

Nephelometric Monitoring Growth of *Candida albicans* Using BMG LABTECH's NEPHELOstar



- Laser nephelometry used for fungal growth determination and for measurement of drug solubility
- Monitoring the growth of *Candida albicans* in presence of antifungal agents
- Phase solubility diagrams of potential drug presented

Introduction

Cyclodextrins (CD) have become useful pharmaceutical excipients, due to their potential to form inclusion complexes with appropriately sized drug molecules¹. 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.².

Econazole-nitrate (EC) and ciclopirox-olamine (CI) are well known antifungal agents suitable for the treatment of many mycotic infections. 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-CD³⁻⁵. By increasing the water solubility of the drug it should be possible to improve its bioavailability, thus enabling improved oral or topical formulations.

Laser nephelometry has been shown to be a reliable technique for the measurement of drug solubility in 96-well plate format^{6,7}. 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 magnitude⁶.

Herein we describe the use of laser nephelometry to investigate the effects of complexation on the drug antimycotic activity using BMG LABTECH's NEPHELOstar (Figure 1). Furthermore nephelometry was used to prepare phase solubility diagrams and to monitor fungal growth.



Fig. 1: BMG LABTECH's laser-based NEPHELOstar

Materials and Methods

- *Candida albicans* DSM 11225
- Sterile and clear 96-well plates, Greiner bio-one, Frickenhausen, Germany
- β-Cyclodextrin, Wacker-Chemie, Burghausen, Germany
- NEPHELOstar, BMG LABTECH, Offenburg, München, Germany

Preparation of cultures

C. albicans were grown on SGA (Sabouraud-Glucose-Agar with gentamycin-chloramphenicol from bioMerieux, Germany) 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 (Sabouraud-Glucose-Bouillon from Oxoid Ltd, UK).

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.

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.⁸ 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, 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.

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 measured on the NEPHELOstar 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 LABTECH NEPHELOstar Evaluation software. The scattered light will remain at a constant intensity until precipitation occurs. At that point it will increase sharply.

Results and Discussion

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 (Figure 2).

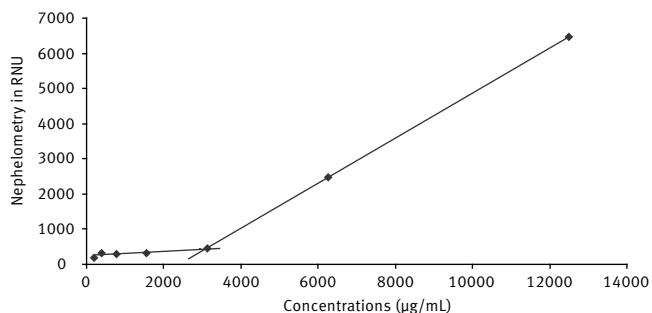


Fig. 2: Solubility diagram of CD-Econazole-nitrate complex

In the absence of CD, the solubility of EC is determined to be ~1.3 mg/mL (graph not shown), while in the presence of CD the solubility increased up to ~3.1 mg/mL for CD-EC complex.

The solubility results for ciclopirox-olamine (CI) differ from the other antifungal agent.

In the absence of cyclodextrin, the solubility of CI is determined to be ~1.2 mg/mL (graph not shown). Linear line was obtained for CD-CI complex and no point of precipitation is found which indicated complete solubility over the concentration ranges (Figure 3).

