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Antimycotic influence of β-cyclodextrin complexes—In vitro measurements using laser nephelometry in microtiter plates

Moustafa M.G. Fouda [a,*], Dierk Knittel [b,1], Uta-Christina Hipler [c,2], Peter Elsner [c], Eckhard Schollmeyer [b]

[a] *Textile Research Division, National Research Centre, Dokki, Cairo, Egypt*
[b] *German Textile Research Institute North-West e.V., Adlerstrasse, 1, D-47798 Krefeld, Germany*
[c] *Department of Dermatology and Allergology, Friedrich Schiller University of Jena, Erfurterstrasse, 35, 07740 Jena, Germany*

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Abstract

To determine the in vitro susceptibility of fungal organisms to β-cyclodextrin (CD) complexes with the antifungal agents econazole-nitrate (EC) and ciclopirox-olamine (CI), a fast, rapid and simple method using laser nephelometry in 96-microtiter plate is used. The antimycotic influence of the complexes against *Candida albicans* DSM 11225 and *Candida krusei* ATCC 6258 species was determined using this method. A rapid inhibition and even killing of both fungi was observed only above certain concentrations of complex ranged between 12.5 and 100 μg/ml for β-CD–econazole complex (CD–EC), while for the complex with ciclopirox-olamine (CD–CI) the range was between 150 and 400 μg/ml. The stability constants of the CD complexes with the two antimycotic derivatives are given. In addition, the nephelometric method allows the determination of solubilities of active agents. Thus, the improvement of solubility of both antimycotic agents in PBS buffer solution was observed by complexation with CD.

Keywords: β-Cyclodextrin; Econazole; Ciclopirox; Antimycotic; Laser nephelometry

1. Introduction

Cyclodextrins, which are cyclic oligosaccharides consisting of six or more α-(1,4)-linked D-glucopyranose units have recently recognized as useful pharmaceutical excipients, due to their potential to form inclusion complexes with appropriately sized drug molecules (Szejtli, 1988). The resulting complexes generally offer a variety of physicochemical advantages over the free drug, including increased water solubility, enhanced bioavailability, improved stability, reduced side effects, etc. (Duchêne et al., 1987). EC and CI (Fig. 1) are antifungal agents suitable for the treatment of many mycotic infections. They are applied topically in the treatment of infections of the skin, hair and mucous membranes, and are given orally mainly for the treatment of candidacies, or intravenously in the treatment of systematic fungal infections (Sawyer et al., 1975; Heel et al., 1978). Previous studies showed that both dissolution properties and consequently microbiological activities of econazole, with very low water solubility (about 3 μg/ml at 25 °C), can be improved by complexation with natural cyclodextrins, particularly with β-CD (Bononi, 1988; Mura et al., 1992; Pedersen et al., 1993). However, alkylated and particularly methylated cyclodextrins demonstrated to be often more effective as solubilizing and complexing agents than parent cyclodextrins (Uekama and Irie, 1987). By increasing the water solubility of the drug it should be possible to improve its bioavailability, thus enabling improved oral or topical formulations. An econazole β-CD and a miconazole β-CD preparation, isolated by freeze-drying, have been patented (Bononi, 1988). According to the patent the preparations containing β-CD were superior to the pure drugs with respect to effectiveness on, e.g., vulvovaginal candidosis (Bononi, 1988). Pedersen studied the formation and antimycotic effect of cyclodextrin inclusion complexes of EC and miconazole (Pedersen et al., 1993). They found that the antimycotic effect of CD–EC against a strain of *Candida*

* Corresponding author. Tel.: +20 2 3371 211; fax: +20 2 3370 931.
E-mail addresses: moustafa_fouda@gmx.net (M.M.G. Fouda), knittel@dtnw.de (D. Knittel), Christina.Hipler@med.uni-jena.de (U.-C. Hipler).
[1] Tel.: +49 2151 8430; fax: +49 2151 843 143.
[2] Tel.: +49 3641 937 355; fax: +49 3641 937 437.

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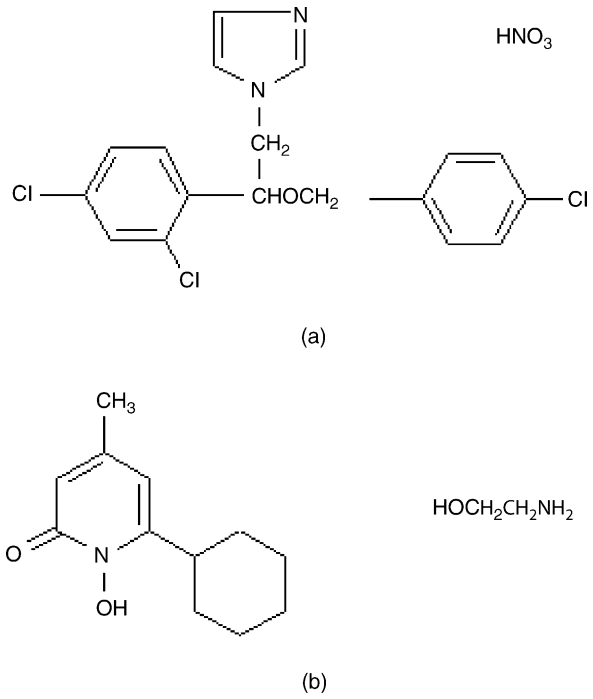

Fig. 1. Chemical structure of: (a) econazole nitrate, (b) ciclopirox olamine.

albicans was superior to the effect of a physical mixture of the two compounds. Laser nephelometry has been shown to be a reliable technique for the measurement of drug solubility in 96-well plate format (Bevan and Lloyd, 2000). Laser nephelometry is the measurement of forward scattered light. When a laser beam is directed through a clear solution, the more particles or turbid suspensions (fungi in this study) in the solution, the greater the amount of forward scattered light (measured as units). The energy of the scattered light is directly proportional to the particle concentration in the suspension for up to three orders of magnitude (Bevan and Lloyd, 2000). The overall aim of the present paper is to use the laser naphelometry, as a new technique, to investigate the effects of complexation on the drug antimycotic activity, in addition to the measurement of phase solubility diagram of both drugs alone and as complexes with CD.

