CARBOHYDE
REVOLUTION OF
CYCLODEXTRINS

Analytical services for cyclodextrin analysis by CarboHyde

How can we ensure the quality of our product?

CarboHyde's analytical portfolio

Cyclodextrin analytics

	Molecule analysis stability	Other molecule structue analysis	Purity inpurity	Structure assay	Degredation tests	Quality, reaction control/monitoring	Residual solvent content	Thermal attributes	Water content	Validation
HPLC			X		X					X
NMR	X	X		X						
MALDI-TOF-MS						X				X
IR						X				X
TGA							X			
CE	X				X					
DLS/SLS	X	X								
Reducing sugar					X					
TLC						X				
DSC								X		
Karl-Fischer titration									X	

CarboHyde's analytical portfolio

Cyclodextrin analytics

	Molecule analysis stability	Other molecule structue analysis	Purity inpurity	Structure assay	Degredation tests	Quality, reaction control/monitoring	Residual solvent content	Thermal attributes	Water containment	Validation
DLS/SLS	X	X								
Reducing sugar					X					
TLC						X				
DSC								X		
Karl-Fischer titration									X	

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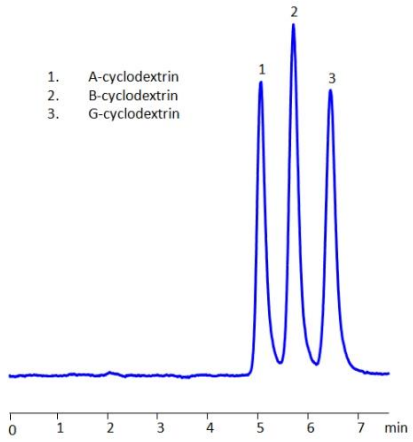
Main analytical methods

Chromatography
Mass Spectrometry (MS)
Nucleic Magnetic Resonance (NMR)

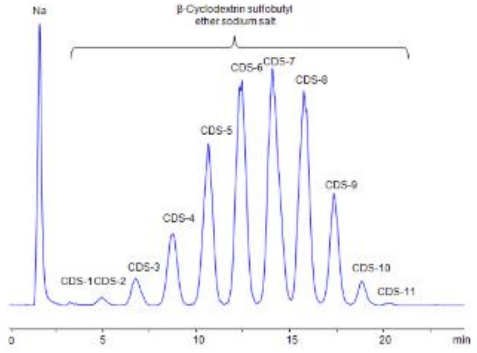
Chromatography methods

CarboHyde can provide services by HPLC

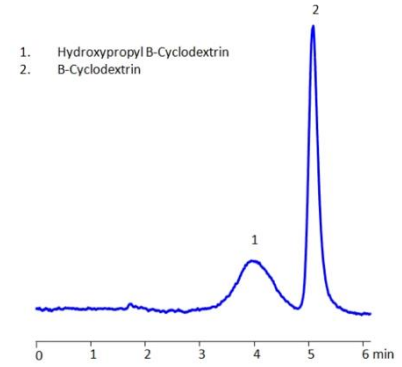
- Qualitative and quantitative determination of CDs
- Determination of impurities, purity, and assay in cyclodextrin samples



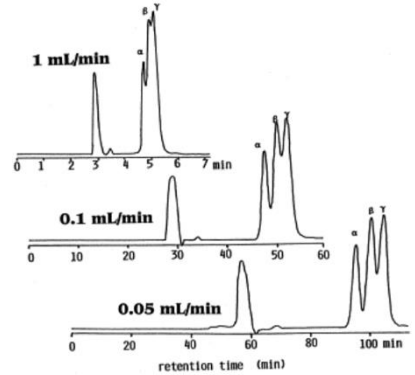
Chromatogram of β-Cyclodextrin, γ-Cyclodextrin and *α*-Cyclodextrin



Chromatogram of β-Cyclodextrins sulfobutyl-ether



Chromatogram of β-Cyclodextrin and Hydroxypropyl β-Cyclodextrin



Chromatograms of the separation of α, β, and γ-CD at different flow rates

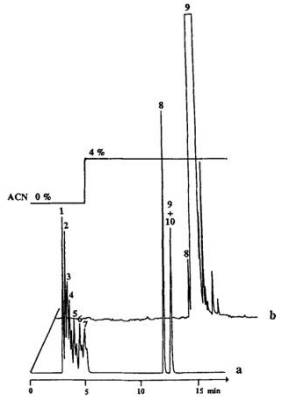
HPLC in real life

1) https://www.google.com/url?sa=i&url=https%3A%2F%2Fsielc.com%2Fhplc-method-for-analysis-of-sulfobutyl-ether-beta-cyclodextrin-sodium-sbecd&psig=AOvVaw0tLSbqhw-FpnaTdeSsUV0m&ust=1752836981317000&source=images&cd=vfe&opi=89978449&ved=0CBUQjRxqFwoTCLCXqd_gw44DFQAAAAAdAAAAABAE
2) https://sielc.com/hplc-method-for-analysis-of-b-cyclodextrin-and-hydroxypropyl-b-cyclodextrin-on-primesep-s2-column_1412
3) https://sielc.com/hplc-method-for-analysis-of-cyclodextrins-on-primesep-s2
4) Berthod, Alain, et al. "Practice and mechanism of HPLC oligosaccharide separation with a cyclodextrin bonded phase." *Talanta* 47.4 (1998): 1001-1012.
5) https://www.materianova.be/en/our-equipment/analysis-characterization/chromatographic-analysis/hplc/

