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# 2,6-Dimethyl betacyclodextrin (DIMEB) in *Biotechnology*



### What are cyclodextrins (CDs)?

• Composed of sugar units

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- Cyclic, doughnut-shaped molecules
- Naturally occurring compounds produced from plants (no animal origin!)
- Used in food, pharmaceuticals, drug delivery, chemical industries, agriculture, etc.



#### **Reversible inclusion complex formation**

#### **Structure and properties of DIMEB**

# Heptakis(2,6-di-O-methyl) beta-cyclodextrin Degree of substitution: 14



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Insoluble in hot water Highly soluble in cold water

**High solubilizing effect:** solubilization of cholesterol extraction of cholesterol from lipid bilayers

Unique potential applications in various fields of biotechnology

Available as pure or DIMEB "enriched" (~40%) material

#### **Composition: DIMEB is NOT RAMEB!**

### HPLC chromatograms of BCD and its methylated derivatives

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RAMEB is a mixture of randomly methylated BCDs with 12-14 methoxy groups in average at random positions;

DIMEB is a single isomer with exactly 14 methoxy groups at positions C2 and C6;

TRIMEB (trimethyl BCD) is a fully methylated single isomer containing 21 methoxy groups in a molecule

> Fenyvesi, É., Szemán, J., Csabai, K., Malanga, M., Szente, L. J. Pharm. Sci. 2014, 103, 1443



- DIMEB has a well-defined structure and consists of a single component

- It can enhance the solubility of complexed substrates (substitute detergents and co-solvents)
- At low concentration it does not damage the microbial cells or the enzymes
- It can intensify the enzymatic conversion of lipophilic substrates
- It can improve the yield of product-inhibited fermentations
- Organic toxic compounds are tolerated by microbes in higher concentrations
- DIMEB complexes can substitute for mammalian serum in tissue cultures

- Unstable and/or insoluble proteins can be dissolved and stabilized in aqueous solution

Why use DIMEB?

# The Cyclodextrin Comparison Appl. 1: Microbiological transformation



CH<sub>3</sub>

 $CH_3$ 

CH<sub>3</sub>

CH<sub>2</sub>

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H₃C Ḫ CH<sub>3</sub> H<sub>3</sub>C н Ĥ Ĥ

 $\bar{C}H_3$ 



**Rapid formation of** ۲ cholesterol complex

 $CH_3$ 

- **Solubilization**
- **Enhanced conversion** rate
- **Decreased product** ٠ inhibition
- **Improved product** stability

Jadoun, J., Bar, R.: Appl. Microbiol. Biotechn. 1993, 40, 477

Effect of DIMEB concentration on the conversion rate

# The Cyclodextrin Company Appl. 2: Biosynthesis by fermentation



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Sacharomyces cerevisiae



Solubility of androstenedione in water at 30°C

- Enhanced production
- Diminished by-products formation
- Decreased product inhibition

Singer, Y., Shity, H., Bar, R. Appl. Microbiol. Biotechnol. 1991, 35, 731-737

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Palmitic acid

Complexation of fatty acids (growth	Bordetella pertussis cell growth					
	DIMEB	Y	β	α	0	Inoculum size cells in 5 µL
inhibitors)	++	-	-	-	-	10 <sup>3</sup>
results in	+++	-	-	-	-	104
i courto in	+++	-	-	-	-	10 <sup>5</sup>
enhanced cell	+++	+	+	++	-	10 <sup>6</sup>
_ growth and	+++	++	++	+++	-	10 <sup>7</sup>
toxin product			+++ full growth	++ 10 <sup>2</sup> to 10 <sup>3</sup> colonies	+ < 100 colonies	- no growth
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#### DIMEB increases pertussis toxin production 100-fold!

Imaizumi et al.: Infect. Immun. 1983, 41, 1138-1143



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# Appl. 4: Serum free culture media



Mycobacterium leprae

Water-soluble lipid/DIMEB complexes: Cultivation of non-cultivable *Mycobacterium leprae*; Serum substitutes for lymphoblast cells;



Lymphoblast cells

Solubilization of lipids (fatty acids, cholesterol, phospholipids)
No threat of prion proteins

Szente *et al.*: J. Incl. Phenom. Mol. Recogn. Chem. 1993, 16, 339-354



- Improvement of the quality of semen by cholesterol supplementation with cholesterol loaded DIMEB (cryopreservation)
- Enhancement of capacitation and fertility rate by preincubation of thawed sperms with DIMEB





CycloLab is the world's only all-around Cyclodextrin Service Provider

**Our services include:** 

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- Supplying DIMEB for commercial products and product development;
- DIMEB also available under cGMP with all supporting documentation
- Upscalable technology to support commercial needs
- Technical support on the proper use of the material
- Extensive knowledge on the analysis of both DIMEB and matrices/products containing DIMEB
- Providing formulation development services, composition optimization, stability assessment;
- Offering analytical services to characterize complexes and products;
- Assisting in compilation of regulatory documentation.



# **Company contacts:**

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