Analysis of negatively charged cyclodextrins on different stationary phases: combination of ion-exchange and complex forming interactions

Julianna Szemán[1], Róbert Iványi[2], Krisztina Ludányi[3], Borbála Dalmadi-Kiss[3], Lajos Szente[1], Vígh Gyula[4], †Gábor Varga[5]

[1]CycloLab Kft, Budapest, Hungary (e-mail: szeman.j@cyclolab.hu)
[2]Bio-Sol-Dex Kft., Kecskemét, Hungary
[3]Semmelweis University, Department of Pharmaceutics, Budapest, Hungary
[4]Texas A&M University, Department of Chemistry, College Station, TX 77843-3255, USA
[5]ChiroQuest Kft., Budapest, Hungary

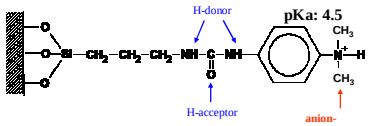
Introduction

The statistically substituted cyclodextrins (CDs) consist of a very large number of components (homologues and isomers bearing different numbers of substituents at different positions). Using even the most sophisticated analytical methods the separation of all components is beyond the possibility, however, the characterization of such mixtures with well-tailored HPLC analysis is possible. The resulting fingerprints are suitable for e.g. monitoring the production process, comparing different batches etc.

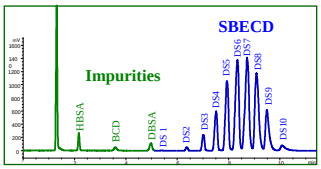
A novel, special stationary phase, CD-Screen-DAP was developed 3 years ago for the analysis of statistically substituted anionic CD derivatives. The separation process combines ion-exchange and complex forming interactions. The component groups of sulfobutylether β-cyclodextrin (SBECD) could be separated according to the number of substituents (DS) on the CD rings. Moreover, the synthesis-related impurities (BCD, hydroxybutane sulfonic acid - HBSA and its dimer - DBSA) of the substance could also be analyzed. However, the reagent used for preparation of the CD-Screen-DAP stationary phase was too toxic, therefore, further development was necessary to find other reagents to prepare stationary phases that have similar separation potency as the CD-Screen-DAP column. The selected new functional groups contain aromatic rings for inclusion complex formation with CDs and the pKa values of the functional groups were taken into consideration. Other point of view was to use less hazardous, easily available reagents.

In this work 4 new stationary phases were prepared using different functional groups, and tested with various CD derivatives. The complex forming abilities of the CD derivatives with these functional groups on the stationary phases were evaluated aiming at the separation of linear degradation products from CDs.

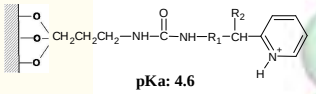
Structure of CDScreen-DAP stationary phase



Separation of anionic CDs is based on:
- **Anion-exchange**
- **H-bonding**
- **Inclusion**
- **Apolar-apolar interaction**

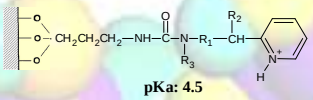


CDS-PEA
(2-piridyl)alkyl-carbamide



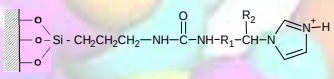
pKa: 4.6

CDS-MAEP
2-(2-piridyl)alkyl-N-alkyl-carbamide



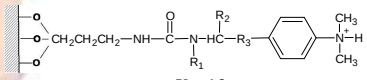
pKa: 4.5

CDS-API
(1-imidazolyl)alkyl-carbamide



pKa: 6.8

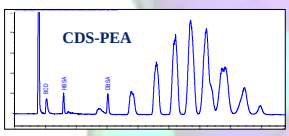
CDS-DAB
((4-dimethylamino)aryl)alkyl-carbamide



pKa: 4.9

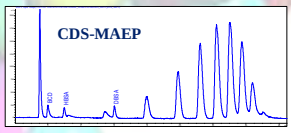
Separation of the components of SBECD and its impurities

Tasks:
- Quantitative analysis of the impurities of SBECD (BCD, HBSA and DBSA)
- Determination of the component distribution of SBECD according to the degree of substitution (DS)



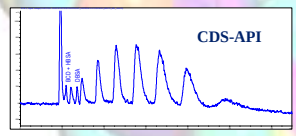
pH 4,5; Gradient: 0 - 12 min B: 3 – 70 %

- weak complex formation ⇨ short retention of BCD
- SBECD separated according to DS
- HBSA and DBSA are well separated



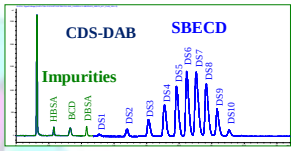
pH 4.5; Gradient: 0 - 15 min B: 3 – 60 %

- weak complex formation ⇨ short retention of BCD
- SBECD separated according to DS
- HBSA and DBSA are well separated



pH 6.2; Gradient: 0 - 15 min B: 3 – 60 %

- very weak complex formation ⇨ BCD, DS=1 and DS=2 are not separated
- the tasks could not be solved with further optimization



pH 4.5, Gradient: 0 - 10 min B: 10 – 40 %, 20 min 60 %

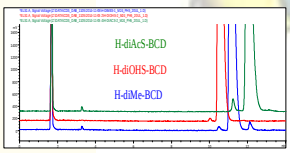
- strong complex formation ⇨ long retention time of BCD
- SBECD separated according to DS
- HBSA and DBSA are well separated
- **the separation is very similar to that obtained with CD-Screen-DAP column**

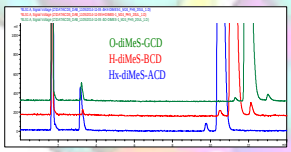
Purity test of single isomer sulfated cyclodextrin derivatives

The single isomer sulfato cyclodextrin derivatives are widely used as chiral selectors in capillary electrophoresis. The purity test of chiral additives is very important during the preparation and purification. Therefore, the separation of isomeric impurities of single isomer sulfato cyclodextrin derivatives was tested on CDS-DAB column. The separation potency of the column was examined with a decomposed sulfated sample and with an in-process sample of single isomer carboxymethylated CD derivative, as well.