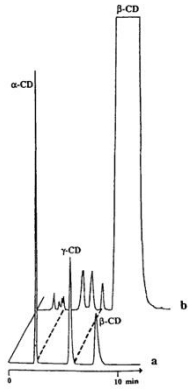
Chromatography methods

CarboHyde provides loads of different types of HPLC detectors

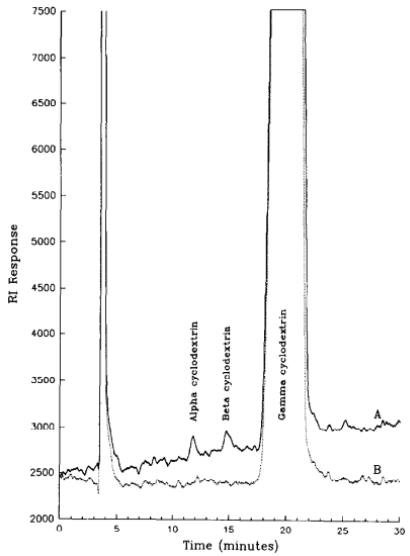
- Evaporative Light Scattering (ELSD)
- Charged Aerosol Detector (CAD)
- Refractive Index (RI)
- Mass Spectrometry (MS) detectors



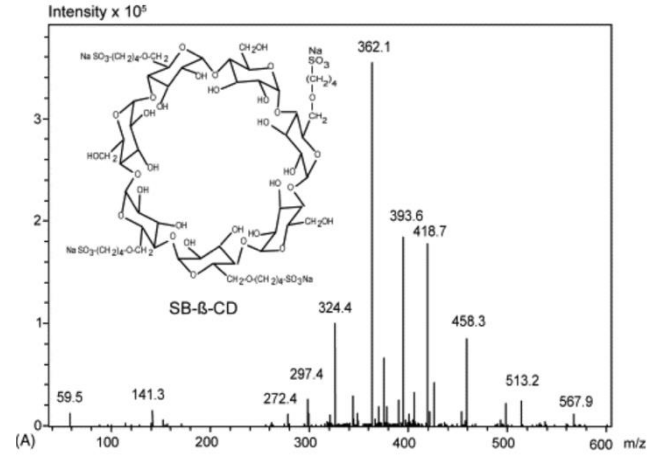
ELSD detector made chromatogram. Chromatogram of a standard mixture of β-cyclodextrin and nine potential impurities. (b) Chromatogram of a commercial β-cyclodextrin



ELSD detector made chromatogram. Chromatogram of a standard mixture of α -, β -, and γ-cyclodextrin. (b) Chromatogram of a commercial β -cyclodextrin



RI detector made chromatogram. Chromatogram of γ-CD raw materials containing (A) α- and β-CD, (B) no impurities



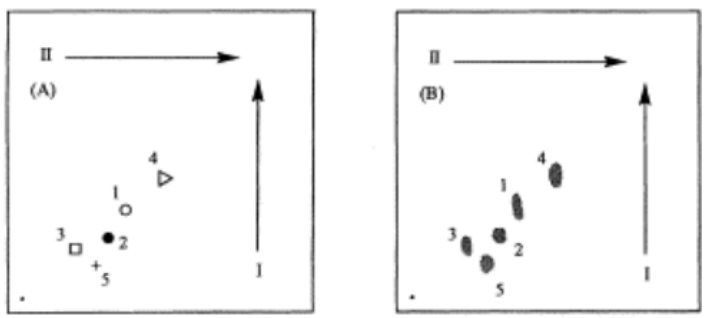
Chromatogram of mass spectrum for a 10 mM infusion of sulfobutylether-β-cyclodextrin (SB-β-CD)

1) Caron, I., C. Elfakir, and M. Dreux. "Advantages of evaporative light scattering detection for the purity control of commercial cyclodextrins." *Journal of liquid chromatography & related technologies* 20.7 (1997): 1015-1035.
2) White, Gregory, et al. "Determination of the impurity profile of γ-cyclodextrin by high-performance liquid chromatography." *Journal of Chromatography A* 625.2 (1992): 157-161.
3) Groom, Carl A., et al. "Detection of nitroaromatic and cyclic nitramine compounds by cyclodextrin assisted capillary electrophoresis quadrupole ion trap mass spectrometry." *Journal of Chromatography A* 1072.1 (2005): 73-82.

