CYCLOLAB

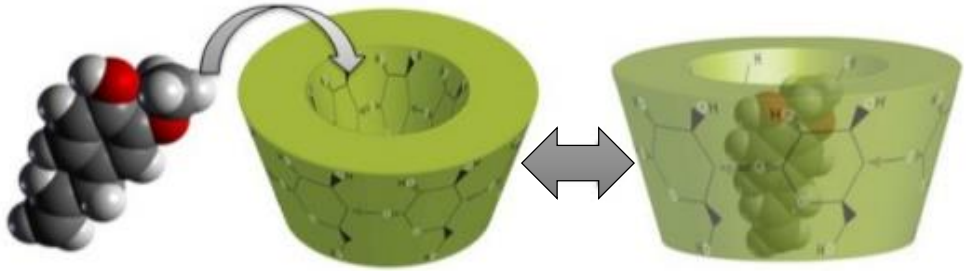
PIONEERS IN CYCLODEXTRIN-BASED INNOVATION

GETTING THE BEST OUT OF CYCLODEXTRINS

Heptakis(2,6-di-*O*-methyl)-beta-cyclodextrin (DIMEB) in Biotechnology

WHAT ARE CYCLODEXTRINS?

- Composed of sugars
- Cyclic molecules
- Naturally occurring compounds
- Used in food, pharmaceuticals, drug delivery, chemical industries, agriculture, etc.
- **Sub-nanometer** sized molecular containers with hydrophilic outer phase and hydrophobic interior properties
- Reversible inclusion complex formation



CYCLO AB

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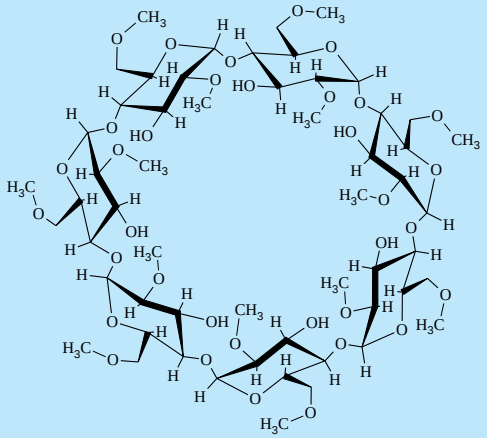
Structure and properties of DIMEB

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

Insoluble in hot water

Highly soluble in cold water



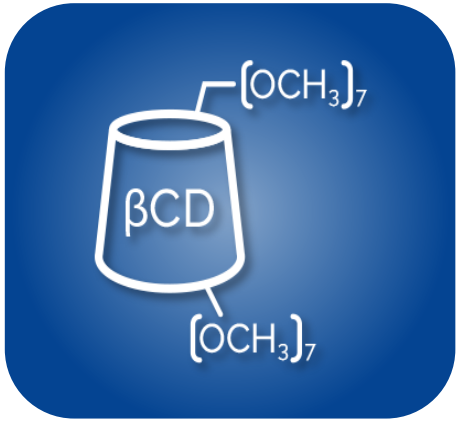
High solubilizing effect:

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



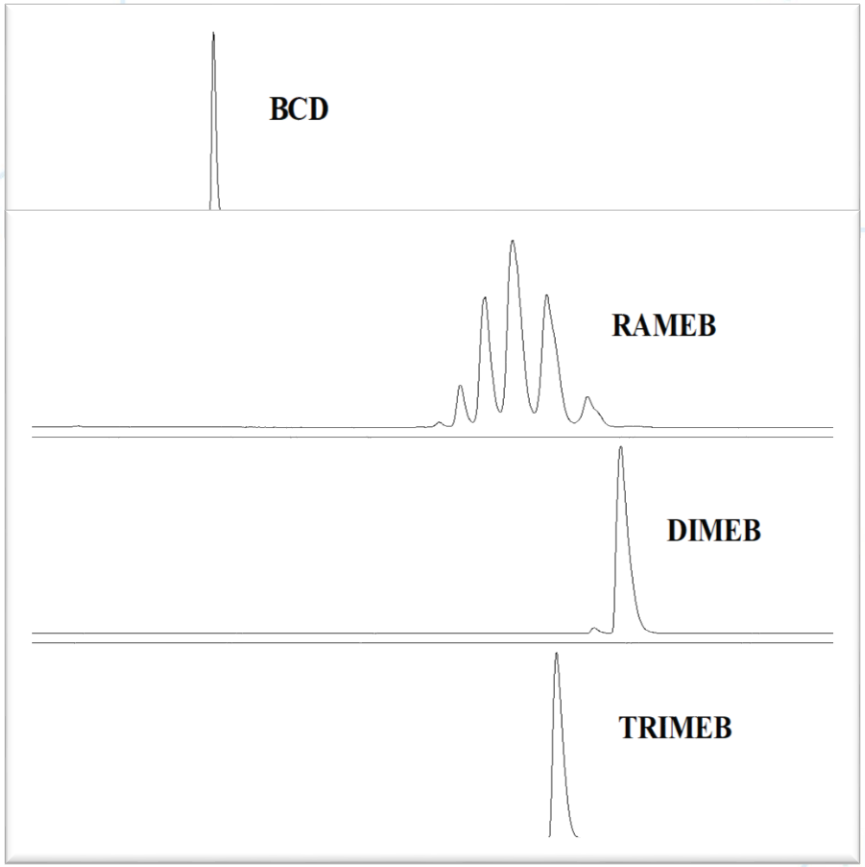
Composition: DIMEB is NOT RAMEB!

