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Single Isomer Cyclodextrin Derivatives as Chiral Selectors in Capillary Electrophoresis

Introduction

It is widely recognized today that chirality is an important modulator of the effects and properties of chiral substances in a variety of fields such as pharmacology, agrochemistry, food chemistry, environmental chemistry, etc. The phenomenon of chirality exists in all biological systems. Therefore, analytical methods for the determination of single enantiomers in different natural and industrial samples are required. Cyclodextrins (CDs) play an important role as chiral selectors in capillary electrophoresis (CE) and other chromatographic techniques. CD assisted CE (CD–CE) has become an attractive alternative to HPLC for chiral analysis, due to the intrinsic properties of both CE (high separation efficiency, speed of analysis, low reagent consumption, and small sample requirement) and CDs (good enantiorecognition abilities, high water solubility, UV transparency, and wide assortment of different neutral, cationic and anionic CDs with different functional groups [1], [2]).

In CD–CE, the enantiomers having identical physico-chemical properties are discriminated based on their different interaction affinity to the CDs present in the BGE.

Although many separation problems can be solved with natural CDs, the use of CD derivatives has several advantages, such as higher solubility and increased selectivity due to the ionic substituents of the molecules. The properties of the selectors can significantly influence their separation potency especially in the electromigration techniques, such as CE and electrokinetic chromatography (EKC). Large number of CD derivatives are now used in CE for chiral analysis.

Application of CDs as chiral selectors for enantiorecognition is fundamentally based on complexation (by inclusion or external) of at least a part of the analyte and various interactions between analyte and functional moieties (hydroxyls or different substituents) of the CD rims. Therefore, position and structure of the substituents can play an important role in the enantioseparation ability of a CD derivative.

CD derivatives

The hydroxyl groups present on the rim of the CD (αCD contains 18, βCD 21 and γCD 24 hydroxyl groups [3]) can be easily modified by chemical reactions with various functional groups (see Fig. 1). The degree of substitution (DS) indicates how many of the hydroxyl groups in the CD are substituted in average; the number starts with 0 for a totally unsubstituted CD up to 18, 21 or 24 for α-, β- and γCD, respectively when hydroxyl groups are completely modified. In the case of statistically substituted CD derivatives the DS is an average number [4]. It is usually not integer as randomly substituted derivatives are mixtures of several hundreds of homologues/isomers of more or less similar structure. The single isomer CD derivatives (SIDs) contain only one isomer. Typical SIDs are the mono-substituted CDs (having one substituent in a molecule) and the persubstituted CDs (having all OH groups substituted or at least all OH groups in the same positions). There are of course other SIDs as well, e.g. heptakis(2,6-di-O-methyl)-βCD. The SID should contain at least 90% of the specific isomer.

The CD derivatives can be distinguished in different ways:

- non-ionic or ionic (neutral, anionic, cationic, amphoteric)
- type of functional group (methyl, sulfate, sulfobutyl, carboxylate, amino etc.)
- monomer or polymer
- substitution pattern: randomly substituted or single isomer CD derivatives (See Fig. 2)

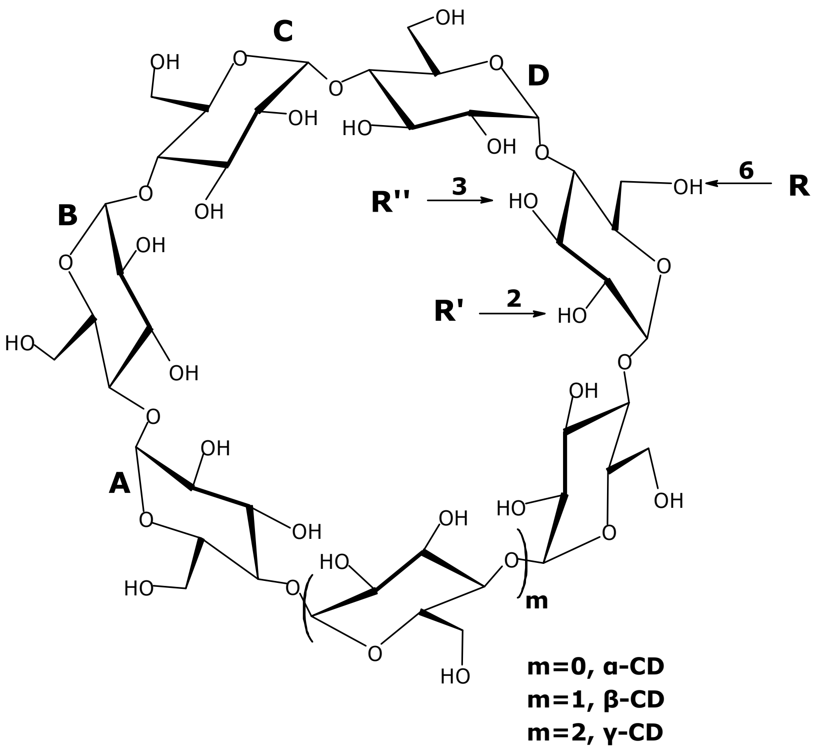

Figure 1: Structure of CD; R, R' and R'' standing for potential functional groups