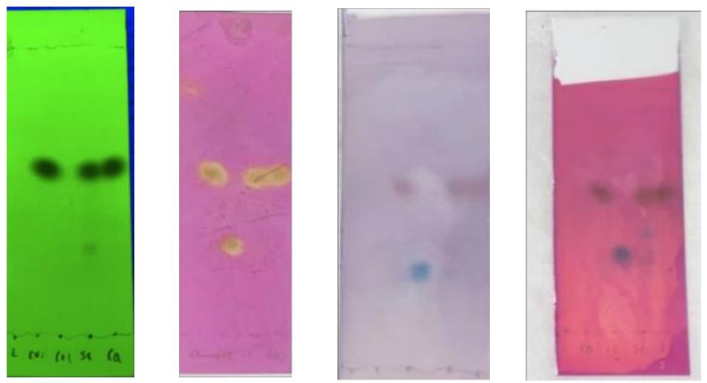
Chromatography methods

Thin layer chromatography (TLC)

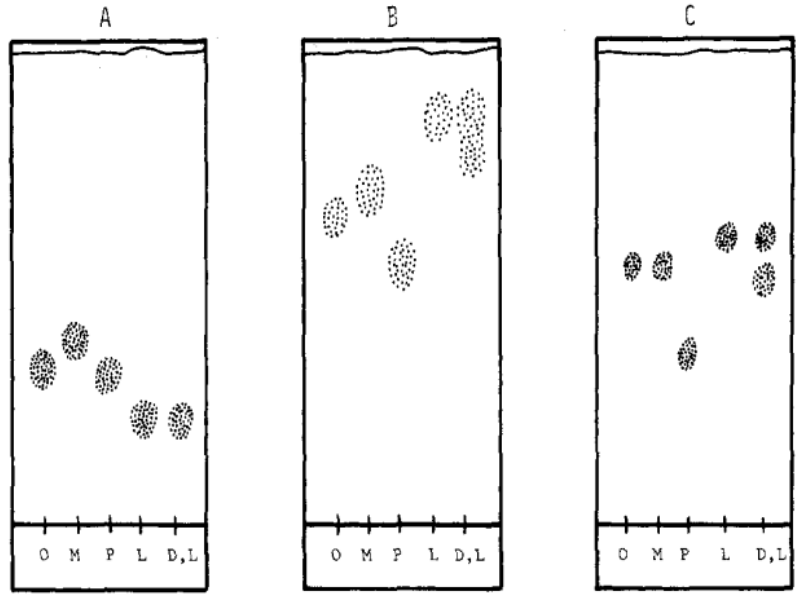
- Track the organic reactions
- Relatively cheap and easy to use



Theoretical (A) and experimental (B) 2D inclusion Reversed-Phase High-Performance Thin-Layer Chromatography with Me-β-CD of a mixture of the taurine conjugated bile acids



Several novel TLC plates are visualized using various techniques.



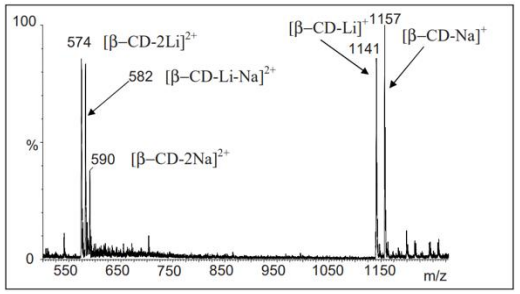
Three planar chromatograms illustrating the effect of different types of silica gels on the β-CD coverage and on-spot size.

1) Momose, Toshiaki, et al. "Method for the separation of the unconjugates and conjugates of chenodeoxycholic acid and deoxycholic acid by two-dimensional reversed-phase thin-layer chromatography with methyl β-cyclodextrin." *Journal of Chromatography A* 811.1-2 (1998): 171-180.
2) https://www.researchgate.net/figure/Developed-TLC-plates-visualized-using-various-techniques-Sequence-of-spots-1-Limonene_fig1_366646310
3) Alak, Ala, and Daniel W. Armstrong. "Thin-layer chromatographic separation of optical, geometrical, and structural isomers." *Analytical Chemistry* 58.3 (1986): 582-584.

