ANTIFUNGAL COTTON FABRIC *VIA* MOLECULAR ENCAPSULATION OF TERBINAFINE

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The aim of the study was to investigate inclusion complex formation of terbinafine, an allylamine based antifungal pharmaceutical agent, by spray drying and kneading, and its grafting onto cotton fabric. Terbinafine was introduced in monochlorotriazine-beta-cyclodextrin in 3 different molar ratios. The products were obtained by spray drying and kneading. The products were analyzed by X-ray diffractometry, differential scanning calorimetry and FTIR spectroscopy. Terbinafine and monochlorotriazine-beta-cyclodextrin in 1:3 molar ratio presented evidence of inclusion complex formation by both spray drying and kneading methods. The inclusion complex was applied to 100% cotton fabric by the padding method. Then the fabric was subjected to different numbers of washing cycles. N percentage of the fabrics was determined by C, H, N, S elemental analysis to evaluate washing durability. Antifungal strength against *Trichophyton rubrum* was observed even after 15 washing cycles.

Keywords: terbinafine, monochlorotriazine-β-cyclodextrin, cotton fabric, spray drying, kneading

INTRODUCTION

Cyclodextrins are natural cyclic oligosaccharides, which consist of 6, 7 and 8 glucopyranose units. β-cyclodextrin, which has 7 glucopyranose units, has been preferred for use in textile applications, such as essential oil, odor and dye encapsulation. The cyclic structure of cyclodextrins presents characteristic 3D structural features, thus having the appearance of a truncated cone. Cyclic glucopyranose units form an apolar cavity, and the hydroxyl groups of the glucopyranose units are oriented outside of the cone. Thus cyclodextrins have the capacity to encapsulate substances in their apolar cavity. As a matter of fact, substances that contain apolar groups and have suitable dimensions are only molecularly encapsulated by cyclodextrins. Molecular encapsulation with cyclodextrin is called inclusion complex, and cyclodextrins screen some physical and chemical features of the encapsulated substances.[1,2]

Cyclodextrins have been used for scent, cosmetic and dye applications in textiles.[3] Some studies have been carried out on the effects of cyclodextrins on dyeing of cotton,[4-6] polyester,[7,8] polyamide,[9] polyacrylic[10] and secondary acetate.[11] Cyclodextrins can also be used for finishing of textiles conferring flame retardancy,[12] scent and insect repellency,[13-16] UV and sun protection[17,18] and in fragrance applications.[19]

In recent years, applications of pharmaceutical agents to textiles have attracted the attention of researchers and industry. Microencapsulated terbinafine is applied to cotton fabrics by the padding method using melamine-formaldehyde microcapsules.[20] Ethyl cellulose microcapsules of both terbinafine and ketoconazole are also used for textile applications.[21] Microencapsulation of diclofenac, an anti-inflammatory agent, has been studied to achieve sport bandages.[22] Tamoxifen, an antineoplastic agent, has been microencapsulated through the complex coacervation method and applied to cotton fabric by coating.[23] Cyclodextrins can be widely used in this field due to their broad applications in the pharmaceutical industry. As an example, the inclusion complex of diclofenac and cyclodextrin is used to wound dressing applications,[24] while the miconazole:cyclodextrin inclusion complex is

used in manufacturing antibacterial fabrics.[25,26] The antifungal properties of cotton fabrics loaded with the inclusion complex of ketoconazole: cyclodextrin have been observed against *Trichophyton rubrum* and *Aspergillus niger.*[27]

The aim of this study has been to obtain an antifungal fabric by encapsulating terbinafine, an allylamine based antifungal pharmaceutical agent (Figure 1), in monochlorotriazine-β-cyclodextrin, using the spray drying and kneading methods. The effects of molar ratio and preparation method on the products obtained were investigated by XRD, DSC, FTIR analyses. The products were then applied to 100% cotton fabric through the impregnation method. The washing durability was determined by C,H,N,S elemental analysis. The antifungal properties against *Trichophyton rubrum* after washing were also determined.

