 300 µm | SeMFi - LIMF - FI - UNLP


A
B
C
D