HPLC chromatograms of BCD and its methylated derivatives

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



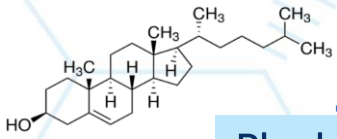
Fenyvesi, É., Szemán, J., Csabai, K., Malanga, M., Szente, L.
J. Pharm. Sci. 2014, 103, 1443

Why use DIMEB?

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

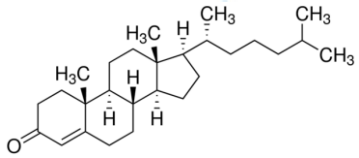
Application 1: microbiological transformation



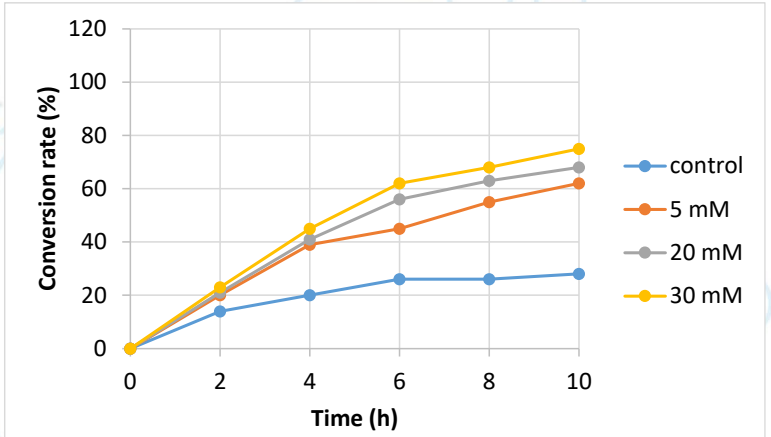
Cholesterol

oxidation

Rhodococcus erythropolis



Cholest-4-en-3-one



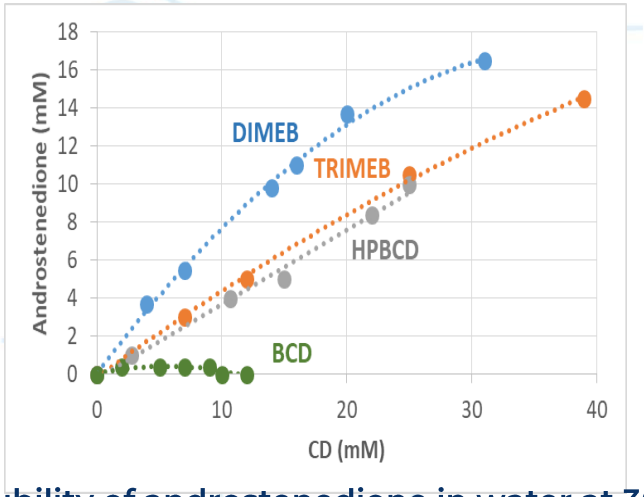
Effect of DIMEB concentration on the conversion rate

- Rapid formation of cholesterol complex
- Solubilization
- Enhanced conversion rate
- Decreased product inhibition
- Improved product stability

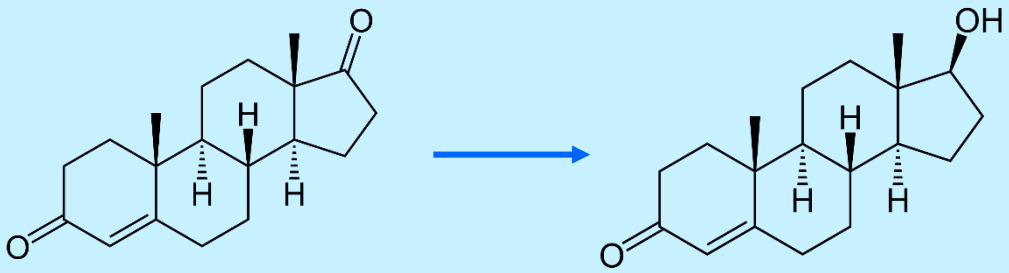
Jadoun, J., Bar, R.: Appl. Microbiol. Biotechn. 1993, 40, 477