EXPERIMENTAL

In this study, terbinafine (TER), an antimicotic agent, was kindly supplied by Nobel İlaç San. A.Ş. (Turkey). Monochlorotriazine-β-cyclodextrin (MCTβCD) was purchased from Wacker Chemie (Turkey). Ethanol and methanol were obtained from Fluka. All chemicals were used without any purification. 100% cotton 1/1 plain weave fabric (125 g m^{-2} unit weight, and 38 cm^{-1} and 35 cm^{-1} density in warp and weft direction, respectively) was used.

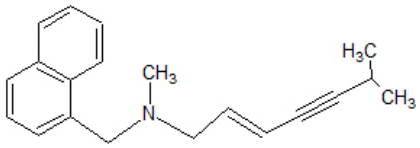

Figure 1: Terbinafine structure

Preparing of complexes

Spray drying

The feed mixtures were spray dried in a Lab Plant SD4 spray drier (Lab Plant Ltd., England) with the main chamber (380 mm × 110 mm). The drier was operated at 90 °C air inlet and 40-50 °C outlet temperatures. The pump power was kept at 20% to maintain a feed flow rate of 5 mL min^{-1}, the maximum capacities of air blowing and compressor were used. During the drying processes, the temperature of the feed mixture was 25 °C (Figure 2).

Kneading

MCTβCD and TER were mixed in a mortar with the addition of methanol:water (1:1 v:v) mixture and the mixture was kneaded continuously until the evaporation of the solvent.

Products were prepared in three molecular ratios (TER:MCTβCD molar ratios = 1:1, 1:2 and 1:3) by both kneading and spray drying methods.

Characterization of complexes

Differential Scanning Calorimetry (DSC)

Measurements of the melting point and melting heat of the obtained microcapsules were performed by a Perkin–Elmer DSC apparatus. The samples were compared with Al pan under nitrogen flow. The measurements were performed varying the temperature in the range from 20 to 250 °C with a heating rate of 10 °C min^{-1}.

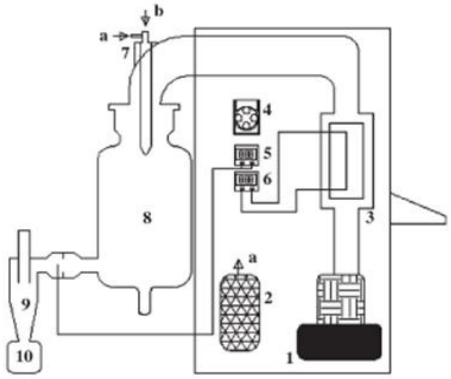

Figure 2: Spray drying apparatus (1-blower and air filter; 2-compressor; 3-heater; 4-perisaltic pump; 5-temperature control; 6-inlet thermocouple; 7-atomizer (a) compressed air, (b) solution feed; 8-drying chamber; 9-cyclone; 10-product collector)

X-ray diffraction (XRD)

XRD patterns of the samples were obtained using an X-ray diffractometer (Rigaku D/Max-2200/PC). The observations were carried out at 2θ and a copper source was employed.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of the melamine-formaldehyde microcapsules at different molar ratios were obtained using Perkin–Elmer Spectrum BX FTIR

(wavenumbers 400-4000 cm^{-1}) at room temperature, according to the KBr tablet method.

Application to textiles

MCTβCD-TER complexes were applied to cotton fabric. First, the products (20 g L^{-1}) were dissolved in water and then the solution pH was adjusted to pH 10 by adding 0.5 N NaOH (Merck). The application was performed according to the impregnation method at 75% pickup in vertical type foulard (Rapid-Labortex, Taiwan). The volume of the trough was 200 mL. Drying was carried out at 150 ^oC for 3 minutes in a laboratory type oven (Nüve, Turkey). Curing was performed at 150 ^oC for 5 minutes in laboratory type stenter (Labortex, Taiwan).