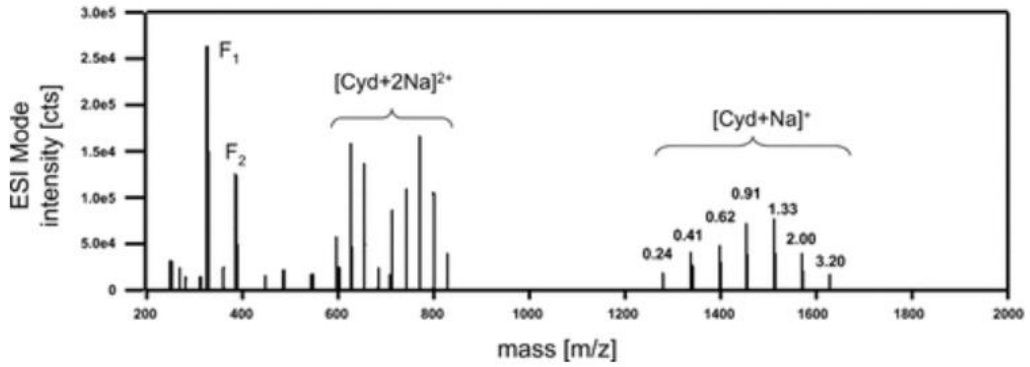
Mass Spectrometry methods

Electron Spray Ionization – Mass spectrometry (ESI-MS)

- Identification of novel CD derivatives
- The CDs decomposition can be measured



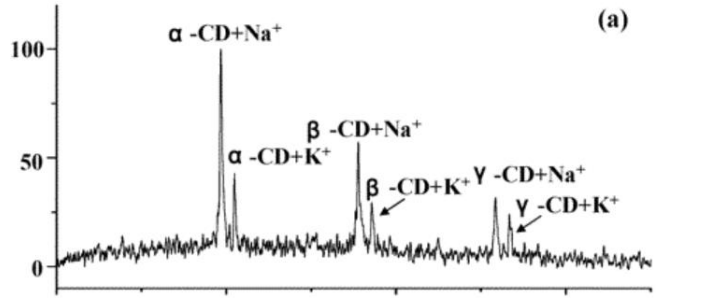
ESI mass spectrum of the β-CD lithium and sodium cations competition experiment.



CD = CyD is measured as a Na^+ (with MS values indicated) or Na_2^{2+} adducts. Fragments F1 and F2 originate from CD (possible impurities)

Matrix Assisted Laser Desorption Ionization – Time of Flight-Mass spectrometry (MALDI-TOF-MS)

- Molecular weight determination of CD derivatives, including polymers
- Distribution of the degree of substitutions



TiO_2-MALDI mass spectra of equal amounts of α-, β-, and γ-CD

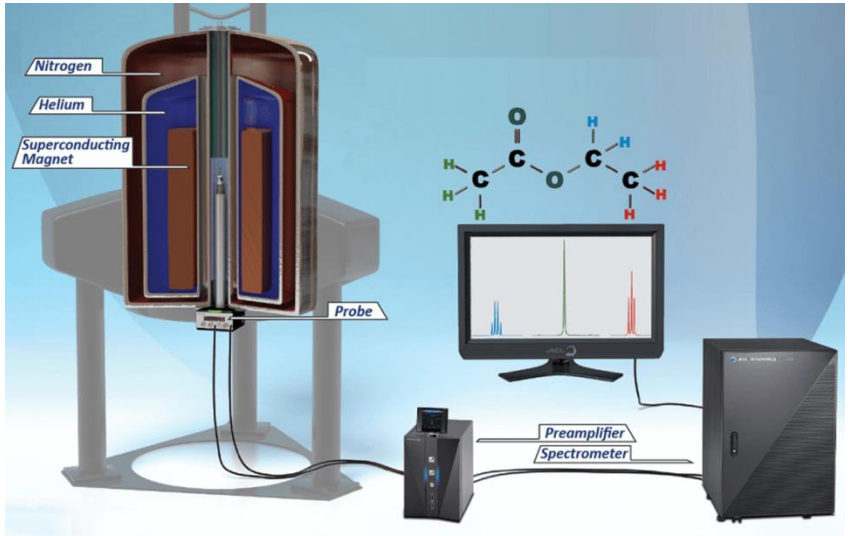
1) Short, Luke C., and Jack A. Syage. "Electrospray photoionization (ESPI) liquid chromatography/mass spectrometry for the simultaneous analysis of cyclodextrin and pharmaceuticals and their binding interactions." *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry* 22.4 (2008): 541-548.
2) Chen, Cheng-Tai, and Yu-Chie Chen. "Molecularly imprinted TiO2-matrix-assisted laser desorption/ionization mass spectrometry for selectively detecting α-cyclodextrin." *Analytical Chemistry* 76.5 (2004): 1453-1457.
3) Reale, Samantha, Erika Teixidò, and Francesco De Angelis. "Study of alkali metal cations binding selectivity of β-cyclodextrin by ESI-MS." *Annali di Chimica: Journal of Analytical, Environmental and Cultural Heritage Chemistry* 95.6 (2005): 375-381.

