

**IMPROVED PROPERTIES OF NATAMYCIN UPON FORMATION OF  
CYCLODEXTRIN INCLUSION COMPLEXES**

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## ABSTRACT

Natamycin is an antimycotic with very low water solubility and extremely high photosensitivity, which is used to extend the shelf life of shredded cheese products. The objectives of this research are: (a) to find a new delivery system for natamycin, which increases its aqueous solubility and (b) to increase the chemical stability of natamycin so that it has a prolonged antifungal effect on the surface of the shredded cheese.

Molecular inclusion complexes of natamycin were formed with  $\beta$ -, hydroxypropyl  $\beta$ -, and  $\gamma$ -cyclodextrins (CDs) which allowed large increases in aqueous solubility without the use of organic co-solvents or surfactants. The water solubility of natamycin was increased 16-fold, 73-fold, and 152-fold with  $\beta$ -CD,  $\gamma$ -CD, and hydroxypropyl  $\beta$ -CD, respectively. The natamycin:CD inclusion complexes resulted in nearly equivalent *in vitro* antifungal activity as natamycin in its free state. Nuclear magnetic resonance (NMR) was utilized to prove the formation of true inclusion complexes.  $^1\text{H}$  NMR shift titrations of *N*-(3'-*N*-dimethylaminosuccimido) natamycin with  $\beta$ - and  $\gamma$ -CDs enabled determination of the stoichiometry of both complexes as 1:1. Aqueous solutions of natamycin (20 mg/L) were found by quantitative HPLC to be completely degraded after 24 hours of exposure to 1000 lux fluorescent lighting at 4 °C. After 14 days of storage in darkness at 4 °C, 92.2% of natamycin remained in active form. Aqueous solutions of natamycin: $\beta$ -CD complex and natamycin: $\gamma$ -CD complex were significantly more stable ( $p < 0.05$ ) than natamycin in its free state when stored in darkness at 4 °C. Clear poly(ethylene terephthalate) packaging with an ultraviolet light absorber allowed 85.0% natamycin to remain after 14 days of storage under 1000 lux fluorescent lighting at 4 °C. Such dramatic increases in water solubility and light stability will enable natamycin to function as a more effective antimycotic in the food industry.

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## LIST OF ABBREVIATIONS AND TERMS

$^1\text{H NMR}$	Proton Nuclear Magnetic Resonance
CAS	Chemical Abstract Service
CD	cyclodextrin
CFR	Code of Federal Regulations
COSY	Correlation Spectroscopy
DMPS	dimethylpolysiloxane
DMSO	dimethyl sulfoxide
DS	degree of substitution
FAO	Food and Agriculture Organization of the United Nations
GRAS	Generally Recognized as Safe
HP $\beta$ -CD	hydroxypropyl $\beta$ -cyclodextrin
IDF	International Dairy Federation
IUPAC	International Union of Pure and Applied Chemistry
$K_{mn}$	stepwise binding constant
L	ligand
$L_t$	total ligand concentration
LD <sub>50</sub>	lethal dose 50%
$m$	stoichiometric coefficient of S
MIC	minimum inhibitory concentration
NF	National Formulary
NMR	Nuclear Magnetic Resonance
$n$	stoichiometric coefficient of L
PDA	photodiode array
PE	poly(ethylene)
PET	poly(ethylene terephthalate)
PVA	poly(vinyl acetate)
S	substrate
$S_t$	total substrate concentration
$s_0$	equilibrium solubility of S in the absence of L (intrinsic solubility)

$T_d$	decomposition temperature
TGA	Thermogravimetric Analysis
TMS	tetramethylsilane
TSP	3-(trimethylsilyl) propionate sodium salt
USP	United States Pharmacopeia
UV	ultraviolet
WHO	World Health Organization
$\alpha$ -CD	$\alpha$ -cyclodextrin
$\beta$ -CD	$\beta$ -cyclodextrin
$\beta_{mn}$	overall binding constant
$\delta$	NMR chemical shift parameter (ppm)
$\delta_0$	NMR chemical shift in the free state
$\delta_c$	NMR chemical shift in the complexed state
$\Delta\delta_{obs}$	observed NMR chemical shift difference
$\gamma$ -CD	$\gamma$ -cyclodextrin