2. Experimental section

2.1. Chemicals and materials

Sterile 96-well microtiter plates were kindly supplied by greiner bio-one GmbH, Germany. Breathe-Easy Gas Permeable Sealing Membrane for Microtiter Plates 6 in. × 3.25 in. were obtained from Carl Roth GmbH, Germany. CASY® cups, CASY® ton (isotonic dilution liquid for cell cultures), CASY® clean were supplied by Schärfe System GmbH, Germany. McFarland standard Kit-0.5,1,2,3,4,5 and Sabouraud-Glucose-Agar with gentamycin-chloramphenicol (SGA) were from bioMerieux, Germany. β-Cyclodextrin was purchased from Wacker-Chemie GmbH, Germany. Sabouraud-Glucose-Bouillon (SGB) was from Oxoid Ltd., England. Phosphate buffered saline tablets (PBS), econazole nitrate, ciclopiroxolamine were from Sigma–Aldrich Chemical Company, Germany. Fluorescent fungal surface labelling reagent FUN®-1 cell strain (F-7030) (300 μl of 10 mM solution in anhydrous dimethylsulfoxide) and GH solution (sterile 2% D-(+) glucose containing 10 mM Na-HEPES, pH 7.2) were from MoBiTec, Germany. All other chemicals and solvents are of analytical grade.

2.2. Cell cultures

Two types of microorganisms were used in this study: *C. albicans* DSM 11225 and *Candida krusei* ATCC 6258.

2.3. Preparation of cell cultures

Both of *C. albicans* and *C. krusei* were grown on (SGA) at 30 °C for 24–48 h. Three to five well-isolated colonies of the same morphological type were selected from an overnight culture using a sterile wire loop and inoculated in 20 ml (SGB). The suspensions were incubated with shaking at 250 rpm/30 °C for 24 h. Then the overnight cell cultures were counted using CASY® 1 and adjusted to a final working concentration of 6×10^5 cells/ml in (SGB).

2.4. Preparation of antifungal agents

Both EC and CI were independently dissolved in a mixture of chloroform/methanol 1:1 to achieve a final stock solution containing 20 mg/ml of antifungal agent. The stock solution of EC was diluted with SGB and adjusted to be 1.25–100 μg/ml while as for CI; it was in the range of 1.25–10 μg/ml. All solutions were stored at −80 °C until used.

2.5. Preparation of the inclusion and antifungal complexes

A solution of EC was prepared by dissolving it in a chloroform/methanol mixture 1:1. CD was dissolved in hot water at 85–90 °C. Equimolar amounts (1:1 molar ratio) of EC and CD solutions were mixed together with stirring for 30 min at 85–90 °C (Buschmann et al., 2001). By cooling, crystallization of the complex was obtained. The complex was filtered using G3 filter and kept in a desiccator overnight. On the other hand, the second complex between CI and CD was also prepared according to the previously mentioned method (Buschmann et al., 2001), in which methanol was used as a proper solvent for CI. Moreover, a molar ratio of 1:2 of CI:CD was also used. The antifungal complex of CD–EC was prepared in a concentration range of 12.5–100 μg/ml using DMSO as a solvent, while the CD–CI was prepared in a concentration range of 150–400 μg/ml using distilled water.

2.6. Phase solubility studies

In this experiment, both of drugs and complexes were diluted in DMSO. Then the drug and complex solutions were

independently pipetted into PBS-buffer with a concentration of DMSO of 1–5 vol.%. All samples were scanned at 30 °C, with an integration time of 0.1 s, so that a plate (96 samples) could be scanned in ∼68 s. A gain of 122 and a laser intensity of 1% were set to allow direct comparison of all results. All raw data were processed using the BMG NEPHELOstar Galaxy® Evaluation software. The scattered light will remain at a constant intensity until precipitation occurs. At that point it will increase sharply.

2.7. Preparation of microtiter plate

Microtiter plate was prepared via method in which SGB was used as medium and 3×10^5 cells/ml as inoculums. Using a multichannel pipette, 100 μl of 2× antifungal concentrations was dispensed into columns 5–12 of sterile disposable 96-well microtiter plates. Columns 5 and 9 contained the highest concentration and columns 8 and 12 the lowest concentration of drug. Each concentration was repeated eight times. Columns 3 and 4 (controls) received 100 μl of diluent (SGB), columns 9–12 (blank; drug + medium) received also 100 μl of diluent. Then, 100 μl of working cells suspension prepared above were dispensed into each well of columns 3–8. The plates were covered by a gas permeable sealing membrane and scanned nephelometrically. The plates were shaked and incubated at 30 °C during measurement. The cell growth curve of each organism was obtained automatically every hour until 24 h in term of units. Then by using the standard curve of *C. albicans* and *C. krusei* determined by McFarland standard Kit these units were quantified into cell counts/ml.