Washing durability

Washing durability of cotton fabrics were determined by using Atlas Linitest apparatus, according to TS EN ISO 105 C06 Test for color fastness - Part C06: Color fastness to domestic and commercial laundering 2001. ECE (A) non-phosphate standard detergent No 2 was used for trials. A1S method was used (40 ^oC, 30 min, 10 steel balls).

C,H,N,S analysis

The common organic elements C (carbon), H (hydrogen), N (nitrogen) and S (sulphur) were analysed by a Vario Elemental C,H,N,S analyzer. The sample (5.0 mg) was taken in a tin capsule for determining its percentage composition as to C, H, N and S, while the percentage of O (oxygen) was determined by difference.

Antifungal test

Antifungal tests were performed according to the study of Gregory *et al*.[28] In this work, the authors modified the AATCC Test Method 30 Antifungal activity - assessment on textile materials: Mildew and rot resistance of textile materials by using pathogen test cultures such as *Trichophyton rubrum (*Athlete's foot*). Trichophyton rubrum* (DSMZ 16111) was used as test culture.

RESULTS AND DISCUSSION

Characterization of MCTβCD-TER products

Kneading method

Figure 3 depicts the DSC thermographs of the products, which were prepared by the kneading method with different molar ratios. The endothermic peak of MCTβCD around 130 ^oC might be caused by boiling of water molecules. When examining the DSC thermograph of native TER, an endothermic peak is observed above 200 ^oC, which may due to the start of TER decomposition. This endothermic peak is a key to the observation of inclusion complex formation. As can be seen in the thermographs, the endothermic peak of TER starts disappearing with increasing the mole fraction of MCTβCD. At 1:3 molar ratio of the product, the characteristic peak of TER disappears completely. Thus we can conclude that there has been formed an inclusion complex between TER and MCTβCD at 1:3 molar ratio.

The proof of inclusion complex formation was double checked by XRD analysis. XRD patterns of 1:1 and 1:3 molar ratios of the products and native TER and MCTβCD are shown in Figure 4. TER presents a more crystalline structure than MCTβCD, and due to this, TER has characteristic peaks around 5, 10, 16, 18, 19.5, 21, 24, 30, and 38^o 2θ. When comparing the patterns of 1:1 and 1:3 molar ratios, the characteristic peaks of TER, especially those at 5 and 10^o, disappear with an increasing mole fraction of MCTβCD. It can be inferred that an inclusion complex has been formed between MCTβCD and TER at 1:3 molar ratio.

The FTIR spectrum of 1:3 molar ratio of TER:MCTβCD is shown in Figure 5. A broad peak between 3500 and 3200 cm^{-1} is assigned to O–H stretching in α-D-glucopyranoside units. A slight peak is observed around 1490 cm^{-1}, which is due to in-plane –C=N– vibrations of the triazine ring system, part of the reactive group of MCTβCD. N–CH_3 stretching of TER is noted at 2473 cm^{-1}. Some shifts between 2200 and 2000 cm^{-1} are attributed to the stretching vibration of carbon-carbon triple bond in TER structure. The band around 770 cm^{-1} in the FTIR spectrum corresponds to the bending vibration of the aromatic C–H bond in the naphthalene ring system of TER. However, the stretching band of C-Cl between 1722 and 1734 cm^{-1} is not observed due to the reaction between triazine and cellulose.

Spray drying method

Figure 6 shows DSC thermographs of spray dried mixtures of MTCβCD and TER. As mentioned before, TER gave an endothermic peak around 200 ^oC due to its decomposition. Similarly as for the kneaded sample, the decomposition of TER at 1:1 molar ratio is clearly seen in the thermograph. The decomposition peak of TER disappears gradually with increasing the molar fraction of MCTβCD and at 1:3 molar ratio it is totally absent.

