

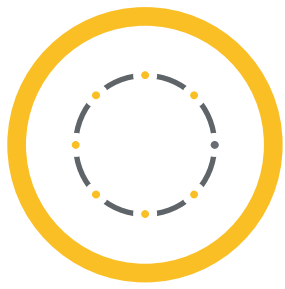


CARBOHYDE
SUGAR IS LIFE



Cyclodextrins

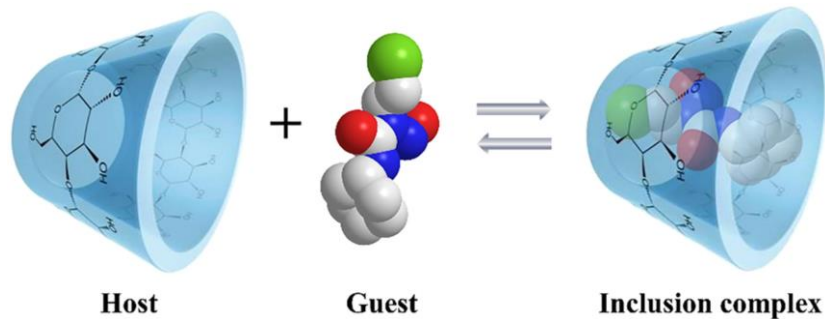
Application in Vaccine



What are Cyclodextrins (CDs)?

Properties

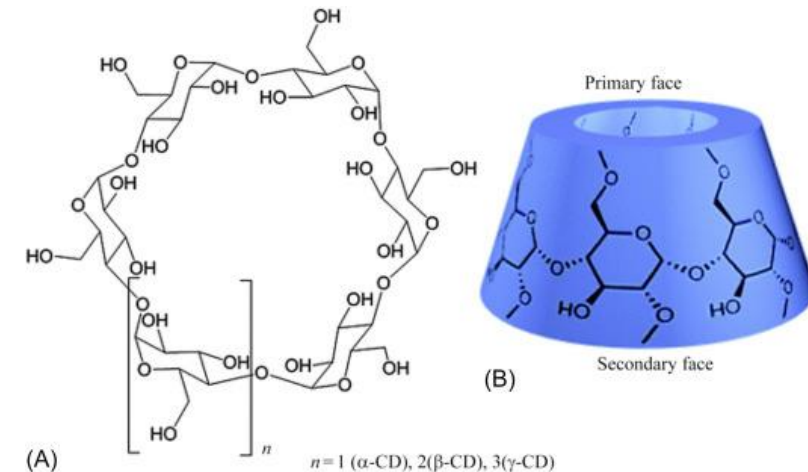
- Naturally occurring cyclic molecules, composed of sugars
- Toroid structure with **interior hydrophobic cavities** of 0.5 to 1.0 nm in diameter and **exterior hydrophilic rims**
- Diverse range of **applications**: food, pharmaceuticals, drug delivery, chemical industries, agriculture, etc.
- Often, the **aim** is to increase the solubility, dissolution rate, and stability of poorly soluble APIs.



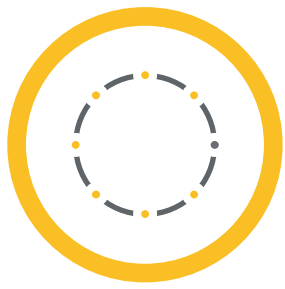
Parenteral CDs

Pharmaceutically accepted CDs and derivatives in parenteral formulations:

- α -CD
- Hydroxypropyl- β -cyclodextrin (HPBCD)
- Sulfobutylether- β -cyclodextrin (SBE- β -CD)