2.8. Staining with fluorescent dye (FUN-1)

To do this staining, overnight cell culture of *C. albicans* DSM 11225 and *C. krusei* ATCC 6258 species were prepared. Cells of both cultures were counted and adjusted using CASY® 1 to a final working concentration of 3×10^5 cells/ml in 20 ml SGB. This number of cells were inoculated with previously detected inhibitory and lethal concentrations of both complexes and without as positive control. These cultures were incubated at 30 °C for 24 h. Then 200 μl from each overnight cell culture was suspended in 1 ml GH solution. The suspension was centrifuged at $14\,000 \times g$ for 5 min. The pellets obtained were resuspended again in 50 μl GH solution. From the last suspension 10 μl was added to 10 μl of FUN-1 (10 μM). After incubation at 30 °C for 30 min 10 μl of cell suspension was trapped between a microscope slides in preparation for microscopy. All microscopy was carried out by means of the Olympus Fluorescence microscope. Epifluorescence illumination was provided by either a 50 or a 100-W mercury arc lamp. An excitation filter of 480 nm and an emission filter ≥530 nm were used. Photomicrographs were acquired with an Olympus Digital Camera. Photographic slides were digitalized electronically and composite figures were assembled from the resulting images with analysis software® (Soft Imaging System).

2.9. Instrumentation analysis

2.9.1. Laser nephelometer

The laser nephelometer instrument used in this study is the NEPHELOstar Galaxy® (BMG LABTECH GmbH, Germany).

2.9.2. Cell counter

The cell count was determined using CASY® 1 Cell counter + Analyzer System Model TT (Schärfe System GmbH, Germany).

2.9.3. Fluorescence microscope

The FUN-1 stained fungi were observed using Fluorescence microscope BX 40. Digital Camera BX40 + C5050 Zoom were from Olympus Optical Co. GmbH, Germany. Analysis software® (Soft Imaging System GmbH, Münster, Germany).

2.9.4. GlpKa Instrument

Stability constants for cyclodextrin–drug complexes may be obtained by the analysis of phase solubility diagrams. But in this study, pH-metric titrations, as a direct method for the measuring of the protonation constant, are used by GlpKa analyzer (Sirius Analytical Instruments Ltd., East Sussex, UK). All titrations are done in aqueous solution. The ionic strength is kept constant at 0.15 M using KCl. The protonation constant (K_a) of the drug alone is measured at first, then titration also are done in the presence of drug and β-cyclodextrin at different concentrations of β-cyclodextrin. The following equations describe the reactions taking place:

$$\text{drug} + \text{H}^+ = \text{drugH}^+ \quad (K_1, \text{ protonation of the drug}) \tag{1}$$

$$\text{drugH}^+ + \text{CD} = \text{drugH}^+\text{CD}$$

$$(K_2, \text{ binding of protonated drug}) \tag{2}$$

$$\text{drug} + \text{CD} = \text{drugCD} \quad (K_3, \text{ binding of unprotonated drug}) \tag{3}$$

The term pK_a refers to the negative logarithm of the equilibrium constant for an acid/base protonation. The stability constants of CD–EC and CD–CI complexes are calculated directly from the difference in titration curves for (K_1) and (K_3) equilibria using the software package Refinement Pro (Version V1.114, Sirius Analytical Instruments, UK).

2.9.5. Differential scanning calorimetry (DSC)

Inclusion complexes, free drug, and β-cyclodextrin were subjected to DSC studies using DSC (Model 2910, TA Instruments, Inc., GmbH, Germany). The temperature ranged between 20 and 400 °C, The scan rate was 20 °C/min. The sample weighed 3–4 mg.

2.9.6. Elemental analysis

The elemental analysis was carried out at the university of Duisburg-Essen using a Carlo-Erbach 1006 and EA 3000 (Hekatech).

2.10. Statistics

Each measurement was performed eight times. Means and standard deviations were calculated by means of the Microsoft® Excel software. Statistical calculations included Student's *t*-test to ascertain whether the mean of the eight signals determined for each concentration was significant or not in comparison with the control.

3. Results and discussion

3.1. Characterization of the prepared inclusion complexes

3.1.1. Stability constant

The stability constants of the two complexes were determined using the pH-metric titrations in aqueous solution. The obtained value of the stability constant for CD–EC complex was ($\log K = 2.92 \pm 0.18$) and this value is in accordance with the previous value obtained by another method used by Pedersen et al. (1993). Other different value was reported by Pedersen for econazole nitrate–β-CD complex depending on the medium. The value for CD–CI complex was ($\log K = 2.09 \pm 0.06$).

3.1.2. Elemental analysis

The elemental analysis of CD–EC and CD–CI complexes was determined. As a result, the molar ratio of the inclusion complexes were confirmed. The data assured that CD–EC complex was prepared in 1:1 molar ratio but the other complex between CI and CD was prepared in 1:2 molar ratio, respectively, as represented in the experimental section. The obtained elemental analysis data are summarized as follows:

- β-CD: $C_{42}H_{70}O_{35}$
  - Calculated: C = 44.44%, H = 6.17%, O = 49.38%
  - Found: C = 44.60%, H = 6.34%, O = 49.60%
- 1:1 CD–EC complex: $C_{60}H_{86}Cl_3N_3O_{39}$
  - Calculated: C = 45.58%, H = 5.44%, N = 2.66%, O = 39.50%
  - Found: C = 45.72%, H = 5.51%, N = 2.61%, O = 39.40%