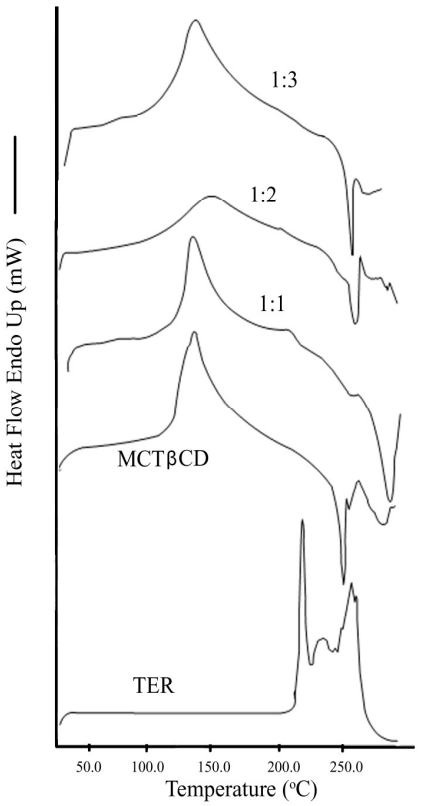

Figure 3: DSC thermograms of kneaded products

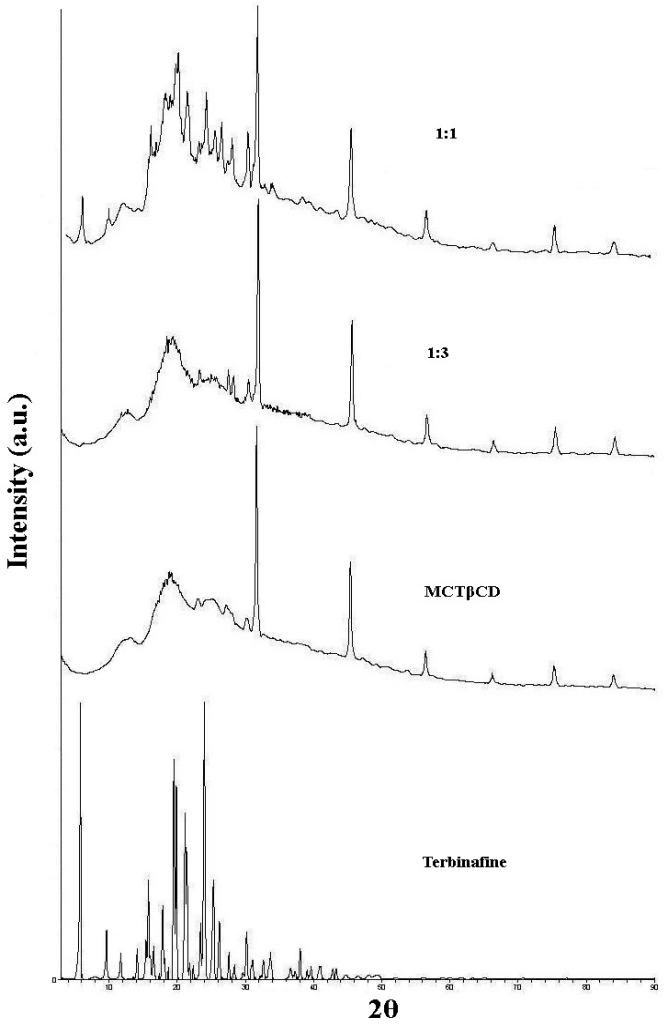

Figure 4: XRD patterns of kneaded products

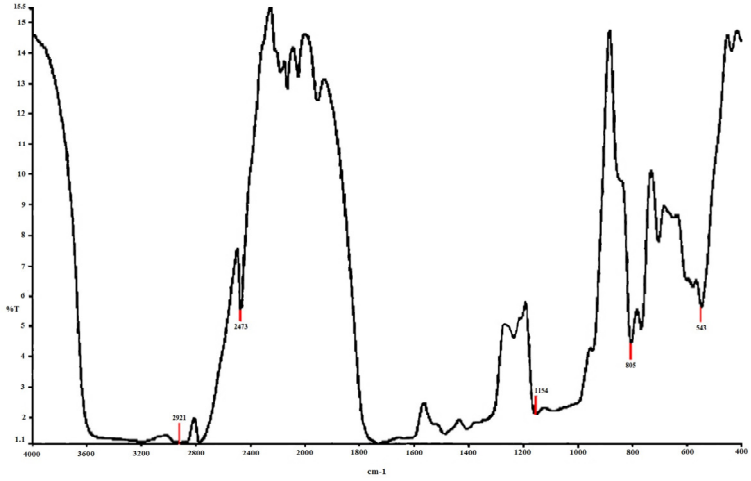

Figure 5: FTIR spectrum of the 1:3 molar ratio kneaded product

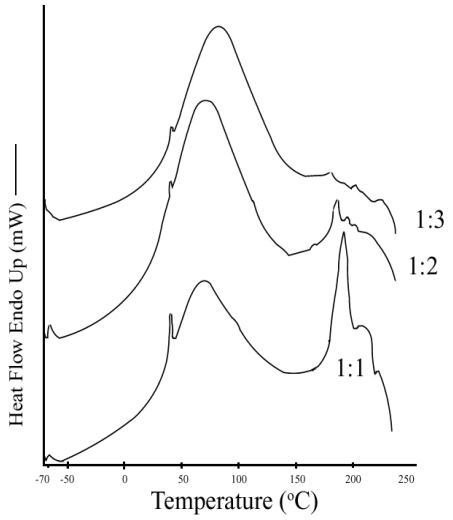

Figure 6: DSC thermograms of spray dried products

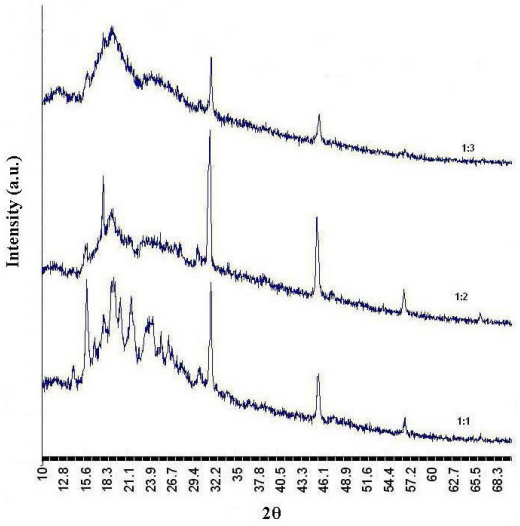

Figure 7: XRD patterns of spray dried products

The XRD patterns of the spray dried samples are shown in Figure 7. Strong peaks are observed in the case of 1:1 molar ratio, which can be referred to the crystalline nature of TER. However, the intensity of the peaks decreases and some of them disappear at 1:2 molar ratio. Thus, it can be concluded that some of TER molecules do not form an inclusion complex with MCTβCD and remain in a crystalline form. In the case of 1:3 molar ratio, an inclusion complex can be observed due to the absence of the peaks characteristic of the crystalline nature of TER. MCTβCD plays a dominant role and screens the characteristic TER peaks at 1:3 molar ratio.