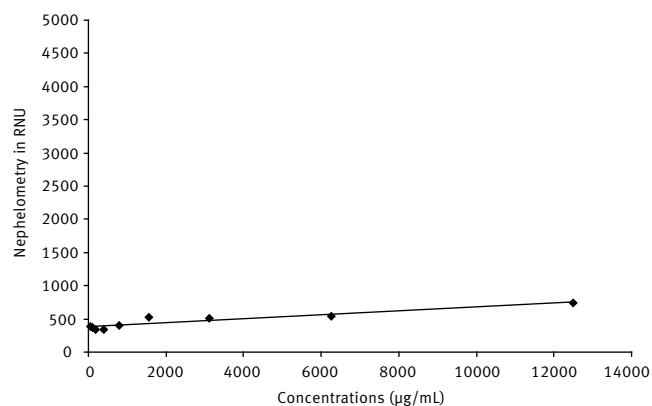


Fig. 3: Solubility diagram of CD-Ciclopirox-olamine complex

Influence of complexed antifungal agents on the growth of *Candida albicans*

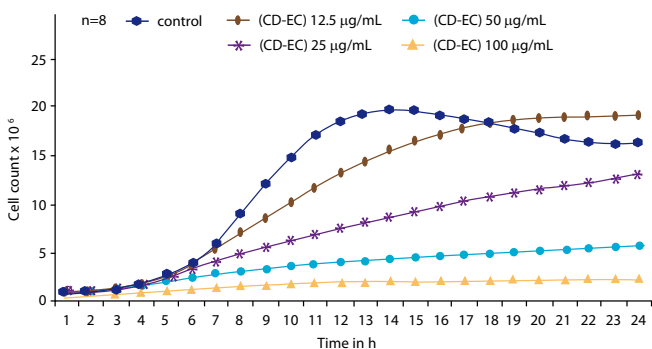


Fig. 4: Influence of econazole nitrate complex (CD-EC) on the growth of *Candida albicans*

Figure 4 shows, that a concentration of 25 µg/mL of CD-EC inhibits the cell growth while at 50 µg/mL cells are killed in comparison with the control. These values obtained with the complex is comparable to the findings for the free EC.

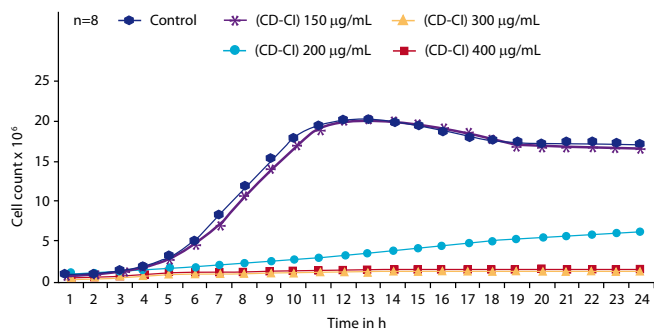


Fig. 5: Influence of ciclopirox complex (CD-CI) on the growth of *Candida albicans*

In contrary to CD-EC the complexed ciclopirox was less effective (Figure 5). At a concentration of 200 µg/mL of the complex, cells were inhibited and at a concentration of 300 µg/mL of the complex, cells were killed. The CD-CI complex was also less effective compared to the free CI that caused cell death already at 10 µg/mL.

Conclusion

This study has proven that laser nephelometry in a 96-well microtiter plate can be used as a 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.

References

- 1) Szejtli, J. (1988) Cyclodextrin Technology. Kluwer, Dordrecht
- 2) Duchene, D., Glomot, F., Vaution, C. (1987) Pharmaceutical applications of cyclodextrins. In: Duchene, D. (Ed.), Cyclodextrins and their Industrial Uses. Editions De Sant'e, Paris, **Cap. 9**.
- 3) Bononi, L.J. (1988) β -Cyclodextrin complexes having anti-mycotic activity. European Patent Application 288.019.
- 4) Mura, P., Liguori, A., Bramanti, G., Campisi, E., Faggi, E. (1992) *Eur. J. Pharm. Biopharm.* **3**, 119–123.
- 5) Pedersen, M., Edelsten, M., Nielsen, V.F., Scarpellini, A., Skytte, S., Slot, C. (1993) *Int. J. Pharm.* **90**, 247–254.
- 6) Bevan, C.D., Lloyd, R.S. (2000) *Anal. Chem.* **72**, 1781–1787.
- 7) Fouda, M.M.G., Knittel, D., Hipler, U.-C., Elsner, P., Schollmeyer, E. (2006) *Int. J. Pharm.* **311**, 113–212.
- 8) Buschmann, H.J., Knittel, D., Jonas, C., Schollmeyer, E. (2001) *Lebensmittelchemie* **55**, 53–84.

Germany: **BMG LABTECH GmbH** Tel: **+49 781 96968-0**

Australia: BMG LABTECH Pty. Ltd. Tel: +61 3 59734744
 France: BMG LABTECH SARL Tel: +33 1 48 86 20 20
 Japan: BMG LABTECH JAPAN Ltd. Tel: +81 48 647 7217
 UK: BMG LABTECH Ltd. Tel: +44 1296 336650
 USA: BMG LABTECH Inc. Tel: +1 919 806 1735

Internet: **www.bmglabtech.com** info@bmglabtech.com