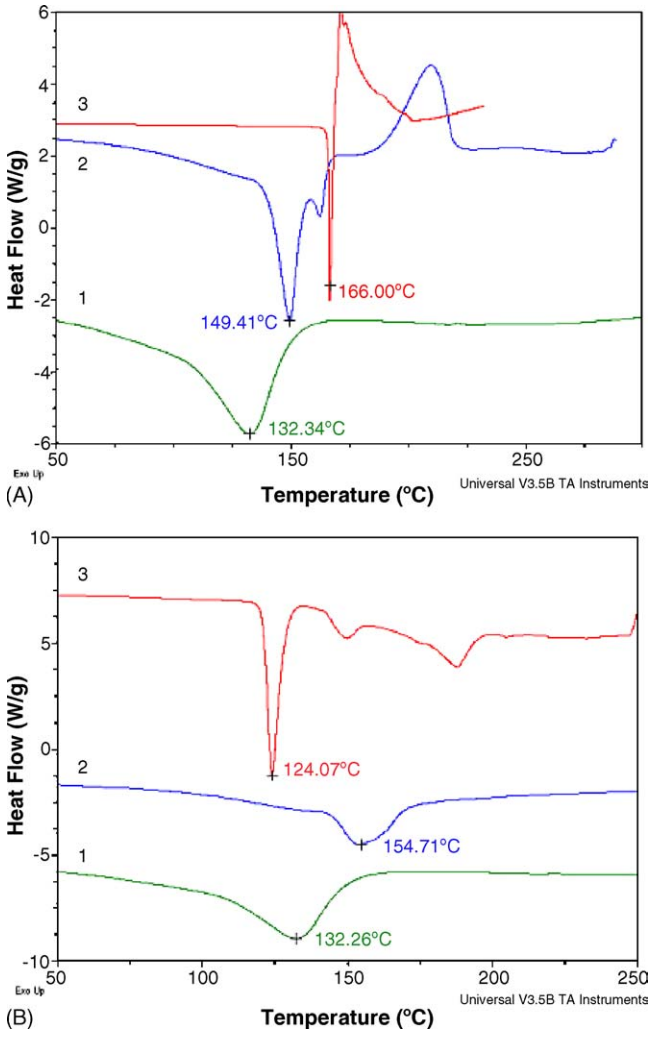

Fig. 2. DSC of econazole (A) and ciclopirox (B) with CD: 1, CD; 2, complex; 3, pure drug.

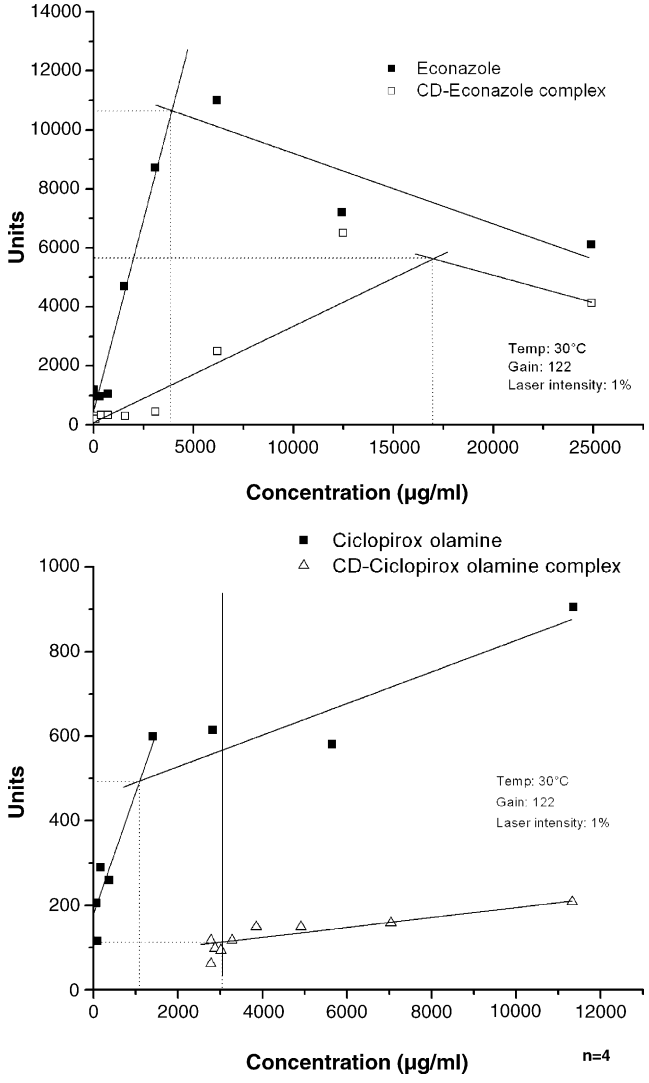

Fig. 3. Solubility diagrams of EC, CD–EC, CI and CD–CI measured by laser nephelometry.

- 2:1 CD–CI complex: $C_{98}H_{164}N_2O_{73}$
  - Calculated: C = 46.33%, H = 6.40%, N = 1.10%, O = 46.01%
  - Found: C = 46.66%, H = 6.17%, N = 0.56%, O = 46.62%

3.1.3. Differential scanning calorimetry (DSC)

CD–EC and CD–CI complexes were characterized by DSC to ensure that the inclusion complexes were formed. The obtained thermograms are shown in Fig. 2A and B. It can be seen (curves 1 in A and B) that the CD has a transition at 132 °C while as each of EC (curve 3 in A) and CI (curve 3 in B) have a transition at 166 and 124 °C, respectively, which were absent in both inclusion complexes (curves 2 in A and B). A small endothermic peak at 162 °C was recorded for CD–EC complex (curve 2 in A) which is due to fusion of very small amount of excess fine crystals of EC. The reduction of transition area of drug in that case assures the formation of the crystalline inclusion complex between CD and EC. This finding is in accordance with other related work (Ahmed et al., 1998). Another exothermic peak is also recorded for CD–EC (curve 2 in A) which is due to oxidation of uncomplexed traces of drug.