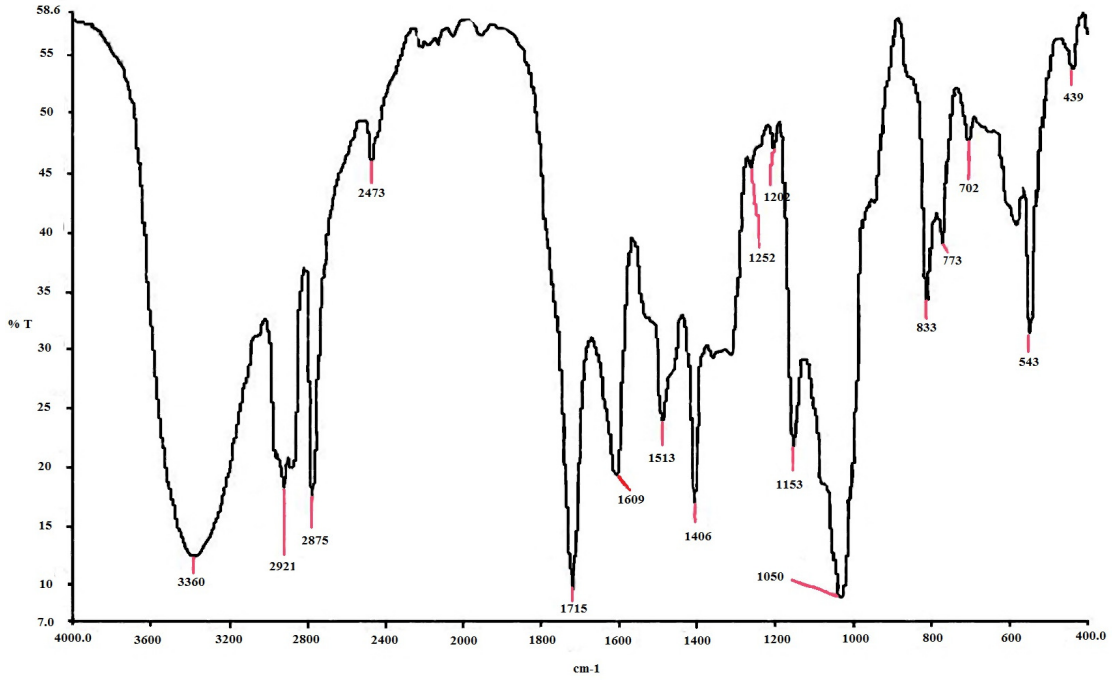

Figure 8: FTIR spectra of spray dried products of 1:3 molar

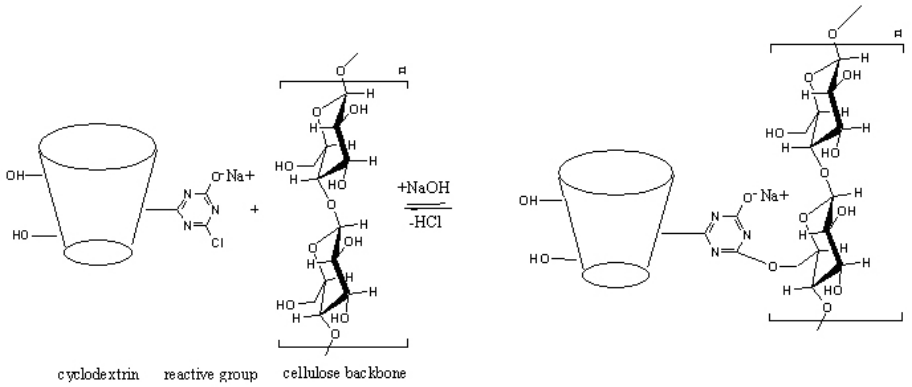

Figure 9: Mechanism of grafting of monochlorotriazine-β-cyclodextrin onto cotton fabrics

From all the data obtained from the DSC thermographs and XRD patterns, it can be inferred that an inclusion complex is formed at 1:3 molar ratio of TER:MCTβCD.