The structure of CDs



Cyclodextrins in Vaccines - Stabilization

Key considerations

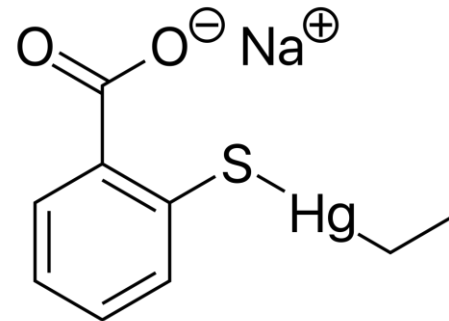
- Cyclodextrins have emerged as promising stabilizers for vaccines, ensuring the **preservation of their immunogenicity**.
- Vaccines often contain fragile antigens that can degrade during storage, leading to reduced efficacy.
- Cyclodextrins can **protect these antigens** by forming inclusion complexes and shielding them from degradation factors such as light, heat, and enzymatic activity.
- This stabilization approach has been explored for various vaccines, including protein-based vaccines, viral vector vaccines, and subunit vaccines, improving their shelf life and efficacy.



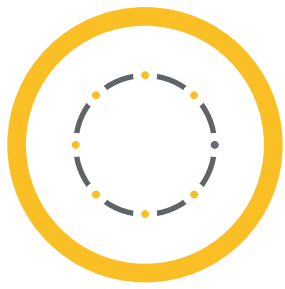
Cyclodextrins in Vaccines - Stabilization

Cyclodextrins may increase the chemical integrity of vaccine components

- It has been found that CDs may protect inactivated polio virus (IPV) from D-antigen titer loss caused by the presence of thiomersal.
- Studied on a limited panel of CDs, β -CD, γ -CD and 2-hydroxypropyl- γ -CD provided protection of IPV from the detrimental effects of the used preservative.



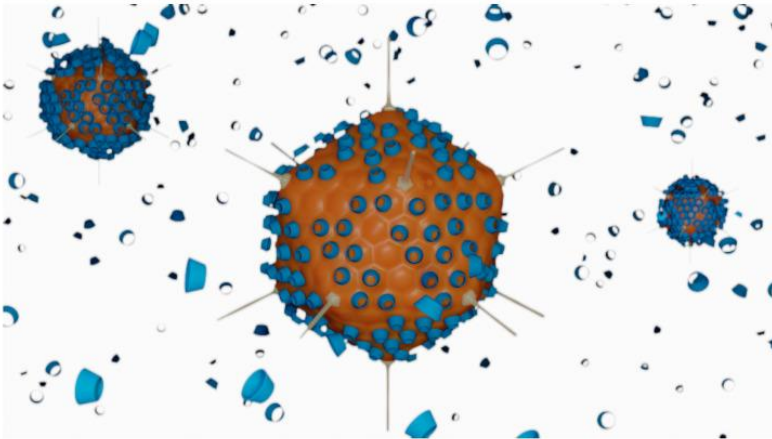
The structure of
thiomersal



Cyclodextrins in Vaccines - Adjuvants

Covid-19 vaccine J&J ad26.cov2.s

- Adenovirus type 26 encoding the SARS-CoV-2 spike glycoprotein

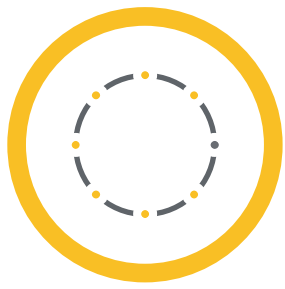


HPBCD acts as a cryopreservative, helping to stabilize the surface of the virus during the freeze-drying step of the vaccine preparation.¹



Janssen COVID-19 vaccine, sold under the brand name Jcovden

¹Braga SS, et al. Cyclodextrins in Antiviral Therapeutics and Vaccines. doi: 10.3390/pharmaceutics13030409. Pharmaceutics. 2022



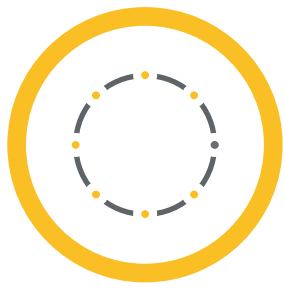
Cyclodextrins in Vaccines - Adjuvants

Sulfolipo CD - Suvaxyn

- Suvaxyn PCV™ contains inactivated recombinant Porcine Circovirus type 1, expressing the Porcine Circovirus type 2 ORF2 protein. This vaccine is used for the active immunization of pigs over the age of 3 weeks against Porcine Circovirus type 2 (PCV2).
- Sulfolipo-cyclodextrin is used as an adjuvant.