3.1.4. Phase solubility diagrams

Solubility diagrams were monitored using laser nephelometry which determine the solubility of potential drug candidates supplied as dimethyl sulfoxide (DMSO) solutions in 96-well plates (Bevan and Lloyd, 2000). The time required to determine the solubility of inclusion complexes varies widely from the usual period of 1–2 days to durations of 1–2 weeks (Connors, 1987). Lipinski et al. (2001) have published protocols for the determination of drug solubility by nephelometric and turbidimetric methods but the author claims quantitative performance at a constant percentage of DMSO cosolvent in static microtiter plate wells. In this study laser nephelometry overcame all problems of solubility. This system has been validated according to Bevan and Lloyd (2000) and Testa et al. (2001). All solubility measurements are carried out at 30 °C, gain 122 and a laser intensity of 1%. The concentration of DMSO co-solvent is constant at 5% so the solubility enhancement by a gradual increase

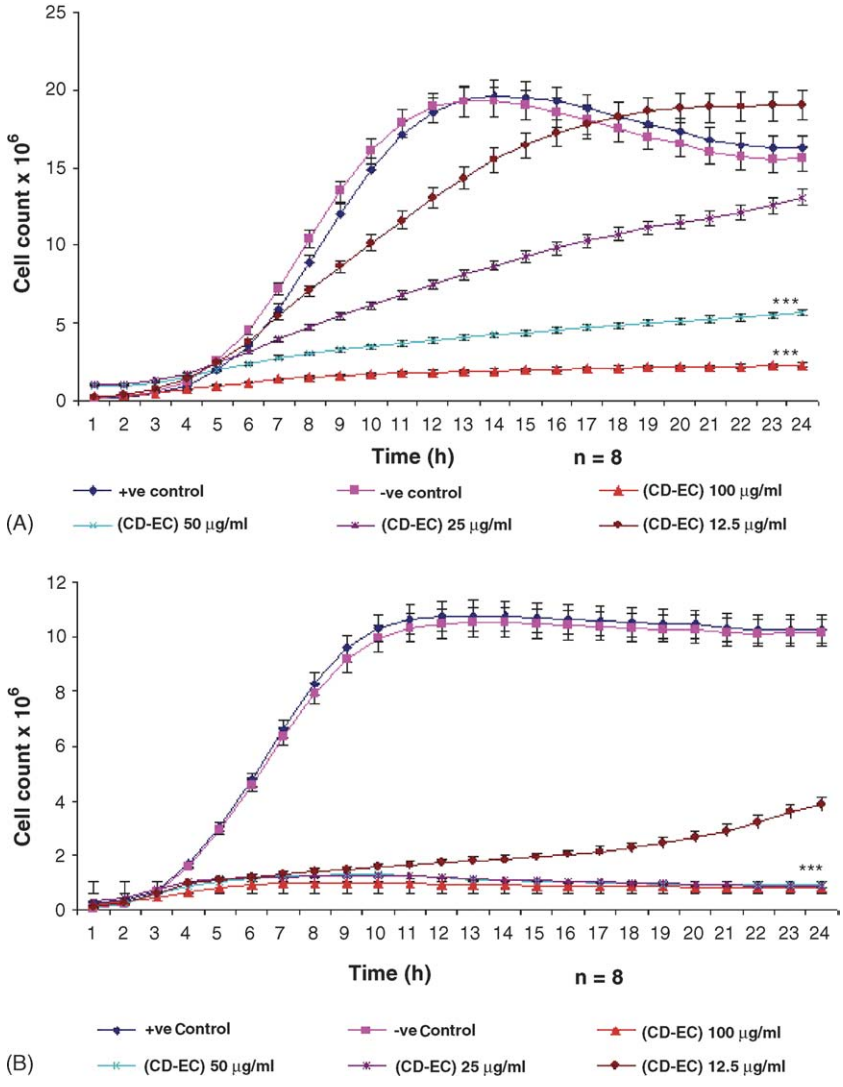

Fig. 4. Effect of CD–EC complex on the cell growth of: (A) *Candida albicans* DSM 11225; (B) *Candida krusei* ATCC 6258.

in DMSO is avoided. In the absence of CD, the solubility of EC and CI is determined to be ∼3.9 and ∼1.2 mg/ml, respectively, while in the presence of CD the solubility increased up to ∼16 mg/ml for CD–EC complex. Linear line was obtained for CD–CI complex and no point of precipitation is found which indicated complete solubility over the concentration ranges as described in Fig. 3.

3.2. Factors affecting the Candida species cell growth

All factors affecting the *Candida* species cell growth were studied and evaluated using laser nephelometry instrument. Given below are the results obtained along with appropriate discussion.

3.2.1. Influence of solvent

The influence of a mixture of chloroform/methanol 1:1 as negative control on the cell growth of both *Candida* species was evaluated to know whether this solvent mixture has an effect on *Candida* species or not. It was found clearly that for all concentrations used ranged between 2.5 and 0.0625%, no significant effect of this solvent on the cell growth of both organisms in comparison with control are found.

3.2.2. Influence of econazole nitrate

The results demonstrated that EC has a pronounced effect against *C. krusei* than *C. albicans*. With respect to *C. albicans* a concentration of 25 μg/ml of EC inhibited the cell growth while at 50 μg/ml cells were killed in comparison with control. On the other hand, using a concentration of 10 μg/ml of EC inhibited the cell growth of *C. krusei* while at 12.5 μg/ml cells were completely killed. Inhibition of the cell growth by EC can be explained either by metabolism of EC or by sequestration of this product by cell constituents like proteins or lipids (Cope, 1980). A preliminary study with ^{125}I iodated EC according to the Greenwood and Hunter method (Greenwood et al., 1963) showed penetration of this product into the cells.