Figure 8 shows the FTIR spectrum of the 1:3 molar ratio product. An intensive peak around 3360 cm^{-1} is attributed to the stretching of the O-H bond in the glucopyranose units of MCTβCD. The absorption bands of 2921 and 2875 cm^{-1} can be assigned to anti-symmetric and symmetric CH_2 vibration. A peak at 2473 cm^{-1} is due to N-CH_3 stretching. Moderate peaks between 2300 and 2100 cm^{-1} are attributed to C triple bond C stretching. The band at 773 cm^{-1} may be assigned to C-H stretching of the naphthalene ring. However, the stretching band of C-Cl between 1722 and 1734 cm^{-1} is not observed due to the reaction between triazine and cellulose.

Application to cotton fabric

Halogenated triazines react with the hydroxyl groups of cellulose, according to the nucleophilic addition-elimination (hetero-aromatic) substitution mechanism. The reaction mechanism is illustrated in Figure 9. First, the most basic form of the nucleophilic groups, in this case the O^- groups of cellulose attack the heterocyclic carbon of the reactive group. Then the nucleophilic group is replaced by the halogen of triazine. Thus a covalent bond is created, which affords good fastness properties.

C,H,N,S analysis

Determining the amount of nitrogen has a key role in observing the properties of fastness to washing due to N groups of both triazine and terbinafine. Thus C, H, N, S analysis was carried out. Figure 10 shows the N percentage of the fabrics, which were subjected to different numbers of washing cycles. The control fabric was untreated cotton fabric. After impregnation of the inclusion complex, the nitrogen percentage increased approximately two fold. N percentage decreased sharply to around 0.25 after one washing and continued to decrease after 5, 10 and 15 washing cycles. From 15 to 25 washing cycles, the N percentage of the fabrics did not show significant changes.

Antifungal peculiarities

Trichophyton rubrum is a highly specialized pathogenic fungus, which is one of the most common agents of superficial mycoses. It infects healthy individuals and is exclusively found in keratinized layers of the skin (e.g. *stratum corneum*), nails, or hair. *T. rubrum* is widespread and very contagious. For this reason, *T. rubrum* was chosen to evaluate the antifungal activity of the fabrics under study. Figure 11 shows the antifungal test results. The antifungal properties were maintained after 15 washing cycles. Nevertheless, *T. rubrum* growth was observed after 20 washing cycles. In our previous work, where TER was microencapsulated in melamine-formaldehyde resin, the antifungal effect of the fabrics was maintained up to 15 washing cycles.[20] The encapsulation of TER by both melamine-formaldehyde and MCTβCD gave the same results as to washing durability and antifungal peculiarities.