CD derivtatives

Randomly substituted CDs

- Non ionic CDs
  - RAMEB
  - HPBCD
  - etc.
- Anionic CDs
  - CMBCD
  - SBEBCD
  - etc.
- Cationic CDs
  - QABCD

Single isomer CDs

- Anionic CDs
  - HSBCD
  - OSGCD
  - HDABCD
  - etc.
- Cationic CDs
  - NH_2-BCD
  - IPABCD
  - PABCD
  - etc.

RAMEB: randomly methylated βCD
HPBCD: hydroxypropylated βCD
CMBCD: carboxymethylated βCD
SBEBCD: sulfobuthylated βCD
QABCD: quaterner-ammonium βCD

HSBCD: heptakis-6-O-sulfo-βCD
OSGCD: octakis-6-O- sulfo-βCD
HDABCD: heptakis-(2,3-di-O-acetyl)-γCD
NH_2-BCD: 6-monodeoxy-6-monoamino-βCD
IPABCD: 6-monodeoxy-6-(2-hydroxy)propylamino-βCD
PABCD: 6-monodeoxy-6-(3-hydroxy)propylamino-βCD

Figure 2: Grouping of CD derivatives as a function of substitution pattern

In the case of the randomly multisubstituted derivatives, besides the limited reproducibility of their synthesis, the dissimilarity of isomeric structures can lead to uncertainty in their practical applications (*e.g.*, changeable enantiorecognition properties) [5].

The kind of CD used for chiral analysis plays an important role for robustness of an analytical procedure. The resolution of racemates, for example, depends not only on the DS but also on the substitution pattern and position as well as on the purity of the CD used [6]. In addition to the separation efficiency also the migration times and migration order of the compounds may be influenced by the degree and the locus of substitution [7]. No wonder that the batch-to-batch variations of random substituted derivatives are also influential.

Both SIDs and randomly substituted CDs are commercially available. SIDs are usually of higher price because of the complexity of the synthesis and purification. SIDs help to understand the interactions between the analyte and the selector.

There can be enormous differences between the separation efficiency of SIDs and randomly substituted CDs having the same substituents.

Anionic single isomer derivatives

In the literature a lot of articles dealing with enantioseparation using CD-CE can be found. In the past twenty years single isomer CD derivatives got more and more attention. The first SIDs for CE were synthetized by Vígh *et al*. in 1997 [8]. These derivatives contain sulfate groups, thus obtaining permanent polyanionic charge.

As a first step, Vigh *et al*. described the synthesis of three SIDs, namely, heptakis-6-O-sulfo-β-CD (HS-βCD) [9], heptakis(2,3-di-O-acetyl-6-O-sulfo)-βCD (HDAS-βCD) [10] and heptakis(2,3-di-O-methyl-6-O-sulfo)-β-CD (HDMS-βCD) [11]. In order to further investigate the role of the cavity size in the enantiomeric separation, they synthesized three SIDs of γCD, namely octakis(2,3-di-O-acetyl-6-O-sulfo)-γCD (ODAS-γCD) [12], octakis-6-O-sulfo-γCD (OS-γCD) [13], and octakis(2,3-di-O-methyl-6-O-sulfo)-γCD (ODMS-γCD) [14], as well as three SIDs of α-CD, namely, hexakis(2,3-di-O-acetyl-6-O-sulfo)-αCD (HxDAS-αCD) [15], hexakis(6-O-sulfo)-αCD (HxS-αCD) [16] and hexakis(2,3-di-O-methyl-6-O-sulfo)-αCD (HxDMS-αCD) [17]. These selectors became commercially available and were successfully used for the separation of a large number of analytes of nonelectrolyte and weak electrolyte character in both low and high pH aqueous background electrolytes (BGEs) and acidified non-aqueous BGEs.