Tested substances:

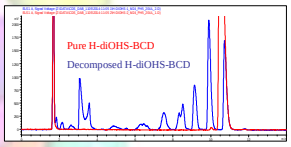
H-diAcS-BCD:	Heptakis (2,3-diacetyl-6-sulfato)-β-cyclodextrin
H-diOHS-BCD:	Heptakis (2,3-dihydroxi-6-sulfato)-β-cyclodextrin
H-diMeS-BCD:	Heptakis (2,3-dimethyl-6-sulfato)-β-cyclodextrin
Hx-diMeS-ACD:	Hexakis (2,3-dimethyl-6-sulfato)-α-cyclodextrin
O-diMeS-GCD:	Octakis (2,3-dimethyl-6-sulfato)-γ-cyclodextrin
O-diMeCM-GCD	Octakis (2,3-dimethyl-6-carboxymethyl)-γ-cyclodextrin



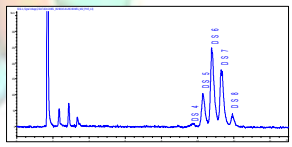


CDS-DAB column, Eluent A: buffer pH 5.0; Eluent B: acetonitrile
Gradient: 0 – 10 min B: 12 - 40 %

- **The selectivity of CDS-DAB column proved to be suitable to separate of isomeric impurities of single isomer sulfated CD derivatives.**
- **CDS-DAB column was successfully used to separate the degradation products of sulfated CDs, as well as to follow the carboxymethylation process of CD derivatives.**



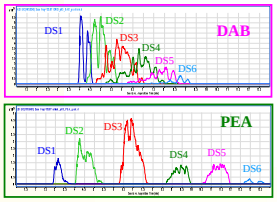
Separation of degradation products of H-diOHS-BCD



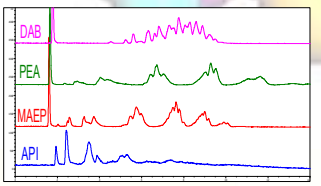
In process control of O-diMe-CM-GCD

Analysis of statistically substituted carboxymethylated β-cyclodextrin (CMBCD) derivative

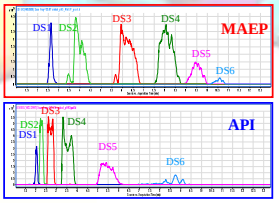
HPLC-MS: Extracted ion chromatograms



HPLC-ELSD analysis



HPLC-MS: Extracted ion chromatograms



- **CMBCD was separated according to the DS on API, MAEP and PEA columns (mainly ion exchange – complex formation is negligible).**
- **The MS spectra of the peaks recorded using CDS-DAB column verified the combined effect of ion exchange and inclusion complex formation. The desired separation of the components can be achieved with changing the pH, ionic strength and solvent gradient (more detailed fingerprint or separation according to the DS)**

Materials and methods

SBECD (Dexolve™), CMBCD (DS 3.5) and single isomer sulfated cyclodextrin derivatives. The solvents and reagents originated from Sigma-Aldrich and Merck Ltd.

HPLC-ELS method: The measurements were performed on an Agilent 1100-1200 instrument, using Alltech 3300 light scattering (ELS) detector. The columns (250 mm x 4.0 mm ID) were filled with the prepared modified silica stationary phases (particle size: 5 µm). The column was thermostated at 25 °C. Mobile phase A was 0.5% triethylamine and acetic acid to set the solution pH to the desired value. Mobile phase B was acetonitrile. The eluent flow rate was 1.4 mL/min. The temperature of ELS detector was 70°C, and 1.5 L/min flow of nebulizer nitrogen gas was applied.

HPLC-MS method: The HPLC/MS measurements were performed on an Agilent 1200 HPLC coupled with an 6460 QQQ instrument. The HPLC separation was performed on the same column as HPLC-ELS measurements. Mobile phase A was 36 mM ammonium-acetate solution to set the solution pH to the desired value. Mobile phase B was acetonitrile. The eluent flow rate was 1.4 mL/min. The ion source was an Jet Stream ESI source in negative mode using the next parameters: m/z 500–3000; fragmentor voltage 135 V, gas temperature 300°C, gas flow 12 L/min, nebulizer gas 45 psi, sheat gas flow 11 L/min, sheat gas temperature 400°C, capillary voltage: 3500 V.

Acknowledgement:
The authors are grateful for their valuable technical assistance of Ms. Zs. Zachár and Ms. O. Nemes.

Summary

- **Four stationary phases containing different functional groups were prepared to characterize anionic cyclodextrin derivatives.**
- **It was proved that CDS-DAB [((4-dimethylamino)aryl)alkyl-carbamide] combine the effect of ion exchange and inclusion complex formation.**
- **The separation properties of the newly developed CDS-DAB stationary phase are very similar to the earlier prepared CD-Screen-DAP stationary phase.**
- **A novel HPLC column with the desired features was successfully manufactured, using less hazardous reagents.**

PBA 2015. Tbilisi, Georgia