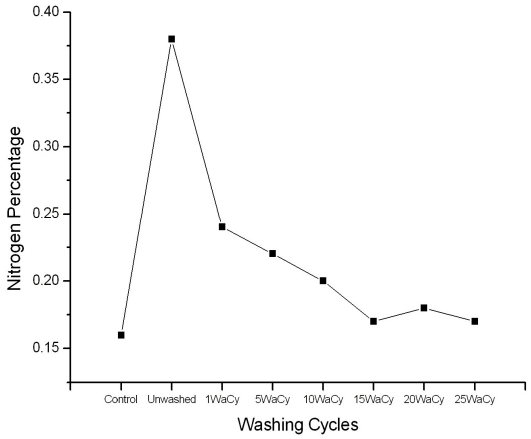

Figure 10: Nitrogen percentage of fabrics after various washing cycles

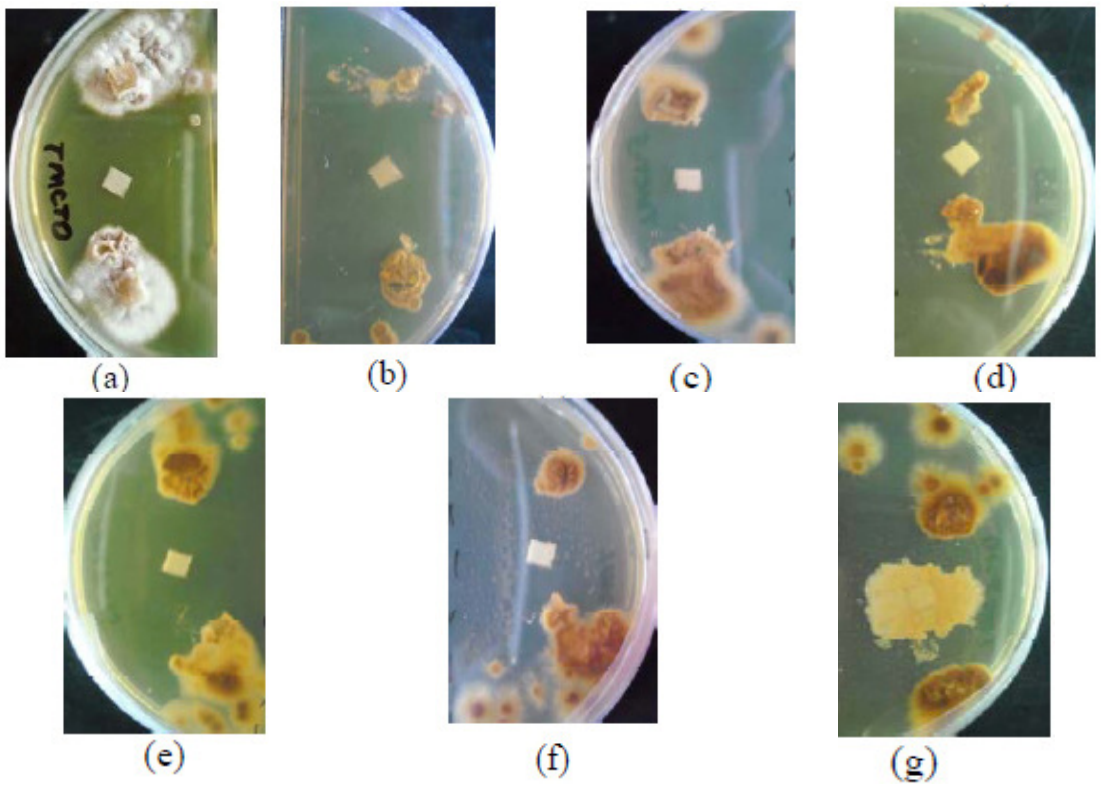

Figure 11: Antifungal properties of grafted fabrics against *T. rubrum* after different washing cycles; (a) unwashed; (b) 1 washing cycle; (c) 5 washing cycles; (d) 10 washing cycles; (e) 15 washing cycles; (f) 20 washing cycles; (g) 25 washing cycles

CONCLUSION

In this work, terbinafine, an allylamine based antifungal agent, was used to form an inclusion complex with monochlorotriazine-β-cyclodextrin by both spray drying and kneading methods. Three products with different molar ratios of monochlorotriazine-beta-cyclodextrin and terbinafine were applied to the textile surface. The thermal behavior and XRD peaks of terbinafine were completely screened by monochlorotriazine-

beta-cyclodextrin at 1:3 molar ratio for both spray drying and kneading methods. The existence of TER was proved by FTIR analysis at 1:3 molar ratio.

The inclusion complexes were successfully applied to cotton fabric by the padding method. Washing durability was checked by C, H, N, S elemental analysis. Nitrogen percentage was a key factor used in evaluating the washing durability. A sharp decrease was observed after the first washing cycle, however, after 15 washing cycles the nitrogen percentage did not change significantly. The amount of nitrogen could be determined by both triazine and the nature of terbinafine. However, the antifungal properties of the loaded fabrics agreed with the results of elemental analysis. The antifungal properties were maintained after 15 washing cycles. These results are in good agreement with those obtained in our previous work, when the fabrics loaded with melamine-formaldehyde microcapsules exhibited antifungal properties even after 15 washing cycles. In conclusion, this work confirms that monochlorotriazine-beta-cyclodextrin can be used in manufacturing antifungal or antimycotic fabrics.

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