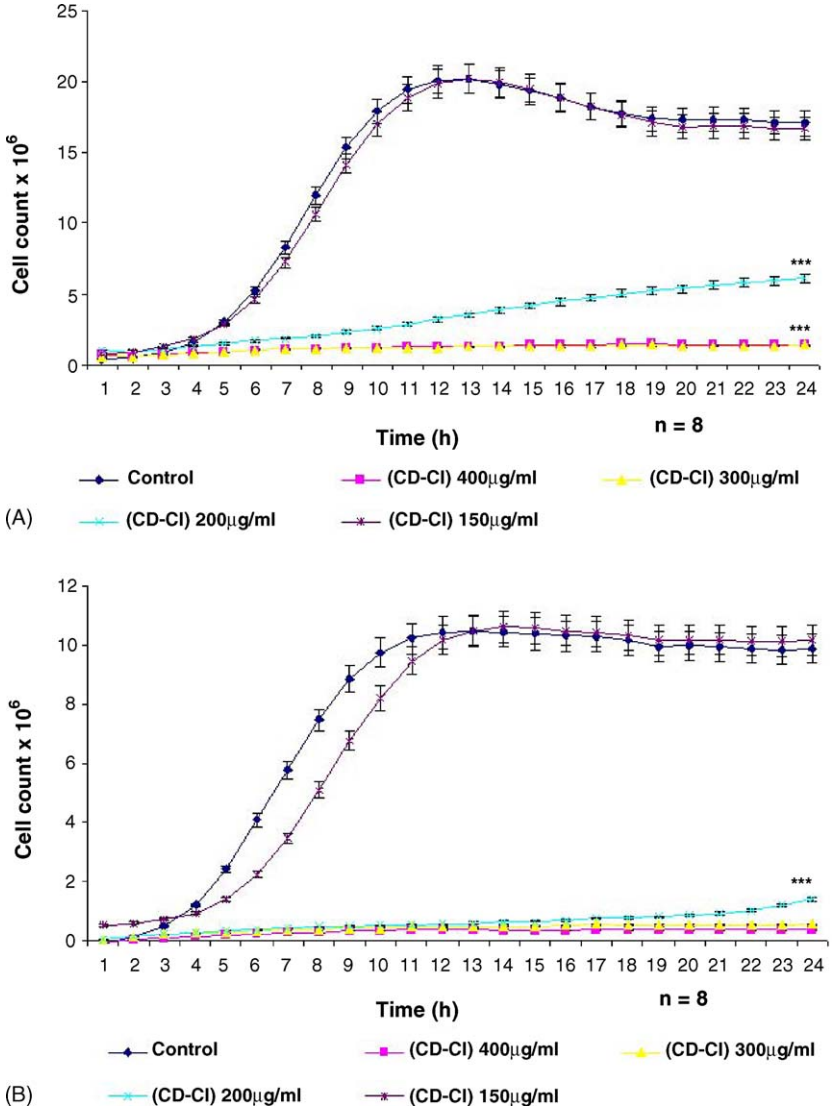

Fig. 5. Effect of CD–CI complex on the cell growth of: (A) *C. albicans* DSM 11225; (B) *C. krusei* ATCC 6258.

3.2.3. Influence of ciclopirox-olamine

Ciclopirox is effective against a broad spectrum of fungal organisms including dermatophytes, yeast and moulds acting in a fungistatic or fungicidal manner in vitro (Gupta, 2000; Jue et al., 1985; Korting and Grundmann-Kollmann, 1997). CI shows a greater in vitro activity towards *C. albicans* than other antimycotics tested (Abrams et al., 1991). CI has also shown in vitro activity against *Malassezia furfur* (del Palacio-Hernandez et al., 1990; Kokjohn et al., 2003). In our study CI was more effective against *C. albicans* at very low concentration (10 μg/ml) where all cells were killed while for other concentration ranges from 1.25 to 5 μg/ml the cells were more active. This finding was in agreement with the above previously mentioned results. For *C. krusei*, the drug was less effective than with other organism. At 12.5 μg/ml cells were killed and at other concentration less than 12.5 μg/ml cells were more active. CI was found to have the highest in vitro activity against both fungi and bacteria when compared to EC and butenafine HCl (Kokjohn et al., 2003).

3.2.4. Influence of β-CD–econazole complex

Econazole nitrate is an antimycotic drug used both locally and systematically. Improvement of solubility and release rate by complexation with CD is therefore essential for rapid antimycotic activity (Ahmed et al., 1998). Fig. 4A and B shows the same results obtained by EC. *C. albicans* was inhibited at a concentration of 25 μg/ml of CD–EC complex, while cells were killed at 50 μg/ml of the same complex in comparison with control. *C. krusei* was inhibited at a concentration of 12.5 μg/ml of the complex and killed at 25 μg/ml. By using laser nephelometry in microtiter plates as a new method, the antimycotic influence was studied every hour until 24 h. In all growth phases, especially the logarithmic and stationary phases, the results obtained were significant and reproducible for the concentration ranges used