Nuclear Magnetic Resonance (NMR)

CarboHyde can provide services by NMR

- Structure identification <- chemical shifts (ppm) + peak height, types
- Degredation test <- avarage degree of substitution
- Hydrodynamic diameter
- Hydrodynamic diameter distribution

Same NMRs, but they have different magnetic power (GHz)



The main parts of NMR

$$\frac{7 \times A_1}{4 \times A_2}$$

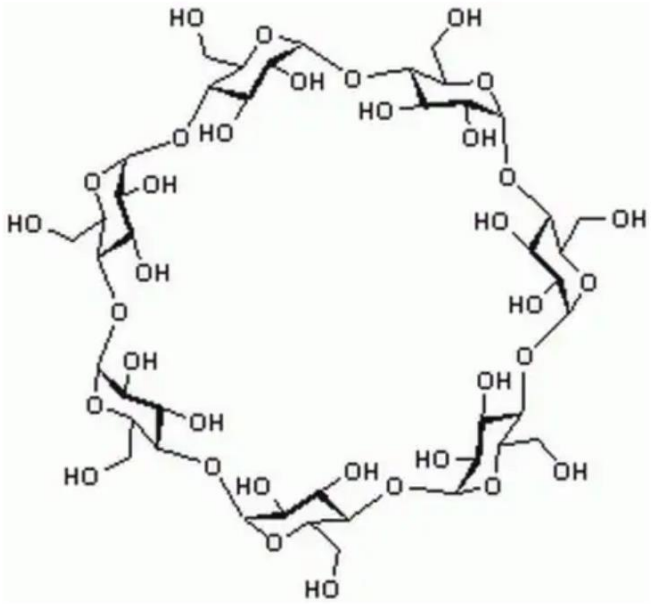
Average degree of substitution equation of SBECD in Ph-Eur.11.4. pharmacopoeia
A1 = area of the peaks due to the inner methylene protons (indicated in bold) of the sulfobutyl ether functional group;
A2 = area of the peaks due to the glycosidic protons (indicated in bold).

1) https://www.bruker.com/en/products-and-solutions/mr/nmr/ascend-nmr-magnets.html
2) https://www.jeolusa.com/RESOURCES/Analytical-Instruments/NMR-Basics

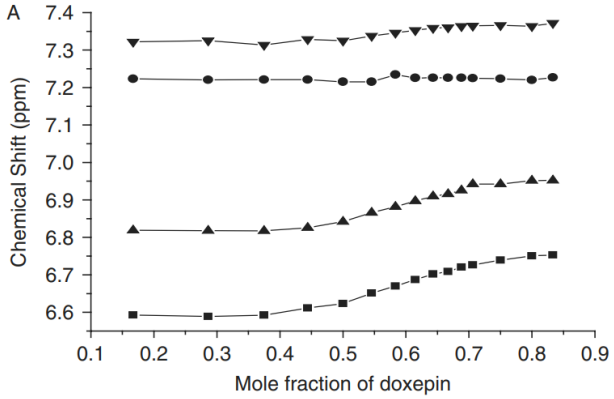
Nuclear Magnetic Resonance (NMR)

CarboHyde provides different type of NMR spectroscopy

- Provided NMR spectroscopies:
  - *TOCSY (Total Correlation Spectroscopy)*
  - *ROESY (Rotating-frame nuclear Overhauser effect correlation spectroscopy)*
  - *COSY (Correlation Spectroscopy)*
  - (NOESY (only for small, or big molecules CarboHyde usually does not have to use it))



βCD



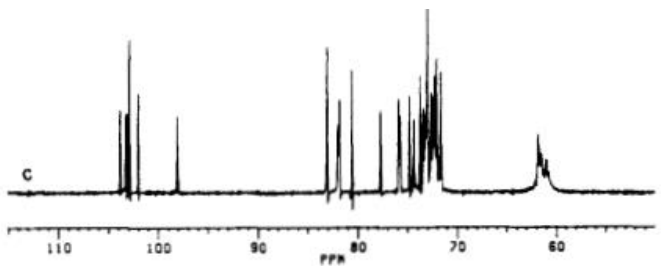
The ROESY spectrum of doxepin with a fivefold excess of βCD

1) Cruz, Jennifer R., et al. "NMR characterization of the host-guest inclusion complex between β-cyclodextrin and doxepin." *Magnetic Resonance in Chemistry* 46.9 (2008): 838-845.
2) https://www.sigmaaldrich.com/HU/hu/product/mm/239760?utm_source=google&utm_medium=cpc&utm_campaign=21891572337&utm_content=171292948458&gad_source=1&gad_campaignid=21891572337&gbraid=0AAAAD8kLQS6x0rn4OdgkODj7ZEJz5-GQ&gclid=CjwKCAjw7fzDBhA7EiwAOqJkh0Ec_AYNhug_qN27_1eqhfnliOEAQsCNmImeU5YiIOcKTmw6ojQWDBoCA3oQAvD_BwE