Application 2: biosynthesis by fermentation

Sacharomyces cerevisiae



Solubility of androstenedione in water at 30°C



Androstenedione → Testosterone

- 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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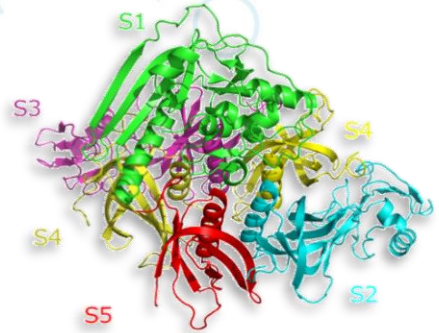
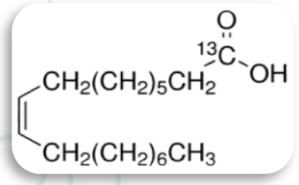
Application 3: vaccine production



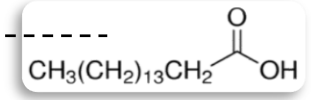
Production

Inhibition

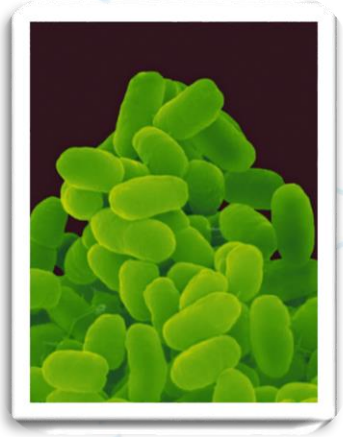
Pertussis toxin



Oleic acid



Palmitic acid



Bordetella pertussis

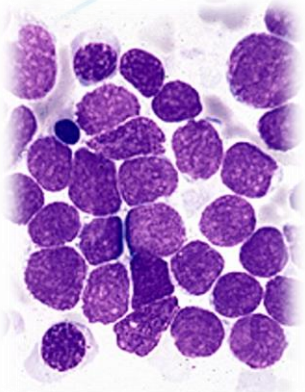
Inoculum size cells in 5 µL	0	α	β	γ	DIMEB
10^3	-	-	-	-	++
10^4	-	-	-	-	+++
10^5	-	-	-	-	+++
10^6	-	++	+	+	+++
10^7	-	+++	++	++	+++
- no growth	+ < 100 colonies	++ 10^2 to 10^3 colonies	+++ full growth		

Complexation of fatty acids (growth inhibitors) results in enhanced cell growth and toxin production

DIMEB increases pertussis toxoid production 100-fold

Application 4: serum-free culture media

Mycobacterium leprae



Lymphoblast cells

Szente et al.: J. Incl. Phenom. Mol. Recogn. Chem. 1993, 16, 339-354
Rajnavölgyi et al. Beilstein J. Org. Chem. 2014, 10, 3152–3160

Water-soluble lipid/CD complexes

Cultivation of non-cultivable Mycobacterium leprae

Serum substitutes for lymphoblast cells

Non-cholesterol interacting fatty acid/CD complexes

Solubilization of lipids (fatty acids, cholesterol, phospholipids)

No threat of prion proteins

Application 5: artificial fertilization

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

Who we are and what we offer?

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

Our services include:

- 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

WHO ARE WE AT CYCLOLAB?

The world's only all-round CYCLODEXTRIN company with over

40-year experience of CD-technology

in pharmaceutical-, cosmetics-, food-, environmental- and analytical applications

Experience

Over 490 technical/scientific papers
and 950 technical reports to customers

200 different cyclodextrin derivatives
130 patents/applications
40 products on the market

Drug Master Files (USA type IV) and eCTD

Over 20,000 citations to CYCLOLAB's papers

Expertise & Technology

Custom synthesis

Drug solubilization and stabilization

Further industrial applications

Cyclodextrin-related analytics

Stability testing

GMP-conform manufacturing

Feasibility studies

DEXOLVE™

CYCLOLAB SERVICE PORTFOLIO
RELATED SERVICES – R&D

Early phase drug development

Customization of CD enabled formulations

Investigation of changes in physico-chemical properties

Life cycle management

Custom cyclodextrin synthesis

Exclusive manufacture, unique synthetic routes

Self-tailored products and characteristics

In vitro bioequivalence studies

Design and performance of in vitro studies to support bioequivalence of a CD enabled formulation

Analytical services

Method development, validation; cGMP release testing of pharma grade CDs

HPLC, GC, CE, UV, MS, NMR, IR, Micro and BET content methods

Stability studies

CD-guest interaction studies

CD-based chiral separations

Assay, impurity tests

Bioanalytical investigations

IP services and consultation

30 years of experience in compilation of CD related patents (synthesis, application, etc.), patent claim analysis, consultancy in CD related projects

Over 62.000 CD related papers

DEXOLVE™

CYCLOLAB SERVICE PORTFOLIO
RELATED SERVICES – R&D

Feasibility study

Running a short feasibility study with your molecule free of charge

Proof of concept to consider CD based formulations

CycloLab Grant

CycloLab offers a unique possibility to collaborate on creating novel and interesting cyclodextrins under the terms of the CycloLab Grant

The proposal after application is thoroughly evaluated by CycloLab

If the application is approved, the cyclodextrin is provided free of charge for the beneficiary

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