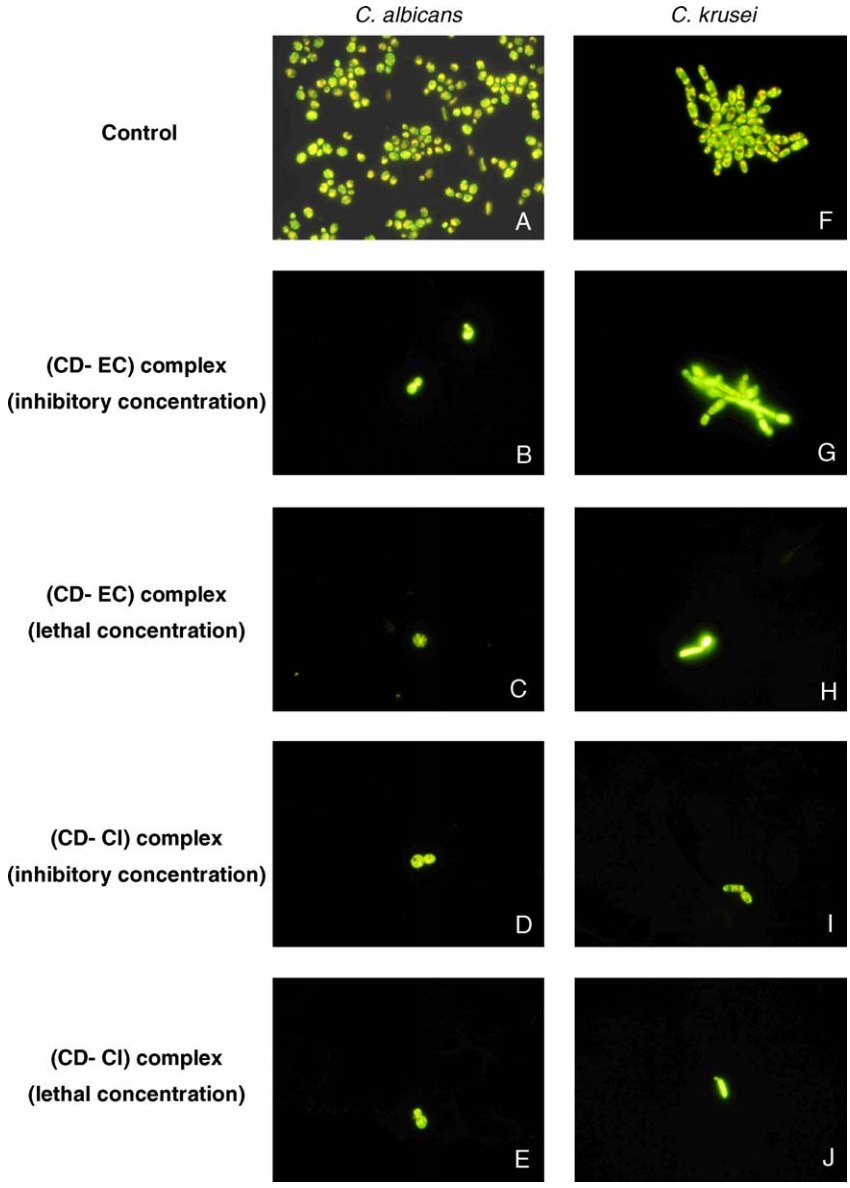

Fig. 6. Metabolic activity of both complexes (inhibitory/lethal concentration) against *C. albicans* and *C. krusei*; stained with FUN-1 dye.

either for drug alone with the challenge organism or with CD complexes. In other study the antimycotic effect of the saturated drug solutions containing various cyclodextrin concentrations was estimated on the basis of inhibition zone size (Ahmed et al., 1998; Pedersen et al., 1993). The largest inhibition zone of growth of *C. albicans* was obtained in case of inclusion complex of the drug with CD. This finding was attributed to the higher dissolution rate of this inclusion complex as compared to the drug alone in correlation with the solubility data, and these reflect the higher antimycotic activity by rapid diffusion through agar medium. These finding results were in accordance with the results in this study.

3.2.5. Influence of β-CD–ciclopirox complex

The efficiency of complexation may sometimes be rather low, and therefore relative large amounts of CD must be used to complex small amounts of drug (Loftsson and Brewster, 1996; Loftsson et al., 1999). The particle size of solid CD complexes can affect the dissolution rate and therefore the bioavailability of the product. The particle size distribution and crystalline properties of the complex are dependent on the method of complex preparation (Barber et al., 1998). In this study, CD–CI complex was less effective against *C. albicans* than other complex. At concentration of 200 μg/ml of the complex, cells were inhibited but at 300 μg/ml of the same complex cells were killed as described in Fig. 5A and B. As for *C. krusei* the complex was more effective. At a concentration of 200 μg/ml cells were killed and for other concentration less than 200 μg/ml cells were active.

3.3. Metabolic activity evaluation by vital staining with FUN-1

A new family of fluorescent probes has been developed for assessing the viability and metabolic activity of fungi. This class of dyes is exemplified by the FUN-1 [2-chloro-4-(2,3-dihydro-3-methyl-(benzo-1,3-thiazol-2-yl)-methylidene)-1-phenyl-quinolinium iodide] stain. A membrane-permeant nucleic acid-binding dye that has been found to give rise to cylindrical intravascular structures (CIVS) in fungi (Millard et al., 1997). Biochemical processing of the dye by active cells yielded CIVS that were markedly red shifted in fluorescence emission and therefore spectrally distinct from the nucleic acid-bound form of the dye. FUN-1 stain is a halogenated unsymmetrical cyanine compound with one fixed positive charge which is virtually not fluorescent in aqueous solution. However, after complexation with DNA or RNA and excited with light between 470 and 500 nm, FUN-1 stain fluorescence at 530 nm increased as much as 400-fold (Roth et al., 1995). The antimycotic influence of CD–drug complexes cannot be evaluated without consideration of the morphology of *Candida* species (Belanger et al., 1997). Also FUN-1 staining helps to characterize azole resistant strains (Kuhn et al., 2002; Pina-Vaz et al., 2001). In this study the metabolic activity of the cells was demonstrated by vital staining with FUN-1 resulting in red fluorescent with (CIVS) structures in both *Candida* species in the controls, which indicated the activity and viability of these cells. But under the influence of CD–EC and CD–CI complexes, cells were green or pale yellow fluorescence. That means, most of the cells lost their metabolic activity. Different images for cells stained with FUN-1 affected by each complex in different inhibitory and lethal concentrations were described in Fig. 6.

4. Conclusion

This study has proven that laser nephelometry in a 96-well microtiter plate can be used as a novel method for the rapid determination of the solubility of potential drug compounds. Laser nephelometry can distinguish between the concentration at which the drug just goes into or just comes out of solution. On the other hand, this technique can be used efficiently for monitoring and evaluating the growth of microorganisms like fungi or bacteria.

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References

Abrams, B.B., Hanel, H., Hoehler, T., 1991. Ciclopirox olamine: a hydroxypyridone antifungal agent. Clin. Dermatol. 9, 471–477.