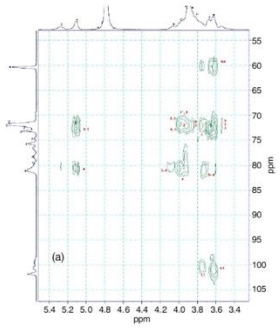
Nuclear Magnetic Resonance (NMR)

CarboHyde provides different type of NMR spectrum

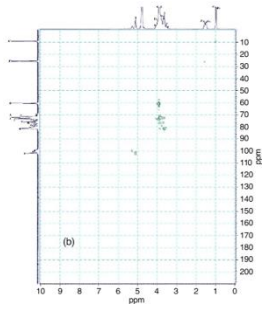
- Provided NMR spectrums:
  - ^{1}H (Hydrogen-1 spectrum): 1D NMR technique
  - ^{13}C (carbon-13 spectrum): 1D NMR technique
  - HSQC (Heteronuclear Single Quantum Coherence): 2D NMR technique
  - HMBC (Heteronuclear Multiple Bond Correlation): 2D NMR technique



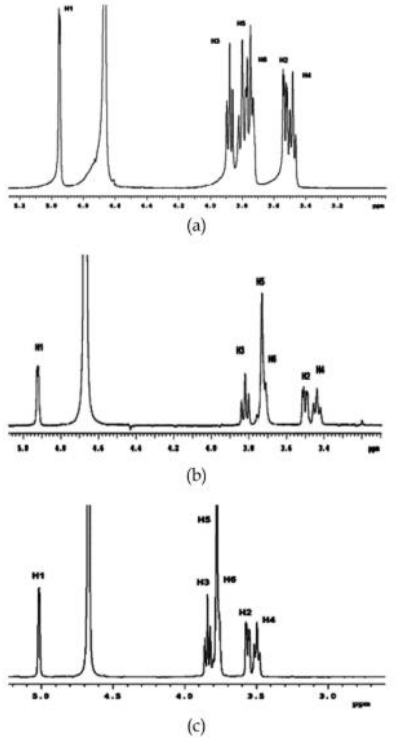
^{13}C NMR spectra of α-CD hexahydrate



HMBC NMR spectrum of hydroxy butyl-β-cyclodextrin measured in D2O.



HSQC NMR spectrum of hydroxy butyl-βCD measured in D2O.



^{1}H NMR spectra of the natural cyclodextrins. (a) α-CD, (b) β-CD, and (c) γ-CD

1) Pessine, Francisco BT, Adriana Calderini, and Guilherme L. Alexandrino. "Cyclodextrin inclusion complexes probed by NMR techniques." *Magnetic resonance spectroscopy* 3 (2012): 264.
2) https://www.researchgate.net/publication/392147825_Synthesis_and_Structural_Characterization_of_Hydroxy_Butyl-b-cyclodextrin
3) Gidley, Michael J., and Stephen M. Bociek. "Carbon-13 CP/MAS NMR studies of amylose inclusion complexes, cyclodextrins, and the amorphous phase of starch granules: Relationships between glycosidic linkage conformation and solid-state carbon-13 chemical shifts." *Journal of the American Chemical Society* 110.12 (1988): 3820-3829.

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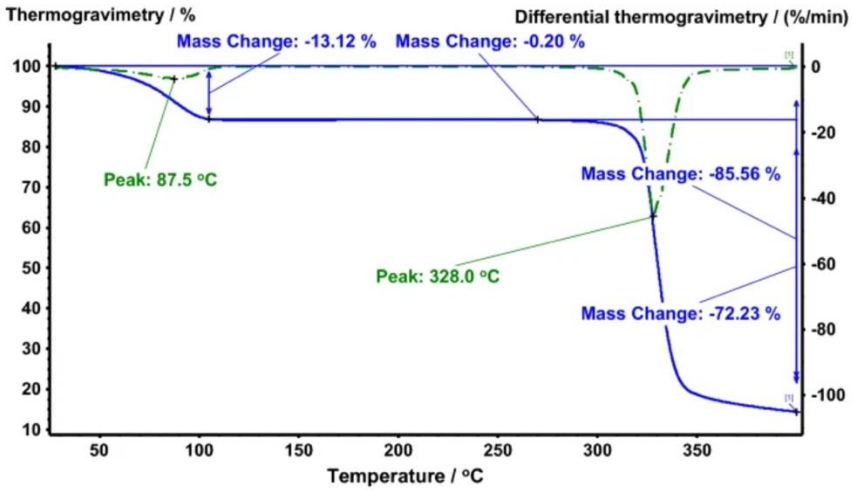
Other useful analytical methods

Other tools

Thermogravimetric analysis (TGA)