The advantage of this kind of selectors lies in assuring a strong interaction with any cationic analyte in addition to the analyte interaction of hydrophobic nature with the CD cavity at any pH value [18]. Sometimes, randomly substituted highly sulfated CDs succeed in the enantiomeric separation, whereas SIDs do not. For example, antiarrhytmic drugs propafenone, diprafenone and their metabolites were better enantioseparated by randomly sulfated βCD than by HDAS-βCD, HDMS-βCD and HS-βCD [19], whereas doxylamine was resolved by HS-βCD only. On the contrary, alprenolol was resolved by the randomly sulfated CD, but not by HS-βCD [20]. The randomly substituted (SCD) and single isomer sulfated CDs (HSCD) investigated in this study differed in the degree of substitution and the position of the anionic substituents. The SCD had a range of substitution from 7–11, the HSCD material had 7 sulfates/ CD. A higher degree of substitution would impart a greater anionic character to the CD that might cause a more significant impact on the electrophoretic mobility of the analyte.

Sulfated γCDs (ODAS-γCD, OS-γCD, ODMS-γCD) were used for CD-EKC enantiomer separations. OS-γCD interacts with many analytes differently than its counterpart, ODAS-γCD, and its analogous βCD derivative, HS-βCD. Often, selectivity values observed with OS-γCD were different from those of other SIDs, like ODAS-γCD, HS-βCD or HDAS-βCD. Adequate, fast separations were obtained with OS-γCD in the high pH background electrolyte for a large number of analytes [21].

HxDAS-αCD, HxS-αCD and HxDMS-αCD show less interaction affinity towards many of the analytes tested than the analogous β- and γCD derivatives [15,16,17].

Carboxymethyl CDs (CM-CDs) represent another group of negatively charged CD derivatives which have already been successfully used for the separation of enantiomers of basic compounds in CE. The currently used routine CE protocols usually apply the commercially available randomly substituted CM derivatives of α-, β- and γCDs [22]. In these randomly substituted derivatives the exact position of the CM moiety on the CD skeleton can be only partially determined and varies from batch-to-batch, therefore the results achieved in CE using different batches of randomly substituted CM-CDs have a limited reproducibility. It has been confirmed experimentally that the location of the CM group plays a significant role in the resulting enantioselectivity [23]. For this reason, Benkovics *et al.* synthesized a new family of single-isomer 2,3-di-*O*-methyl-6-*O*-carboxymethyl CDs with a high isomeric purity [24]. Although the use of these novel SIDs as chiral resolving agents has not been published yet, one can assume that the tunable ionization state of the carboxymethyl groups may result in special enantioselectivity.

Cationic single derivatives

Cationic CDs as chiral selectors have been much less used compared to anionic CDs. Cationic CDs are either strong-electrolytes, like those functionalized with quaternary ammonium groups, or weak electrolytes. Just as other CD derivatives, cationic CDs can be randomly substituted or SIDs. The first examples of cationic SIDs in the literature for CD-EKC applications were aminofunctionalized βCDs, such as the 6-A,D-dimethylamino-βCD [25], 6-amino-βCD [26], 6-deoxy-6-N-histamino-β-CD and 6-deoxy[4-(2-aminoethyl)imidazolyl]-6-N-histamino-β-CD [27].

In CycloLab Iványi *et al*. synthetized the family of single-isomer amino-β-CD derivatives containing an amino or (hydroxy)alkylamino group in one of the primary positions and applied them successfully in chiral CE. Three racemic model compounds (mandelic acid, *cis*-permethrinic acid, and *cis*-deltamethrinic acid) were separated in the experiments. One hydroxyalkyl group attached to the primary amino *N*-atom significantly increased both the enantioselectivity and the resolution compared to the primary amino-βCD, while two hydroxyalkyl moieties decreased them due to the predominance of steric hindrance [28].

Nonaqueous capillary electrophoresis (NACE) was successfully applied to the enantiomeric purity determination of *R*-flurbiprofen using 6-monodeoxy-6-mono(2-hydroxy)propylamino-β-CD (IPA-β-CD) as chiral selector. The nonaqueous BGE was made up of 20 mM IPA-β-CD, 20 mM ammonium camphor sulfonate and 40 mM ammonium acetate in methanol (Fig. 3) [29].