Ahmed, M.O., El-Gibaly, I., Ahmed, S.M., 1998. Effect of cyclodextrins on the physicochemical properties and antimycotic activity of clotrimazole. Int. J. Pharm. 171, 111–121.

Barber, D., Keuter, J., Kravig, K., 1998. A logical stepwise approach to laser diffraction particle size distribution analysis methods development and validation. Pharm. Dev. Technol. 3, 153–161.

Belanger, P., Nast, C.C., Fratti, R., Sanati, H., Ghannoum, M., 1997. Voriconazole (UK-109, 496) inhibits the growth and alters the morphology of fluconazole-susceptible and -resistant *Candida* species. Antimicrob. Agents Chemother. 41, 1840–1842.

Bevan, C.D., Lloyd, R.S., 2000. A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry in microtiter plates. Anal. Chem. 72, 1781–1787.

Bononi, L.J., 1988. β-Cyclodextrin complexes having anti-mycotic activity. European Patent Application 288.019.

Buschmann, H.J., Knittel, D., Jonas, C., Schollmeyer, E., 2001. Einsatzmöglichkeiten von Cyclodextrinen im Lebensmittelbereich. Eine kurze Übersicht. Lebensmittelchemie 55, 53–84.

Connors, K.A., 1987. Binding Constants: The Measurement of Molecular Complex Stability. Wiley, New York.

Cope, J.E., 1980. Mode of action of miconazole on *Candida albicans*: effect on growth, viability and K+ release. J. Gen. Microbiol. 119, 245–251.

del Palacio-Hernandez, A., Guarro-Artigas, J., Figueras-Salvat, M.J., 1990. Changes in fungal ultrastructure after short-course ciclopiroxolamine therapy in pityriasis versicolor. Clin. Exp. Dermatol. 15, 95–100.

Duchêne, D., Glomot, F., Vaution, C., 1987. Pharmaceutical applications of cyclodextrins. In: Duchêne, D. (Ed.), Cyclodextrins and their Industrial Uses. Editions De Santé, Paris, Cap. 9.

Greenwood, F.C., Hunter, W.M., Glover, J.S., 1963. The preparation of I-131-labelled human growth hormone of high specific radioactivity. Biochem. J. 89, 114–123.

Gupta, A.K, 2000. Ciclopirox: an overview. Int. J. Dermatol. 40, 1–7.
Heel, R.C., Brogden, R.N., Speight, T.M., Avery, G.S., 1978. Econazole: a review of its antifungal activity and therapeutic efficacy. Drugs 16, 177–201.
Jue, S.G., Dawson, G.W., Brogden, R.N., 1985. Ciclopirox olamine 1% cream. A preliminary review of its antimicrobial activity and therapeutic use. Drugs 29, 330–341.
Kokjohn, K., Bradley, M., Griffiths, B., Ghannoum, M., 2003. Evaluation of in vitro activity of ciclopirox olamine, butenafine HCl and econazole nitrate against dermatophytes, yeasts and bacteria. Int. J. Dermatol. 42, 11–17.
Korting, H.C., Grundmann-Kollmann, M., 1997. The hydroxypyridones: a class of antimycotics of its own. Mycoses 40, 243–247.
Kuhn, D.M., Chandra, J., Mukherjee, P.K., Ghannoum, M.A., 2002. Comparison of biofilms formed by *Candida albicans* and *Candida parapsilosis* on bioprosthetic surfaces. Infect. Immun. 70, 878–888.
Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 46, 3–26.
Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.
Loftsson, T., Masson, M., Sigurjonsdottir, J.F., 1999. Methods to enhance the complexation efficiency of cyclodextrins. S.T.P. Pharm. Sci. 9, 237–242.
Millard, P.J., Roth, B.L., Thi, H.P., Yue, S.T., Haugland, R.P., 1997. Development of the FUN-1 family of fluorescent probes for vacuole labeling and viability testing of yeasts. Appl. Environ. Microbiol. 63, 2897–2905.
Mura, P., Liguori, A., Bramanti, G., Campisi, E., Faggi, E., 1992. Improvement of dissolution properties and microbiological activity of miconazole and econazole by cyclodextrin complexation. Eur. J. Pharm. Biopharm. 3, 119–123.
Pedersen, M., Edelsten, M., Nielsen, V.F., Scarpellini, A., Skytte, S., Slot, C., 1993. Formation and antimycotic effect of cyclodextrin inclusion complexes of econazole and miconazole. Int. J. Pharm. 90, 247–254.
Pina-Vaz, C., Sansonetty, F., Rodrigues, A.G., Costa-de-Oliveira, S., Martinez-de-Oliveira, J., Fonseca, A.F., 2001. Susceptibility to fluconazole of *Candida* clinical isolates determined by FUN-1 staining with flow cytometry and epifluorescence microscopy. J. Med. Microbiol. 50, 375–382.
Roth, B.L., Millard, P.J., Yue, S.T., Haugland, R.P., 1995. Intravacuolar stains for yeast and other fungi. U.S. Patent 5,445,946.
Sawyer, P.R., Brogden, R.N., Pinder, R.M., Speight, T.M., Avery, G.S., 1975. Miconazole: a review of its antifungal activity and therapeutic efficacy. Drugs 9, 406–423.
Szejtli, J., 1988. Cyclodextrin Technology. Kluwer, Dordrecht.
Testa, B., Van de Waterbeemd, H., Folkers, G., Guys, R., 2001. Pharmacokinetic Optimization in Drug Research. Verlag Helvetica Chimica Acta, Zürich, pp. 257–273.
Uekama, K., Irie, T., 1987. Pharmaceutical applications of methylated cyclodextrin derivatives. In: Duchêne, D. (Ed.), Cyclodextrins and their Industrial Uses. Editions De Santé, Paris, p. 393.