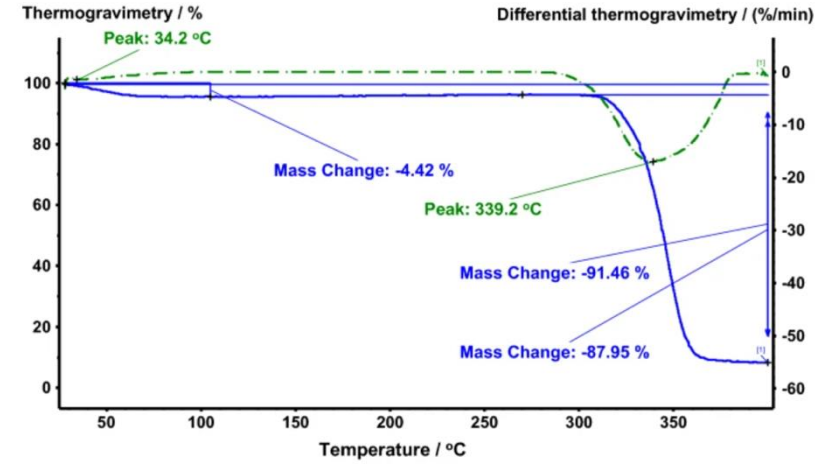
- Residual solvent content <- mass decrease <- solvent evaporate <- temperature rise

Fig. 5



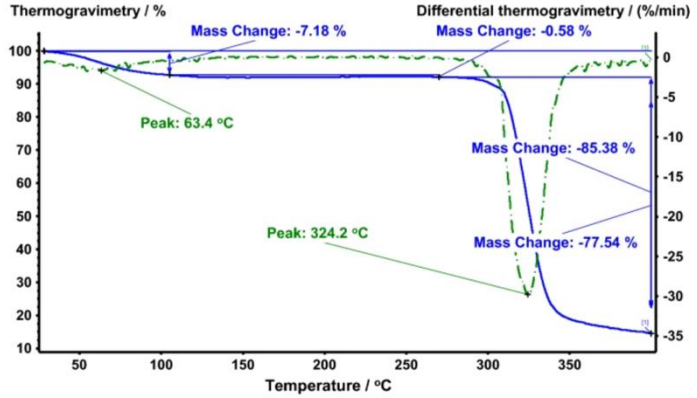
Thermogravimetry-differential thermogravimetry analysis of βCD.

Fig. 9



Thermogravimetry-differential thermogravimetry analysis of 2-hydroxypropyl-β-CD.

Fig. 7



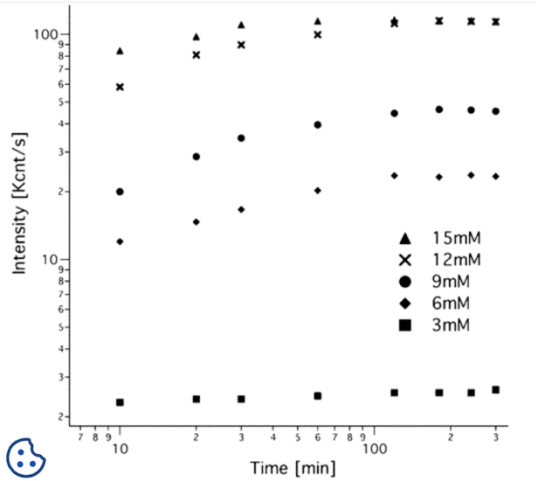
Thermogravimetry-differential thermogravimetry analysis of γCD.

1) Hădărugă, Nicoleta G., et al. "A review on thermal analyses of cyclodextrins and cyclodextrin complexes." *Environmental Chemistry Letters* 17.1 (2019): 349-373.

Other tools

Dynamic, Static Light Scattering (DLS,SLS)

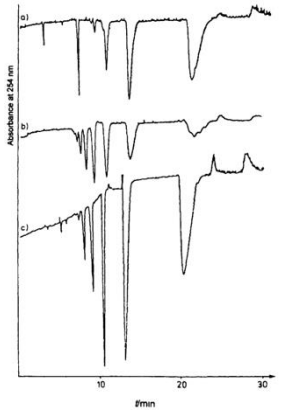
- Polymer molecule mass
- Complex diameter
- Complex diameter distribution



Scattered intensities of βCD samples as a function of the time elapsed after filtration.

Capillary electrophoresis (CE)

- Determination of interaction strength between API and cyclodextrins
- Molecule stability



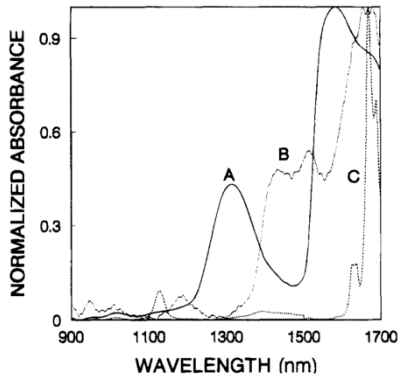
Scattered intensities of βCD samples as a function of the time elapsed after filtration.