Vaccine containing sulfolipo-CD against Porcine Circovirus for veterinary use



Cyclodextrins in Vaccines - Adjuvants

HPBCD - Cevac BI L

- Cevac BI L Live freeze-dried vaccine, Massachusetts B48 and Hitchner B1 strains for the active immunization of chickens against Newcastle Disease and Infectious Bronchitis.
- HPBCD is used as an adjuvant.

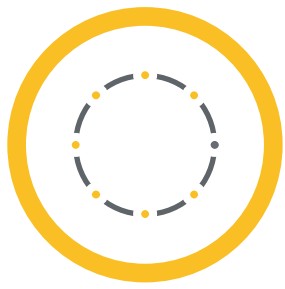


Live freeze dried vaccine, Massachusetts, B48 and Hitchner B1 strains

For the active immunization of chickens against Newcastle Disease and Infectious Bronchitis



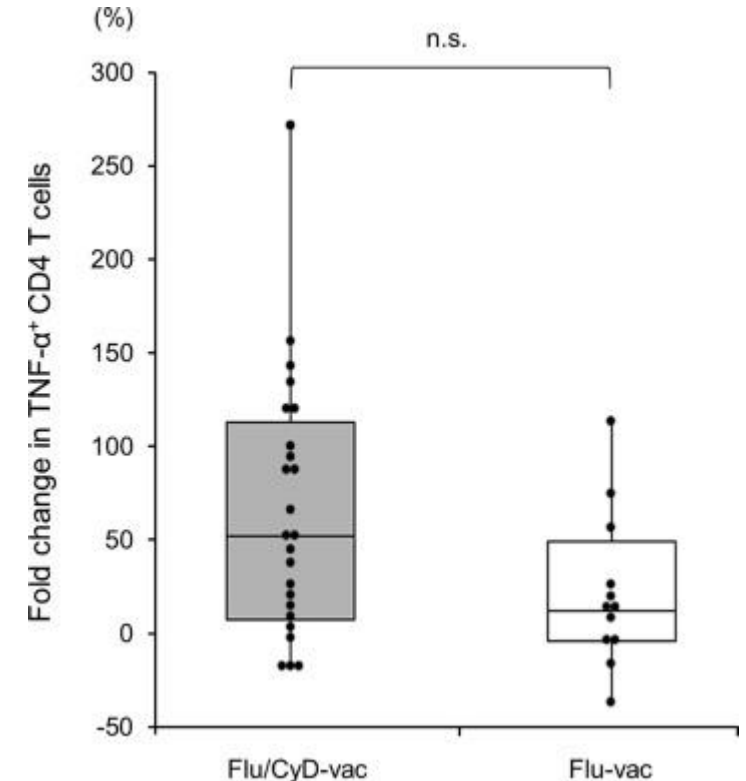
Vaccine containing HPBCD against Newcastle disease for veterinary use

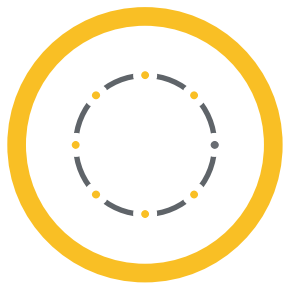


Cyclodextrins in Vaccines - Adjuvants

Daichii Sankyo - intranasal influenza vaccine

- HPBCD-adjuvanted intranasal influenza vaccine
- Able to induce lymphocyte proliferation
- Maintain a longer immune response
- Reduce the allergenic risk of the vaccine
- Boost T-cell related immune response
- Flu/CyD-vac is safe and sufficiently immunogenic despite a reduced antigen dose.
- Phase I results published in 2022 - Despite the HA antigen dose in Flu/CyD-vac being reduced by 40%, it is as immunogenic as the conventional HA vaccine, indicating the dose-sparing effect of HP- β -CyD