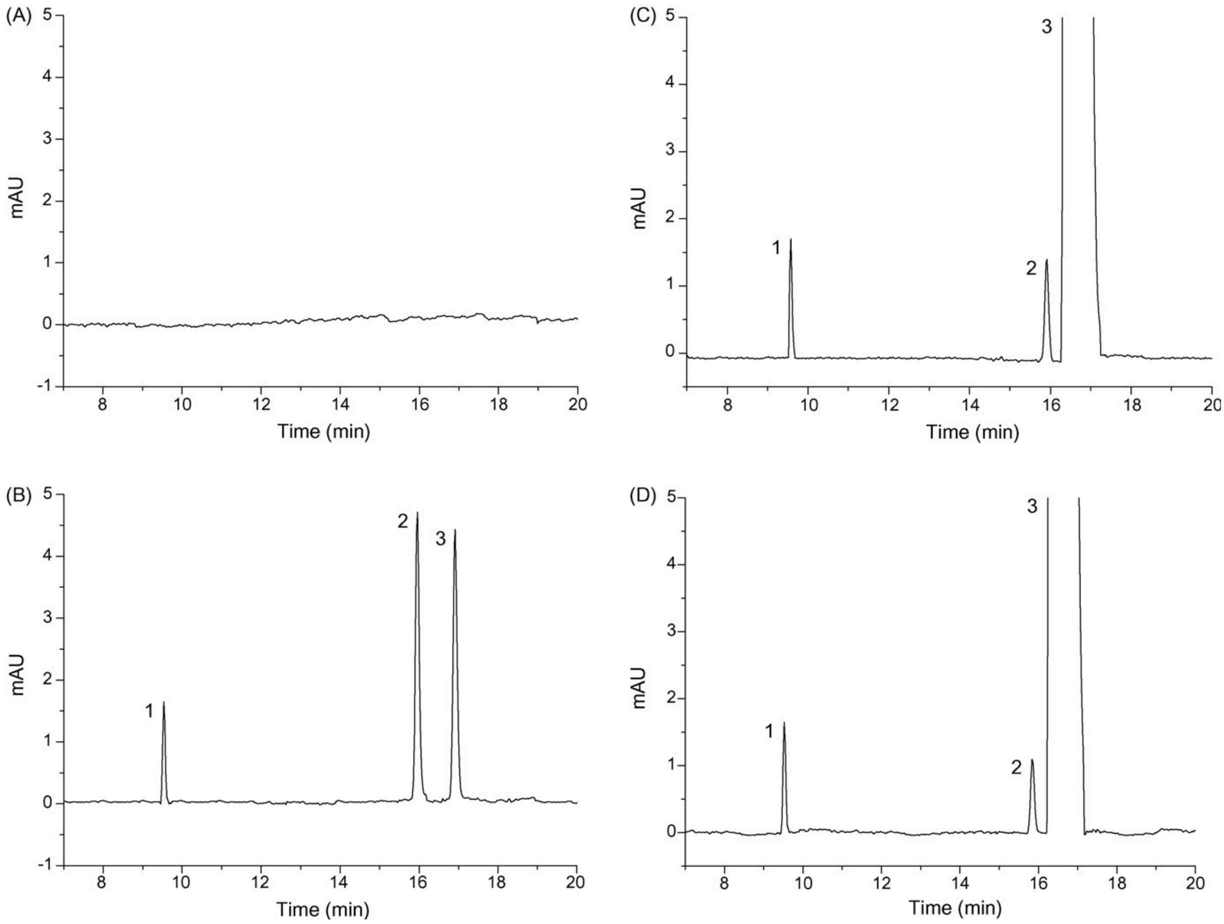

Fig. 3. Typical electropherograms of methanol (A), a methanolic solution of flufenamic acid (50 µg/mL, peak 1) and racemic flurbiprofen (48 µg/mL, peak 2-3) (B), a methanolic solution of R-flurbiprofen (2 mg/mL, peak 3) containing S-flurbiprofen (5 µg/mL, peak 2) and flufenamic acid (50 µg/mL, peak 1) (C) a methanolic solution of R-flurbiprofen (2 mg/mL, peak 3) containing S-flurbiprofen (2 µg/mL, peak 2) and flufenamic acid (50 µg/mL, peak 1) (D) in the presence of IPA-β-CD chiral selector [29]

Summary

In the case of the randomly multisubstituted derivatives, besides limited reproducibility of their synthesis, the dissimilarity of isomeric structures can lead to uncertainty in their practical applications (*e.g.*, changeable enantiorecognition properties). Only the use of single-isomer derivatives can solve this problem. However, both random and single isomer derivatives have advantages and disadvantages. Generally, the use of SIDs led to more reproducible and reliable results than the randomly substituted cyclodextins. SID cyclodextrins can be suitable to develop validated methods either in pharmaceutical or in research area.

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Review, Monomeric species, Dinuclear systems, Homo- and heterometallic sandwich-type complexes, Supramolecular chemistry

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Physical mixing, Kneading, Sonication, Amorphous form

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Endocytic uptake, Controlling plasma cholesterol levels, Lysosomal lipid storage diseases

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Nanohybrids, Glassy carbon electrode, Supramolecular host–guest recognition, Thiol-β-cyclodextrin

Analytica Chimica Acta, 2015, 892, 85-94; DOI:10.1016/j.aca.2015.08.046

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