1) Bonini, Massimo, et al. "Self-assembly of β-cyclodextrin in water. Part 1: Cryo-TEM and dynamic and static light scattering." *Langmuir* 22.4 (2006): 1478-1484.
2) Chankvetadze, B., G. Endresz, and G. Blaschke. "Charged cyclodextrin derivatives as chiral selectors in capillary electrophoresis." *Chemical Society Reviews* 25.2 (1996): 141-153.

Other tools

Infrared Spectroscopy (IR)

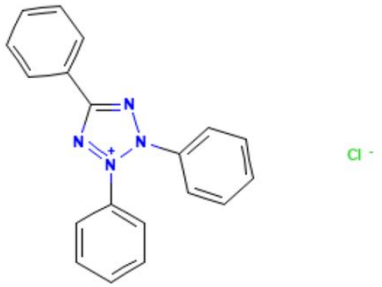
- Polymer molecule mass
- CD inclusion diameter
- CD inclusion diameter distribution



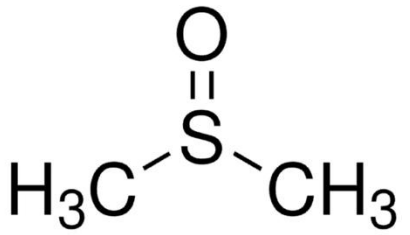
Normalized NIR absorption spectra of DzO (A), benzene (B), and α-CD in D20 (C)

Reducing sugar

- Testing for degredation
- Maximum limitation: 0,05%
- test solution: triphenyltetrazolium chloride R
  methanol R, dimethyl sulfoxide R, sodium hydroxide R [1]



Triphenyltetrazolium chloride



Dymethly-sulfoxid

1) https://pubchem.ncbi.nlm.nih.gov/compound/9283#section=2D-Structure
2) Politi, M. J., Chieu D. Tran, and Guan-Hong Gao. "Near-infrared spectroscopic investigation of inclusion complexes between cyclodextrins and aromatic compounds." *The Journal of Physical Chemistry* 99.38 (1995): 14137-14141.
3) https://www.sigmaaldrich.com/HU/hu/product/sigma/d2438?utm_source=google&utm_medium=cpc&utm_campaign=21891572337&utm_content=171292948458&gad_source=1&gad_campaignid=21891572337&gbraid=0AAAAD8kLQTbMqkZswXS32kj2W51KcWNW&gclid=CjwKCAjw7fzDBhA7EiwAOqJkhz4nfNFoZkR99mY4TquBaXsY7mspTswcPgnWWj9KcGt7EJolAsdBUBoCk-cQAvD_BwE
4) [1] Monograph of Sulfobuthylbetadex Sodium in Ph.Eur.11.4.

Other tools

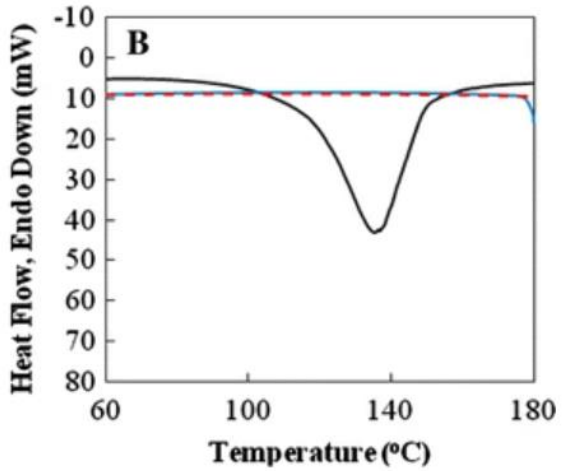
Karl Fischer titration

- It is substantial for all of the CDs complexes water containment measurements
- Easy to use
- Precise and reliable

Volumetric Karl Fischer Titrator

Differential Scanning Calorimetry (DSC)

- Thermical attributes
- Thermical stability



Pure component differential scanning calorimetry first heating (*black*, *solid*), cooling (*blue*, *solid*) and second heating (*red*, *dotted*) scans for **B** βCD.

1) https://uk.hach.com/p-kf1000-series-volumetric-karl-fischer-titrator-with-1-burette-and-2-pumps/KF1121.98?srsltid=AfmBOoqQhGR1JCkv4UOXFEvi8juOuQv503-seGSG-M7IHdVeiC_3cd8Z
2) Grandelli, Heather E., et al. "Inclusion complex formation of β-cyclodextrin and Naproxen: a study on exothermic complex formation by differential scanning calorimetry." *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 77.1 (2013): 269-277.

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For any questions:

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