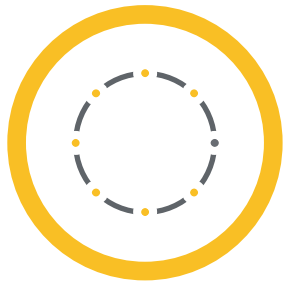




CHD-401 - A Novel CD-Based Vaccine Adjuvant

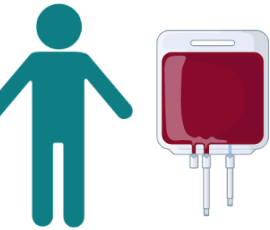
Background

- Typical adjuvants are aluminium salts, oil-in-water emulsions containing squalene, virosomes or liposome-based adjuvant system
- Only a few adjuvants have sufficient potency and low enough toxicity or lack of side-effects for clinical use
- The adjuvants market for the vaccines was estimated at USD 770 million by 2021, and is expected to grow by a CAGR of 10.5% over the forecast period
- The use of carbohydrates as vaccine adjuvants is an emerging field: a number of natural and synthetic carbohydrate structures have been used as adjuvants in clinical trials, and two have recently been approved in human vaccines: MPLA and QS-21
- While they have high biocompatibility and tolerability and a strong safety profile, naturally derived carbohydrate adjuvants are heterogeneous, difficult to obtain and, in some cases, unstable
- Cyclodextrins have also been recently discovered for vaccine adjuvant indications (J&J Covid vaccine, Daiichi Sankyo intranasal influenza vaccine in clinics) - they are chemically made, if single isomers well understood and simple/cheap to make



CHD-401 - A Novel CD-Based Vaccine Adjuvant

Methodology for screening

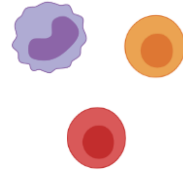


Whole blood from
Healthy donors

Centrifugation over
Ficoll-Hypaque



Peripheral blood
mononuclear cells
(PBMC)

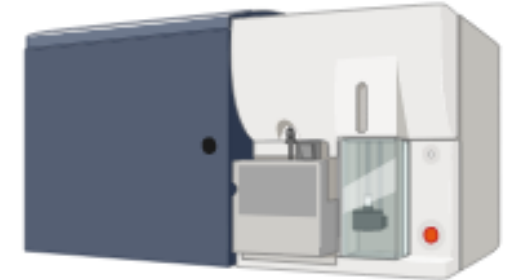


1×10^6 cells/ mL
Complete medium



24 h

Flow Cytometry

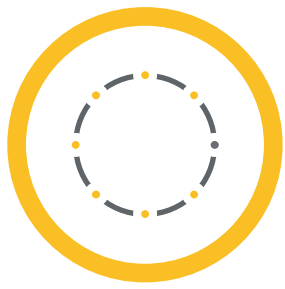


BD LSRFortessa™ X-20 Cell Analyzer

40+ CDs and two positive
controls: E. coli LPS and
purified Beta1,2 cyclic glucan
from Brucella

Activation markers

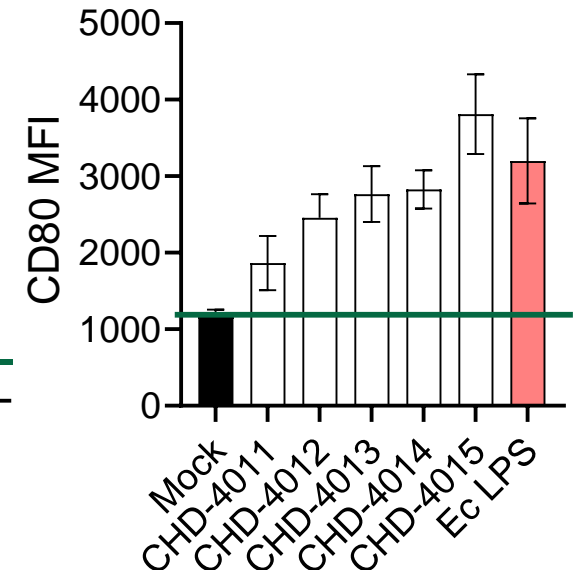
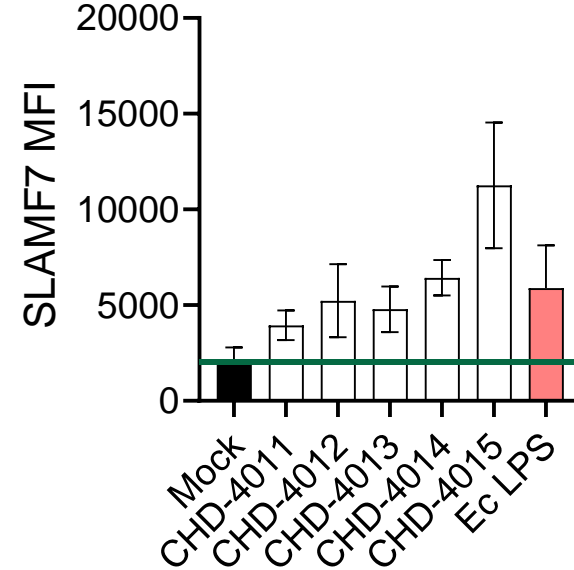
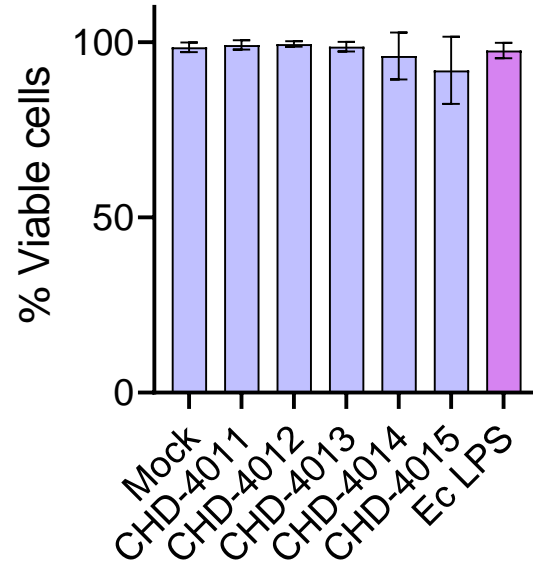
- CD14⁺ monocytes
 - SLAMF7
 - SLAMF8
 - CD80
 - CD86
 - HLA-DR (MHC-II)
- CD3⁺ T lymphocytes
 - SLAMF7
 - SLAMF8
 - HLA-DR (MHC-II)
- Viability



CHD-401 - a novel cyclodextrin-based vaccine adjuvant

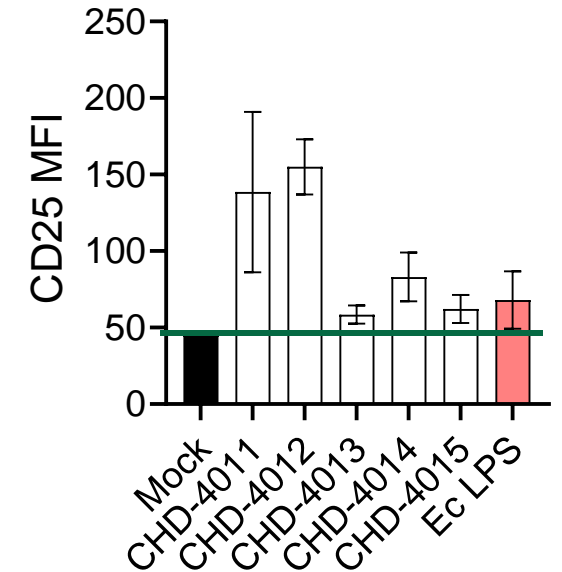
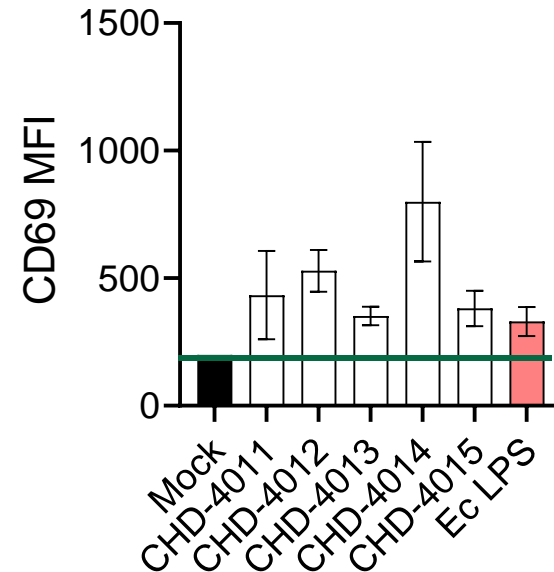
Activation of CD14+ Monocytes

Markers: SLAMF7 and CD80
(flow cytometry analysis)



Activation of CD3+ Lymphocytes

Markers: CD69 and CD25
(flow cytometry analysis)

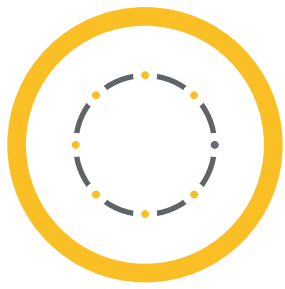




Cyclodextrins in vaccines - cryoprotection

Features of cryoprotection with CDs

- Cryoprotectants, safeguard biological samples from the damaging effects of freezing and thawing. They help maintain the integrity of cellular structures, prevent osmotic stress, and minimize the harmful effects of ice crystal formation, thereby preserving the biological material's viability.
- Cyclodextrins can protect cells and tissues by **encapsulating and shielding sensitive biomolecules**, such as proteins and enzymes, from freezing-induced damage.
- Cyclodextrins can also help **maintain the osmotic balance** during freezing and thawing processes, further enhancing cryopreservation success.
- CDs can also **improve the post-thaw recovery** of biological samples. They can act as chelators, capturing and removing ions that could cause cellular damage during thawing.
- CDs can also enhance the stability and activity of certain enzymes and proteins, allowing them to **retain their functionality after thawing**.
- The application of cyclodextrins in cryopreservation extends to various fields, including biobanking, regenerative medicine, and reproductive technologies. They have been utilized in the preservation of stem cells, sperm, oocytes, and embryos, as well as in the storage of tissues and organs for transplantation.



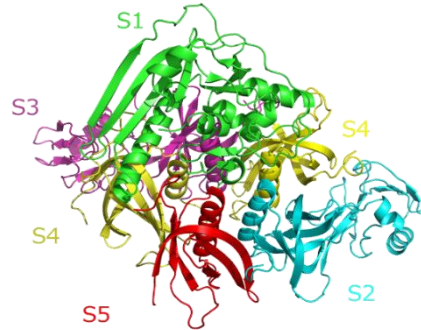
Cyclodextrins in vaccine production



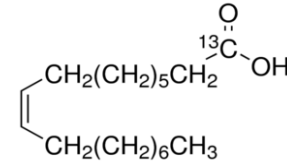
Bordetella pertussis

Production →

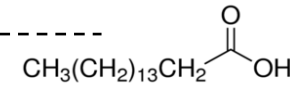
← inhibition



Pertussis toxin



Oleic acid



Palmitic acid



Bordetella pertussis cell growth

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

++ 10² to 10³ colonies

+++ full growth

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

DIMEB increases pertussis toxin production 100-fold!



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